2nd Special Conference

EACR AACR SIC

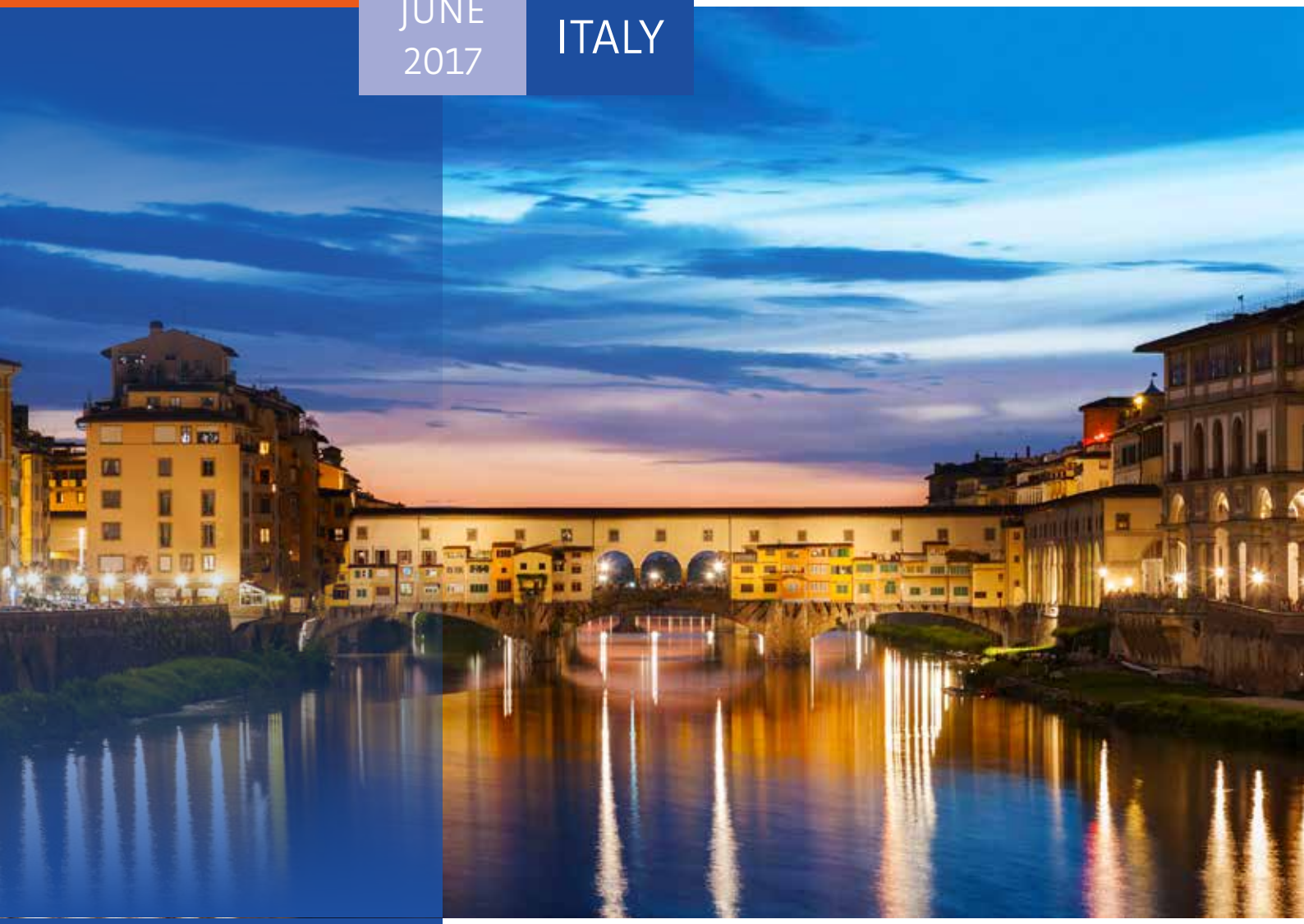
EACR-AACR-SIC SPECIAL CONFERENCE 2017

The Challenges of Optimising Immuno and Targeted Therapies

From Cancer Biology to the Clinic

24-27 JUNE 2017

FLORENCE ITALY



EACR European Association for Cancer Research

AACR American Association for Cancer Research FINDING CURES TOGETHER™

SOCIETA' ITALIANA DI CANCEROLOGIA

PROCEEDINGS BOOK

ORGANISING & SCIENTIFIC COMMITTEE

- Anton Berns - Conference Co-Chair
- Nancy E. Davidson - Conference Co-Chair
- Silvia Giordano - Conference Co-Chair
- Paola Chiarugi
- Riccardo Dolcetti
- Olivera J. Finn
- Margaret Foti
- Richard Marais
- Daniel Peeper
- Dean Post
- Jane Smith
- David Solit
- Gabriella Sozzi

24-27 JUNE 2017

FLORENCE ITALY

CONTENTS

ACKNOWLEDGEMENTS

EACR, AACR and SIC would like to thank the following organisations for their generous support of the Conference:

PROFESSIONAL EDUCATIONAL GRANTS:

- AbbVie
- AstraZeneca
- Lilly
- Pfizer

EACR, AACR and SIC express sincere thanks for the generous support of the organisations sponsoring Symposia, Keynote and Award Lectures.

EACR, AACR and SIC also wish to thank the following companies and organisations for their support of the Conference by taking part in the exhibition:

- Adaptive Biotechnologies
- ATCC - LGC Standards
- Bio-Rad Laboratories S.r.l.
- Biofield Innovation
- ChemoMetec A/S
- Don Whitley Scientific Ltd
- ENVIGO RMS
- EPHORAN Multi Imaging Solutions
- Fujifilm VisualSonics
- JPT Peptide Technologies GmbH
- Menarini Silicon Biosystems
- Merck KGaA

LETTER OF WELCOME

It is our pleasure to welcome you to the second EACR-AACR-SIC Special Conference "The Challenges of Optimising Immuno and Targeted Therapies: From Cancer Biology to the Clinic" to be held on 24-27 June 2017 in Florence, Italy.

Hosted by the European Association for Cancer Research (EACR), the American Association for Cancer Research (AACR) and the Italian Cancer Society (SIC), the EACR-AACR-SIC Special Conference 2017 will build on the success of the previous Conference, which was widely praised for the quality of its programme and inspirational contributions.

The Conference format is designed to bring together basic, translational and clinical researchers, as well as researchers working on the development of new targeted therapeutics. Leading experts will present the latest achievements of multidisciplinary research dealing with drug action and resistance and highlight challenges that require a concerted multidisciplinary effort. The programme will comprise Keynote Lectures, Plenary Symposia, Scientific Symposia, Meet the Expert sessions, Oral and Poster presentations with ample time for discussion.

We trust you will share our excitement about the exceptional scientific programme of this meeting.

Prof Anton Berns
(Conference Co-Chair EACR)

Dr Nancy E. Davidson
(Conference Co-Chair AACR)

Dr Silvia Giordano
(Conference Co-Chair SIC)

EACR
European Association for Cancer Research

EACR 25

30 June - 3 July 2018 • Amsterdam

SAVE THE DATE

for this landmark congress celebrating 50 years of the EACR

25th Biennial Congress of the

European Association for Cancer Research

Celebrating 50 years

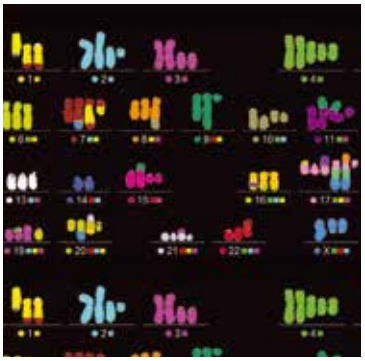
From Fundamental Insight to Rational Cancer Treatment

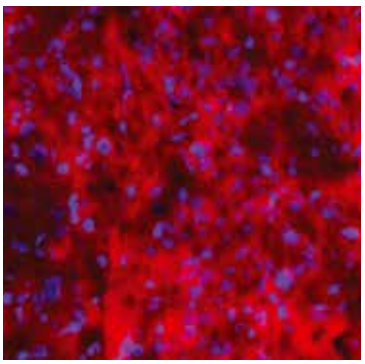
SPEAKERS CONFIRMED AS OF APRIL 2017

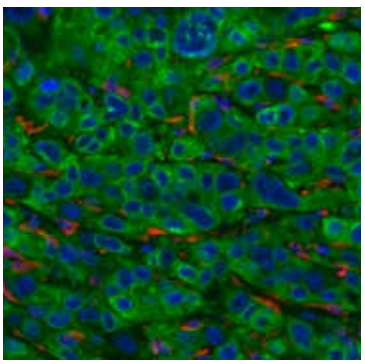
Uri Alon (Israel)
Angelika Amon (USA)
Khusru Asadullah (Germany)
Allan Balmain (USA)
Mariano Barbacid (Spain)
Alberto Bardelli (Italy)
Rene Bernards (Netherlands)
Anton Berns (Netherlands)
Jannie Borst (Netherlands)
Carlos Caldas (United Kingdom)
Hans Clevers (Netherlands)
Hugues de Thé (France)
Federica Di Nicolantonio (Italy)
Caroline Dive (United Kingdom)
Neta Erez (Israel)
Gerard Evan (United Kingdom)
Amanda Fisher (United Kingdom)
Richard A. Flavell (USA)
Richard J Gilbertson (United Kingdom)
Romina Goldszmid (USA)
Eyal Gottlieb (Israel)
J.H. Hoeijmakers (Netherlands)
Nada Jabado (Canada)
Stephen P. Jackson (United Kingdom)
Olli Kallioniemi (Finland)
Jan Korbel (Germany)
Guido Kroemer (France)
Xiale Shirley Liu (USA)
Nuria Lopez-Bigas (Spain)
Richard Marais (United Kingdom)
Frank McCormick (USA)
Ultan McDermott (United Kingdom)
Sean J. Morrison (USA)
Julia A. Newton Bishop (United Kingdom)
Klaus Pantel (Germany)
Dana Pe'er (USA)
Klaus Rajewsky (Germany)
Yardena Samuels (Israel)
Ton Schumacher (Netherlands)
Andrea Sottoriva (United Kingdom)
Michael Speicher (Austria)
Ravid Straussman (Israel)
Charles Swanton (United Kingdom)
Andreas Trumpp (Germany)
Maarten van Lohuizen (Netherlands)
Karen Vousden (United Kingdom)
Sabine Werner (Switzerland)

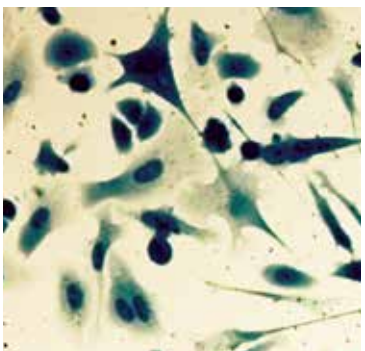
#EACR25

www.eacr25.org









2017 SCIENTIFIC CONFERENCES

Presenting the most significant research on cancer etiology, prevention, diagnosis, and treatment

AACR International Conference on Translational Cancer Medicine
Held in cooperation with the Latin American Cooperative Oncology Group (LACOG)
Conference Cochairs: Carlos L. Arteaga and Carlos Gil M. Ferreira
May 4-6, 2017 | São Paulo, Brazil

Hematologic Malignancies: Translating Discoveries to Novel Therapies
Conference Chair: Jonathan D. Licht
Conference Cochairs: Lucy A. Godley, Louis M. Staudt, and Catherine J. Wu
May 6-9, 2017 | Boston, MA

Advances in Sarcomas: From Basic Science to Clinical Translation
Conference Cochairs: Irene L. Andrulis, Ping Chi, Jonathan A. Fletcher, and Lee J. Helman
May 16-19, 2017 | Philadelphia, PA

International Conference on Malignant Lymphoma (ICML)
ICML President: Franco Cavalli
Chair, Local Organizing Committee: Michele Ghielmini
June 14-17, 2017 | Lugano, Switzerland

EACR-AACR-SIC Special Conference 2017: The Challenges of Optimizing Immuno- and Targeted Therapies: From Cancer Biology to the Clinic
Conference Cochairs: Anton J. M. Berns, Nancy E. Davidson, and Silvia Giordano
June 24-27, 2017 | Florence, Italy

Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference
Conference Cochairs: Stanley Riddell, Robert D. Schreiber, Christoph Huber, and Guido Kroemer
September 6-9, 2017 | Mainz/Frankfurt, Germany

Advances in Modeling Cancer in Mice: Technology, Biology, and Beyond
Conference Cochairs: Cory Abate-Shen, Kevin M. Haigis, Katerina A. Politi, and Julien Sage
September 24-27, 2017 | Orlando, FL

Tenth AACR Conference on The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved
Conference Cochairs: John M. Carethers, Rick A. Kittles, Christopher I. Li, and Electra D. Paskett
September 25-28, 2017 | Atlanta, GA

Tumor Immunology and Immunotherapy
Conference Cochairs: James P. Allison, Carl H. June, Miriam Merad, and Giorgio Trinchieri
October 1-4, 2017 | Boston, MA

Addressing Critical Questions in Ovarian Cancer Research and Treatment
Conference Cochairs: Robert C. Bast, Jr., Ursula A. Matulonis, and Anil K. Sood
October 1-4, 2017 | Pittsburgh, PA

Advances in Breast Cancer Research
Conference Cochairs: Myles A. Brown, Tak W. Mak, Ramon E. Parsons, and Laura J. van 't Veer
October 7-10, 2017 | Hollywood, CA

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
Scientific Committee Cochairs: Antoni Ribas, James L. Gulley, and Charles Swanton
October 26-30, 2017 | Philadelphia, PA

Prostate Cancer: Advances in Basic, Translational, and Clinical Research
Conference Cochairs: Johann S. de Bono, Karen E. Knudsen, Peter S. Nelson, and Mark A. Rubin
December 2-5, 2017 | Orlando, FL

Pediatric Cancer
Conference Cochairs: Peter C. Adamson, Nada Jabado, and Charles W. M. Roberts
December 3-6, 2017 | Atlanta, GA

San Antonio Breast Cancer Symposium
Codirectors: Carlos L. Arteaga, Virginia G. Kaklamani, and C. Kent Osborne
December 5-9, 2017 | San Antonio, TX

Learn more and register at
AACR.org/Calendar

AACR American Association for Cancer Research
FINDING CURES TOGETHER®

SOCIETA' ITALIANA DI CANCEROLOGIA

60TH ANNUAL MEETING OF THE ITALIAN CANCER SOCIETY

MILAN, 19-22 SEPTEMBER 2018

CARE AND CURE OF CANCER PATIENTS:

BRIDGING BASIC RESEARCH INTO CLINICAL SETTING

Dear colleagues,

We are pleased to invite you to the 60th Annual Meeting of the Italian Cancer Society (SIC), which will be held in Milan, 19-22 September 2018.

The meeting will include lectures, oral presentations and poster discussions highlighting the best cancer research and medicine from Italian institutions bridging basic and translational studies to clinical practice.

The meeting will focus on emerging areas of cancer research and specific disease sites and will be enriched by the presence of national and international scientists who will share new concepts, tools and techniques for a better knowledge and cure of cancer.

Senior and early career investigators will benefit of exciting science and dynamic interactions with the opportunity to establish collaborative networks and team building among Italian cancer scientists.

Traditionally, the lecture in memory of Prof. Giorgio Prodi will be given by an internationally recognised Italian investigator.

The Meeting will take place at the historic home of the University of Milan. This location is very close to the city centre, at walking distance from the most famous historical and cultural attractions in Milan.

We look forward to welcoming you all in Milan,

Gabriella Sozzi

ACCREDITATION INFORMATION

ACCME ACCREDITATION INFORMATION - CONTINUING MEDICAL EDUCATION (CME)

ACCREDITATION STATEMENT

The American Association for Cancer Research (AACR) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education activities for physicians.

CREDIT DESIGNATION STATEMENT

AACR has designated this live activity for a maximum of 20,25 *AMA PRA Category 1 Credit(s)™*. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Credit certification for individual sessions may vary, dependent upon compliance with the ACCME Accreditation Criteria. The final number of credits may vary from the maximum number indicated above.

CLAIMING (CME) CREDIT

Physicians and other health care professionals seeking *AMA PRA Category 1 Credit(s)™* for this live continuing medical education activity must complete the CME Request for Credit Survey. Certificates will only be issued to those who complete the survey. The Request for Credit Survey will be available via a link on the CME tab of the ECCO website and via email. *Your CME certificate will be sent to you via email after the completion of the activity.*

STATEMENT OF EDUCATIONAL NEED, TARGET AUDIENCE, AND LEARNING OBJECTIVES

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012. The most common causes of cancer death are cancers of:

- *lung (1.59 million deaths)*
- *liver (745 000 deaths)*
- *stomach (723 000 deaths)*
- *colorectal (694 000 deaths)*
- *breast (521 000 deaths)*
- *oesophageal cancer (400 000 deaths).*

There have been unprecedented improvements in immunotherapies, opening the avenue to develop combinatorial targeted and immunotreatment.

The conference will seek to educate physicians on the landscape of emerging cancer treatments and the use of novel profiling methods as a means for optimizing treatment selection. Physicians participating in this CME activity will gain knowledge on the standard use of targeted therapies and immunotherapies for patients with cancer.

This Special Conference will bring together basic, translational and clinical scientists, as well as physicians engaged in the development of new therapeutics. It will provide up-to-date information on both targeted and immunological approaches to the treatment of patients with cancer, highlighting areas of significant advancement and diseases that remain underserved. The program will focus on the challenges to the development of precision medicine approaches including tumor heterogeneity, the development of drug resistance, and ongoing efforts to better stratify patients for targeted therapy and to increase the numbers of patients with durable clinical responses.

After participating in this CME activity, physicians should be able to:

1. Demonstrate an increased understanding of how particular targeted agents and immunotherapies are selected for use in patients with advanced cancer.
2. Integrate the use of molecular profiling methods to select the most appropriate treatment.
3. Evaluate the emerging cancer treatments and their underlying biology.
4. Establish a greater understanding of mechanisms of drug resistance and how combination therapies may increase treatment response or durability.

DISCLOSURE STATEMENT

It is the policy of the AACR that the information presented at AACR CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, AACR will provide information that Scientific Program Committee members and speakers have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity. This disclosure information is available on page 10.

ACKNOWLEDGMENT OF FINANCIAL OR OTHER SUPPORT

This activity is supported by professional educational grants and will be disclosed at the activity.

QUESTIONS ABOUT CME?

Please contact the Office of CME at +1 215 440 9300 or cme@aacr.org.

DISCLOSURE OF FINANCAL RELATIONSHIPS

In compliance with the standards set by the Accreditation Council for Continuing Medical Education (ACCME), it is the policy of the American Association for Cancer Research (AACR) that the information presented at CME activities will be unbiased and based on scientific evidence. To help participants make judgments about the presence of bias, the AACR has provided information that planning committee members, speakers, and abstract presenters have disclosed about financial relationships they have with commercial entities that produce or market products or services related to the content of this CME activity.

Relationships are abbreviated as follows: E, Employee of listed company, G, Grant/research support recipient, A, Advisor or review panel member, C, Consultant, S, Stock Shareholder, SB, Speakers' Bureau, H, Honoraria, O, Other.

Last Name	First Name	Company	Relationships	Type	Role
Akkari	Leila	Mem. Sloan Kettering Cancer Ctr.	No Relationships		Speaker
Arthur	Ronald	American Association for Cancer Research	No Relationships		Program Committee
Azmi	Asfar	Wayne State Univ. School of Medicine	Karyopharm Therapeutics	O	Speaker
Bernards	René	Netherlands Cancer Inst.	Agendia, Qameleon Therapeutics	E,S	Speaker
Berns	Anton	Netherlands Cancer Inst.	No Relationships		Program Committee
Bertotti	Andrea	University of Turin	No Relationships		Speaker
Boletta	Alessandra	Fondazione San Raffaele del Monte Tabor	No Relationships		Speaker
Chiarugi	Paola	University of Florence	No Relationships		Program Committee, Speaker
Coussens	Lisa	OHSU Knight Cancer Inst.	Plexxikon Inc. , Pharmacyclics, Inc., Acerta Pharma, Janssen R&D, Deciphera Pharmaceuticals, Genentech, Inc. Aduro Biotech, Inc., Eisai, Inc., ImClone, Abbott Labs, Becton Dickinson, AZ, Celgene	A,O,G	Speaker
Davidson	Nancy	Fred Hutchinson Cancer Research Ctr.	No Relationships		Program Committee
Dolcetti	Riccardo	CRO-IRCCS, Natl. Cancer Inst.	No Relationships		Program Committee, Speaker
Engelman	Jeffrey	Novartis	Novartis	E	Speaker
Finn	Olivera (Olja)	Univ. of Pittsburgh School of Medicine	Opus Bio	G	Program Committee, Speaker
Foti	Margaret	American Association for Cancer Research	No Relationships		Program Committee
Gajewski	Thomas	Univ. of Chicago	Merck, BMS, Roche/Genentech, Incyte, Jounce, Aduro, Ono, Seattle Genetics, Bayer, Evelo, Janssen, Pfizer	A,C,G,O	Speaker
Garraway	Levi	Eli Lilly and Company	Novartis, Foundation Medicine, Warp Drive, Boehringer Ingelheim, Lilly	C,A,S,G,E	Speaker
Giordano	Silvia	Universita'di Torino	No Relationships		Program Committee
June	Carl	Univ. of Pennsylvania	No Relationships		Speaker
Kalluri	Raghu	UT MD Anderson Cancer Ctr.	No Relationships		Speaker
Lanfrancone	Luisa	European Inst. of Oncology	No Relationships		Speaker
Lauffenburger	Douglas	MIT	Merrimack Pharmaceuticals, Applied BioMath, Immuneering, Torque Therapeutics, Janssen Pharmaceuticals	A,G	Speaker
Marais	Richard	Cancer Research UK Manchester Inst.	No Relationships		Program Committee
Martins	Carla	Univ. of Cambridge	No Relationships		Speaker
Merad	Miriam	Icahn School of Medicine at Mount Sinai	No Relationships		Speaker
Peeper	Daniel	Netherlands Cancer Institute	MSD, Genmab	G	Program Committee, Speaker

Last Name	First Name	Company	Relationships	Type	Role
Post	Dean	American Association for Cancer Research	No Relationships		Program Committee
Quezada	Sergio	Univ. College London Cancer Inst.	No Relationships		Speaker
Rescigno	Maria	European Institute of Oncology	No Relationships		Speaker
Sahai	Erik	The Francis Crick Institute	No Relationships		Speaker
Samuels	Yardena	Weizmann Inst. of Science	No Relationships		Speaker
Schreiber	Robert	Washington Univ. School of Medicine	Igenica Biotherapeutics, Jounce Therapeutics, Neon Therapeutics, BioLegend, NGM	S	Speaker
Smith	Jane	European Association for Cancer Research	No Relationships		Program Committee
Solit	David	Mem. Sloan Kettering Cancer Ctr.	Pfizer, Loxo Oncology	A	Program Committee, Speaker
Sozzi	Gabriella	Fondazione IRCCS Ist. Nazionale dei Tumori	No Relationships		Program Committee
Swanton	Charles	The Francis Crick Institute and UCL Cancer Institute	No Relationships		Speaker
Thompson	Craig	Mem. Sloan Kettering Cancer Ctr.	Agios Pharmaceuticals, Merck, CRL	A,O	Speaker
Trumpp	Andreas	German Cancer Research Ctr. and HI-STEM	No Relationships		Speaker
Vander Heiden	Matthew	David H. Koch Institute for Integrative Cancer Research at MIT	Agios Pharmaceuticals, Aeglea Biotherapeutics	A,S	Speaker
Wargo	Jennifer	UT MD Anderson Cancer Ctr.	No Relationships		Speaker
Werner	Sabine	ETH Hönggerberg - Institute for Cellbiology	No Relationships		Speaker
Zitvogel	Laurence	Gustave Roussy Cancer Center	No Relationships		Speaker

SIC ACCREDITATION INFORMATION - ITALIAN CONTINUING MEDICAL EDUCATION (CME): 1783-194611

ACCREDITATION STATEMENT

We applied for CME accreditation (Italian Physicians only) for the following disciplinary areas: Medical Surgeon (Disciplines: Haematology, Medical Genetics, Pathological Anatomy, Pharmacology and Clinical Toxicology, Clinical Pathology, Internal Medicine, Clinical Biochemistry, Oncology); Biologist, Pharmacist, Chemist (Discipline: Analytical Chemistry); Physicist (Discipline: Health Physics); Veterinary Surgeon.

CREDIT DESIGNATION STATEMENT

No. 4,8 Italian Ministry of Health CME (Continuing Medical Education) credits have been assigned with a participation of 16 hours.

CLAIMING (CME) CREDIT

Participants who wish to claim Italian CME accreditation, need to be registered to the conference. At the conference venue in Florence they should show their badge at the Italian CME desk where they will get access to the CME form and will need to sign the attendance sheet.

QUESTIONS ABOUT CME

For any further questions kindly contact the Italian CME desk at the venue.

GENERAL INFORMATION

CONFERENCE SECRETARIAT

c/o ECCO – the European CanCer Organisation
Avenue E. Mounier 83, B-1200 Brussels
EAS2017@ecco-org.eu

The Secretariat can be reached at tel. +39 055 4973 461 during the Conference.

CONFERENCE VENUE

Firenze Fiera (Florence Conference & Exhibition Centre)
Piazza Adua 1
50123 Firenze, Italy
Tel. +39 055 497 21
www.firenzefiera.it/en

BADGES

For security reasons, delegates are requested to wear their badge at all times during the Conference. Delegates having lost their badge can obtain a new badge at the registration helpdesk. A fee of 75 EUR per participant will be charged to print a new badge.

CATERING

Coffee Breaks:

Coffee breaks, courtesy of the organisers, have been scheduled as follows:

SATURDAY 24 JUNE:	15:30–16:00
SUNDAY 25 JUNE:	10:15–10:45 / 16:15–16:45
MONDAY 26 JUNE:	10:15–10:45 / 16:15–16:45
TUESDAY 27 JUNE:	10:00–10:30

Lunches:

Lunches, courtesy of the organisers will be offered to delegates at the following times:

SUNDAY 25 JUNE:	12:30 – 14:30
MONDAY 26 JUNE:	12:30 – 14:30

All delegates are invited to attend the official EACR-AACR-SIC 2017 Exhibitor Reception to enjoy networking with peers and some light refreshments – this reception will be held on Saturday 24 June, 18:00 – 19:30.

All catering will be served in the exhibition area.

CERTIFICATE OF ATTENDANCE

Certificates of Attendance will be accessible upon completion of an online Conference Satisfaction Survey. Following the Conference, you will receive an email link to the questionnaire which also provides the link for you to print your Certificate of Attendance.

We kindly ask you to keep your Conference badge as you will need the unique badge code to print your Certificate of Attendance. The Conference Secretariat will not mail Certificates of Attendance to participants after the Conference. For information on CME accreditation see page 9.

CITY INFORMATION

All delegates will receive practical information about Florence, including a city map, in their Conference bag. Delegates are also invited to download the free Florence Conference Card which provides special offers and discounted fees for museums, restaurants, car rental, taxis and other services. www.bit.ly/FCBcard

CLOAKROOM

A cloakroom is located on the ground floor.

Cloakroom Opening Hours:

SATURDAY 24 JUNE	09:30 – 20:00
SUNDAY 25 JUNE	07:00 – 19:30
MONDAY 26 JUNE	07:00 – 20:00
TUESDAY 27 JUNE	08:00 – 14:30

CONFERENCE DINNER:

SUNDAY 25 JUNE 20:00

A seated dinner will take place at Terrazza Brunelleschi, the rooftop terrace of Grand Hotel Baglioni. Join us at this unique venue for a warm and friendly networking evening.

The dinner is accessible for all delegates who have a ticket. Price per person: 65 EUR.

A limited number of tickets may be for sale at the registration helpdesk at the Conference Centre (not onsite at the dinner venue). Ticket holders will be asked to present their ticket upon arrival at the venue.

EXHIBITION

The EACR-AACR-SIC 2017 Exhibition is an essential part of the Conference and provides an opportunity to network and review important innovations.

The exhibition will be held in the Passi Perduti area located around the Auditorium of the Conference Centre on level -1. Entrance is free for registered delegates but limited to researchers, oncology professionals, press and exhibitors.

Exhibition Opening Hours:

SATURDAY 24 JUNE:	15:30–19:30
SUNDAY 25 JUNE:	10:15–17:00
MONDAY 26 JUNE:	10:15–17:00

For the exhibition floorplan and list of exhibitors, please see the exhibition section (page 17) of this Proceedings Book.

FIRST AID

No first aid room is available in the Conference Centre. In case of medical emergency, please refer to the registration helpdesk at the entrance of the congress centre.

INSURANCE

The organisers do not accept liability for individual medical, travel or personal insurance. Participants are strongly advised to make their own arrangements regarding health and travel insurance. The organisers of the EACR-AACR-SIC Special Conference 2017 accept no responsibility for loss due to theft or negligence.

INTERNET WI-FI ACCESS

General Wi-Fi access is available throughout the Conference Centre. For access, activate the Wi-Fi network on your laptop or device, select the network listed as EAS2017, and enter the user name and password: EAS2017.

INTERNET ZONE

The official EACR-AACR-SIC 2017 Internet Zone is available free of charge during the Conference. The terminals provide you with the following services: internet browsing, access to web-based mail, the Conference searchable programme and exhibitor information.

LANGUAGE & TRANSLATION

The official language of the Conference is English. Simultaneous translation will not be provided.

LOST & FOUND

All enquiries should be directed to the registration helpdesk in the entrance hall. The organisers accept no responsibility for loss due to theft or negligence.

POSTER SESSIONS

Posters will be on display for one day in the dedicated poster areas: on the Ballatoi, the mezzanine level above the exhibition, and in the Limonaia building (Sunday or Monday, across the various topics; for details please refer to the Scientific Programme).

Poster presenters will be able to mount their poster on the day their poster is to be presented as of 08:30. Posters must be removed by 18:15 on the day the poster was presented. Any posters remaining after this time will be removed by the organisers and cannot be reclaimed.

REGISTRATION

The EACR-AACR-SIC Special Conference 2017 is open to all registered participants. Your official name badge is required for admission to the Conference Centre and all Conference events. For security reasons, participants are requested to wear their badge at all times.

Registration Opening Hours:

SATURDAY 24 JUNE:	09:00–19:00
SUNDAY 25 JUNE:	07:00–18:00
MONDAY 26 JUNE:	07:00–18:00
TUESDAY 27 JUNE:	08:00–12:00

Registration Package

The full Conference registration package includes:

- Entry to all scientific sessions and Satellite Symposia;
- Entry to the Exhibition (restricted to researchers, oncology professionals and media);
- Proceedings Book;
- Coffee breaks and lunches, as well as the Exhibitor Reception on Saturday 24 June;
- Wi-Fi access in the Conference Centre and access to the Internet Zone terminals;
- Conference bag including a city map, information leaflets about Florence and a discount brochure of the Barberino Designer Outlet.

The day registration package includes:

- Access to all scientific sessions and Satellite Symposia on that day;
- Entry to the Exhibition (restricted to researchers, oncology professionals and media);
- Proceedings Book (subject to availability);
- Coffee breaks and/or lunches on that day;
- Wi-Fi access in the Conference Centre and access to the Internet Zone terminals;
- Conference bag including a city map, information leaflets about Florence and a discount brochure of the Barberino Designer Outlet (subject to availability).

EACR GENERAL ASSEMBLY:

SATURDAY 24 JUNE 13:15

The General Assembly of EACR will be held in the Sala Verde of the Congress Centre. This event is open to EACR members only. A buffet lunch will be available from 12:30 for those attending the meeting.

Register to attend: www.eacr.org/general-assembly

SIC GENERAL ASSEMBLY:

MONDAY 26 JUNE 19:00

The General Assembly of SIC will be held in the Sala Verde of the Congress Centre. This event is open to SIC members only.

SOCIAL MEDIA

Twitter is available during the Conference – tweet, network and follow updates using hashtag #EAS17.

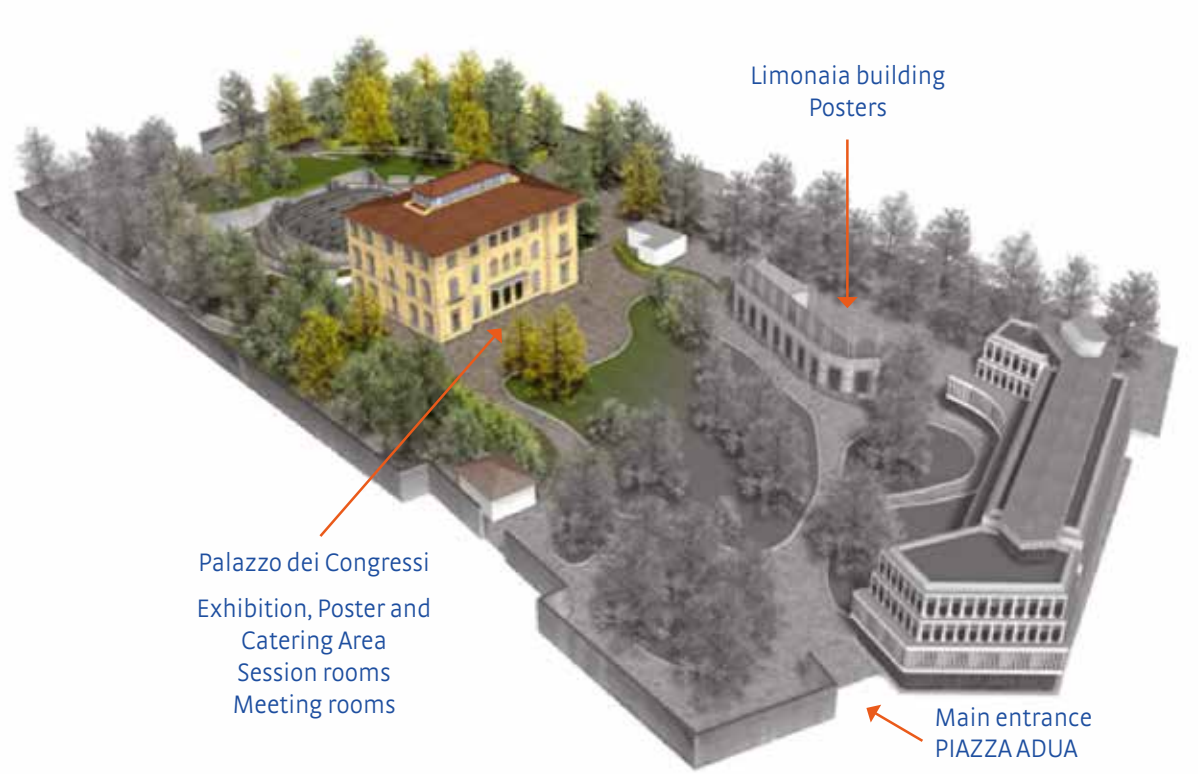
SPEAKER PREVIEW ROOM

The Speaker Preview Room is located in room 11 (ground floor). Speakers are requested to bring their PowerPoint presentations to the Speaker Preview Room at least 4 hours before their session starts or one day in advance if the session starts early in the morning. Session rooms are not equipped for laptop presentations.

Speaker Preview Room Opening Hours

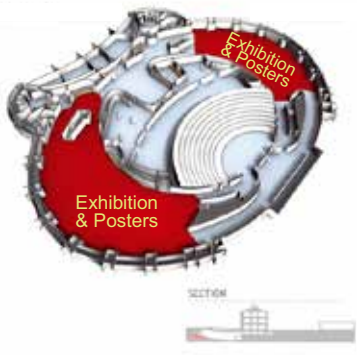
SATURDAY 24 JUNE	10:00 – 17:30
SUNDAY 25 JUNE	07:00 – 18:30
MONDAY 26 JUNE	07:00 – 18:30
TUESDAY 27 JUNE	08:00 – 13:00

VENUE FLOORPLANS



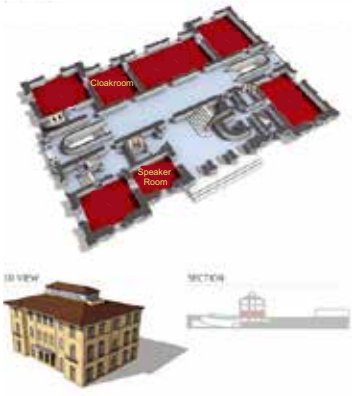
PALAZZO DEI CONGRESSI

lower floor

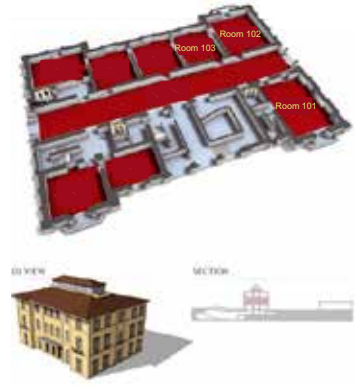


lower floor

ground floor

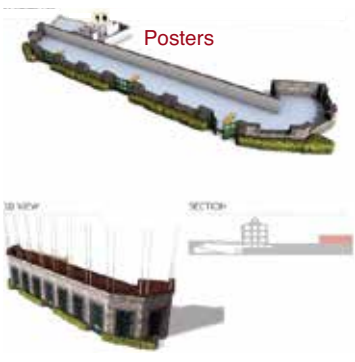


first floor



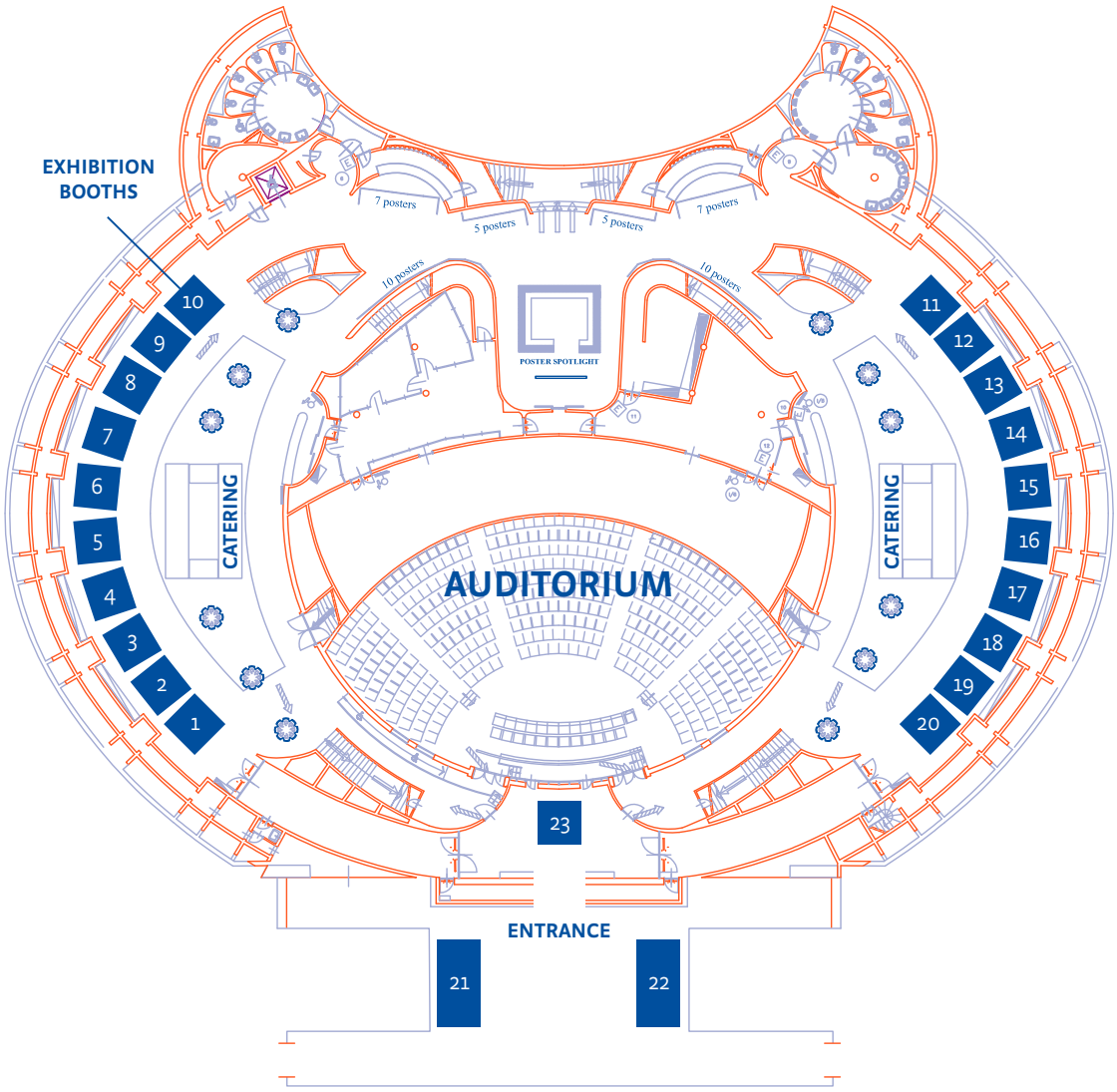
second floor

limonaia



EXHIBITION

EXHIBITION FLOORPLAN



LIST OF EXHIBITORS

Exhibitor:	Booth number
ADAPTIVE BIOTECHNOLOGIES	20
AMERICAN ASSOCIATION FOR CANCER RESEARCH (AACR)	22
ATCC - LGC STANDARDS	18
BIO-RAD LABORATORIES S.R.L.	15
BIOFIELD INNOVATION	1
CHEMOMETEC A/S	4-3
DON WHITLEY SCIENTIFIC LTD	11
EUROPEAN ASSOCIATION FOR CANCER RESEARCH (EACR)	21
ENVIGO RMS	10
EPHORAN MULTI IMAGING SOLUTIONS	14
FUJIFILM VISUALSONICS	13
JPT PEPTIDE TECHNOLOGIES GMBH	19
MENARINI SILICON BIOSYSTEMS	5-6
MERCK KGAA	7
SOCIETÀ ITALIANA DI CANCEROLOGIA (SIC)	23

EXHIBITOR PROFILES

Adaptive Biotechnologies
Booth number 20
www.adaptivebiotech.com

Adaptive Biotechnologies is at the forefront of immune-based discoveries, combining high-throughput sequencing and expert bioinformatics to profile T-cell and B-cell receptors. We bring the accuracy and sensitivity of our immunosequencing platform into laboratories around the world to drive groundbreaking research in cancer and other immune-mediated diseases. Adaptive also translates immunosequencing discoveries into clinical diagnostics and therapeutic development to improve patient care.

American Association for Cancer Research (AACR)
Booth number 22
www.aacr.org

The mission of the American Association for Cancer Research is to prevent and cure cancer through research, education, communication, and collaboration. Through its programs and services, the AACR fosters research in cancer and related biomedical science; accelerates the dissemination of new research findings among scientists and others dedicated to the conquest of cancer; promotes science education and training; and advances the understanding of cancer etiology, prevention, diagnosis, and treatment throughout the world.

ATCC - LGC Standards
Booth number 18
www.lgcstandards-atcc.org

ATCC is the premier global biological materials resource and standards organization whose mission focuses on the acquisition, authentication, production, preservation, development, and distribution of standard reference microorganisms, cell lines, and other materials. While maintaining traditional collection materials, ATCC develops high quality products, standards, and services to support scientific research and breakthroughs that improve the health of global populations.

Bio-Rad Laboratories S.r.l
Booth number 15
www.bio-rad.com

Bio-Rad Laboratories is a world leader in providing products for the life science research and diagnostic markets. In our Life Science Group, we build the industry leading solutions for oncology research, including the highly sensitive Droplet Digital™ PCR technology and our new technology for single-cell sequencing, the ddSEQ™ Single-Cell Isolator.

Biofield Innovation
Booth number 1
www.biofieldinnovation.it

BIOFIELD INNOVATION is an innovative start-up that was founded in July 2015. It was born as a joint project of a group of experienced professionals and young researchers. BIOFIELD INNOVATION operates in the fields of biotechnology, mechatronics and Information and Communication Technology (ICT) and is distinguishes itself through a strong dedication to basic and applied research in life sciences.

ChemoMetec A/S
Booth number 4-3
www.chemometec.com

Providing automated Image Cytometer's within cell counters and advanced cell analyzers to streamline processes for maximum efficiency. We have specialized assays for aggregated cells, cells on microcarriers and adipose derived stem cells. Our products are known for their high quality and precision as well as the "ease of use". No service agreements, high level of support and free software updates.

Don Whitley Scientific Ltd
Booth number 11
www.dwscientific.co.uk

Don Whitley Scientific is a leading international supplier of innovative scientific equipment and services to tissue culture and microbiology laboratories. The Hypoxystation was designed specifically for scientists wanting to strictly maintain physiologically relevant incubation conditions for cell culture research. It is ideal for those who require the ability to accurately control oxygen as well as carbon dioxide, temperature and humidity.

European Association for Cancer Research (EACR)
Booth number 21
www.eacr.org

The EACR is Europe's professional membership association for those working and studying in cancer research, with over 10,000 members worldwide. Our mission is "The advancement of cancer research: from basic research to prevention, treatment and care."

ENVIGO RMS

Booth number 10

www.envigo.com

Envigo provides essential products and research services for pharmaceutical, crop protection, and chemical companies as well as universities, governments, and other research organizations. With over 3,800 employees across 50+ locations worldwide, Envigo provides comprehensive scientific expertise and a full service offering in non-clinical research and development, research models and services, regulatory consulting, and analytical support to our customers.

EPHORAN Multi Imaging Solutions

Booth number 14

www.ephoran-mis.com

EPHORAN Multi Imaging Solutions, a CRO based in Italy, provides a complete toolset of imaging techniques to study, develop, and promote the application of imaging technologies in in vivo pre-clinical drug research and development.

Ephoran can provide preclinical imaging services covering all imaging techniques: MRI, PET, CT, SPECT, US, Optical Imaging, and Photoacustic imaging, to assure a link between the preclinical and clinical studies.

Fujifilm VisualSonics

Booth number 13

www.visualsonics.com

FUJIFILM VisualSonics specifically focuses on developing ultrasound technology that has been scaled to much higher frequencies than commonly found in many of the conventional ultrasound systems on the market today. As a result, our ultrasound platform provides images at resolutions that far exceed any other system available; as fine as 30 micrometers, clearly differentiating VisualSonics from its competitors.

JPT Peptide Technologies GmbH

Booth number 19

www.jpt.com

JPT is the leading provider of innovative peptide based products and services for the development of new immune therapies. Those include PepTrack™ - peptide libraries for fast neo-epitope prioritization; PepMixTM - peptide pools for clinical immune monitoring and the GxP peptides for economical access to high quality peptides in individualized therapies using adoptive cell transfer or dendritic cell pulsing.

Menarini Silicon Biosystems

Booth number 5-6

www.siliconbiosystems.com

Menarini Silicon Biosystems, develops technologies and products that help researchers understand the biological complexity of disease through the study of single cells. The company's DEPArray NxT is the only image-based digital cell-sorting and isolation platform that enables clinical researchers to conduct molecular analyses on live or fixed cells with single-cell precision. Thanks to the acquisition of the CELLSEARCH® Circulating Tumor Cell System in 2017, the company now provides an end-to-end workflow solution for the enumeration, isolation, and molecular characterization of CTCs from a simple blood test in the clinical research setting.

Merck KGaA

Booth number 7

www.merckgroup.com

Merck KGaA, Darmstadt, Germany, is a leading science and technology company for innovative and top-quality high-tech products. With a catalog of more than 300,000 products, our Life Science delivers many of the most highly-respected brands in the industry, such as Millipore, Milli-Q, SAFC and BioReliance. Our Life Science business brings together the legacy expertise of the life science portfolio of Merck KGaA and Sigma-Aldrich.

Società Italiana di Cancerologia (SIC)

Booth number 23

www.cancerologia.it

The Italian Cancer Society (SIC) was established in 1952 and is the longest-lived national association dedicated to oncology operating in Italy. SIC focuses on experimental, clinical and social oncology, helping to develop different aspects of these fields, such as the promotion of translational research, by stimulating interactions between preclinical and clinical investigators and propelling the transfer of knowledge from bench to the bedside, as well as by establishing relations with similar international associations. SIC organizes its activity through dedicated working groups set up to develop specific lines of research. The Society particularly encourages national cancer research in Italy, by supporting young researchers.

PROGRAMME AT A GLANCE

Saturday 24 June 2017

AUDITORIUM

Annual Meeting of SIC Young Investigators
10:00-10:30
Room: SALA VERDE

SIC Pre-Conference Workshop
10:30-12:30
Scholarly and Scientific Communication
Before and After the Internet
Room: SALA VERDE

EACR General Assembly
13:15-14:00
Room: SALA VERDE

Opening Address
14:15-14:30

Opening Lecture
14:30-15:30
Tumour Metabolism
Craig Thompson (US)

Coffee Break

Keynote Lecture
16:00-17:00
Immunotherapy
Robert Schreiber (US)

Plenary Symposium
17:00-18:00
Immunogenomics
Carl June (US)
2 Presenters from Best Abstracts

Exhibitor Reception
18:00-19:30

Exhibition 15:30 - 19:30

Sunday 25 June 2017

AUDITORIUM

Meet the Expert
07:30-08:30
Oxidative and metabolic stress signals within tumour microenvironment
Paola Chiarugi (IT)

Keynote Lecture
08:30-09:15
Immunogenomics
Levi Garraway (US)

Proffered Papers I
09:15-10:15

Poster Viewing / Coffee Break
10:15-10:45

Symposium
10:45-12:30
Immunotherapy Resistance
Sergio Quezada (UK)
*Laurence Zitvogel (FR)
2 Presenters from Best Abstracts
*Yardena Samuels (Israel)

Lunch 12:30
Poster Defence 13:15 - 14:30

Keynote Lecture
13:30-13:15
The RAS Pathway
Richard Marais (UK)

Symposium
14:30-16:15
Microenvironment & Microbiome
*Sabine Werner (CH)
Tom Gajewski (US)
2 Presenters from Best Abstracts
*Leila Akkari (NL)

Poster Viewing / Coffee Break
16:15-16:45

Symposium
16:45-18:15
New Therapeutic Approaches
*Olivera Finn (US)
*Luisa Lanfrancone (IT)
2 Presenters from Best Abstracts

Keynote Lecture
18:15-19:00
Resistance to Targeted Cancer Drugs
René Bernards (NL)

Conference Dinner / Networking Event
20:00

SALA VERDE

Meet the Expert
07:30-08:30
Functional oncogenomics for development of combinatorial therapies
Daniel Peeper (NL)

Posters & Exhibition 10:15 - 17:00

Monday 26 June 2017

AUDITORIUM

Meet the Expert
07:30-08:30
Challenges of combination cancer immunotherapy
Riccardo Dolcetti (IT)

Keynote Lecture
08:30-09:15
Combination Therapies in Lung Cancer
Jeffrey Engelman (US)

Proffered Papers II
09:15-10:15

Poster Viewing / Coffee Break
10:15-10:45

Symposium
10:45-12:30
Drug Resistance
*Jennifer Wargo (US)
*Maria Rescigno (IT)
2 Presenters from Best Abstracts
Douglas Lauffenburger (US)

Lunch 12:30
Poster Defence 13:15 - 14:30

Symposium
14:30-16:15
Tumour Cell Metabolism
*Carla Martins (UK)
Matthew Vander Heiden (US)
2 Presenters from Best Abstracts
*Alessandra Boletta (IT)

Poster Viewing / Coffee Break
16:15-16:45

Symposium
16:45-18:15
Mouse Models
*Lisa Coussens (US)
Andrea Bertotti (IT)
2 Presenters from Best Abstracts

Keynote Lecture
18:15-19:00
Exosomes
Raghu Kalluri (US)

SALA VERDE

Meet the Expert
07:30-08:30
Defining the actionable genome
David Solit (US)

Satellite Symposium
12.30-13.30
Menarini Silicon Biosystems

SIC General Assembly
19:00-20:00

Posters & Exhibition 10:15 - 17:00

Tuesday 27 June 2017

AUDITORIUM

Special Lecture
08:30-09:00
IMvigor211: A Phase III Randomized Study Examining Atezolizumab Versus Chemotherapy for Platinum-Treated Advanced Urothelial Carcinoma
Thomas Powles (UK)

Giorgio Prodi Award Lecture
09:15-10:00
The soldiers and the enemies in the cancer battlefield: A 30-year story of defeats and successes in tumour immunology
Mario P. Colombo (IT)

Coffee Break
10:00-10:30

Symposium
10:30-12:15
Cell Plasticity/Single Cell Analysis
Andreas Trumpp (DE)
Erik Sahai (UK)
2 Presenters from Best Abstracts

Keynote Lecture
12:15-13:00
Immunotherapy
Charles Swanton (UK)

13:00-14:00
"Piero Trivella"
Award for the Best Posters

"Guido Berlucchi Foundation"
Awards for the Best Posters

"Guido Berlucchi Foundation"
Award for the Best Oral Presentation

"Elena Cappannini"
Award for the Best 2016 Publication

"Pezcoller Foundation"
Scholarship Award

Travel Grants
Conference Highlights & Closing Remarks

SCIENTIFIC PROGRAMME

SATURDAY 24 JUNE 2017

Opening Address:

14:15 - 14:30 | **AUDITORIUM**

14:15 Welcome by EACR
Speaker: A. Berns *(Netherlands)*

14:20 Welcome by AACR
Speaker: N.E. Davidson *(USA)*

14:25 Welcome by SIC
Speaker: S. Giordano *(Italy)*

Opening Lecture: Tumour Metabolism

14:30 - 15:30 | **AUDITORIUM**

Chair: N.E. Davidson (USA)

14:30 Metabolic modulation of cancer immunotherapy
Speaker: C.B. Thompson (USA)

Keynote Lecture: Immunotherapy

16:00 - 17:00 | **AUDITORIUM**

Chair: R. Marais (United Kingdom)

16:00 Tumor neoantigens as targets for cancer specific immunotherapy
Speaker: R. Schreiber *(USA)*

Plenary Symposium: Immunogenomics

17:00 - 18:00 | **AUDITORIUM**

Chair: S.A. Quezada (United Kingdom)

17:00 CAR T cells enter mainstream oncology
Speaker: C. June *(USA)*

17:30 Proffered Paper: The T Cell Repertoire during tumor formation* 1
S. Efroni

17:40 Proffered Paper: Impact of intratumoral clonal heterogeneity on immune checkpoint inhibitor response* 2
E.E. Vietsch, A. Javaid, J. McCutcheon, G. Giaccone, A.T. Riegel, A. Wellstein

17:50 Discussion and roundup

Exhibitor Reception

18:00 - 19:30 | **AUDITORIUM**

SUNDAY 25 JUNE 2017

Meet the Expert Session: Oxidative and metabolic stress signals within tumor microenvironment

07:30 - 08:30 | **AUDITORIUM**

07:30 Speaker: P. Chiarugi *(Italy)*

Meet the Expert Session: Functional Oncogenomics for Development of Combinatorial Therapies

07:30 - 08:30 | **SALA VERDE**

07:30 Speaker: D. Peeper *(Netherlands)*

Keynote Lecture: Immunogenomics

08:30 - 09:15 | **AUDITORIUM**

Chair: P. Chiarugi (Italy)

08:30 New direction in molecular oncology
Speaker: L.A. Garraway *(USA)*

Proffered Papers: Proffered Papers 1*

09:15 - 10:15 | **AUDITORIUM**

Chair: M. Macagno (Italy)

09:15 Proffered Paper: Structural basis of HuR inhibition by Dihydrotanshinone-I 3
A. Provenzani, P. Lal, L. Cerofolini, I. Bonomo, V. D'Agostino, M. Gorospe, D. Dixon, P. Seneci, L. Marinelli, M. Fragai

09:25 Proffered Paper: GDE2 promotes neuroblastoma differentiation through GPI-anchor cleavage and is a marker of clinical outcome 4
E. Matas-Rico, M. Van Veen, D. Leyton-Puig, J. Van den Berg, J. Koster, K. Kedziora, A. Perrakis, K. Jalink, R. Versteeg, W. Moolenaar

09:35 Proffered Paper: POPX2 phosphatase regulates apoptosis through the TGF-beta activated kinase pathway 5
C.G. Koh, T. Weng

09:45 Proffered Paper: Stage-dependent therapeutic efficacy in PI3K/MTOR-driven squamous cell carcinoma of the skin 6
C. Darido, C. Cullinane, R. Pearson, S. Jane

09:55 Proffered Paper: Perfusion-based bioreactor culture of primary cancer tissue maintains tumor microenvironment complexity and allow in-vitro testing of immune blockade therapy 7
M.G. Muraro, S. Muenst, C. Manfredonia, V. Mele, S. Däster, W.P. Weber, G.C. Spagnoli, G. Iezzi, I. Martin, S. Soysal

*** Not designated for CME credit**

10:05 Proffered Paper: Clinical translation of nuclear export inhibitors in pancreatic cancer 8
A. Azmi, P. Philip, M. Kauffman, Y. Landesman, W. Senapedis, S. Shacham, A. Mahipal, E. Baloglu, I. Muqbil, R. Mohammad

Symposium: Immunotherapy Resistance

10:45 - 12:30 | **AUDITORIUM**

*Chair: O. Finn **(USA)***

10:45 Deciphering and targeting immune regulation at the tumour site
Speaker: S.A. Quezada *(United Kingdom)*

11:10 Mechanisms of secondary resistance to immune checkpoint blockade
Speaker: L. Zitvogel *(France)*

11:35 Proffered Paper: Using implantable microdevices to systemically identify optimal combinations of immunotherapy and chemotherapy in situ* 9
O. Jonas

11:45 Proffered Paper: Targeting type I interferon activity to the tumor microenvironment or to dendritic cells as a novel, generic and safe cancer immunotherapy* 10
A. Cauwels, S. Van Lint, F. Paul, G. Garcin, S. De Koker, S. Gerlo, Y. Bordat, G. Uze, J. Tavernier

11:55 Towards deciphering the mutational and neo-antigenic landscape in melanoma
Speaker: Y. Samuels *(Israel)*

12:20 Discussion and roundup

Keynote Lecture: The Ras Pathway

12:30 - 13:15 | **AUDITORIUM**

*Chair: A. Berns **(Netherlands)***

12:30 Precision medicine for melanoma
Speaker: R. Marais *(United Kingdom)*

Symposium: Microenvironment & Microbiome

14:30 - 16:15 | **AUDITORIUM**

*Chair: L. Zitvogel **(France)***

14:30 Activin - a major regulator of the wound and skin cancer microenvironment
Speaker: S. Werner *(Switzerland)*

14:55 Tumor and host factors regulating anti-tumor immunity and immunotherapy efficacy
Speaker: T. Gajewski *(USA)*

15:20 Proffered Paper: Stromal cell immunomodulatory potential in the tumour microenvironment is regulated by inflammatory signalling* 11
G. O'Malley, S. Naicker, K. Lynch, P. Lohan, T. Ritter, L. Egan, A. Ryan

15:30 Proffered Paper: Cabozantinib eradicates advanced murine prostate cancer by activating anti-tumor innate immunity* 12
A. Patnaik, K. Swanson, E. Csizmadia, A. Solanki, N. Landon-Brace, H. Ye, J. Karp, S. Sabina, S. Balk, L. Cantley

15:40 Modulation of the myeloid cell response in radiation-treated gliomas circumvents tumor recurrence
Speaker: L. Akkari *(Netherlands)*

16:05 Discussion and roundup

Symposium: New Therapeutic Approaches

16:45 - 18:15 | **AUDITORIUM**

*Chair: D. Peeper **(Netherlands)***

16:45 Cancer immunoprevention
Speaker: O. Finn *(USA)*

17:15 Functional targeting of novel vulnerabilities in melanoma
Speaker: L. Lanfrancone *(Italy)*

17:45 Proffered Paper: Harnessing the spatially regulated tyrosine phosphorylation mechanisms for precision medicine* 13
V.k. Ulaganathan, A. Ullrich

17:55 Proffered Paper: Suppression of oncogene transcription with PNA-delivery peptide conjugates –potential therapy for BRAF-V600E and KRAS-G12D driven tumors* 14
J. Rothman, O. Surriga, G. Ambrosini, G. Schwartz

18:05 Discussion and roundup

Keynote Lecture: Resistance to Targeted Cancer Drugs

18:15 - 19:00 | **AUDITORIUM**

*Chair: R. Marais **(United Kingdom)***

18:15 Targeting drug resistant cancers
Speaker: R. Bernards *(Netherlands)*

*** Not designated for CME credit**

MONDAY 26 JUNE 2017

Meet the Expert Session: Challenges of Combination Cancer Immunotherapy

07:30 - 08:30 | **AUDITORIUM**

07:30 Speaker: R. Dolcetti *(Italy)*

Meet the Expert Session: Defining the actionable genome

07:30 - 08:30 | **SALA VERDE**

07:30 Speaker: D. Solit *(USA)*

Keynote Lecture: Combination Therapies in Lung Cancer*

08:30 - 09:15 | **AUDITORIUM**

Chair: G. Sozzi ***(Italy)***

08:30 Targeted therapies and resistance: Where are we going?
Speaker: J.A. Engelman *(USA)*

Proffered Papers: Proffered Papers 2*

09:15 - 10:15 | **AUDITORIUM**

Chair: S. Ventura ***(Italy)***

09:15 Proffered Paper: Immunologic reshaping of cancer by stimulation of innate nucleic acid sensor RIG-I — 15
C. Schuberth-Wagner, M. Niewel, M. Renn, C. Jakobs, A. Schwickart-Halbe, J. Vollmer

09:25 Proffered Paper: A cross-tumors approach identifies the transcription factor FOSL1 as a KRAS oncogene dependency in lung and pancreatic cancer — 16
A. Vallejo, N. Perurena, E. Guruceaga, P.K. Mazur, K. Valencia, M. Ponz-Sarvise, A. Bozec, J. Sage, F. Lecanda, S. Vicent

09:35 Proffered Paper: A blood-based multi-marker panel for pancreatic cancer early detection — 17
M. Capello, L. Bantis, G. Scelo, R. Brand, M.A. Firpo, M.H. Katz, P. Brennan, Z. Feng, A. Taguchi, S.M. Hanash

09:45 Proffered Paper: Role of PD-L1 immunoregulatory protein in breast cancer cells metabolic reprogramming — 18
J. Berthe, J. Kluza, H. El Bouazzati, I. Briche, X. Thuru, S. Galiègue-Zouitina, B. Quesnel

09:55 Proffered Paper: Human bone marrow-derived mesenchymal stem cells promote invasiveness and transendothelial migration of osteosarcoma cells through a mesenchymal to ameboid transition — 19
L. Pietrovito, E. Giannoni, V. Gori, F. Bambi, P. Chiarugi, M.L. Taddei

10:05 Proffered Paper: NFIB and YBX1 bind to and repress ESR1, revealing a therapeutically relevant regulatory loop in breast cancer — 20
K.B. Meyer, T.M. Campbell, M.A.A. Castro, B.A.J. Ponder

Symposium: Drug Resistance

10:45 - 12:30 | **AUDITORIUM**

Chair: J.A. Engelman ***(USA)***

10:45 Understanding resistance to cancer therapy: From bedside to bench, and back again
Speaker: J. Wargo *(USA)*

11:10 Role of the immune system in antibody targeted therapy and resistance
Speaker: M. Rescigno *(Italy)*

11:35 Proffered paper: Response to targeted therapy in melanomas expressing ALK fusions and other ALK variants* — 21
K. Couts, J. Bemis, J. Turner, S. Bagby, D. Murphy, J. Christiansen, J. Hintzsche, T. Medina, R. Doebele, W. Robinson

11:45 Proffered Paper: STAT3 mediates resistance to BRAF inhibitors in thyroid carcinoma cells* — 22
T. Notarangelo, L. Sisinnni, V. Condelli, M. Landriscina

11:55 Analysis of tumor microenvironment factors effects on drug responses
Speaker: D.A. Lauffenburger *(USA)*

12:20 Discussion and roundup

Symposium: Tumour Cell Metabolism

14:30 - 16:15 | **AUDITORIUM**

Chair: D. Peeper ***(Netherlands)***

14:30 Exploiting lung tumor metabolic heterogeneity for improved therapy
Speaker: C. Martins *(United Kingdom)*

14:55 Role of metabolism in tumor growth
Speaker: M. Vander Heiden *(USA)*

15:20 Proffered Paper: Global transcriptional analysis reveals miR23b-3p and amino acids transport as a key metabolic hub of endocrine therapy resistance in ER+ breast cancer* — 23
M. Bacci, M. Ferracin, M. Ramazzotti, G. Comito, E. Giannoni, L.A. Martin, P. Chiarugi, A. Morandi

15:30 Proffered Paper: A chemical-genetic CRISPR screen identifies cancer vulnerabilities to perturbation of mitochondrial respiration* — 24
M. Chandrashekhar, M. Aregger, T. Hart, J. Moffat

15:40 mTORC1-driven metabolic Reprogramming in Renal cell carcinoma
Speaker: A. Boletta *(Italy)*

16:05 Discussion and roundup

*** Not designated for CME credit**

Symposium: Mouse Models

16:45 - 18:15 | **AUDITORIUM**

Chair: A. Berns ***(Netherlands)***

16:45 Therapeutic strategies for neutralizing protumor inflammation: Lessons learned from preclinical mouse models
Speaker: L.M. Coussens *(USA)*

17:15 From mouse to bedside: Preclinical strategies for precision medicine in colorectal cancer
Speaker: A. Bertotti *(Italy)*

17:45 Proffered Paper: Targeting immunotherapy to the tumor microenvironment using anti-PDL1 VHH* 25
M. Dougan, J. Ingram, H. Ploegh, S. Dougan

17:55 Proffered Paper: The immunoreceptor NKG2D promotes tumorigenesis in models of inflammation-driven cancer* 26
J. Guedes, S. Sheppard, N. Guerra

18:05 Discussion and roundup

Keynote Lecture: Exosomes

18:15 - 19:00 | **AUDITORIUM**

Chair: S. Giordano ***(Italy)***

18:15 The biology and function of exosomes in diagnosis and treatment of cancer
Speaker: R. Kalluri *(USA)*

TUESDAY 27 JUNE 2017

Giorgio Prodi Award Lecture

09:15 - 10:00 | **AUDITORIUM**

Chair: G. Sozzi ***(Italy)***

09:15 The soldiers and the enemies in the cancer battlefield: A 30-year story of defeats and successes in tumor immunology
Speaker: M.P. Colombo (Italy)

Cell Plasticity/Single Cell Analysis

10:30 - 12:15 | **AUDITORIUM**

Chair: L.M. Coussens ***(USA)***

10:30 Metabolic pathways control malignant stem cells and therapy resistance
Speaker: A. Trumpp *(Germany)*

11:00 Imaging therapy failure
Speaker: E. Sahai *(United Kingdom)*

11:30 Proffered Paper: Investigating epithelial and mesenchymal triple negative breast cancer plasticity: identification of duel Wnt and YAP susceptibility for effective tumor targeting* 27
A. Sulaiman, S. McGarry, A. Arnaout, C. Nessim, L. Wang

11:40 Proffered Paper: Intra-tumoral heterogeneity in Glioblastoma is a result of stochastic reversible plasticity rather than a hierarchical differentiation process* 28
A. Golebiewska, A. Dirkse, T. Buder, N.H.C. Brons, N. Sauvageot, S. Leite, C. Herold-Mende, A. Deutsch, A. Voss-Böhme, N. Simone P.

11:50 Discussion and roundup

Keynote Lecture: Immunotherapy

12:15 - 13:00 | **AUDITORIUM**

Chair: N.E. Davidson ***(USA)***

12:15 Chromosomal chaos and order during lung cancer evolution
Speaker: C. Swanton *(United Kingdom)*

Closing Session: Closing Remarks

13:00 - 14:00 | **AUDITORIUM**

Chair: G. Sozzi ***(Italy)***

13:00 **"Piero Trivella"**
Award for the Best Posters

"Guido Berlucchi Foundation"
Award for the Best Posters
Award for the Best Oral Presentation

"Elena Cappannini"
Award for the Best 2016 Publication

"Pezcoller Foundation"
Fellowships Award

Travel Grants, Conference Highlights & Closing Remarks

*** Not designated for CME credit**

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ABSTRACTS

SATURDAY 24 JUNE 2017

17:00-18:00: PLENARY SYMPOSIUM: IMMUNOGENOMICS

1 Proffered Paper: The T Cell Repertoire during tumor formation

S. Efroni[1]

[1] Bar-Ilan University, The Mina & Everard Goodman Faculty of Life Sciences, Ramat Gan, Israel

BACKGROUND: To see how the T cell repertoire changes during 6 months of breast cancer progression in mice, and to learn if we can utilize these changes to learn about the tumor, we quantified this repertoire and then, using machine learning, identified the T cells clones that can tell us which mouse is developing breast cancer, and whether or not that mice is currently sick.

MATERIALS AND METHODS: We followed 10 female mouse of a transgenic mouse strain that expresses the un-activated rat neu (Erbb2) oncogene, along with 5 control mice.

These mice develop mammary tumors spontaneously over 5-8 months. To quantify the peripheral T cell repertoire, we extracted T cells from blood, every month, over the period of 9 months. Cells from these samples were sorted and later processed through a cDNA TCR α and β library preparation protocol using single-molecule barcoding and then NGS sequenced.

We then used the output of these experiments, a large dataset of 250000 T cell clones, over 90 temporal samples, as input to a set of machine learning algorithms.

RESULTS: A careful analysis of the sequences demonstrated a connection between the behavior of public clones and their convergent recombination behavior, in a similar manner to the findings we have reported before (System-wide Analysis of the T Cell Response. Cell Reports 2016). Most importantly, we were able to use the repertoire to classify tumor and non-tumor mice, using their immunological repertoire. Using feature selection algorithms, we were able to provide superior classification using a small subset (3 to 6 clones) of the T cell repertoire. Thus, machine learning and feature selection allowed us to reduce the hundreds of thousands of TCR alpha and beta sequences obtained during repertoire sequencing, to a set of six clones, with which we can identify the source of a blood sample as tumor or control. We can further stratify older transgenic mice (older than 5 months) and those of older control mice, using the same small T cell clones subset. This latter classification has been obtained with as little as three T cell clones.

CONCLUSIONS: sing samples over time point during tumor progression, and employing machine learning methods to observe these big data, we can now tag blood samples according to their tumor predisposition and/or tumor stage. based only on repertoire data.

NO CONFLICT OF INTEREST

2 Proffered Paper: Impact of intratumoral clonal heterogeneity on immune checkpoint inhibitor response

E.E. Vietsch[1], A. Javaid[1], J. McCutcheon[1], G. Giaccone[1], A.T. Riegel[1], A. Wellstein[1]

[1] Georgetown University, Oncology, Washington DC, USA

INTRODUCTION: Cancer cells are subjected to evolutionary selection of clonal populations by changes in the microenvironment as well as their response to drug treatment. We wished to evaluate how this heterogeneity impacts efficacy of checkpoint inhibition.

MATERIALS AND METHODS: Cancer cells are subjected to evolutionary selection of clonal populations by changes in the microenvironment as well as their response to drug treatment. To understand the contribution of clonal subpopulations to the malignant progression and to the response to drugs, we established a model of tumor heterogeneity from six syngeneic, clonal primary cancer cells isolated from a mutant Kras/P53 mouse pancreatic cancer (KPC). The clones were characterized molecularly and tumors reconstituted from mixes of the clonal cell lines.

RESULTS AND DISCUSSION: These clonal cells formed invasive and metastatic lesions when grafted into hosts. The original tumor and clonal cell lines harbored common mutations in 99 genes suggesting their common ancestry. Additional unique mutations in the clonal lines were used to identify and quantitate clones in heterogeneous cell pools. The clones showed different levels of MAP kinase signaling, unique morphologies, different growth rates in vitro and tumor growth rates in immune competent mice. Moreover, the sensitivity to ~200 anticancer drugs revealed an up to 25-fold varying in vitro sensitivity of the clones to signal transduction inhibitors and cytotoxic drugs.

To our surprise, drug sensitivity of individual clones when included in a heterogeneous cell population was strikingly different from their drug sensitivity when growing on their own. In particular the sensitivity of clones to MEK or PI3K inhibition was not predictive of their sensitivity when grown in a pool with the other clones. Furthermore, the sensitivity of clones to an anti-PD1 checkpoint inhibitor was distinct across the clonal cells growing in the heterogeneous mixture. Some clones were resistant and others highly sensitive to the checkpoint inhibition. We will discuss pathways and drivers of resistance in the different subpopulations.

CONCLUSIONS: We conclude that malignant progression and selection of checkpoint inhibitor sensitive cancer cell subpopulations is impacted by the crosstalk between clonal cell populations present in heterogeneous tumors and the host environment.

NO CONFLICT OF INTEREST

SUNDAY 25 JUNE 2017

09:15-10:15: PROFFERED PAPERS: PROFFERED PAPERS 1

3 Proffered Paper: Structural basis of HuR inhibition by Dihydrotanshinone-I

A. Provenzani[1], P. Lal[2], L. Cerofolini[2], I. Bonomo[2], V. D'Agostino[2], M. Gorospe[3], D. Dixon[4], P. Seneci[5], L. Marinelli[6], M. Fragai[2]

[1] University of Trento, CIBIO, Trento, Italy
[2] University of Florence, CERM, Florence, Italy
[3] National Institutes of Health, National Institute on Aging, Baltimore, USA
[4] University of Kansas Medical Center, 5 Department of Cancer Biology, Kansas City, USA
[5] University of Milan, Department of Chemistry, Milan, Italy
[6] University of Naples, Department of Pharmacy, Naples, Italy

INTRODUCTION: The human antigen R protein (HuR) is a RNA-binding protein that recognizes U/AU-rich elements in diverse RNAs through two RNA-recognition motifs, RRM1 and RRM2, and post-transcriptionally regulates the fate of target RNAs. Many of the HuR-target transcripts encode for key oncogenic drivers and inflammatory genes. The natural product Dihydrotanshinone-I (DHTS) prevents the association of HuR and target RNAs in vitro and in cultured cells by interfering with the binding of HuR to RNA.

MATERIAL AND METHOD: We performed NMR titration of HuR with DHTS and Molecular Dynamic (MD) simulation to identify the key residues within RRM1 and RRM2 responsible for the interaction between DHTS and HuR. RNA Electromobility Shifts and Alpha Screen Assays with truncated form of the protein and site specific mutants confirmed the NMR and MD indication. By HuR ribonucleoprotein immunoprecipitation followed by microarray (RIP-chip) analysis during DHTS treatment on HeLa cells we identified the transcriptome-wide modulation of HuR binding. By the utilization of CRISPR/CAS9 mediated-HuR knock-out cells xenografted cancer cells we evaluated the HuR dependency of DHTS antitumor effects

RESULTS AND DISCUSSION: We identify the structural determinants of the interaction between DHTS and HuR. DHTS interacts with HuR through the same binding regions as target RNAs, and stabilizes HuR in a loop conformation that blocks HuR association with target RNAs, competitively. The impact of DHTS on HuR binding to target mRNAs transcriptome-wide showed that DHTS treatment of HeLa cells paradoxically enriched HuR binding to mRNAs with longer 3'UTR and with higher density of U/AU-rich elements, suggesting that DHTS inhibits the association of HuR to weaker target mRNAs. In vivo, DHTS potently inhibited xenograft tumor growth in a HuR-dependent cell model without systemic toxicity.

CONCLUSION: We show that DHTS is a competitive inhibitor of HuR by interacting with the same region of HuR-RNA binding, we describe the transcriptome-wide effects of HuR inhibition and and we provide evidences for the antitumor efficacy of an anti-HuR therapy.

NO CONFLICT OF INTEREST

4 Proffered Paper: GDE2 promotes neuroblastoma differentiation through GPI-anchor cleavage and is a marker of clinical outcome

E. Matas-Rico[1], M. Van Veen[1], D. Leyton-Puig[1], J. Van den Berg[1], J. Koster[2], K. Kedziora[1], A. Perrakis[3], K. Jalink[1], R. Versteeg[2], W. Moolenaar[1]

[1] The Netherlands Cancer Institute, Cell Biology, Amsterdam, Netherlands
[2] Academic Medical Center, Oncogenomics, Amsterdam, Netherlands
[3] The Netherlands Cancer Institute, Biochemistry, Amsterdam, Netherlands

BACKGROUND: Neuroblastoma is a childhood cancer characterized by impaired differentiation of immature neuroblasts. A better understanding of differentiation regulatory pathways is essential for the development of new therapies for this often fatal malignancy. GDE2 is a multi-pass membrane glycerophosphodiesterase with a catalytic ectodomain known to promote embryonic neurogenesis. Here we examine a possible role of GDE2 in regulating neuroblastoma differentiation.

METHODS: cell biological, biochemical and biophysical assays; live-cell imaging; RhoA biosensor; inducible overexpression; knockdown and CRISPR-based knockout studies; RNA-seq transcriptome analysis; neuroblastoma patient survival analysis.

RESULTS: We find that high GDE2 expression is strongly associated with favorable outcome in independent neuroblastoma patient cohorts. Elevated GDE2 expression induces differentiation of neuroblastoma cells, suppresses cell motility, and opposes RhoA-driven neurite retraction. GDE2 alters the Rac-RhoA activity balance and the expression of multiple differentiation-associated genes, as revealed by overexpression and knockdown studies. A single point mutation in the ectodomain abolishes GDE2 function. We show that, mechanistically, GDE2 acts by cleaving (in cis) and releasing glycosylphosphatidylinositol (GPI)-anchored glypican-6, a putative co-receptor or ligand of an as-yet-unidentified transmembrane receptor, thereby promoting neuroblastoma differentiation in a cell-autonomous manner.

CONCLUSIONS: Our result establish GDE2 as a cell-intrinsic inducer of neuroblastoma differentiation with prognostic significance. In a broader context, our work highlights GPI-anchor cleavage as a signaling mechanism to suppress the malignant phenotype. Enhancing GDE2 activity is a candidate therapeutic approach for improving clinical outcome in neuroblastoma, and possibly other malignancies.

NO CONFLICT OF INTEREST

5 Proffered Paper: POPX2 phosphatase regulates apoptosis through the TGF-beta activated kinase pathway

C.G. Koh[1], T. Weng[1]

[1] Nanyang Technological University, School of Biological Sciences, Singapre, Singapore

INTRODUCTION: We have earlier reported that high POPX2 phosphatase levels positively correlated to cancer cell motility and invasiveness. Through our proteomics studies, we have implicated the mitogen-activated kinase pathway in POPX2-regulated signaling. We have also found that POPX2 affects kinesin trafficking leading to the impairment of cell-cell adhesion. The loss of cell-cell adhesion is an indication of epithelial to mesenchymal transition and onset of metastasis. All these earlier findings suggest that POPX2 could be a target for therapeutic intervention. However, in this study, we discover that POXP2 interacts with TAK1 (TGF-beta activated kinase 1). TAK1 is essential for several important biological functions including innate immunity, development and cell survival. We find that POPX2 dephosphorylates and inactivates TAK1 leading to increased apoptosis when the cells suffer DNA damage induced by etoposide. Knocking down POPX2 or inhibiting the activity of POPX2 can lead to enhanced cell survival.

MATERIAL AND METHOD: Possible POPX2 interacting proteins were identified through a pull-dwon/mass spectrometry analysis using Flag-POPX2 as bait. We further validated protein interaction through pulldown and western blot analysis as well as immunoprecipitation of endogenous proteins. In our experiment VP16, a topoisomerase inhibitor, was used to treat U2-OS cells to induce DNA double stranded breaks and apoptosis. The extent of apoptosis was analysed through western blot using anti-caspase 3 and PARP antibodies. Specific anti-phospho-TAK1 antibodies were used to examine the phosphorylation and activation status of the kinase.

RESULTS AND DISCUSSION: We identified TAK1 as an interacting partner of POPX2. We also found that POPX2 can dephosphorylate TAK1 and affect the activity of the TAK1 kinase. It is well-known that TAK1 regulates NF-κB mediated transcription via IKK complexes. In POPX2 knockdown cells, elevated nuclear translocation of NF-κB and increased mRNA levels of NF-κB mediated genes further support our hypothesis that POPX2 negatively regulates TAK1-IKK-NF-κB signaling.

CONCLUSION: Our data demonstrate that cells with higher levels of POPX2 are more vulnerable to apoptosis induced by etoposide. We found that POPX2 is a negative regulator of TAK1 signaling pathway and modulates apoptosis through the regulation of TAK1 activity. Hence the levels of POPX2 in the tumor cells can influence the therapeutic outcome of cytotoxic drugs used in chemotherapy.

NO CONFLICT OF INTEREST

6 Proffered Paper: Stage-dependent therapeutic efficacy in PI3K/MTOR-driven squamous cell carcinoma of the skin

C. Darido[1], C. Cullinane[1], R. Pearson[1], S. Jane[2]

[1] Peter MacCallum Cancer Centre, Oncology, Melbourne, Australia
[2] Monash University, Medicine, Melbourne, Australia

INTRODUCTION: The incidence of Squamous Cell Carcinoma (SCC) of the skin is rising alarmingly for up to five times that of all other cancers combined, and it is particularly very high in immunosuppressed patients, especially those undergoing organ transplants and patients being treated for other malignancies (e.g. melanoma therapy with B-Raf inhibitors). Moreover, the lack of model systems to investigate the targeting of cancer drivers at different stages of SCC development precludes efficient therapeutic interventions in patients.

MATERIAL AND METHOD: Here, we have used mice with a conditional deletion of the transcription factor Grainyhead-like 3 (Grhl3) in the skin to induce loss of PTEN and activation of the PI3K/mTOR pathway, which in the context of chemical carcinogen treatment, promotes aggressive SCC development. In parallel, GRHL3/PTEN deficiency in human SCC occurs as a result of transcriptional inhibition by an oncogenic miR-21, driving PI3K/mTOR hyperactivation. Using these preclinical models we trialled inhibition of oncogenic PI3K/mTOR and miR-21 during the initiation, promotion/progression and establishment stages of skin SCC.

RESULTS AND DISCUSSION: We first discovered that treatment with PI3K/mTOR inhibitors completely ablated tumor initiation in mice. Importantly, the PI3K/mTOR inhibitors also induced a significant delay in the course of papilloma progression to malignancy following initiation with carcinogens. However, established SCC did not show any growth regression, indicating that this therapy is ineffective in established cancers. Mechanistically, we found that resistant SCCs displayed increased miR-21 expression in Grhl3-deficient mice. Similar result were seen in human SCC in which antagonist of miR-21 rescued expression levels of GRHL3/PTEN, leading to inactivation of PI3K/mTOR signaling, however the combination of miR-21 antagonist with PI3K/mTOR inhibitors did not bypass cancer resistance. The mechanism of SCC resistance to combined inhibitors was acquired in party via c-Myc and Oct-4 upregulation.

CONCLUSION: Our result are the first to provide molecular evidence for the efficacy of targeting oncogenic drivers in the initiation and promotion stages and to indicate that combination therapy may induce an aggressive phenotype when applied in the establishment stage of skin SCC.

NO CONFLICT OF INTEREST

7 Proffered Paper: Perfusion-based bioreactor culture of primary cancer tissue maintains tumor microenvironment complexity and allow in-vitro testing of immune blockade therapy

M.G. Muraro[1], S. Muenst[2], C. Manfredonia[1], V. Mele[1], S. Däster[3], W.P. Weber[3], G.C. Spagnoli[1], G. Iezzi[2], I. Martin[1], S. Soysal[3]

[1] Department of Biomedicine, University and University Hospital of Basel, Basel, Switzerland
[2] Institute of Pathology, University Hospital of Basel, Basel, Switzerland
[3] Department of Surgery, University Hospital of Basel, Basel, Switzerland

In vitro culture of primary cancer tissue is still very limited and the generation of patient derived xenograft determine the loss of human-cancer associated stroma. In this context, the use of 3D in vitro systems based on human tissue may be an innovative system to be exploited for keeping the tumor microenvironment (TME) complexity of the tissue in vitro.

Freshly excised colorectal (CRC) and breast cancer (BrCa) specimens were fragmented and cultured in 3D 'sandwich-like format" between porous collagen scaffolds under perfusion flow (U-CUP, Cellec Biotek AG). The maintenance of tumor and immune-infiltrating cells, survival and phenotypic characterization were histologically assessed. In a second step cancer treatment were tested.

U-CUP culture allowed the preservation, viability and expansion of tumor tissue with concomitant stromal and immune cells. Expanding cancer cells were viable after 10 and 21 days (CRC and BrCa, respectively). Administration of anti-ER treatment to Lumina A ER+ BrCa was associated with decreased expansion of cancer tissue into the scaffold after 21 days. The maintenance of immune-infiltrating cells allowed testing of immune blockade therapy. Administration of anti-PDL1 antibody, alone or in combination with anti-CTLA4, to the culture medium was associated with increased expression of markers of immune-activation (i.e. IFNg) and decreased expression of immunosuppressive cytokine IL10.

Preserving malignant, interstitial and immunocompetent cells comprised in surgically excised tumor specimens might allow a direct evaluation of the effects of various treatments on the complex TME. This engineered in vitro model could allow animal-free testing and it could be extended as a platform allowing the testing of innovative approaches for the treatment of human malignancies. Our findings shed the light on a promising system for selecting personalized treatment based on a patient's tumor specific microenvironment.

CONFLICT OF INTEREST

Ownership: MGM, GCS, and IM are shareholder of Cellec Biotek AG Board of Directors: IM is member of the board of directors of Cellec Biotek AG

8 Proffered Paper: Clinical translation of nuclear export inhibitors in pancreatic cancer

A. Azmi[1], P. Philip[1], M. Kauffman[2], Y. Landesman[2], W. Senapedis[2], S. Shacham[2], A. Mahipal[3], E. Baloglu[2], I. Muqbil[1], R. Mohammad[1]

[1] Wayne State University School of Medicine- Karmanos Cancer Institute, Department of Oncology, Detroit, USA
[2] Karyopharm Therapeutics, Research and Development, Newton Massacheusettes, USA
[3] Mayo Clinic, Oncology, Rochester, USA

BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) remains a deadly disease in urgent need of newer therapeutic modalities. In PDAC, over-expression of the nuclear exporter protein exportin 1 (XPO1) also known as chromosome maintenance region 1 leads to functional inactivation of multiple tumor suppressor proteins TSPs through their mislocalization to the cytoplasmic compartment.

MATERIAL AND METHODS: 91 PDAC and 71 normal pancreatic ductal tissues were analyzed histologically for expression of XPO1. Specific inhibitor of nuclear export (SINE) compounds that bind to cys528 NES recognizing domain of XPO1 and –ve control KPT-301 were tested for their synergistic activity with gemcitabine and nab-paclitaxel in PDAC cellular and cancer stem cell derived models. CRISPR/Cas9 genome editing was performed to create mutants for SINE specificity analysis. Agilent HT12 microarrays and pathway analysis was performed post combination treatment. Anti-tumor efficacy of combination regimen was tested in several xenograft and KPC mice model.

RESULTS: XPO1 was found to be over-expressed in PDAC not normal pancreas tissue. SINE compounds not inactive analog KPT-301 induced PDAC cell death and synergized with gemcitabine (GEM) and nab-paclitaxel leading to enhanced PDAC growth inhibition, apoptosis, and spheroid disintegration of PDAC derived cancer stem cells (CSCs) (CI<1). The observed synergy was due in part to enhanced nuclear localization of TSPs and suppression of CSC markers alongside reversal of EMT markers. Crispr Cas9 mutant cells lacking SINE binding cys528 were not responsive to treatment confirming specific role of XPO1 in the reaction. Pathway analysis showed global nuclear retention of TSPs, reversal of immune suppressive signaling and CSC sustaining networks and activation of fibroblast specific cell death pathways. Selinexor as single agent drastically inhibited the growth of sub-cutaneous xenograft ($p<0.01$) and synergistically enhanced the anti-tumor efficacy

of gemcitabine or nab-paclitaxel. Anti-tumor efficacy studies in Pdx organoid derived orthotopic xenograft and KPC mice models are ongoing.

CONCLUSIONS: Our multi-model pre-clinical work has led to the approval of a Phase Ib/II clinical study involving GEM-nab-paclitaxel-Selinexor for metastatic PDAC (NCT02178436).

CONFLICT OF INTEREST

Ownership: Karyopharm Therapeutics
Advisory Board: Karyopharm Therapeutics
Board of Directors: Karyopharm Therapeutics
Corporate-sponsored Research: Karyopharm Therapeutics
Other Substantive Relationships: Karyopharm Therapeutics

10:45-12:30: SYMPOSIUM: IMMUNOTHERAPY RESISTANCE

9 Proffered Paper: Using implantable microdevices to systemically identify optimal combinations of immunotherapy and chemotherapy in situ

O. Jonas[1]

[1] *Harvard Medical School / Brigham & Women's Hospital, Radiology & Medical Oncology, Boston, USA*

Both immuno- and chemotherapies typically exhibit diverse responses across patients, and there are few clinically validated biomarkers available to predict responders accurately a priori.

Using miniaturized implantable microdevices (Jonas et al, Science Translational Medicine 2015) for high-throughput testing of multiple drugs and combinations within the native tissue, we are able to test the combinatorial response of up to 100 distinct combinations of immuno- and chemotherapies within a single tumor, and without systemic toxicities. Our analytical methods include a combination of multi-plexed immunohistochemistry to assess multiple immune markers, and MALDI mass spectrometry tissue imaging to directly measure the metabolomic and proliferative balance between immune and cancer cells.

Our studies show significant tumor model-dependent synergies between immune checkpoint inhibitors and multiple cytotoxic and targeted chemotherapies, including cisplatin and PI3K inhibitors. These synergies can be potentiated by optimizing the relative dose and timing of the immuno- and chemotherapies.

We demonstrate a clinically feasible method for rapidly identifying and validating optimal personalized combinations of immuno- and chemotherapies from the currently available set of agents. Furthermore, we are able to show that pharmacological perturbation of certain microenvironmental and metabolic factors in the tumor can significantly enhance the efficacy of immune checkpoint inhibitors.

NO CONFLICT OF INTEREST

10 Proffered Paper: Targeting type I interferon activity to the tumor microenvironment or to dendritic cells as a novel, generic and safe cancer immunotherapy

A. Cauwels[1], *S. Van Lint*[1], *F. Paul*[2], *G. Garcin*[2], *S. De Koker*[1], *S. Gerlo*[1], *Y. Bordat*[2], *G. Uze*[2], *J. Tavernier*[1]

[1] *Ghent University and VIB, Department of Medical Protein Research, Gent, Belgium*
[2] *Montpellier University, CNRS UMR 5 2 3 5 , Montpellier, France*

INTRODUCTION: Recently, immunotherapy was put forward as a fourth pillar for cancer treatment, next to surgery, chemo- and radiotherapy. Immunotherapeutics include antibodies, cellular therapies and cytokines. Type I IFN, for instance, is approved for the treatment of several hematological and solid cancers. However, its systemic toxic side effects are severely dose-limiting. To curtail toxicity, we developed AcTakines, Activated-by-Targeting Cytokines, improved (mutated) immunocytokines fused to cell-specific targeting domains.

MATERIALS AND METHODS: As a murine AcTaferon (type I IFN based AcTakine), we use hIFNa2-Q124R, which breaches the cross-species barrier and is thus weakly active on mouse cells. Selective targeting is achieved by coupling the hIFNa2-Q124R to single domain antibodies. C57BL/6J or Balb/c female mice were used for the melanoma and carcinoma/lymphoma tumor models, respectively. Tumor progression was monitored via caliper measurements, hematological parameters using a Hemavet analyzer.

RESULTS AND DISCUSSION: Fusion of hIFNa2-Q124R to anti-CD20 single domain antibody restored full IFN activity on CD20+ cells, such as B lymphocytes or A20 B cell lymphoma. In vivo treatment of A20 or B16-CD20+ tumors with CD20-targeted AcTaferon drastically reduced tumor growth, similar to high dose wild-type mIFN immunocytokine. In sharp contrast to the latter, however, tumor-targeted AcTaferon did not cause any systemic toxicity (evaluated via body weight, temperature, and blood cell counts). The AcTaferon antitumor effect was lost in IFNAR-deficient, Batf3-deficient or CD8-depleted animals, as well as in mice lacking IFNAR1 on CD11c+ cells, indicating involvement of conventional cross-presenting DC (cDC1). Furthermore, selective targeting of AcTaferon to cDC1 was sufficient to induce tumor stasis in both melanoma and breast carcinoma models. Additionally, when combined with chemotherapy, low-dose TNF, or immune checkpoint blockade, complete tumor regressions and long-lasting tumor immunity were observed, still without adverse effects.

CONCLUSION: Collectively, our findings indicate that targeting type I IFN activity to the tumor microenvironment in general, or to cDC1 specifically, can eradicate tumors and establish a memory response, without concomitant toxic side effects. As such, AcTaferons provide a safe and generic addition to current cancer (immuno) therapies.

CONFLICT OF INTEREST

Board of Directors: J.T. and G.U. are scientific co-founders, and J.T. is CTO of Orionis Biosciences. J.T. holds stock-options in the company.

14:30-16:15: SYMPOSIUM: MICROENVIRONMENT &MICROBIOME

11 Proffered Paper: Stromal cell immunomodulatory potential in the tumour microenvironment is regulated by inflammatory signalling

G. O'Malley[1], *S. Naicker*[1], *K. Lynch*[1], *P. Lohan*[1], *T. Ritter*[1], *L. Egan*[2], *A. Ryan*[1]

[1] *National University of Ireland Galway, Regenerative Medicine Institute, Galway, Ireland*
[2] *National University of Ireland Galway, Pharmacology and Therapeutics, Galway, Ireland*

BACKGROUND: The colon tumour microenvironment (TME) is highly stromal in composition and a greater stromal cell density correlates with a poor prognosis for patients. The majority of these stromal cells are of mesenchymal origin (MSCs) and are known contributors to tumour angiogenesis and invasiveness. Little is known about the role of their immunosuppressive potential in the TME. We investigated the molecular regulation of the induced immunosuppressive, tumour-promoting phenotype of tumour-associated MSCs, and the effect of inflammation on this process.

MATERIALS AND METHODS: Balb/c bone marrow derived MSCs were treated with conditioned medium from untreated CT26 tumour cells (MSCTCM) or TNF-α treated CT26 cells (MSCTNF-TCM). In an immunocompetent Balb/c syngeneic mouse model, we assessed tumour growth and anti-tumour immune responses following sub-cutaneous injection of CT26 cells alone or co-injection with MSCControl or MSCTNF-TCM

RESULTS: Treatment with conditioned medium resulted in increased expression of TCR ligands MHC-I, MHC-II and PD-L1 compared to MSCControl. This was significantly enhanced by TNF-α induced tumour cell inflammation. MSCTCM co-cultured with syngeneic activated T cells displayed an enhanced ability to suppress CD8+ T cell proliferation, which was further potentiated by inflammatory activation of CT26 (MSCTNF-TCM). This effect was dependent on induced PD-L1 expression on MSCs as PD-1 blockade restored CD8+ T cell proliferation and granzyme B secretion. In our in vivo model, co-injection of MSCControl significantly promoted tumour growth, and this was further potentiated by the co-injection of MSCTNF-TCM. We showed that this stromal cell mediated tumour promotion could be reversed by administration of a PD-1 blocking antibody, via restoration of granzyme B secreting CD8+ T cells.

CONCLUSION: We show for the first time that stromal cells in the TME directly modulate anti-tumour immune responses via PD-L1. This data will lead to better stratification of patients for immunotherapeutic regimens resulting in more targeted and durable responses

NO CONFLICT OF INTEREST

12 Proffered Paper: Cabozantinib eradicates advanced murine prostate cancer by activating anti-tumor innate immunity

A. Patnaik[1], *K. Swanson*[2], *E. Csizmadia*[3], *A. Solanki*[4], *N. Landon-Brace*[4], *H. Ye*[5], *J. Karp*[4], *S. Sabina*[6], *S. Balk*[7], *L. Cantley*[8]

[1] *The University of Chicago/Beth Israel Deaconess Medical Center/Harvard Medical School, Medicine-Hematology/Oncology, Chicago- IL, USA*
[2] *Beth Israel Deaconess Medical Center/Harvard Medical School, Neurology, Boston, USA*
[3] *Beth Israel Deaconess Medical Center, Medicine-Gastroenterology, Boston, USA*
[4] *Brigham and Women's hospital, Medicine, Boston, USA*
[5] *Beth Israel Deaconess Medical Center/Harvard Medical School, Pathology, Boston, USA*
[6] *Brigham and Women's hospital, Pathology, Boston, USA*
[7] *Beth Israel Deaconess Medical Center/Harvard Medical School, Medicine, Boston, USA*
[8] *Weill Cornell Medical College, Meyer Cancer Center, New York, USA*

Several kinase inhibitors that target aberrant signaling pathways in tumor cells have been deployed in cancer therapy. However, their impact on the tumor immune microenvironment remains poorly understood. The tyrosine kinase inhibitor cabozantinib showed striking responses in cancer clinical trial patients across several malignancies. Here we show that cabozantinib rapidly eradicates large, poorly-differentiated tumors that form in the context of PTEN/p53 deficient murine prostate cancer. This was associated with enhanced release of neutrophil chemotactic factors from tumor cells, including CXCL12 and HMGB1, resulting in robust infiltration of neutrophils into the tumor microenvironment. Critically, cabozantinib-induced tumor clearance in mice was abolished by antibody-mediated granulocyte depletion or HMGB1 neutralization or blockade of neutrophil chemotaxis with the CXCR4 inhibitor, plerixafor. Collectively, these data demonstrate that cabozantinib triggers a neutrophil-mediated anti-cancer innate immune response, resulting in rapid tumor clearance.

(Manuscript summarizing this work has been favorably reviewed, revised and submitted back to Cancer Discovery for publication)

NO CONFLICT OF INTEREST

16:45-18:15: SYMPOSIUM: NEW THERAPEUTIC APPROACHES

13 Proffered Paper: Harnessing the spatially regulated tyrosine phosphorylation mechanisms for precision medicine

V.k. Ulaganathan[1], A. Ullrich[1]

[1] Max Planck Institute of Biochemistry, Department of Molecular Biology, Martinsried, Germany

Variations in the germline and somatic genomes are important determinants of cancer pathogenesis and disease progression. However, the limited availability of the target-specific drugs and the prevalence of the inter & intratumor genetic heterogeneity pose enormous challenges in identifying the right drug for the right patient. Hence a serious rethinking on the conventional 'one drug fits all' approach in the drug discovery process is urgently needed. Protein-coding mutations that alter the quality and quantity of tyrosine phosphorylations often impact protein-protein interactions such as the tyrosine-phosphorylated juxtamembrane motifs recruiting STAT3 to the inner cell membranes (Ulaganathan et al Nature 528, 2015). Recently, we made a remarkable observation that polypeptides or proteins of interest when anchored to the inner cell membrane underwent chemical modification by tyrosine phosphorylation, suggesting a spatially determined regulatory mechanism for tyrosine phosphorylation in living cells. Here, by exploiting the spatially controlled protein tyrosine phosphorylation phenomenon, we demonstrate innovative genotype-centric approaches to tackling non-druggable targets in cancer, namely, the direct modulation of transcription factor activity and sequence specific inhibition of protein variants in living cells. Specific transcription factors when recruited to inner cell membrane leaflet by either attaching to a transmembrane helical domain sequence, lipid modification sequence (such as myristoylation) or by trans-acting membrane-proximal interactions, all induced enhanced phosphorylation of the transcription factor of interest resulting in the modulation of its function. Next, when high-affinity modular domains capable of sequence-specific interactions (such as point mutation-specific monoclonal antibodies) were anchored to the inner cell membranes, effective sequence-specific knock-down of the target protein and mRNA expression occurred in an allele- or mutation-specific manner. These result demonstrate a prelude to a novel drug discovery approach in precision medicine for cell-type specific transcription modulation and sequence variant specific inhibition of protein expression.

NO CONFLICT OF INTEREST

14 Proffered Paper: Suppression of oncogene transcription with PNA-delivery peptide conjugates –potential therapy for BRAF-V600E and KRAS-G12D driven tumors

J. Rothman[1], O. Surriga[1], G. Ambrosini[1], G. Schwartz[1]

[1] Columbia University Medical Center, Division of Oncology, New York, USA

INTRODUCTION: We have developed and evaluated a strategy to block transcription of oncogenes such as BRAF V600E and KRAS G12D directly by using modified complementary peptide nucleic acid (PNA) oligomers that target oncogenesis specifically and effectively causing inhibition of tumor growth. These proof of principle trials against BRAF V600E and KRAS G12D in vitro and in vivo should provide a new means to develop PNA-delivery peptide conjugates as targeted drug therapeutics across a broad range of oncogenes that drive cancer cell growth.

MATERIALS AND METHODS: Obstruction of KRAS G12D expression was evaluated through suppression of cell proliferation in an array of cell lines and specific mRNA transcription. Likewise we have assessed obstruction of BRAF V600E transcription specifically in variety of cell lines by monitoring suppression of cell proliferation, BRAF V600E protein expression, and mRNA transcription. Tumor reduction was assessed through xenograft mouse models.

RESULTS: Our result indicate that exposure of the melanoma cell lines to a modified PNA-peptide conjugate complementary to BRAF V600E mutation sequence result in a concentration-dependent and time-dependent inhibition of cell growth that is specific for the BRAF V600E-driven mutant melanoma cell lines with inhibition of mRNA and protein expression. Xenograft mouse trials show tumor growth delay and necrosis compared to PNA controls. This 50mg/kg dose was well tolerated without associated weight loss. By H&E staining, tumor tissue from trials shows ablation and extensive scarring upon exposure to BRAF V600E-complementary PNA-peptide conjugate whereas saline and scramble PNA sequence controls do not. Similarly quantitative measurement shows a 2.5-fold decrease in Ki67 and a 3-fold increase in TUNEL expression.

Exposure of KRAS G12D-dependent cell line to modified PNA-peptide conjugate complementary to KRAS G12D mutation sequence also result in concentration-dependent and time-dependent inhibition of cell growth and concomitant decrease in mRNA transcription. Cell lines expressing KRAS WT and KRAS G12C, both differing from KRAS G12D by a single nucleobase, show no suppression.

CONCLUSION: Our result indicate that these PNA-peptide derivatives could represent a novel and promising new therapy for patients with genes specific for and causative of tumorigenesis. This strategy could be applied to a multitude of cancers either with specific translocations or mutations differing from wild-type cells even by only a single base pair.

NO CONFLICT OF INTEREST

MONDAY 26 JUNE 2017

09:15-10:15: PROFFERED PAPERS: PROFFERED PAPERS 2

15 Proffered Paper: Immunologic reshaping of cancer by stimulation of innate nucleic acid sensor RIG-I

C. Schuberth-Wagner[1], M. Niewel[2], M. Renn[1], C. Jakobs[1], A. Schwickart-Halbe[3], J. Vollmer[1]

[1] Rigontec GmbH, Research, Martinsried, Germany
[2] Rigontec GmbH, Clinical Development, Martinsried, Germany
[3] Rigontec GmbH, CMC, Martinsried, Germany

INTRODUCTION: We describe a novel immunotherapy approach in which a viral defense system is harnessed to stimulate anti-tumor immunity. Cancer immunotherapy has revolutionized oncology in recent years, yet many tumors become resistant or do not respond to current treatments such as checkpoint inhibitors. Stimulation of the innate immune system opens a new therapeutic strategy that could be combined effectively with other immunotherapeutic regimens. The ubiquitously expressed cytosolic RNA receptor retinoic acid inducible gene I (RIG-I) recognizes double-stranded RNA bearing a 5'-triphosphate. RIG-I plays a prominent role in antiviral defense. Its activation induces apoptosis preferentially in tumor cells and simultaneously activates the innate immune system via type I interferon (IFN) signaling.

MATERIAL & METHOD: We developed an optimized, fully synthetic oligonucleotide, designated RGT100, which is a RIG-I selective ligand. RGT100 activates the RIG-I pathway leading to the induction of cytokines, including IFN-a and IFN-b and apoptosis in tumor cells.

RESULTS: The treatment of tumor-bearing mice with RGT100 demonstrated potent anti-tumor activity in a variety of tumor models. Histological and flow cytometric analysis of the tumors revealed infiltration and activation of immune cells after RGT100 treatment. Treatment of subcutaneous tumors by intratumoral injection led to efficacy of both the treated tumors as well as untreated contralateral tumors. Furthermore, systemic delivery of RGT100 was efficacious against both local subcutaneous B16 melanoma as well as its lung metastases. Data support both natural killer (NK) cell-mediated and T cell-mediated anti-tumor activities, and resistance to tumor re-challenge has been demonstrated.

CONCLUSION: In summary, Rigontec's RIG-I-selective ligand RGT100 shows strong anti-tumor activity in several clinically relevant mouse tumor models, while bearing an advantageous safety profile. RGT100 has entered clinical evaluation in advanced cancer patients in Q1 2017. This agent will be clinically evaluated for single-agent activity as well as in combination with checkpoint inhibitors.

NO CONFLICT OF INTEREST

16 Proffered Paper: A cross-tumors approach identifies the transcription factor FOSL1 as a KRAS oncogene dependency in lung and pancreatic cancer

A. Vallejo[1], N. Perurena[1], E. Guruceaga[2], P.K. Mazur[3], K. Valencia[1], M. Ponz-Sarvise[4], A. Bozec[5], J. Sage[3], F. Lecanda[2], S. Vicent[1]

[1] Center for Applied Medical Research, Program in Solid Tumors and Biomarkers, Pamplona, Spain
[2] Center for Applied Medical Research, Proteomics- Genomics and Bioinformatics Core Facility, Pamplona, Spain
[3] Stanford University School of Medicine, Department of Genetics and Pediatrics, Stanford, USA
[4] University of Navarra Clinic, Department of Medical Oncology, Pamplona, Spain
[5] University of Erlangen-Nuremberg, Department of Internal Medicine 3 and Institute of Clinical Immunology, Erlangen, Germany

INTRODUCTION: KRAS is often mutated in human cancers. However, the vast majority of mutant KRAS cancers remain refractory to current clinical therapies. Thus, a deeper understanding of the molecular mechanisms triggered by KRAS oncogene may yield alternative strategies to neutralize KRAS-mediated effects. Gene-expression profiling has unveiled KRAS signatures from single experimental systems or tumor types that classify patients according to KRAS genotype and dependency, but they show little overlap. This raises the question whether a core of genes relevant for mutant KRAS biology is preserved across different tumor types.

MATERIAL AND METHODS: A meta-analysis on gene-expression data from in vitro and in vivo experimental systems of epithelial/mesenchymal origin with either wild-type or mutant KRAS allele, as well as from publicly-available data from lung adenocarcinoma (LAC) and pancreatic ductal adenocarcinoma (PDAC) colorectal cancer, cholangiocarcinoma and multiple myeloma tumors, was performed. Follow-up experiments included panels of human cancer cell lines with known RAS status, patient derived xenografts (PDXs), genetically-engineered mouse models (GEMMs) of Kras-driven LAC and PDAC, and lung cancer cell lines and 3D organoid cultures from these GEMMs.

RESULTS AND DISCUSSION: We report the identification of a common transcriptional signature across mutant KRAS cancers of distinct tissue origin that includes the transcription factor FOSL1. Lung and pancreatic cancers expressing oncogenic KRAS can be targeted by genetic inhibition of FOSL1. Additionally, high FOSL1 expression identifies a group of mutant KRAS lung and pancreatic cancer patients with the worst survival outcome. Mechanistically, FOSL1 links the KRAS

oncogene to transcriptional regulation of components of the mitotic machinery, a pathway previously postulated to function orthogonally to oncogenic KRAS. FOSL1 targets included AURKA, whose genetic inhibition impaired viability of mutant KRAS cells. Lastly, combination of AURKA and MEK inhibitors induced a genotype-dependent deleterious effect on mutant KRAS cells in vitro and in vivo.

CONCLUSION: We have devised an integrative strategy to expose common core elements of KRAS signaling that unveiled FOSL1 as a relevant gene in KRAS-driven LAC and PDAC. FOSL1 functions by transcriptionally regulating a subset of genes involved in mitotic fitness that may provide new avenues to treat KRAS-driven cancers.

NO CONFLICT OF INTEREST

17 Proffered Paper: A blood-based multi-marker panel for pancreatic cancer early detection

M. Capello[1], L. Bantis[2], G. Scelo[3], R. Brand[4], M.A. Firpo[5], M.H. Katz[6], P. Brennan[3], Z. Feng[2], A. Taguchi[7], S.M. Hanash[1]

[1] University of Texas MD Anderson Cancer Center, Clinical Cancer Prevention, Houston, USA
[2] University of Texas MD Anderson Cancer Center, Biostatistics, Houston, USA
[3] International Agency for Research on Cancer IARC, Genetic Epidemiology, Lyon, France
[4] University of Pittsburgh, Division of Gastroenterology- Hepatology & Nutrition, Pittsburgh, USA
[5] University of Utah, Department of Surgery, Salt Lake City, USA
[6] University of Texas MD Anderson Cancer Center, Surgical Oncology, Houston, USA
[7] University of Texas MD Anderson Cancer Center, Translational Molecular Pathology, Houston, USA

BACKGROUND: Strategies for the detection of early-stage pancreatic ductal adenocarcinoma (PDAC) are urgently needed. We previously developed and sequentially validated a circulating plasma protein biomarker panel consisting of TIMP1, LRG1, and CA19-9 with improved performance compared with CA19-9 alone for detecting early-stage PDAC. Since a serologic response in the form of autoantibodies to tumor antigens is triggered at early stages of tumor development, we have investigated the contribution of autoantibodies as additional types of potential circulating biomarkers which might improve the performance of the three-protein anchor panel.

MATERIAL AND METHOD: Microarray slides coated with 187 previously described tumor-associated antigens were applied to evaluated the autoantibody reactivity of three combined plasma sample cohorts consisting of 73 early-stage PDAC cases, 60 healthy controls (HCs), and 74 benign pancreatic disease (BPD) cases. Antigens mRNA and protein expression was evaluated through analysis of The Cancer Genome Atlas RNA-seq data for PDAC (N=112) and through in-depth mass spectrometry analysis of a panel of PDAC cell lines (N=11), respectively.

RESULTS: Sixty-five antigens were selected as potentially more relevant to PDAC based on their mRNA and protein expression in PDAC tissues and cell lines. IgG reactivity against 18 antigens was significantly elevated in PDAC compared to both HCs and BPD patients (AUC>0.60 and P<0.05). The autoantibodies with a top performance were utilized to develop an autoantibody panel able to significantly improve the three-protein anchor panel based on a logistic regression model. A panel consisting of four autoantibodies and the validated three protein biomarker anchor panel yielded AUC (95% CI) of 0.969 (0.946-0.991) and a cross-validation related average AUC of 0.968 in discriminating PDAC from HCs, with in a significant improvement over anchor panel alone (P =0.044). In the comparison between PDAC and BPD cases the same protein and autoantibody panel yielded an AUC (95% CI) of 0.902 (0.857-0.947), also with significantly improved performance compared to anchor panel (P=0.011).

CONCLUSION: An autoantibody signature significantly improved performance characteristics of the validated three-protein anchor panel in discriminating early-stage PDAC from both HCs and BPD cases, confirming the complementary nature of these different types of biomarkers.

NO CONFLICT OF INTEREST

18 Proffered Paper: Role of PD-L1 immunoregulatory protein in breast cancer cells metabolic reprogramming

J. Berthe[1], J. Kluza[1], H. El Bouazzati[1], I. Briche[1], X. Thuru[1], S. Galiègue-Zouitina[1], B. Quesnel[1]

[1] Univ. Lille- Inserm- CHU Lille- UMR-S 1 1 7 2 - JPArc - Centre de Recherche Jean-Pierre AUBERT Neurosciences et Cancer, Team "Persistance factors of leukemic cells", Lille, France

When normal cells progressively evolve to a neoplastic state, they acquire many characteristics. Indeed, tumor cells follow abnormal metabolic pathways and exhibit the ability to avoid immune destruction, in part by exploiting immune checkpoints. Many of these are currently under clinical investigation for new cancer treatments, among them the PD-1/PD-L1 immune checkpoint.

PD-L1 (Programmed Death-Ligand 1) is a transmembrane protein, that is overexpressed on tumor cells and that belongs to the B7 immunoregulatory proteins family. Its expression is associated with poor cancer outcome in many types of cancer. Indeed, PD-L1 binding to its T cell receptor PD-1 delivers an immunosuppressive signal and inhibits T cell functions. Recently, it has been described that PD-L1 induces cancer cells resistance to apoptosis and chemotherapy. However, cellular mechanisms modulated by PD-L1 and involved in these functions are still unclear. It is known that abnormal metabolic pathways contribute to tumor growth and therapy resistance; therefore, in this study, we investigated the role of PD-L1 in cancer cell metabolic reprogramming that could explain resistance to apoptosis.

For this, a knock-out was generated in the breast cancer cell line MDA-MB-231 by genome editing targeting the PD-L1 encoding gene, as well as the same line with PD-L1 overexpression. We have found that PD-L1 induces a shift from oxidative phosphorylation to glycolysis of tumor cells, indicating that PD-L1 promotes the Warburg effect. Moreover, mitochondrial reactive oxygen species (ROS) production following pro-oxidant treatment is increased in PD-L1 overexpressing cells. Transcriptomic analysis were done and suggest that NRF2-mediated oxidative stress response pathway is suppressed by PD-L1. Furthermore, human breast cancer xenograft experiments in NUDE mice demonstrated that PD-L1 overexpression increases tumor growth.

Together, our result show that PD-L1 overexpression regulates cancer cells metabolism and more especially ROS production through the NFR2 pathway. Besides, the Warburg effect, as promoted by PD-L1, is known to contribute to tumor progression and therapy resistance. We are now currently examinating the impact of PD-L1 metabolism deregulation on resistance to apoptosis.

Thus, our study evidences novel mechanisms underlying the impact of PD-L1 in cancer cell progression. Anti-PD-L1 therapies might be used to target cancer cell metabolism and this way to improve breast cancer treatment.

NO CONFLICT OF INTEREST

19 Proffered Paper: Human bone marrow-derived mesenchymal stem cells promote invasiveness and transendothelial migration of osteosarcoma cells through a mesenchymal to ameboid transition

L. Pietrovito[1], E. Giannoni[1], V. Gori[2], F. Bambi[2], P. Chiarugi[1], M.L. Taddei[3]

[1] University of Firenze, Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Firenze, Italy
[2] Meyer Children's Hospital, Transfusion Medicine and Cell Therapy, Firenze, Italy
[3] University of Firenze, Dipartimento di Medicina Sperimentale e Clinica, Firenze, Italy

BACKGROUND: Bone marrow mesenchymal stem cells (BM-MSCs) represent critical players of tumor stroma. Osteosarcoma (OS) is the most common primary bone sarcoma, characterized by high local aggressiveness and rapid metastasis. Here, we investigate the in vitro effects induced by the complex interplay between BM-MSCs and OS cells on tumor progression.

MATERIALS AND METHODS: BM-MSCs were provided by the Meyer Hospital, Florence. OS cells (Saos-2, MG-63 and HOS) and endothelial cells (HUVECs) were from ATCC. ELISA and cytokines arrays of conditioned medium (CM) were performed. Boyden chambers were used for evaluating cells' migration, invasion and transendothelial migration. BM-MSCs transdifferentiation was assayed by WB analysis, immunofluorescence and functional assays. Pull-down assays, PCR analysis and capillary morphogenesis were performed to evaluate RhoA-GTPases activation and tumor angiogenesis.

RESULTS: BM-MSCs showed an avid tropism for OS cells. We identified for the first time, GRO-α, MCP-1 and TGF-β1 as pivotal factors for this migration. Once in contact with OS cells, BM-MSCs trans-differentiate into cancer associated fibroblasts (CAFs)-like cells and increase the secretion of GRO-α and MCP-1 in tumor microenvironment. These cytokines, in concert with IL-6 and IL-8, boost invasiveness and intravasation capacity of OS cells, in addition to confer anoikis resistance. The increase of the metastatic potential of OS cells is mediated by a mesenchymal to ameboid transition (MAT). Indeed, following contact with CM from tumor-activated BM-MSCs, OS cells show a rounded morphology, insensibility to the MMPs inhibition, activation of RhoA and downregulation of active Rac1 GTPases, all markers of MAT. Finally, we found that the cross-talk between BM-MSCs and OS cells stimulates secretion of pro-angiogenic factors by OS cells and promotes migration and invasion of endothelial cells, thus supporting tumor vascularisation.

CONCLUSIONS: Our result reveal homing properties of BM-MSC for different OS cell lines and emphasize their pro-tumorigenic activity in OS progression. In particular, a complex interchange of signals mediated by IL-6, IL-8, GRO-α and MCP-1 produced by tumor-activated BM-MSCs determines a MAT of tumor cells, promoting an increase of their transendothelial migration and invasion. Therefore, therapeutic approaches aim to block or impair these cytokines could potentially reduce primary tumor growth and metastatic dissemination of OS.

NO CONFLICT OF INTEREST

20 Proffered Paper: NFIB and YBX1 bind to and repress ESR1, revealing a therapeutically relevant regulatory loop in breast cancer

K.B. Meyer[1], T.M. Campbell[1], M.A.A. Castro[2], B.A.J. Ponder[1]

[1] University of Cambridge, CRUK Cambridge Institute, Cambridge, United Kingdom
[2] Federal University of Parana UFPR, Bioinformatics and Systems Biology Lab, Parana, Brazil

INTRODUCTION: Studying transcription factor (TF) interactions and gene regulatory networks in breast cancer, we recently identified two distinct and opposing clusters of TFs associated with estrogen receptor-positive (ER+) and -negative (ER-) breast cancer and breast cancer risk. The relative activity of these two groups of TFs has a dramatic effect on patient outcomes and is likely to influence the phenotypic plasticity observed in breast cancer. This plasticity is likely to be relevant in the development of resistance to therapy.

METHODS: Here, we examine the molecular mechanisms underlying the opposing functions of the two groups of TFs by studying protein-protein interactions between TFs and their functional consequences. We also examine the effect of cell signalling pathways, in particular signaling by FGFR2, on the relative activity of the two groups of TFs.

RESULTS AND DISCUSSION: Using Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins (RIME), co-immunoprecipitation experiments and FRET microscopy studies we identify NFIB and YBX1 as two novel interactors of the ESR1 (estrogen receptor alpha) protein. We demonstrate that NFIB and YBX1, two TFs associated with ER- disease progression, interact with the ESR1-FOXA1 complex and are able to repress transcriptional activation by ESR1.

We have previously shown that FGFR2, a known breast cancer risk gene, is able to repress ESR1 activity. We now demonstrate that FGFR2 signalling has wide-ranging effects on the two TF-clusters and induces many regulons that are also activated in basal-like breast cancer. The effects of FGFR2 signalling are possibly mediated via increased phosphorylation of YBX1 and its interaction with ESR1. In line with these findings, we demonstrate in a breast cancer cell line that FGFR2 inhibitors may increase the efficacy of estrogen deprivation therapy.

CONCLUSION: We therefore show that members of two opposing TF-clusters, driving ER+ and ER- diesease, respectively, physically interact, postulate that this interaction is affected by FGFR2 signalling and provide first evidence that this can potentially be exploited therapeutically.

NO CONFLICT OF INTEREST

10:45-12:30: SYMPOSIUM: DRUG RESISTANCE

21 Proffered paper: Response to targeted therapy in melanomas expressing ALK fusions and other ALK variants

K. Couts[1], J. Bemis[1], J. Turner[1], S. Bagby[1], D. Murphy[2], J. Christiansen[2], J. Hintzsche[1], T. Medina[1], R. Doebele[1], W. Robinson[1]

[1] University of Colorado Anschutz Medical Campus, Division of Medical Oncology, Aurora, USA
[2] Ignyta- Inc., Diagnostics, San Diego, USA

INTRODUCTION: Anaplastic Lymphoma Kinase (ALK) is an oncogenic kinase which is not typically expressed in melanomas and other cancers, but can be expressed and activated by mechanisms such as gene fusions. ALK fusions have been reported in several cancers, but they have not been identified in melanomas. Recently a novel alternate ALK transcript (ALKATI) was found to be expressed in 10% of melanomas however its response to targeted therapy has not been well studied.

MATERIALS AND METHODS: We used qRT-PCR and western blotting to screen for ALK expression in 45 melanoma patient-derived xenograft (PDX) tumors, including tumors from distinct melanoma subtypes. A potential ALK fusion was characterized using fluorescence in-situ hybridization and targeted RNA sequencing. PDX tumors, PDX-derived cell lines, and NIH3T3 cells expressing ALK fusions, ALKATI, or wild-type (wt) ALK were treated with ALK inhibitors (crizotinib, ceritinib) and cell viability or tumor growth was analyzed. Additionally, a melanoma patient whose tumor expressed ALKATI was enrolled in a phase I basket trial of a ROS1/TRK/ALK inhibitor (entrectinib).

RESULTS: We observed ALK expression in 12/45 (26.7%) melanoma PDX tumors. We identified an EML4-ALK fusion in a mucosal melanoma, which is the first report of a characterized ALK fusion in melanoma. ALKATI was expressed in 8/45 PDX models (18%), and 2 of these tumors co-expressed ALKATI and wt ALK. Three tumors (6.7%) expressed wt ALK only. The melanoma with an EML4-ALK fusion showed strong inhibition of cell viability and tumor growth upon treatment with ALK inhibitors. Melanomas expressing ALKATI or wt ALK did not respond to ALK inhibitors, and ALK did not appear to be phosphorylated/activated in these melanomas. We observed the same responses in NIH3T3 cells, where cells expressing an EML4-ALK fusion, but not ALKATI or wt ALK, responded to ALK inhibitors. Additionally, the melanoma patient whose tumor expressed ALKATI did not respond to targeted therapy with entrectinib.

CONCLUSION: Although we observed ALK expression at a high frequency across melanomas, only those with an ALK fusion appear to be activated and respond to treatment with ALK inhibitors. These result suggest melanoma patients should be screened for ALK fusions and targeted therapy with ALK inhibitors would be an option for these patients, whereas patients expressing ALKATI or wt ALK may not respond to ALK targeted therapies.

NO CONFLICT OF INTEREST

22 Proffered Paper: STAT3 mediates resistance to BRAF inhibitors in thyroid carcinoma cells

T. Notarangelo[1], L. Sisinnni[1], V. Condelli[1], M. Landriscina[1,2]

[1] IRCCS- Referral Cancer Center of Basilicata, Laboratory of Pre-Clinical and Translational Research, RIONERO IN VULTURE, Italy
[2] University of Foggia, Medical Oncology Unit, Foggia, Italy

BACKGROUND: Thyroid carcinomas (TC) bearing BRAF mutations represent approximately 36–53% of all thyroid tumors. Differently from melanomas, BRAF-mutated thyroid cancers are poorly sensitive to BRAF inhibitors (BRAFi) and develop acquired resistance, through activation of alternative pathways. In this study, gene expression profiling was used to identify novel mechanisms of resistance to vemurafenib and activation of STAT3 pathway was investigated as potentially responsible for poor response to BRAFi in BRAF-mutated thyroid cancer cells.

MATERIAL AND METHODS: A full-genome gene expression profiling was performed in TC BRAF V600E FRO cells exposed to vemurafenib for 4, 15, 24 h. The Ingenuity Pathway Analysis (IPA) was used to identify genes and/or pathways modulated in response to vemurafenib and likely responsible of drug resistance.

RESULTS: The whole-genome gene expression analysis and IPA identified STAT3 among the most significantly upregulated genes in response to vemurafenib, with maximal induction 15 h after treatment, in parallel with modulation of several genes associated to STAT3 pathway (i.e., IL6, CEBPb, STAT1, STAT3, SOCS2, cFOS). The upregulation of STAT3 and STAT3-related genes was confirmed by quantitative PCR and immunoblot analysis in several BRAF V600E TC cell lines and in TC cells chronically adapted to vemurafenib, this supporting the hypothesis that STAT3 pathway is activated in response to BRAFi. Interestingly, the simultaneous blockade of STAT3 (by siRNA or pharmacological inhibition) and BRAF (by vemurafenib) induced a significant attenuation of S phase, with arrest of cells in the G0/G1 phase, in BRAF V600E cell lines and, with less extent, in vemurafenib-resistant TC cells. Furthermore, since it has been proposed that STAT3 signaling/expression is activated upon stimulation with IL6, the humanized anti-human IL-6 receptor antibody, tocilizumab was used to revert resistance to BRAFi. Preliminary data suggest that combined exposure to tocilizumb and vemurafenib result in G0-G1 arrest, with attenuation of S phase, in BRAF V600E TC cells

CONCLUSION: These data support the role of STAT3 signaling in resistance to vemurafenib and candidate IL6 secretion in response to BRAFi as a mechanism responsible for STAT3 pathway activation. Thus, dual blockade of IL6 and BRAF may represent a strategy to potentiate vemurafenib single agent activity and prevent/delay resistance.

NO CONFLICT OF INTEREST

14:30-16:15: SYMPOSIUM: TUMOUR CELL METABOLISM

23 Proffered Paper: Global transcriptional analysis reveals miR23b-3p and amino acids transport as a key metabolic hub of endocrine therapy resistance in ER+ breast cancer

M. Bacci[1], M. Ferracin[2], M. Ramazzotti[1], G. Comito[1], E. Giannoni[1], L.A. Martin[3], P. Chiarugi[1], A. Morandi[1]

[1] University of Florence, Department of Experimental and Clinical Biomedical Sciences, Florence, Italy
[2] University of Bologna, Department of Specialised- Experimental- and Diagnostic, Bologna, Italy
[3] Institute of Cancer Research, Breast Cancer Now Research Centre, London, United Kingdom

BACKGROUND: Aromatase inhibitors (AI) are the first-line endocrine treatment choice for postmenopausal women with ER+ breast cancers. However, resistance remains a major challenge. We have recently demonstrated that miR155 controls central carbon metabolism of AI resistant cells. To further elucidate whether this plasticity may involve additional metabolic pathways and other miRNAs, we performed global transcription analysis of parental and long-term oestrogen deprived (LTED) cells, a model that mimics AI resistance.

MATERIAL AND METHOD: MCF7 ER+ breast cancer cells and their LTED derivatives were subjected to miRNA and mRNA microarray profiling. Data were then subjected to (i) Gene Set Enrichment and (ii) Magia2 (Bisognin et al., 2012) analysis to identify post-transcriptional regulatory networks. Metabolic characterization was performed using radioactive assay, confocal microscopy, qRT-PCR and Western Blotting analyses.

RESULTS: Hierarchical cluster analysis showed that the samples are divided into parental and LTED groups and ~3K genes and ~60 miRNAs are differentially regulated between MCF7 and MCF7-LTED cells. Gene sets related to amino acids transport (MSigDb M188; MSigDb M15239) are inversely associated with the LTED profile. Crucially, integrative analysis showed the deregulation of a key hub involving miR23b-3p (upregulated in LTED) and the amino acid transporter SLC6A14 (downregulated in LTED).

Radioactive tracing analysis revealed an overall reduced amino acids uptake in LTED cells and concomitant activation of autophagy (i.e. enhanced LC3-II and beclin-1 expression together with mTOR and AMPK pathways analysis). The central role of miR23b-3p in orchestrating this reprogramming was confirmed by miRNA interference in vitro.

Importantly, Kaplan-Meier analysis of ER+ patients undergoing endocrine therapy revealed that lower levels of SLC6A14 are associated with poor survival (n=1225; HR=0.8; P=0.01) and, accordingly, higher miR23b-3p expression is associated with worse prognosis in a TGCA derived ER+ breast cancer patients cohort (n=328; HR=2.8, P=0.02). This hub might be important also in resistance to other endocrine agents, since high miR23b-3p and low SLC6A14 are also features of tamoxifen and fulvestrant resistant cells.

CONCLUSION: Endocrine therapy resistant cells undergo a strategic adaptive mechanism characterised by high metabolic plasticity and independency from the extracellular environment (e.g. exogenous amino acids). miR23b-3p has prognostic value and its higher expression is a feature of endocrine therapy resistant cells. Identifying miR23b-3p controlled pathways, such as autophagy, may offer an array of potential targetable pathways to be exploited as monotherapies or combinatorial approaches to combat or delay endocrine therapy resistance in ER+ breast cancers.

NO CONFLICT OF INTEREST

24 Proffered Paper: A chemical-genetic CRISPR screen identifies cancer vulnerabilities to perturbation of mitochondrial respiration

M. Chandrashekhar[1], M. Aregger[2], T. Hart[3], J. Moffat[1]

[1] University of Toronto- Donnelly CCBR, Molecular Genetics, Toronto, Canada
[2] University of Toronto, Donnelly CCBR, Toronto, Canada
[3] The University of Texas MD Anderson Cancer Center, Department of Bioinformatics and Computational Biology, Houston, USA

BACKGROUND: CRISPR-Cas9 genetic screening enables the identification of cellular fitness genes that operate either globally (core fitness genes) or specifically within a particular genetic background: or environmental context (context-specific fitness genes) at an unprecedented depth. In tumors, this is the foundation for the concept of synthetic lethality as genes required in tumor cells but not in adjacent normal tissues should make ideal therapeutic targets with high effectiveness and minimal side effects.

METHODS: Towards this goal, we have developed a second-generation CRISPR guide RNA library of 176,500 guides targeting 17,661 human protein-coding genes, and used it to screen human cell lines to identify genes whose knockouts induce significant fitness defects.

RESULTS: Our screens accurately recapitulate pathway-specific genetic vulnerabilities induced by known oncogenes and identify novel context-specific vulnerabilities. Furthermore, we identified a specific dependency on mitochondrial activity in specific cell lines and validated this using various complex I inhibitors. This observation strongly supports the idea that some tumors are dependent on mitochondrial respiration (MR), a clear exception to the Warburg effect, and this could be exploited as a targetable weakness. In order to identify vulnerabilities upon perturbation of MR, we performed a genome-wide CRISPR screen with an inhibitor of MR. Our screen revealed that inhibition of MR sensitizes cells to loss of genes involved in other metabolic pathways such as glycolysis and lipid biosynthesis, and also with genes in the aspartate-malate and the glycerol-phosphate shuttle. We also find a novel uncharacterized gene whose loss suppresses the affect of the MR inhibitor and show that this protein localizes to the mitochondria.

CONCLUSIONS: Thus, we show that chemical-genetic CRISPR screens can be applied to identify novel genetic interactions with MR, while also delineating critical drug resistance mechanisms. Our findings demonstrate that CRISPR-Cas9 screens enable a high-resolution view of the genetic vulnerabilities of a cell that may represent therapeutic opportunities in cancer.

NO CONFLICT OF INTEREST

16:45-18:15: SYMPOSIUM: MOUSE MODELS

25 Proffered Paper: Targeting immunotherapy to the tumor microenvironment using anti-PDL1 VHH

M. Dougan[1], J. Ingram[2], H. Ploegh[3], S. Dougan[2]

[1] Massachussets General Hospital, Gasteroenterology, Boston, USA
[2] Dana Farber Cancer Institute, Cancer Immunology and Virology, Boston- MA, USA
[3] Boston Children's Hospital, Pediatrics, Boston, USA

Targeted cytokine delivery to the tumor microenvironment can have powerful effects on the immune landscape in tumors. Most cytokines act in autocrine or paracrine fashion and have short half-lives. Concentration of cytokines in the right location is critical; yet, the few cytokine based therapies used in patients are given systemically, such as IL-2, often resulting in severe dose-limiting toxicities. Efficacy of cytokine-based therapies is limited by an inability to deliver them to the proper location, and an incomplete knowledge of which cytokines combine to induce positive changes in the cellular infiltrates in various cancer types.

Cytokine-based therapies can be particularly promising as a means of recruiting T cells to immunologically cold tumors, which respond poorly to checkpoint blockade therapies. Among the least responsive tumor types to anti-CTLA-4 and PD-1 blockade is pancreatic ductal adenocarcinoma (PDAC). PDAC is characterized by a dense stromal cell reaction, resulting in a nutrient-poor immunosuppressive environment. The majority of immune infiltrates are immunosuppressive myeloid cells. A robust means of targeting the tumor microenvironment and an understanding of the effects of combination therapy on a coordinated immune response to pancreatic cancer are still lacking.

We have developed an anti-PD-L1 VHH-based cytokine delivery system that exploits the presence of PD-L1 in the tumor microenvironment to concentrate IL-2 or IFNg in the tumor and avoid systemic side effects. VHHs are tiny – approximately 1/10th the size of full sized antibodies, and we show by PET imaging that our anti-PDL1 VHH can penetrate dense pancreatic tumors and deliver therapeutically active cytokines. Targeted delivery of IL-2 or IFNg reduced tumor burden by 50%, while isotype control-conjugated cytokines or blockade of PD-L1 alone showed little effect. IL-2 increased the number of intratumoral CD8 T cells, while IFNg reduced myeloid-derived suppressor cells and reprogrammed intratumoral macrophages. Orthotopically implanted PDAC organoids develop extensive fibrosis and are minimally infiltrated by CD8 T cells, thus better modeling the human disease. VHH-targeting of IFNg reduces tumor burden by more than 50% in mice harboring PDAC organoid-derived tumors, and correlates with a reduction in MDSCs and a reprogramming of tumor-associated macrophages.

NO CONFLICT OF INTEREST

26 Proffered Paper: The immunoreceptor NKG2D promotes tumorigenesis in models of inflammation-driven cancer

J. Guedes[1], S. Sheppard[1], N. Guerra[1]

[1] Imperial College London, Life Sciences, London, United Kingdom

BACKGROUND: NKG2D is one of the most potent stimulatory receptor expressed on effector cells of the innate and adaptive immune system. The binding of the activating receptor NKG2D to its multiple ligands creates a powerful system for sensing cellular stress. Ligands for NKG2D are expressed at low level on healthy tissue and upregulated in various type of cancer. The current paradigm is that NKG2D acts solely as a tumor suppressor and a phase I trial for NKG2D CAR T-cell therapy is currently recruiting patients. Nonetheless, a comprehensive understanding of NKG2D function in vivo is still missing due to the scarcity of model studies that recapitulate human cancer. Based on accumulating evidence that NKG2D contribute to inflammatory and autoimmune disorders, we postulated that upon long-lasting injury, transforming cells would benefit from NKG2D-ligand expression to create a tumor environment that promotes tumorigenesis.

METHOD: Hepatocellular carcinoma (HCC) was chemically induced in NKG2D-KO and NKG2D-WT mice. Intestinal polyposis and colon cancer were studied in the Apcmin mouse sufficient or deficient for NKG2D. Survival, tumour burden and composition of the tumor environment were assessed at end-point. All animal work was carried out in compliance with the British Home Office Animals Scientific Procedures Act 1986 (Project licence number 70/7129).

RESULTS: Our result directly implicate NKG2D in the promotion of a tissue environment that favors tumorigenesis in both models. Specifically, a higher mortality associated with an overall increased tumor burden was observed in NKG2D-WT compared to NKG2D-KO mice. In the HCC model, the large recruitment of memory CD8+T cells observed in damaged livers was significantly increased in the presence of NKG2D and associated with a higher expression of the chemokines CXCL9, CXCL10, CCL5 and CCL3 (MIP1-a). CD8+T cells were functional and appear to have been chronically stimulated in vivo, showing a mild yet consistent down regulation of NKG2D and CD3 and an increased PD-1 expression. We observed increased expression of pro-inflammatory cytokines including IL-6 and TNFa associated with increased cycles of liver damage/hepatocyte proliferation, in an NKG2D-dependent manner.

CONCLUSION: Our findings provide genetic evidence of a novel and unexpected role for NKG2D as tumor promoting factor. This conceptual shift unravel the need to selectively target the types of cancer that will benefit from NKG2D-based immunotherapy.

NO CONFLICT OF INTEREST

TUESDAY 27 JUNE 2017

10:30-12:15: SYMPOSIUM: CELL PLASTICITY/SINGLE CELL ANALYSIS

27 Proffered Paper: Investigating epithelial and mesenchymal triple negative breast cancer plasticity: Identification of duel Wnt and YAP susceptibility for effective tumor targeting

A. Sulaiman[1,2,3], S. McGarry[1], A. Arnaout[4], C. Nessim[4], L. Wang[1,2,3,5]

[1] University of Ottawa Faculty of Medicine, Biochemistry- Microbiology and Immunology, Ottawa, Canada
[2] China-Canada Centre of Research for Digestive Diseases, Longhua Hospital- Shanghai University of Traditional Chinese Medicine, Shanghai, China
[3] Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, Canada
[4] Centre for Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, Canada
[5] Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, Canada

BACKGROUND: Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancers but disproportionately accounts for the majority of breast cancer related deaths stemming from the lack of specific therapy. Within TNBC, cancer stem cells (CSCs) are thought to exist in interconvertible mesenchymal or epithelial phenotypes and together drive disease progression and resistance to chemotherapy. Chemotherapy fails to target these sub-populations and development of a specific therapy is an unmet medical need.

MATERIALS AND METHODS: Initially, TNBC cell lines were transduced with E-cadherin to model mesenchymal to epithelial transition or vice versa via knockdown. Activation of Wnt and YAP signaling pathways was analyzed and confirmed via shRNA knockdown. A database of 2509 invasive breast cancer patients correlated patient prognosis with CSC-related gene expression and Wnt and YAP expression. ICG-001 and simvastatin (targeting Wnt and YAP pathways respectively) were tested on fresh patient samples. Finally, athymic mice were injected with TNBC cells into the mammary fat pad and treated daily with the treatment, followed by secondary transplantation to test tumorigenesis.

RESULTS: Epithelial and mesenchymal TNBC showed differential CSC phenotypes (e.g. epithelial ALDH+ vs. mesenchymal CD44+/CD24- CSCs). Importantly, Wnt and YAP signalling was enriched in the epithelial or mesenchymal TNBC respectively and shRNA knockdown of Wnt and YAP pathways inhibited both bulk epithelial and mesenchymal TNBC and their corresponding CSC sub-populations. Patient database analysis revealed that enriched Wnt and YAP signalling was associated with increased ALDH1A1 and CD44 CSC gene expression and diminished patient prognosis. Dual inhibition of Wnt and YAP pathways using ICG-001 and simvastatin significantly induced apoptosis and inhibited proliferation of both epithelial

and mesenchymal TNBC bulk and CSC populations in patient TNBC fragments. Epithelial and mesenchymal TNBC tumor growth was markedly retarded in a human xenograft model after 15 days of treatment and both CSC subpopulations were diminished. Tumorigenicity was significantly hampered in both epithelial and mesenchymal TNBC after secondary transplantation.

CONCLUSION: We identified a novel model to induce mesenchymal to epithelial or vice-versa conversion in TNBC, identified enriched Wnt and YAP signalling while also identifying a novel therapy to inhibit bulk TNBC growth, CSC enrichment and tumorigenicity.

NO CONFLICT OF INTEREST

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28 Proffered Paper: Intra-tumoral heterogeneity in Glioblastoma is a result of stochastic reversible plasticity rather than a hierarchical differentiation process

A. Golebiewska[1], A. Dirkse[2], T. Buder[2], N.H.C. Brons[3], N. Sauvageot[4], S. Leite[4], C. Herold-Mende[5], A. Deutsch[2], A. Voss-Böhme[2], N. Simone P.[1]

[1] Luxembourg Institute of Health LIH, Department of Oncology- Norlux Neuro-Oncology laboratory, Luxembourg, Luxembourg
[2] Technische Universität Dresden, Zentrum für Informationsdienste und Hochleistungsrechnen ZIH, Dresden, Germany
[3] Luxembourg Institute of Health LIH, Core Facility Flow Cytometry, Luxembourg, Luxembourg
[4] Luxembourg Institute of Health LIH, Centre of Competence for Methodology and Statistics CCMS, Luxembourg, Luxembourg
[5] University of Heidelberg, Division of Experimental Neurosurgery- Department of Neurosurgery, Heidelberg, Germany

INTRODUCTION: Glioblastoma (GBM) is a highly malignant brain tumor where no curative treatment is available. The cancer stem cell (CSC) hypothesis posits that GBMs rely on a small subpopulation of cancer cells with stem-like properties responsible for tumor progression and recurrence. Recent experimental data from GBM and other cancers however suggest that CSCs may not be a stable entity. The question arises whether such cells represent a defined subpopulation of tumor cells or whether they represent a changing identity that any cancer cell can adopt depending on the environmental conditions.

MATERIAL AND METHODS: Tumor cell subpopulations were classified based on their expression of four chosen cell membrane markers (CD133, CD15, A2B5 and CD44). The resulting 16 subpopulations were FACS isolated and analyzed for self-renewal and ability to reform the original heterogeneous population in different environments. Mathematical Markov modelling was applied to calculate state transitions between cell states. Intra-tumoral heterogeneity was further interrogated at the single cell transcriptomic level and combined the gene expression profiles and cell membrane marker expression patterns.

RESULTS AND DISCUSSION: Similar to GBM biopsies, we found that stem cell associated markers are heterogeneously expressed in glioma patient-derived xenografts, GBM stem-like cells and primary cultures. GBM subpopulations were able to proliferate and carried stem-cell properties including self-renewal potential. All subpopulations had the capacity to adapt their marker expression profiles to give rise to the original subpopulations. Mathematical modeling revealed a different propensity in reforming the original heterogeneity over time, which was independent of the proliferation index. We observed a phenotypic shift following a change in the environment, resulting from a strong adaptation towards the most favorable phenotypic states. Although subpopulations varied in their potential to adapt to the environment, all were able to reach a steady state equilibrium.

CONCLUSION: Our result suggest that GBM CSCs do not represent a stable entity and that intra-tumoral heterogeneity result from a high adaptation capacity. GBM cells undergo reversible stochastic state transitions over time, which does not follow a hierarchical organisation. This implies that GBM treatment should take into account the strong propensity of cancer cells for phenotypic state transitions and reversible plasticity.

NO CONFLICT OF INTEREST

POSTER SESSIONS

SUNDAY 25 JUNE AND MONDAY 26 JUNE 2017

POSTER SESSION: CANCER GENOMICS, EPIGENETICS AND GENOME INSTABILITY I

100 CD74 is a novel transcription regulator

N. Gil[1], I. Shachar[2]

[1] The Weizmann Institute of Science, Immunology, Rehovot, Israel
[2] Weizmann, Immunology, Rehovot, Israel

INTRODUCTION: CD74 is a cell-surface receptor for the cytokine macrophage migration inhibitory factor (MIF). MIF binding to CD74 induces its cleavage and a release of its cytosolic intracellular domain (CD74–ICD), which regulates cell survival. CD74 id highly up-regulated in CLL, as well as in other cancers, and might be a target for genetic therapy in those cancers. Better understanding the mechanism in which CD74 regulates cell-survival in health and disease might unable developing a therapeutic approach targeting the CD74 pathway. In this study we focused on CD74 function as a transcription regulator.

MATERIALS AND METHODS:B-CLL cells were activated using CD74 Ab for 1h, fixed and subjected to ChIP-seq using Abs for CD74, methylation and acetylation. In parallel, cells were activated for 2h and 8h with CD74 or IgG control Ab and RNA-seq was preformed.

To validate CD74 role in transcription regulation, CD74 levels were down-regulated by small-interfering RNA, and levels of mRNA of target genes was evaluated using RT-PCR.

Changes in protein levels were determined 48h following CD74 activation using FACS.

Complex formation of CD74 and NfKb or RUNX was analyzed using Co-IP.

RESULTS AND DISCUSSION: We characterized the transcriptional activity of CD74–ICD in B-CLL cells. We show that following CD74 activation, CD74–ICD binds to transcription binding sites that control the immune response, apoptosis and cell migration, and are over expressed in leukemia and other immune diseases, indicating that CD74 has a specific role in regulating genes controlling those processes. RNA-Seq indicate that CD74-ICD binding in the proximity of those genes leads to regulation of their expression, thus controlling immune and survival processes. In addition, we found that CD74 interacts with the transcription factors RUNX and NF-κB, inducing their activity both by forming a transcription complex and by up regulating transcription of RUNX and NF-κB genes. This study indicate the mechanism in which CD74 controls many key processes in the cell, such as migration and apoptosis.

CONCLUSION: CD74 is a transcription regulator. In B-CLL cells it binds in the proximity of many CLL-related genes, Immune response genes and apoptosis genes, regulating their expression. CD74 binding to specific sites in the chromatin have major affect on cell survival, among other cell properties.

NO CONFLICT OF INTEREST

101 A path towards determining tumor mutation burden and identifying neoantigens using next-generation sequencing (NGS)

A. So[1], S. Kaplan[1], N.Y. Wang[1], S. Zhang[1], A. Wise[1], K. Kruglyak[1], K. Gutekunst[1]

[1] Illumina, Oncology, La Jolla, USA

BACKGROUND: The recent clinical demonstrations that cancer immunotherapy is effective for some patients, but not others has led to widespread interest in identifying cancer genetic factors that can predict positive response. Importantly, it has been shown by various studies that tumor mutation burden (TMB) and predicted neoantigen load correlate with patient response to checkpoint inhibitors. This project aims to develop a clinical next-generation sequencing (NGS) assay that utilizes TMB and neoantigen information to guide cancer immunotherapy selection.

MATERIALS AND METHODS: Whole exome sequencing (WES) and whole transcriptome sequencing (WTS) libraries were generated using Illumina's TruSeq® Exome and RNA Access library preparation kits, respectively. The samples were pair-end sequenced (2x75bp) using HiSeq® 2000 and 2500 instruments.

RESULTS: Here, we demonstrate a workflow using tumor/normal WES and tumor-only WTS to determine expressed TMB. Using the WES data, we were also able to accurately identify human leukocyte antigen (HLA) major histocompatibility complex (MHC) Class I genes at four-digit resolution. We then identified putative neoantigens, defined as peptides with mutated amino acids, that were expressed in the tumor samples and predicted to bind to the respective HLA sequences. In addition, as a path towards developing a clinical assay for TMB and neoantigen determination, we tested the ability of reagents developed for Illumina's Trusight® Tumor 170 panel to be used for an exome panel. With workflow modifications, we achieved comparable quality of sequencing metrics as compared to the TruSeq® Exome kit.

CONCLUSION: Our data demonstrate a path towards developing a clinical assay that can be used to assess TMB and neoantigen candidates.

CONFLICT OF INTEREST

Other Substantive Relationships: All authors are employed by Illumina.

102 Isobolographic analysis of interactions between cisplatin and histone deacetylase inhibitors in human breast cancer cell lines

A. Wawruszak[1], J. Luszczki[2], A. Grabarska[1], E. Gumbarewicz[1], M. Dmoszynska-Graniczka[1], K. Polberg[3], E. Okon[1], A. Stepulak[1]

[1] Medical University of Lublin, Department of Biochemistry and Molecular Biology, Lublin, Poland
[2] Medical University of Lublin, Department of Pathophysiology, Lublin, Poland
[3] MSW Hospital, Department of Otolaryngology, Lublin, Poland

INTRODUCTION: Histone deacetylase inhibitors (HDIs) are promising anticancer drugs, which play important role in the transcriptional regulation of gene expression, by epigenetic mechanism, including histone acetylation. HDIs induce apoptosis and cell cycle arrest in wide variety of cancer cells including breast carcinoma.

Material and Method. In the present study, we investigated the influence of suberoylanilide hydroxamic acid (SAHA) and valproic acid (VPA), alone or in combination with cisplatin (CDDP), on proliferation, induction of apoptosis and cell cycle progression in T47D, MCF7 and MDA-MB-231 human breast cancer cell lines. The type of interaction between HDIs and CDDP was determined by an isobolographic analysis. The isobolographic analysis is a very precise and rigorous pharmacodynamic method, to determine the presence of synergism, addition or antagonism between different drugs with using variety of fixed dose ratios.

RESULTS: Our experiments show that the combinations of CDDP with VPA or SAHA at a fixed-ratio of 1:1 exerted additive interaction in the viability of MCF7 cells, while in T47D cells there was a tendency to synergy. In contrast, sub-additive (antagonistic) interaction was observed for the combination of CDDP with VPA in MDA-MB-231'triple-negative" (i.e. estrogen receptor negative, progesterone receptor negative, and HER-2 negative) breast cancer cells, whereas combination of CDDP with SAHA in the same MDA-MB-231 cell line yielded additive interaction. Additionally, combined HDIs/CDDP treatment resulted in increase in apoptosis and cell cycle arrest in all tested breast cancer cell lines in comparison with a single therapy.

CONCLUSION: The additive interaction of CDDP with SAHA or VPA suggests that HDIs could be combined with CDDP in order to optimize treatment regimen in some human breast cancers.

NO CONFLICT OF INTEREST

103 Isobolographic analysis demonstrates additive effect of interactions between cisplatin and histone deacetylase inhibitors combined treatment in lung cancer cell lines

E. Gumbarewicz[1], J. Luszczki[2], A. Wawruszak[1], M. Dmoszynska-Graniczka[1], A. Grabarska[1], A. Jarzab[1], K. Polberg[3], A. Stepulak[1]

[1] Medical University of Lublin, Department of Biochemistry and Molecular Biology, Lublin, Poland
[2] Medical University of Lublin, Department of Pathophysiology, Lublin, Poland
[3] MSW Hospital, Department of Otolaryngology, Lublin, Poland

INTRODUCTION: Histone deacetylase inhibitors (HDIs) are a new class of drugs which affect the activity of HDACs resulting in epigenetic changes in many proteins. They regulate target genes expression playing critical role in development and proliferation of cells. HDIs are able to affect cell survival, cell signaling, transport, and gene expression, can induce differentiation, cell growth arrest, apoptosis, inhibit proliferation and angiogenesis in cancer, whereas normal cells are comparatively resistant to the action of HDIs.

MATERIAL AND METHOD: The aim of this study was to investigate the combined effect of a well-known cytostatic agent-cisplatin (CDDP) and a histone deacetylase inhibitors-either suberoylanilide hydroxamic acid (SAHA, vorinostat) or valproic acid (VPA), on the proliferation of lung cancer cells, as well as induction of apoptosis and inhibition of the cell cycle progression. The anti-proliferative activity of VPA or SAHA used alone, or in combination with CDDP were determined by means of MTT test. The type of pharmacologic interactions between HDAC inhibitors and CDDP was assessed using isobolographic analysis.

RESULTS: We observed additive interactions for the CCDP with SAHA, as well as for the CDDP with VPA combinations with respect to their anti-proliferative effects on three different lung cancer cell lines (A549, NCI-H1563 and NCI-H2170). Such additive effects were observed regardless of the histologic type (adenocarcinoma or squamous cell carcinoma) and sensitivity for the drugs applied. Combination treatment also augmented the induction of apoptosis and cell cycle perturbation mediated by CDDP alone, thereby enhancing anti-cancer effect of tested drugs.

CONCLUSION: The combined therapy of HDIs and CDDP may be a promising therapeutic tool in the treatment of lung cancer.

NO CONFLICT OF INTEREST

104 Reappraisal of cancer modeling for more efficacious oncologic interventions

U. Rovigatti[1]

[1] University of Florence, Experimental and Clinical Medicine, Firenze, Italy

BACKGROUND: Over the past two decades, a great deal of molecular mechanisms have been clarified in human cancer cells [1] [2]. Our progress in detailing individual aberrations of specific tumors was certainly facilitated and speeded up by the new sequencing technologies, so called Next Generation Sequencing (NGS). Despite a very fast pace in its accrual and an exorbitant amount of data, the actual theoretical modeling remained essentially unchanged. This has generated obvious paradoxes between an extremely precise and specific definition of genetic (or epigenetic/ chromosomal) aberrations [2] and models of totally stochastic (i.e. casual) origin of malignant cells [3].

MATERIALS, METHODS AND RESULTS: In a 2015 Review on these issues, I suggested that instead of subscribing to the only classical or canonical Hallmarks model, it may be heuristically more efficient to consider several different cancer models [4]. The issue is far from trivial: despite frequent triumphant titles in the mass media, the prognostic outcomes of several metastatic cancers (i.e. the four killers) are still grim. Oncologist Vinay Prasad and colleagues have recently argued in the pages of Nature and elsewhere that today 'Lazarus-effects" are almost non-existent and that 'Precision-Oncology" is still an illusion. With similar but molecular-biology perspective, I have argued in 2015 and 2016 [4] [5] that the entire scaffold of Targeted Gene Therapy (TGT) is based upon the axiom of 'Oncogene-Addiction" (OA). However, OA appears to be a misconception, as it is not validated by any biological reason or principle [4]. Furthermore, genetic aberrations must have an UPSTREAM origin (what I called UPCAN), which has been investigated only in its recessive component (so-called Care-Takers, similar to Tumor Suppressor Gene, TSGs).

CONCLUSIONS: UPCAN mechanisms are more relevant for generation of genomic aberrations: they were called GENOME-STRIPERS and include several new areas of investigation such as chromothripsis [6], chromoplexy [7], kataegis [8], MFV infection [9], big-bang models [10] etc.. In the future, better focusing on GENOME-STRIPERS will allow to understand the origin of genomic aberrations, as well as devising more successful strategies for halting or inhibiting the progress of lethal metastatic cancers.

NO CONFLICT OF INTEREST

105 Genetic screens and transcriptional profiling with CRISPR/Cas9 sgRNA libraries

D. Tedesco[1], D. Deng[2], A. Chenchik[3]

[1] Cellecta- Inc., R&D, Mountain View- CA, USA
[2] Cellecta Inc., R&D, Mountain View CA, USA
[3] Cellecta- Inc., R&D, Mountain View CA, USA

Genome-wide loss-of-function screening is a fundamental method to identify genes responsible for driving biological processes. Complex pooled lentiviral-based libraries expressing large numbers of genetic disruptors, such as shRNAs (RNAi) or sgRNAs (CRISPR), make large-scale cell screening practical.

Although loss-of-function shRNA and sgRNA pooled library screens are similar in concept, the gene loss of function is achieved by different mechanisms (mRNA degradation with RNAi, full gene disruption with CRISPR-KO, transcriptional inhibition with CRISPRi), so some divergences are expected and indeed observed when comparing result obtained using one method versus the other. Furthermore, contrary to RNAi, CRISPR technology can be modified to activate gene expression (CRISPRa), thus enabling the use of genome-wide gain-of-function screening in gene function studies.

We will present and compare unpublished data from genetic screens in human Cancer cell lines using CRISPR-KO, CRISPRi, CRISPRa and RNAi technologies.

We will also present recent advances in a new technology for medium-to-high-throughput transcriptional profiling of CRISPR-modified cells, based on clonal barcoding and single-cell transcriptome analysis. We will show how this novel technology can be applied to systematically analyze the transcriptional changes associated to specific gene activation/inactivation events in a library of multiple genes across multiple cell lines.

CONFLICT OF INTEREST

Other Substantive Relationships: All authors are employed by Cellecta, Inc.

106 Characterizing the role of Thymine DNA Glycosylase (TDG) mediated epigenetic regulation in metastatic liver cancer in vivo

M. Hassan[1], B. Kolendowski[1], M. Isovic[1], M. Underhill[2], J. Torchia[1]

[1] London Regional Cancer Centre, Biochemistry, London, Canada
[2] University of British Columbia, Cellular & Physiological Sciences, Vancouver, Canada

BACKGROUND: Cytosine methylation (5mC) is essential for transcriptional control and genomic stability and is often used as a prognostic marker in cancer. Recent studies have demonstrated that 5mC can be reversed enzymatically by TET proteins which oxidize 5mC into 5-hydroxymethylcytosine (5-hmC), and then to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5caC). This mechanism is known as active DNA demethylation and the base excision repair enzyme Thymine DNA Glycosylase (TDG) plays an essential role in this process by removing 5-fC and 5-caC which are subsequently replaced by the unmethylated cytosine. Importantly, homozygous loss of TDG in mice causes early embryonic lethality and the observed defects are consistent with a central role for TDG in retinoic acid (RA) signaling and epigenetic stability. I hypothesize that TDG mediated active DNA demethylation is necessary for RA signaling events and that TDG functions as a tumor suppressor in vivo by maintaining epigenetic stability in cells.

MATERIALS AND METHODS: This study was performed in mouse embryonic fibroblasts (MEFs) and conditional TDG knockout mouse models (C57BL6). ChIP-qPCR was utilized to asses the binding of proteins to specific DNA sites. Bisulfite sequencing and methylase assisted bisulfite sequencing were used to analyze changes in DNA methylation.

RESULTS: I demonstrate that the Hypermethylated in Cancer 1 (Hic1) gene is a novel retinoic acid receptor (RAR) target and examine the mechanism of dynamic DNA methylation at its promoter. I have found that RA treatment leads to a rapid recruitment of an RAR complex consisting of TDG, the lysine acetyltransferase CBP and activated RNA pol II to the Hic1 locus. The binding of this complex precedes active DNA demethylation of the Hic1 promoter and upregulation of its mRNA transcript. Importantly, I have also shown that Tdg deletion in MEFs abrogates HIC1 induction and DNA demethylation by RA. Remarkably, the conditional deletion of Tdg in adult mice leads to HIC1 protein ablation and an onset of metastatic hepatocellular carcinoma (HCC) (n = 9) compared to age/ sex matched control mice (n = 0). Surprisingly, the HIC1 protein was found to be silenced in the tumors analyzed via promoter DNA hypermethylation.

CONCLUSIONS: My research provides the first line of evidence for TDG as a tumor suppressor and constitutes an important link between its regulation, loss of sensitivity to RA, epigenetic instability in the genome and HCC progression in vivo.

NO CONFLICT OF INTEREST

107 Discovery of a functional long non-coding RNA regulated by breast cancer stem cell marker ALDH1A3

D. Vidovic[1], T. Huynh[1], B. Cruickshank[1], K. Coyle[1], M. Sultan[1], M. Thomas[1], P. Marcato[1]

[1] Dalhousie University, Pathology, Halifax- NS, Canada

BACKGROUND: Aberrant epigenetic changes such as DNA methylation, histone modifications, and functional regulatory long non-coding RNAs (lncRNAs) result in dysregulated gene expression critical for cancer development, cancer stem cell (CSC) maintenance, and response to therapy. The importance of lncRNAs, in particular, in these processes is becoming increasingly evident. We have identified a functional lncRNA, termed retinoic acid-induced non-coding RNA (RAINR), that mediates breast cancer cell growth and may be a novel therapeutic target.

METHODS: Microarray gene expression analyses revealed RAINR as being potentially regulated by breast CSC marker aldehyde dehydrogenase 1A3 (ALDH1A3) and its enzymatic product, retinoic acid (RA). Quantitative PCR (QPCR) was performed to quantify expression of RAINR in different cells and treatment conditions. Knockdown of RAINR was achieved using antisense oligonucleotides (ASOs), and knockdown efficiency was quantified using QPCR. Knockdown or overexpression of ALDH1A3 was achieved using shRNA or ALDH1A3 cDNA-bearing vectors, respectively. Cell proliferation experiments were performed by normalizing endpoint cell counts to a known number of seeded cells.

RESULTS: RAINR expression is regulated by ALDH1A3, as ALDH1A3 knockdown cells had significantly decreased RAINR expression, while ALDH1A3-overexpressing cells had significantly increased RAINR expression. Exogenous RA induced RAINR expression in the majority of cell lines in a breast cancer cell line panel. Furthermore, ASO-mediated knockdown of RAINR dramatically decreased the proliferation of MDA-MB-468 and MCF7 breast cancer cell lines in vitro, demonstrating the requirement for RAINR in breast cancer cell growth.

CONCLUSION: This work identifies an oncogenic functional lncRNA, RAINR, in breast cancer. ASO-RAINR antagonists may show promise for in vivo treatment of breast cancer, possibly by preferentially targeting ALDH1A3+ breast CSCs, which are resistant to many therapies.

NO CONFLICT OF INTEREST

109 Characterization of the genetic heterogeneity and clonal dynamics of a triple negative breast cancer

V. Angeloni[1], C. De Marco[1], L. Cleris[1], M.G. Daidone[1], M. Callari[2]

[1] Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Experimental Oncology and Molecular Medicine, Milan, Italy
[2] UK Cambridge Institute, Department of Oncology and Cancer Research, Cambridge, United Kingdom

INTRODUCTION: The heterogeneity of breast cancer (BC) is broadly proven and categorized by tumor segregation into different molecular subtypes, defined by gene expression profile, that correlate with clinical behavior and are used to define therapeutic strategies. Nowadays, the development of advanced sequencing technologies and bioinformatic tools, enabling a deep characterization of the genomic landscape highlighted that BC is highly heterogeneous not only in terms of histopathologic features and clinical outcome but also at genomic level. Moreover, recent studies revealed the existence of multiple subclones in a single tumor subjected to a darwinian selection causing expansion and decline of subclonal populations over time. Here, we sought to characterize genetic heterogeneity and clonal dynamics in a triple negative BC (TNBC) during progression.

MATERIAL AND METHOD: A cell subpopulation (MS186) of cancer-initiating cells (BCICs) able to grow and metastatize in in vivo serial transplantation experiments

was isolated from a TNBC surgical specimen. From this preclinical model we derived: tumor xenografts, BCICs from primary and secondary xenografts, BCICs from lung and lymph node metastases. Patient primary tumor, xenografts and all isolated BCICs were subjected to whole exome sequencing (WES) and shallow whole genome sequencing. Single nucleotide variants, insertions, deletions, and copy number variation were identified through an ad hoc designed bioinformatic pipeline.

RESULTS AND DISCUSSION: WES revealed overlapping variants between all the samples tested. However, even if the intra-tumor clonal architecture present in the clinical specimen was mostly preserved, upon initial BCIC xenograft in mice, a clonal selection was seen, probably due to the shift human-mouse host environment. The fact that the vast majority of mutations and relative allele frequencies are shared between BCICs and serial xenografts suggests that a dynamic transition between stem-like and differentiated cells, more than a clonal expansion, occurs. Finally, we demonstrated that BCICs isolated from metastatic sites share most molecular and architectural features with their originating xenografts, suggesting that colonization of distant sites may be the result of a polyclonal dissemination.

CONCLUSION: Our findings supports the BCIC plasticity, underlying the importance of drug therapies able to counteract the de-differentiation of BCIC in order to contrast TNBC growth and progression.

NO CONFLICT OF INTEREST

110 Identification of methylome alterations in pilocytic astrocytoma as potential diagnostic and prognostic biomarkers

E. Loi[1], *A. Fadda*[1], *L. Moi*[1], *M. Antonelli*[2], *M. Badiali*[3], *F. Giangaspero*[2,4], *P. Zavattari*[1]

[1] *Univuersity of Cagliari, Department of Biomedical Sciences- Biology and Genetics Unit, Cagliari, Italy*
[2] *Sapienza Univuersity, Department of Radiological- Oncological and Pathological Science, Rome, Italy*
[3] *Microcitemico Children's Hospital, Bone Marrow Transplantation Unit, Cagliari, Italy*
[4] *IRCCS, Neuromed Institute, Pozzili, Italy*

BACKGROUND: Pilocytic astrocytoma (PA) is the most frequent brain tumor in childhood. Since little is known about the potential role of epigenetic mechanisms in the pathogenesis of PA, we analyzed genome-wide DNA methylation profiles in 20 tumors and 4 normal brain samples. Besides, we performed a differential methylation analysis between tumoral samples subdivided according to two criteria: tumor localization (supratentorial (SVT) vs posterior cranial fossa (FCP) and age of onset (PA with onset before (≤ 3 yo) vs after (> 3 yo) 3 years of age).

MATERIALS AND METHODS: Illumina's Infinium HumanMethylation27K BeadChips were used to characterize the DNA methylation profiles of 20 PAs and 4 non-tumoral brain samples. The differential methylation analysis were performed by using statistical tools in R environment. The unsupervised hierarchical clustering (UHC) was carried out through hClust. Ingenuity Pathway Analysis (IPA) was used for the Pathway Enrichment Analysis. The expression of specific genes was examined by qRT-PCR.

RESULTS: The differential methylation analysis between tumoral and non-tumoral samples revealed alterations at genes that, as expected, are mostly belonging to pathways involved in carcinogenesis. The UHC shows how loci significantly differently methylated are able to identify distinct clusters that reflect the subgroups establish a priori. The pathways with the highest number of differently methylated genes in ≤ 3 yo vs > yo and FCP vs SVT subgroups are p53-signaling and c-AMP signaling, respectively.

In order to assess the impact of CpG island promoter methylation on gene expression, we designed qRT-PCR assays for 4 significant differentially methylated genes in SVT vs FCP. As expected, 3 genes were both hypermethylated and downregulated in SVT PAs. Conversely, 1 gene showed a promoter hypermethylation but also an upregulation in the FCP subgroup. Interestingly, 3 out of 4 genes are yet downregulated in the normal brain tissue, confirming the emerging evidence that transcriptionally repressed genes are prone to be affected by aberrant hypermethylation in cancer.

CONCLUSIONS: The identification of brain-region and age-related specific methylation patterns suggests that there are different molecular pathways involved in the pathogenesis of PA. Thus, methylome alterations may be interesting diagnostic and prognostic biomarkers and the molecular characterization of PA may be helpful for the development of targeted therapies.

NO CONFLICT OF INTEREST

111 An easy to use data analytic workflow for DNA methylation-based biomarkers discovery

A. Fadda[1], *P. Zavattari*[1]

[1] *Univuersity of Cagliari, Department of Biomedical Sciences- Biology and Genetics Unit, Cagliari, Italy*

BACKGROUND: A key challenge in cancer prevention is its early detection. No conventional methods meet all of the criteria of an ideal screening tool so, there is the need to design rationally alternative approaches. A biomarker should be easy to detect, non invasive, highly sensitive and highly specific, cost-effective. Aberrant DNA methylation has been recognized as common and early event in carcinogenesis and therefore a potentially early indicator of disease. Technological advances in methylome analysis enabled the identification of new biomarkers. However, the analysis and interpretation of the result could be challenging for non-advanced users. Here we present an easy to use data analytic workflow for DNA methylation-based biomarkers discovery.

MATERIAL AND METHODS: The present work takes advantage of the use of open-source software (RnBeads) and freely available dataset to set up an R language based workflow which interfaces with open access web-based tool (ToppGene Suite).

RESULTS: We used the case/control differentially methylated CpG islands (CGI) as possible source of biomarkers. Given the possible repressive role of the transcription if a hypermethylated CGI overlap a promoter, our search strategy give a meaningful biological framework to the biomarker discovery, ensuring more interpretable results. The CGIs were annotated using two different criteria: a 'proximity criterion" and 'functional criterion". The proposed criteria have their benefits and drawbacks that have to be evaluated to find the most suited one. The gene list, thereby created, was used to perform a pathway enrichment analysis to find the most affected pathways by aberrant DNA methylation. The biomarker selection was performed intersecting the genes belonging to the most altered pathways of two different conditions (e.g. cancerous vs. precancerous lesion). The final step includes a validation on independent dataset and a calculation of the most used biomarker performance parameters (i.e. specificity, sensitivity and ROC curve).

CONCLUSIONS: Our workflow makes the biomarkers discovery easy accessible even to users with no strong background: on DNA methylation. The search strategy, based on a biological framework, ensures meaningful and more interpretable results. Future prospects will be creating a stand-alone package. Encouraging possible future collaborations, we suggest the use of our workflow to everyone who is interested in DNA methylation-based biomarkers discovery.

NO CONFLICT OF INTEREST

112 Novel epigenetic drivers of drug resistance linked to gene expression modulation in colorectal carcinomas: Preliminary analysis

V. Condelli[1], *G. Calice*[1], *V. Simeon*[1], *M.G. Rodriquenz*[2], *L. Sisinni*[1], *M. Landriscina*[1,3]

[1] *IRCCS- Referral Cancer Center of Basilicata, laboratory of preclinical and translational research, Rionero in Vulture PZ, Italy*
[2] *IRCCS- Referral Cancer Center of Basilicata, medical oncology unit, Rionero in Vulture PZ, Italy*
[3] *University of Foggia, medical oncology, Foggia, Italy*

INTRODUCTION: Colorectal carcinoma (CRC) is the second leading cause of cancer-related death worldwide. Besides significant improvements in treatment strategies, its prognosis remains poor and the main cause of treatment failure is drug resistance. Thus, several attempts are still ongoing to validate novel biomarkers and gene signatures predictive of drug resistance to design personalized treatments. Epigenetic events, such as gene promoter DNA hypo/hypermethylation, that are linked to changes in gene expression, have been shown to be responsible for resistance to chemotherapeutics in tumors.

MATERIAL AND METHODS: KRAS G13V HCT116 and BRAF V600E HT29 CRC cell lines were chronically adapted to oxaliplatin (l-OHP) and irinotecan (IRI). The methylation profile was analyzed by Illumina 850K DNA methylation array. Gene expression analysis was performed, in parallel, by Illumina HumanHT12 v4.0 Expression BeadChip. Differently expressed genes were used to evaluate the functional behavior in terms of Biological Processes with Ingenuity Pathway Analysis (IPA).

RESULTS: Drug-resistance was responsible for a wide reprogramming of gene expression in both l-OHP- and IRI-resistant CRC cell lines. IPA identified a shift toward glycolytic and stem-like phenotype and identified several overexpressed genes implicated in epithelial–mesenchymal transition in HCT116 drug-adapted cells. Genome-wide profiling of drug-adapted HT29 cells identified the overexpression of epigenetic biomarkers (i.e. Polycomb group genes). DNA methylation status of l-OHP- and IRI-resistant CRC cell lines was compared with corresponding gene expression profiles. The analysis showed a different overlapping between methylated/modulated genes: 7 and 66 genes in, respectively, l-OHP and IRI-resistant HCT116 cells, 225 and 60 genes in l-OHP and IRI-resistant HT29 cells.

CONCLUSION: A panel of differentially methylated genes was identified, as candidate biomarkers potentially responsible for gene expression modulation and acquisition of drug-resistance.

NO CONFLICT OF INTEREST

113 The complex relationship between DNA methylation and gene expression

E. Loi[1], *A. Fadda*[1], *L. Moi*[1], *M. Antonelli*[2], *M. Badiali*[3], *F. Giangaspero*[2,4], *M.G. Ennas*[5], *P. Cocco*[6], *A. Columbano*[7], *P. Zavattari*[1]

[1] *Univuersity of Cagliari, Department of Biomedical Sciences- Biology and Genetics Unit, Cagliari, Italy*
[2] *Sapienza Univuersity, Department of Radiological- Oncological and Pathological Science, Rome, Italy*
[3] *Microcitemico Children's Hospital, Bone Marrow Transplantation Unit, Cagliari, Italy*
[4] *IRCCS, Neuromed Institute, Pozzili, Italy*
[5] *University of Cagliari, Department of Biomedical Sciences- Cytomorphology Unit, Cagliari, Italy*
[6] *University of Cagliari, Department of Public Health- Clinical and Molecular Medicine Unit, Cagliari, Italy*
[7] *University of Cagliari, Department of Biomedical Sciences- Oncology and Molecular Pathology Unit, Cagliari, Italy*

BACKGROUND: DNA methylation is the most studied epigenetic modification in cancer. It is widely accepted that aberrant CpG island promoter DNA methylation represents a primary event responsible for tumor suppressor genes silencing. Today, an increasing number of evidences suggest that DNA methylation is an early event in cancer development and it targets promoters of genes already repressed in the normal tissue where the tumor arises. In order to investigate this phenomenon, we performed genome-wide methylation analysis on: 18 colorectal cancer (CRC)

samples and 10 peritumoral samples, 12 blood samples from chronic lymphocytic leukemia (CLL) patients and 12 blood samples from healthy donors; 20 pilocytic astrocytomas (PAs) and 4 non-tumoral brain samples. Then, we compared the expression levels of genes that show promoter hypermethylation with patterns of expression in normal tissues.

MATERIAL AND METHODS: Illumina 27K and 450K arrays were used to establish global methylation profiles on the collected samples. Gene expression analysis were performed by using qRT-PCR assays. The tissue-specific gene expression levels were retrieved from GTExPortal.

RESULTS: We found that the majority of genes that show an aberrant promoter hypermethylation in cancer, are normally repressed in the originator tissues. As expected, we observed a strong negative correlation between promoter methylation and gene expression in PAs and colon cancer. On the other hand, we detected a positive or poor correlation between promoter methylation and expression in CLL, probably due to the high heterogeneity of the samples analyzed. Conversely, we found that one gene showed a significant gene body hypermethylation and downregulation in CLL. Interestingly, it has recently suggested that gene body methylation may be correlated with alternative transcript isoforms and it may regulate cell context-specific alternative promoters in gene bodies.

CONCLUSIONS: Our result confirm that normally repressed genes are prone to aberrant methylation in cancer. Although the role of this hypermethylation in the first stages of cancer development has yet to be clarify, methylome alterations may be promising diagnostic biomarkers. Clearly, further studies are needed to investigate the role of promoter and gene body methylation in the regulation of gene expression with the final aim to create targeted therapeutic strategy to restore the corrected methylation patterns.

NO CONFLICT OF INTEREST

114 Cancer astrocytes have a more conserved molecular status in long survival glioblastoma patients: New emerging cancer players

S. Franceschi[1], F. Lessi[2], P. Aretini[2], V. Ortenzi[2], M. La Ferla[1], S. Cristian[2], C. Francesco G[2], R. Vannozzi[3], P. Civita[1], F. Pasqualetti[4], G. Naccarato[2], C.M. Mazzanti[1]

[1] Fondazione Pisana per la Scienza, Genomics Section, Pisa, Italy
[2] University of Pisa, Department of Translational Research and New Technologies in Medicine and Surgery, Pisa, Italy
[3] Azienda Ospedaliera Universitaria Pisana, Neurosurgery Department, Pisa, Italy
[4] Azienda Ospedaliera Universitaria Pisana, Radiology Department, Pisa, Italy

INTRODUCTION: Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumor with a median patient survival of 14.6 months. Despite decades of research and the advent of new therapies, GBM etiology and pathogenesis is still unclear and patients with GBM continue to have a very poor prognosis, almost always related to intracranial progression after surgery or radio-chemotherapy. However, 3–5% of the patients survives for more than 2 years and are referred to as long-term survivors. The aims of this work are to identify a genetic landscape that might be associated with long term survival and to understand the reasons why histologically identical tumors might behave less aggressively than others.

MATERIALS AND METHODS: 13 human glioblastoma subjects were selected to have same histology, similar condition and treatment. All cases had a diagnosis of GBM IV with no previous history of any brain neoplasia. Subjects were grouped depending on time of recurrence free survival (RFS) after first surgery: 6 Short (S) less than 6 months, 3 Medium (M) between 16 and 23 months and an exceptional group of 4 Long (L) over 25 months. Whole exome and transcriptome analysis was performed using NGS technology.

RESULTS: Mutational analysis revealed a much higher number of mutations in the S group. Transcriptome data shows that the higher number of differentialy expressed genes (DEG) is found comparing the two extreme groups of patients' tumors (S and L).

CONCLUSIONS: Gene mutational status, significantly changing between the two extreme groups of patients, revealed that the genetic constitution of less aggressive GBM is clearly more stable. Transcriptional data revealed that the functional state of cancer astrocytes in patients with long survival seems to remain closer to what is the normal astrocyte cell functionality. Finally combining Copy Number Variation analysis with transcriptome data, we found new emerging cancer players, which, confirmed at the RNA and DNA level, become possible oncodrivers.

NO CONFLICT OF INTEREST

115 Circulating miRNAs reflect a pro-tumorigenic and immunosuppressive microenvironment in lung cancer

O. Fortunato[1], C. Borzi[1], G. Centonze[1], M. Boeri[1], V. Huber[1], C. Camisaschi[1], L. Rivoltini[1], V. Cancila[2], U. Pastorino[2], G. Sozzi[1]

[1] Fondazione IRCCS Istituto Nazionale dei Tumori, Dept. Experimental Oncology, Milan, Italy
[2] University of Palermo, Dept. of Health Science, Palermo, Italy

BACKGROUND: miRNAs play a role in the complex network of signaling between cancer cells and tumor microenvironment. We previously reported a 24-miRNA plasma signature with diagnostic value in lung cancer screening cohorts.

MATERIAL AND METHODS: To evaluate the potential origin and the release of the 24 miRNAs we analyzed their expression by real-time or digital PCR in both cells and conditioned medium (CM) from cancer and different cell types of the lung microenvironment. Lung tissues and cell-blocks were analyzed by miRNAs in situ hybridization (ISH). Modulation of miRNAs after in vitro treatments known to induce changes associated with cancer progression, was assessed and correlated to changes observed in circulating miRNAs signatures.

RESULTS: 24-miRNAs analysis showed higher abundance in specific cellular components such as mir-145 in fibroblasts, mir-126 in endothelial cells, mir-133a in skeletal muscle cells or mir-451 and 142-3p in blood cells. Generally, tumor cells showed lower levels of miRNAs compared to epithelial cells. We observed by ISH that mir-451 is specifically expressed in lung interstitial alveolar walls while mir-126 by endothelial cells outside tumor bulk; miR-145 is characteristic of fibroblast and muscle cells and miR-142-3p of hematopoietic cells, fibroblast and muscle whereas mir-21 is over-expressed in the tumor.

The analysis of miRNAs in CM showed that miRNAs secretion is correlated with cellular expression for most cell types (Pearson correlation range: 0.59-0.80). Interestingly, platelets and granulocytes were the components that mostly secreted miRNAs.

In vitro experiments showed that hypoxic endothelial cells up-regulate mir-126 and that mir-145 was up-regulated and secreted in lung cancer-associated compared to normal fibroblasts. Interestingly, during conversion of T lymphocytes into T regulatory cells up-regulation of mir-15b and mir-320 was observed whereas a set of miRNAs were up-regulated in the conversion of macrophages into M2 phenotype. Modulation of miRNAs in immune and stromal cells was consistent with up-regulation of the same miRNAs observed in plasma.

CONCLUSION: Our findings support the conclusion that plasma miRNAs are heterogeneous and secreted by different cellular components of lung microenvironment rather than by tumor cells. In particular, we demonstrated that a pro-tumorigenic and immunosuppressive microenvironment contributes to the de-regulation of miRNAs observed in plasma of lung cancer patients.

NO CONFLICT OF INTEREST

POSTER SESSION: CANCER GENOMICS, EPIGENETICS AND GENOME INSTABILITY II

116 Implementation of a precision medicine program in pediatric oncology - result of the pilot study

C. Robledo[1], C. Rodríguez-Martín[2], G. Gómez-Mariano[2], A. Sastre[3], J.J. Pozo-Kreilinger[4], C. Mata[5], J. Huerta[5], M.R. Manuel Ramírez[6], D. Azorín[7], J. Alonso[1]

[1] Instituto de Salud Carlos III, Instituto de Investigación de Enfermedades Raras, Madrid, Spain
[2] Instituto de Salud Carlos III, Servicio de Diagnóstico Genético. Instituto de Investigación de Enfermedades Raras, Madrid, Spain
[3] Hospital Infantil La Paz, Servicio de Hemato-Oncología Pediátrica., Madrid, Spain
[4] Hospital Universitario La Paz, Servicio de Anatomía Patológica, Madrid, Spain
[5] Hospital General Universitario Gregorio Marañón, Sección de Oncohematología infantil- Servicio de pediatría, Madrid, Spain
[6] Hospital Universitario Niño Jesús, Servicio de Oncología, Madrid, Spain
[7] Hospital Universitario Niño Jesús, Servicio de Anatomía Patológica, Madrid, Spain

BACKGROUND: Childhood cancer is the leading cause of death due to disease in children and adolescents. Although there has been a significant increase in survival rates since the application of modern chemotherapeutic treatments, in the last two decades there has been a stagnation in survival rates. Therefore, it is essential to develop and establish more specific and targeted therapies for each tumor and patient (precision medicine) in order to increase the number of children cancer survivors and to decrease the serious side effects associated with current therapies. To aim of this study was evaluate the next generation sequencing (NGS) to characterize in detail the tumor genetic profile of patients treated in three reference hospitals of pediatric oncology in Spain. The ultimate goal is to make available to pediatric oncologists the technologies and information that enable them to implement precision medicine to treat childhood cancer patients.

MATERIAL AND METHODS: A total of 34 patients diagnosed with different types of childhood cancer (19 leukemias, 8 sarcomas and 7 other tumors) have been analyzed with a panel of 160 genes frequently mutated in cancer, which includes most genes that have been associated with biological therapies (GeneRead Comprehensive Cancer, Qiagen®).

RESULTS: 53% of patients presented mutations in at least one of the genes analyzed by NGS (18/34). A total of 43 mutations were detected in the 34 patients. The most frequently mutated genes were KRAS (5/43), TP53 (4/43), NRAS (3/43) and PTEN (3/43). The p.G12D mutation in KRAS was found in three patients. Several of the alterations identified have been associated with biological therapies (Everolimus (PIK3CA, PTEN), Trametinib (NRAS/KRAS), Trastuzumab (ERBB2), Sorafenib (FLT3)). A mutation in CTNNB1 gene was identified by NGS studies in a sample and this finding contributed to the diagnosis of cranial fasciitis in the patient. In other patient a constitutional mutation in DICER1 gene was detected contributing to characterize the DICER1 associated syndrome.

CONCLUSIONS: The results obtained in this pilot study carried out in three reference hospitals in pediatric oncology show that the genetic characterization of childhood tumors using NGS techniques is also useful in the context of childhood cancer, allowing the identification of alterations with diagnostic, prognosis and therapeutic value, opening the door to use targeted biological drugs in refractory tumors

ACKNOWLEDGMENTS: this work has been funded by 'La fundación de la Sonrisa de Alex", 'La Hucha de Tomás-ASION" and 'La Asociación Pablo Ugarte"

NO CONFLICT OF INTEREST

117 Identification of novel cancer biomarkers of prognostic value using specific gene regulatory networks (GRN): A novel role for RAD51AP1 for ovarian and lung cancers

D. Chudasama[1], V. Bo[2], M. Hall[3], A. Vladimir[4], G. Pados[5], A. Tucker[6], E. Karteris[7]

[1] Royal Brompton & Harefield NHS Foundation Trust, Thoracic Surgery, Harefield, United Kingdom
[2] Brunel University London, Department of Computer Science, London, United Kingdom
[3] Mount Vernon Cancer Centre, Oncology, London, United Kingdom
[4] Harefield Hospital, Thoracic Surgery, London, United Kingdom
[5] University of Thessaloniki Medical School, Oncology, Thessaloniki, United Kingdom
[6] Department of Computer Science, Brunel University London, London, United Kingdom
[7] Brunel University London, Department of Life Sciences, London, United Kingdom

INTRODUCTION: Gene regulatory networks (GRN) are an assembly of regulators that interact at molecular level to influence transcriptional and translational responses. To date, microarray analyses of cancer patients over the past years have led to the discovery of numerous individual 'molecular signatures" associated with specific cancers. However, adoption of these multi-gene signatures in the clinical environment for diagnostic or prognostic testing remains controversial. In this study, we conduct analyses -based on a GRN- to reveal distinct and common genetic features across cancer types and to explore whether these genes can be used as biomarkers.

MATERIALS AND METHODS: For the construction of a GRN we used microarray data from multiple studies in non-small-cell lung (NSCLC), breast (triple negative/ medullary) and ovarian cancers and a combination of glasso and bayesian networks. siRNA employed for silencing RAD51AP1 in vitro, followed by validation of the knockdown of the gene using qRT-PCR and Western blotting. Quantitative RT-PCR was also used for gene expression studies in cancer and control groups, mTOR components and metastatic genes.

RESULTS AND CONCLUSIONS: From the GRN small proline-rich protein 1A (SPRR1A), follistatin like 1 (FSTL1), collagen type XII alpha 1 (COL12A1) and RAD51 associated protein 1 (RAD51AP1) were identified. RAD51AP1 and FSTL1 are significantly overexpressed in ovarian cancer patients but only RAD51AP1 is upregulated in lung cancer patients compared to healthy controls. KM plots predict poorer overall survival for ovarian and lung cancer patients with high expression of RAD51AP1. Transfecting with RAD51AP1 siRNA reduces cell proliferation in ovarian (SKOV3) and lung (A549) cancer cell lines at 72 hr. This effect appears to be modulated by reductions in mTOR signalling and pro-metastatic candidate genes. Discussion: Collectively, our data describe how an initial in silico approach can generate novel biomarkers that may be used diagnostically and prognostically in clinical practice.

NO CONFLICT OF INTEREST

118 Identification of new KEAP1 isoforms – a potential biomarker in HCC?

S. Blois[1], S. Menegon[2,3], L. Moi[1], S. Giordano[2,3], A. Columbano[4], P. Zavattari[1]

[1] University of Cagliari, Department of Biomedical Sciences - Biology and Genetics Unit, Cagliari, Italy
[2] University of Torino, Department of Oncology, Torino, Italy
[3] IRCCS, Candiolo Cancer Institute - FPO, Torino, Italy
[4] University of Cagliari, Department of Biomedical Sciences - Oncology and Molecular Pathology Unit, Cagliari, Italy

BACKGROUND: The KEAP1 (Kelch-like ECH-associated protein 1) protein is a main negative regulator of the cell adaptative response to oxidative and xenobiotic stresses mediated by NRF2 (nuclear factor (erythroid-derived 2)-like 2) transcription factor. Under physiological conditions, NRF2 binds to the Kelch domain of KEAP1 and is mantained at a reduced level in the cytoplasm by the KEAP1-dependent ubiquitination and proteasomal degradation systems. Under oxidative stress, the KEAP1-dependent ubiquitin ligase activity is inhibited and NRF2 can translocate into the nucleus where it specifically recognizes the antioxidant response elements (ARE) located in the promoter of redox balancing genes, phase II detoxifying gene and drug transporters. Although NRF2 showed its protective role in tumorigenesis, accumulating evidence has started to point out the 'dark side" of NRF2 in cancers. The high NRF2 protein levels found in multiple types of human cancers were associated with cancer development, progression and resistance to chemotherapy. Overall, KEAP1 may act as a tumor suppressor gene and loss of KEAP1 functions confers tumorigenic potential to the cells.

MATERIAL AND METHODS: Total RNA was isolated from HepG2, Huh7, SKHep1, PLC cell lines, as well as 11 peritumoral and 14 tumoral from human liver tissues. The full CDS of KEAP1 was amplified by RT-PCR. PCR products were agarose gel purified and bidirectionally sequenced.

RESULTS: Our preliminary results show the presence of multiple isoforms of KEAP1 in human cancer cell lines. Interestingly, we observed a different expression profile between some peritumoral and tumoral liver tissues, suggesting a molecular signature in HCC. A real time RT-qPCR assay specific to the new isoforms was designed and will confirm the significantly lower expression of these new transcripts, as previously observed on agarose gel and by sequencing. In order to elucidate the role of the new isoforms, two of the most expressed transcripts were isolated, sequenced and will be cloned.

CONCLUSIONS: Here, we identified for the first time new isoforms of the KEAP1 gene. Our preliminary result suggest that these new isoforms could be biomarkers of human HCC, however, these promising data deserve further investigation. Western blot analysis and molecular dynamics simulation are in progress to establish whether the new isoforms are able to modulate the NRF2/KEAP1 pathway.

NO CONFLICT OF INTEREST

119 NF-kB-dependent regulation of TET1 in breast cancers

A. Canale[1], E. Collignon[2], C. Al Wardi[2], A. Noel[1], F. Fuks[2]

[1] Giga Cancer, Département des sciences biomédicales et précliniques / Biologie cellulaire et moléculaire- Bât. B2 3 Labo des tumeurs et du développement, Liège, Belgium
[2] Université Libre de Bruxelles-ULB, Laboratory of Cancer Epigenetics, Bruxelles, Belgium

BACKGROUND: Breast cancer is a heterogeneous disease characterized by different clinical behaviors, molecular and histopathological features, risk factors, response to therapy and patient outcome. An interesting anti-cancer approach is targeting epigenomic components as they regulate different oncogenic function. Newly discovered Ten eleven translocation enzymes (TET1-3) convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and are involved in DNA demethylation and gene regulation. 5hmC and TET expression are involved in many physiological and pathological processes including the cancer disease. Recent studies have shown that 5hmC levels and TET expression are dysregulated in cancer but the mechanisms underlying this process have not yet been identified. The aim of this study is to investigate TET1 protein and 5hmC alterations in breast cancer.

MATERIALS AND METHODS: Gene expression data sets were obtained from GEO (Gene Expression Omnibus) and TCGA (The Cancer Genome Atlas). Functional enrichment analysis was performed with DAVID (The Database for Annotation, Visualization and Integrated Discovery). In order to activate NF-κB pathway, MDA-MB231 cells were treated with TNFα and TET1 expression was quantified by RT-qPCR. The binding of NF-κB to TET1 promoter was confirmed by luciferase assay, Electrophoretic Mobility Shift Assay, streptavidin-agarose pulldown assay and Chromatin Immunoprecipitation (ChIP).

RESULTS: Firstly, TET1 expression has been assessed by gene expression microarray and RNA-seq data on human breast cancers, classified in four main subtypes (Luminal A, Luminal B, HEB2 and basal-like). Compared to normal tissues, TET1 expression was found decreased in Luminal A, B and HER2 subtypes, but increased in basal-like cancers. Gene ontology analysis and in vitro data have shown an anti-correlation between TET1 expression and different immune and defense markers, including RelA, member of NF-κB family. To confirm these observations, we have conducted in vitro studies revealing the downregulation of TET1 expression upon NF-κB stimulation in three different basal-like breast cancer cell lines. We next focused on the binding of NF-κB to TET1 promoter and we identified potential binding sites. Finally, we intent to investigate the functional impact of TET1 regulation on 5hmC in breast cancer.

CONCLUSION: To conclude, our study establishes a link between TET1 expression and inflammation in basal-like breast cancer.

NO CONFLICT OF INTEREST

120 Expression analysis of miR-21, miR-205, EGFR, MINA53 and mTOR in Bulgarian patients with non-small cell lung cancer

V. Petkova[1], A. Mitkova[1], G. Stancheva[1], D. Kachakova[1], S. Giragosyan[1], D. Marinova[2], S. Yanina[3], V. Mitev[2], R. Kaneva[1]

[1] Medical University-Sofia, Department of medical chemistry and biochemistry- Center of molecular medicine, Sofia, Bulgaria
[2] Medical University-Sofia, Clinical Center for Lung Diseases, Sofia, Bulgaria
[3] University Hospital for Pulmonary Diseases "St. Sofia", Pathology, Sofia, Bulgaria

INTRODUCTION: MicroRNAs (MiRNAs) are widely studied molecules because their expression is changed in variety of pathophysiological mechanisms in comparison to normal tissue. MiRNAs can help understanding the carcinogenesis of NSCLCs and served as potential biomarkers for tumour diagnosis.The aim of the present study is to analyse the expression patterns of mir-21 and mir-205, as well as EGFR, MINA53 and mTOR in two major NSCLC subtypes: adenocarcinoma(AC) and squamous cell lung carcinoma(SCC) and explore their correlations significance.

MATERIAL AND METHODS: Tissue specimens from 26NSCLC patients were examined:12AC and 14SC together with histological subtype, TNM stage. The expression of EGFR, MINA53, mTOR and two microRNAs – miR-21 and miR-205 were evaluated by RT-qPCR. The statistical analysis was performed by SPSSv20.

RESULTS: MiR-21 and miR-205 demonstrated statistically significant overexpression in 80.77% and 84.61% of all tumour samples, respectively, while both of them were underexpressed in 7.69% of all tumours. No difference in the expression of miR-21 and miR-205 was observed in 11.54% and 7.69% tumours, respectively, in comparison with the normal tissue samples. EGFR was overexpressed in 35%(5SCC&4AC), MINA53 in 50%(8SCC&6AC), and MTOR in 50%(5SCC&6AC) of the tumour samples. Decreased expression of EGFR, MINA53 and MTOR was found in 27%(3SCC&4AC), 3%(1SCC) and 12%(2SCC&1AC).

In SCC statistically significant correlations were observed between miR-21&MTOR(rs= 0,528;p=0.05), miR-21&MINA(rs=0,559;p=0.03) and miR-205& MINA(rs=-0,569;p=0.03). Moreover, we found significant correlations between EGFR&MTOR (rs=0,644;p=0.01), MINA53&MTOR(rs=0,640;p=0.01) and MINA53&EGFR(rs=0,675;p=0.008).No correlations with age, sex, TNM stage and N status were found.

In AC no statistically significant correlations were found between the studied miRNAs and EGFR, mTOR, MINA53, but a positive correlation was discovered between EGFR&MINA (rs=0,818;p=0.001). They didn't demonstrate correlations with age, sex and TNM stage. Only miR-205 showed significant association with N status (p=0,04).

CONCLUSION: The current findings suggest that miR-21 and miR-205 take part in cancerogenesis of NSCLCs, and they demonstrate different role in SCC and AC. EGFR, MINA53 and MTOR are often overexpressed in NSCLCs and there are positive correlations between their expression. Further analysis of enlarged sample is necessary in order to ascertain the correlation between the studied biomarkers.

NO CONFLICT OF INTEREST

121 microRNAs in immunogenic cancer cell death

M. Ravo[1], B. Montico[2], D.A. Faè[2], D. Martorelli[2], E. Muraro[2], R. Tarallo[3], G. Giurato[1,4], A. Weisz[3], R. Dolcetti[5], J. Dal Col[6]

[1] University of Salerno, Department of Medicine Surgery and Dentistry 'Scuola Medica Salernitana'- Laboratory of Molecular Medicine and Genomics, Baronissi, Italy
[2] C.R.O. National Cancer Institute - IRCCS- Aviano, Cancer Bio-Immunotherapy Unit, Aviano, Italy
[3] University of Salerno, Laboratory of Molecular Medicine and Genomics - Department of Medicine Surgery and Dentistry 'Scuola Medica Salernitana', Baronissi, Italy
[4] Genomix4 Life S.r.l., Laboratory of Molecular Medicine and Genomics - University of Salerno, Baronissi, Italy
[5] The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia
[6] University of Salerno, Department of Medicine Surgery and Dentistry 'Scuola Medica Salernitana', Baronissi, Italy

BACKGROUND: Immunogenic cell death (ICD) is a recently described new type of drug-induced apoptosis that stimulates host immune system and enhances immunological responses to immunotherapy. A well-defined combination of damage-associated molecular patterns (DAMPs), including molecules secreted or exposed on dying cell surface characterizes ICD, has been identified while evidence supporting a potential involvement of microRNAs (miRNAs) in this process is scarce. Given the regulatory actions of miRNAs not only in apoptosis but also in the control of autophagy, another important component of ICD, we sought to assess whether they might represent new ICD markers.

MATERIALS AND METHODS: ICD was induced in Mino, SP53, DOHH2 and MDA-MB-231 cells representative, respectively, mantle cell lymphoma, diffuse large B-cell lymphoma and triple negative breast cancer. A combination of retinoic acid and interferon-α (RA/IFNα) and the anthracycline doxorubicin were used as ICD inducers, as we recently showed that RA/IFNα combination is novel ICD inducer in lymphoma cells promoting calreticulin, hsp70 and hsp90 and decreasing CD47 expression on the cell surface. Expression of canonical ICD markers and their exposure on the cell surface were evaluated by multispectral imaging, flow cytometry and immunoblotting. HMGB1 release in the culture medium was assessed by ELISA. As a first step to evaluate the role of miRNAs in ICD, small non-coding RNAs expression profiling was performed, before and after treatments with RA/IFNα, doxorubicin or γ-irradiation (inducing necrosis) by small RNA sequencing (sncRNA-Seq).

RESULTS AND DISCUSSION: RA/IFNα treatment promoted uptake of apoptotic tumor cells by dendritic cells, as demonstrated by phagocytosis assay. A significant increase of HMGB1 protein was also observed in the culture supernatants of all RA/IFNα-treated cell lines. sncRNA-Seq identified a series of miRNAs differentially expressed in treated, respect to untreated, cells (FDR ≤0.05 and |FC|>1.5). Interestingly, this analysis led to the identification of a miRNAs signature characteristic of Mino and MDA-MB-231 cells undergoing ICD, absent in untreated or γ-irradiated/necrotic cells.

CONCLUSION: These result identified sncRNAs modulated in cell lines, representing different cancer models, by both ICD inducers used, suggesting their potential involvement in immunogenic apoptosis.

Supported by Italian Ministry of Health (GR-2011-02350476) and AIRC (IG-17426)

NO CONFLICT OF INTEREST

122 Combined use of NGS and dPCR for liquid biopsy of non-metastatic colorectal cancer patients at surgery

M. Allegretti[1], G. Cottone[2], E. Melucci[3], S. Buglioni[3], F. Carboni[4], A. Garofalo[4], L. Conti[5], E. Pescarmona[3], P. Giacomini[1], F. Spinella[2]

[1] Regina Elena National Cancer Institute, Oncogenomics and Epigenetics, Roma, Italy
[2] Genoma Group, Oncogenomics, Rome, Italy
[3] Regina Elena National Cancer Institute, Pathology, Roma, Italy
[4] Regina Elena National Cancer Institute, Digestive Surgery, Roma, Italy
[5] Regina Elena National Cancer Institute, Clinical Pathology, Roma, Italy

BACKGROUND: Next Generation Sequencing (NGS) and digital PCR (dPCR) have widely been applied to the analysis of tumor tissue DNA (tDNA), and circulating tumor DNA (ctDNA) in the liquid biopsy setting. NGS is optimal for the multiplexed detection of tumor aberrations, while dPCR attains unsurpassed limits of detection (LOD). Monitoring ctDNA in advanced cancer is quite easy, since mutated allele frequencies (AFs) are often high, whereas in the non-metastatic setting few copies per ml must be detected. Herein, we performed a cross-sectional analysis of matched tDNAs and ctDNAs from 32 colorectal cancer (CRC) patients (T1N0M0 to T4N2M0) at surgery by an integrative NGS/dPCR approach.

MATERIAL AND METHODS: Tissues and blood were obtained, after informed consent, from non-metastatic CRC patients undergoing surgery (n=32), and from two control metastatic patients. ctDNAs were extracted from plasma by the QiAMP CNA kit (Qiagen). Both tDNAs and ctDNAs were analyzed by NextSeq (Illumina) and QuantStudio 3D dPCR (LifeTechnologies). dPCR assays were custom-designed to screen for 12 KRAS, BRAF, PIK3CA and TP53 point mutations detected by a 15-gene NGS panel.

RESULTS: The LOD of NGS was set at AF ≥0.2% in spiking experiments. A total of 33 point mutations (21 on tDNAs, 12 on ctDNAs) were detected by NGS/dPCR on 18/34 (52.9%) CRC patients. Mutation AF in tDNAs was 28.5%±15.2% (range 3.7-74.0%), and 10.1%±15.2% (range 0.4-44.0%) in ctDNAs. As expected, high AF (>40%) were detected in ctDNAs from metastatic samples. NGS and dPCR of tDNAs were fully in agreement, since 21/21 mutations (100%) were seen by both, with a negligible difference in estimated AF values (1.3%±1.2; range 0.0-4.1%). Correlations in ctDNAs were lesser (17/20, 85.0%), with AF calling odds of 0.8%±1.1% (range 0.0-2.8%). Only in 8/18 (44.5%) ctDNAs NGS could confirm mutations seen in the corresponding tDNA. Concordance was higher (12/18, 66.7%) with dPCR. No correlation was evident between ctDNAs and TNM staging.

CONCLUSIONS: Sensitive detection of ctDNAs in CRC patients is feasible even in non-metastatic disease. Integrative NGS/dPCR is essential to correctly assign WT/mutated status in this extremely low ctDNA bracket. Remarkably, there is no obvious correlation between ctDNA levels and clinical features. Widening mutation panels may further improve LOD, so as to detect ctDNA in early-stage cancer for population screening.

Supported by EU 633937 – ULTRAPLACAD, and AIRC (IG 19052, and Nuvenia Fellowship to MA)

NO CONFLICT OF INTEREST

123 Correlation of CIMP status with clinico-pathological and molecular features of histological variants of colorectal carcinoma

M.C. Turpín Sevilla[1], J. García Solano[2], P. Carbonell[3], D. Torres Moreno[2], E. Estrada[4], C. Martín[5], A. Tuomisto[6], M.J. Mäkkinen[6], M. Pérez-Guillermo García[2], P. Conesa-Zamora[2]

[1] Santa Lucia University Hospital / Universidad Francisco de Vitoria, Pathology department/ Biotechnology department, Cartagena, Spain
[2] Santa Lucia University Hospital, Pathology department, Cartagena, Spain
[3] Hospital Uniuersitario Virgen de la Arrixaca, Genomic department, Murcia, Spain
[4] University Camilo Jose Cela, Psychology, Madrid, Spain
[5] Universidad Francisco de Vitoria, Evaluación de tecnologias sanitarias, Madrid, Spain
[6] University of Oulu, Pathology department, Oulu, Finland

INTRODUCTION: DNA methylation is the most important epigenetic regulation described and mainly affects CpGs islands, which are DNA regions around 200 base-pair long whose GC content is > 50% and are located at regulatory elements.

Serrated adenocarcinoma (SAC), is more frequently KRAS or BRAF mutated, usually microsatellite stable and associated with a bad prognosis. We analyse the relationship between CIMP status and molecular and histological features of SAC with the view of discerning whether a typical CIMP pattern can be associated with SAC or with some other histological variants of CRC.

MATERIAL AND METHOD: A total of 117 cases from two institutions (Santa Lucía University Hospital, Cartagena, Spain and Oulu University Hospital, Oulu, Finland) were studied. SACs were diagnosed on the basis of prior established criteria and so for CCs and MSI tumors.

Automatic DNA extraction was performed using the Qiacube equipment and the QiaAmp DNA Mini Kit (Qiagen®). Genomic DNA (1000 ng) from each sample was bisulfite converted with the EZ DNA Methylation Kit (Zymo Research®, Orange, CA)

BRAF mutation was determined byTaqMan for BRAF V600E detection and cases with no V600E mutation were direct sequenced for BRAF exon 15. KRAS mutations at codons 12 and 13 were determined by dHPLC and mutation status of 9 and 20 exons in the PI3KCA by direct sequencing after a nested-PCR.

MSI was evaluated in 79 out of 82 tumor cases using the kit MSI Analysis System, version 1.2 (Promega ® Madison, USA)

The CIMP status of the panel genes CACNA1G, CDKN2A, CRABP1, IGF2, MLH1, NEUROG1, RUNX3 and SOCS1 were evaluated by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA).

RESULTS AND DISCUSSION: The methylation data were compared according BRAF, KRAS and/or CIMP status, it were also analyzed following Ogino and Weisemberb criteria for CIMP status.

When panel genes were analyzed individually, all the genes except SOCS1 did show a relation between methylation status and histological subtypes.

KRAS mutated cases were less frequently CIMP-H. BRAF mutated cases were significantly associated with the methylation of all genes included in the panel. Similarly, MSI status was significantly associated with the methylation of all genes except for SOCS1.

CONCLUSION: CIMP status in SACs is different that CCs and a specific combination of methylated genes is more common for SAC that for CC (CDKN2A, MLH1 and IGF2) and it could be a way to identify SAC

NO CONFLICT OF INTEREST

124 Genome-wide analysis of liquid biopsies reveals novel layer of tumor heterogeneity in stage 4/M neuroblastoma

T. Geber[1], L.J. Barber[2], S. Huetter[1], R. Abbasi[1], R. Ladenstein[3], L. Chesler[4], I.M. Ambros[1], M. Gerlinger[2], P.F. Ambros[1]

[1] CCRI- Children's Cancer Research Institute- St. Anna Kinderkrebsforschung, Tumor Biology, VIENNA, Austria
[2] The Institute of Cancer Research, Translational Oncogenomics Lab, London, United Kingdom
[3] CCRI- Children's Cancer Research Institute- St. Anna Kinderkrebsforschung, SiRP, VIENNA, Austria
[4] The Institute of Cancer Research, Paediatric Cancer Biology, London, United Kingdom

BACKGROUND: Intra-tumoral heterogeneity (ITH) is a major challenge for the detection of the relevant genomic aberrations in tumor biopsies. In neuroblastoma, recent data show that genomic changes are frequently sub-clonal and may be either missed due to sampling error or they are indeed not present at all in the

primary tumor. Hence, there is an unmet need for alternative analytic procedures to obtain a more complete picture of the genomic landscape and to minimize sampling error in tumor patients. Analyzing cell-free tumor DNA (ctDNA) from more easily accessible patient biomaterial could surmount these challenges. However, in order to apply ctDNA-based result in the clinics we need to find out how representative this DNA is.

MATERIAL AND METHODS: In this study, we compared the genomic result (SNP array and low coverage WGS) from different DNA sources: primary tumor, disseminated tumor cells (DTCs) and cell-free DNA isolated from peripheral blood (PB) and bone marrow (BM) plasma from stage M neuroblastoma patients.

RESULTS: In this patient cohort ctDNA derived from PB or BM allowed the identification of gene amplifications and segmental chromosomal aberrations in 11/15 patients. However, besides a high number of identical genomic aberrations, we found discordances resulting in more genomic aberrations in the PB-ctDNA as compared to the corresponding primary tumors. On top of this, the DTCs showed, besides the expected genomic aberrations concordant with the tumor and PB-ctDNAs, unique aberrations that were shared only with the ctDNA from the BM. Thus, unexpectedly, the genomic make up of DTCs and ctDNA within the BM seems to be identical, indicating an independent compartment.

CONCLUSIONS: With these data at hand we will be able to better understand ITH and tumor progression and draw conclusions on the most informative type of DNA to address the most relevant clone for a possible relapse.

NO CONFLICT OF INTEREST

125 Novel hypermethylated tumor suppressor genes as indicators of decitabine sensitivity in breast cancer

M. Thomas[1], B. Cruickshank[2], K. Coyle[1], M. Sultan[1], I. Weaver[3], C. Giacomantonio[4], P. Marcato[1]

[1] Dalhousie University, Pathology, Halifax, Canada
[2] Saint Mary's University, Biology, Halifax, Canada
[3] Dalhousie University, Psychology and Neuroscience, Halifax, Canada
[4] Dalhousie University, Surgery, Halifax, Canada

INTRODUCTION: DNA methylation is important in many types of cancer, and is a well-accepted mediator of breast cancer growth. Aberrant hypermethylation drives tumor growth by silencing expression of tumor suppressor genes. Hypermethylation has been observed and treated in myelodysplastic syndromes using the de-methylating drug decitabine; this drug is also being investigated in clinical trials in solid tumors. This presents an opportunity to exploit a known breast tumor growth mechanism using a drug that is already in clinical use. If the key methylated tumor suppressor genes in breast cancer are revealed, then patients with that hypermethylation profile will likely benefit from decitabine treatment.

METHODS: To identify these genes we used a genome-wide knockdown screen approach and identified four putative novel hypermethylated tumor suppressor genes in breast cancer. Hypermethylation of these genes was assessed in decitabine-treated MDA-MB-231 breast cancer cells, normal breast tissue, and patient breast cancer samples (GSE69914).

RESULTS: & Discussion: Gene expression analyses show that decitabine treatment induces expression of candidate genes in MDA-MB-231 cells and that their expression is significantly reduced in breast cancer compared to normal breast tissue (Oncomine, Richardson 2). The candidate genes also fit a tumor suppressor gene expression profile, with low expression in tumors associated with worse patient survival (TCGA Cell, 2015). A panel of breast cancer cell lines showed that there is a methylation pattern of candidate genes that is associated with decitabine sensitivity. This methylation pattern predicted the high sensitivity of a patient-derived breast tumor xenograft to decitabine.

CONCLUSIONS: These result suggest that we have identified hypermethylation 'biomarkers" that may be used to stratify breast cancer patients for decitabine response.

NO CONFLICT OF INTEREST

126 Discovery of novel methylated biomarkers for early colorectal cancer

P. Zavattari[1], A. Fadda[1], D. Gentilini[2], L. Moi[2], L. Barault[3,4], C. Zavattari[5], L. Varesco[6], S. Giordano[3,4], F. Di Nicolantonio[3,4], A. Columbano[7]

[1] University of Cagliari, Department of Biomedical Sciences- Biology and Genetics Unit, Cagliari, Italy
[2] Italian Auxologico Institute of Milan, Molecular Biology Lab, Milan, Italy
[3] University of Torino, Department of Oncology, Torino, Italy
[4] IRCCS, Candiolo Cancer Institute-FPO, Torino, Italy
[5] Independent Researcher, Machine Learning, Lucca, Italy
[6] Istituto Nazionale per la Ricerca sul Cancro, Unit of Hereditary Cancer- Department of Epidemiology-Prevention and Special Functions- IRCCS San Martino IST, Genova, Italy
[7] University of Cagliari, Department of Biomedical Sciences- Oncology and Molecular Pathology Unit, Cagliari, Italy

BACKGROUND: Colorectal cancer (CRC) arises as a consequence of the accumulation of genetic and epigenetic alterations in colonic epithelial cells. While genetic alterations are already used as prognostic and predictive biomarkers, epigenetic alterations are less characterized, although they are recognized as common molecular alterations in the early steps of CRC tumorigenesis.

The aims of the present study were to identify signature alterations in the CRC methylome, to test whether these alterations represent early events in CRC development, to explore the use of non-invasive techniques (stool and cell free plasma DNA) to reveal altered methylation and to correlate the mRNA gene expression of CRCs with the altered DNA methylation.

MATERIAL AND METHODS: Methylome analysis was conducted by HumanMethylation450 BeadChip. Raw data were analysed using the R/Bioconductor package RnBeads, to perform a differential methylation analysis. We focus on CpG islands results, annotating them based on 450k manifest to create a gene list. Pathways enrichment analysis was conducted using the web portal ToppGene. The in silico validation was performed in publicly available datasets (TCGA, GSE48684, GSE52270, GSE53051). Specificity, sensitivity and AUC values were calculated using the 'OptimalCutpoints" R package. Methylation analysis of three selected markers was performed in 78 additional tumoral and matched peritumoral samples by pyrosequencing, and in 24 stool DNA and 45 cell free plasmaDNA of CRC patients by digital PCR.

RESULTS: We identified and validated in over 600 samples a panel of 74 altered CpG islands, annotating genes belonging to the most significantly involved pathways: Wnt and cadherin signaling, neuroactive ligand-receptor interaction. The panel discriminates CRCs and adenomas from peritumoral and normal mucosa, with very high specificity (1) and sensitivity (0.9992). We evaluated the mRNA gene expression of the 74 genes finding that over 70% of the hypermethylated islands resulted in downregulation of gene expression. To establish the usefulness of these findings as non-invasive markers for detection of CRC, we selected and tested three biomarkers in stool DNA and ctDNA from plasma, confirming the presence of altered methylation in affected patients.

CONCLUSIONS: In conclusion, our study identified a panel of genes with altered methylation in both adenomas and CRCs candidating its use as biomarker for adenomas and early CRC detection through non-invasive techniques.

NO CONFLICT OF INTEREST

127 Ultra-deep sequencing of cell-free DNA for screening and monitoring gynecological cancers

C. Schumacher[1], N. Nair[2], O. Camacho-Vanegas[2], J. Irish[1], L. Kurihara[1], P. Dottino[2], M. Schwartz[2], T. Harkins[1], J. Martignetti[2], V. Makarov[2]

[1] Swift Biosciences, Research and Development, Ann Arbor, USA
[2] The Mount Sinai Hospital, Human Genetics, New York, USA

BACKGROUND: Acellular DNA in blood and other bodily fluids is known as cell-free DNA (cfDNA). cfDNA arises from apoptosis or necrosis of healthy and cancerous cells. For cancer, noninvasive collection and analysis of cfDNA can be used to screen for tumor derived mutations. Here we present a method using targeted, PCR-based next generation sequencing (NGS) to identify low frequency somatic mutations in cfDNA from women with uterine and ovarian cancers. In two pilot studies we demonstrate how ultra-deep targeted sequencing enables variant detection down to 1%. This technique also includes amplicons which target germline SNPs to ensure proper sample tracking by generating each individual's unique genetic fingerprint. The first study was a retrospective examination of circulating cfDNA from blood while the second study examined cfDNA derived from uterine lavage as a potential screening method to detect early stage uterine cancer.

MATERIAL AND METHOD: In the first study, 11 women had previously undergone tumor resection and the tumor profile was determined using an NGS-based amplicon panel targeting 56 oncology-related genes. cfDNA samples were then isolated from blood at two or more time points ranging from 7 to 64 months apart, and sequenced using the same NGS-based amplicon panel. In the second study, 104 women underwent uterine lavage. cfDNA and DNA derived from cellular material were isolated from the aspirated lavage. The samples were then sequenced with an NGS-based amplicon panel that represented a subset of the previous panel, focusing only on the gynecological-related oncology genes.

RESULTS AND DISCUSSION: In the first study, tumor-specific mutations were found in the cfDNA of 8 of 11 women. In one case, the frequency of mutation detected in the cfDNA was nearly three times that detected in the tumor, and the patient died 4 days later. In the second study, 7 women were identified by histopathology to have uterine cancer, and in parallel, oncology-related mutations were discovered in all of the corresponding cfDNA samples. Additionally, 51 of the 104 women not identified by histopathology to have cancer had cfDNA bearing oncology-related mutations. All mutations in this study were further validated using digital droplet PCR.

CONCLUSION: We have provided preliminary evidence for the use of ultra-deep sequencing of cfDNA derived from both blood and uterine lavage fluid as a means of screening for and monitoring gynecologic cancers in the research setting.

CONFLICT OF INTEREST

Other Substantive Relationships: CS, JI, LK, TH, and VM are paid employees of Swift Biosciences

128 Genome-wide profiling identifies the THYT1 signature as a distinctive feature of widely metastatic Papillary Thyroid Carcinomas

G. Gandolfi[1], M. Ragazzi[2], D. De Biase[3], V. Sancisi[1], M. Gugnoni[1], G. Manzotti[1], A. Frasoldati[4], S. Piana[2], A. Ciarrocchi[1]

[1] Arcispedale S. Maria Nuova-IRCCS, Laboratory of Translational Research, Reggio Emilia, Italy
[2] Arcispedale S. Maria Nuova-IRCCS, Pathology Unit, Reggio Emilia, Italy
[3] University of Bologna, Dipartimento di Farmacia e BioTecnologie, Bologna, Italy
[4] Arcispedale S. Maria Nuova-IRCCS, Endocrinology Unit, Reggio Emilia, Italy

BACKGROUND: Papillary Thyroid Carcinomas (PTCs) are generally indolent tumors. However, a small but significant percentage of PTCs behaves aggressively, progressing to a diffuse metastatic spreading and leading to patient's death. Due

to their rarity, the genomic landscape of widely metastatic PTCs has remained so far unexplored, limiting the identification of prognostic markers to foresee aggressiveness and progression of these lesions. Furthermore, lack of reliable markers for predicting the metastatic behavior of PTCs prevents a correct risk based stratification of the disease, thus contributing to the issue of patients' overtreatment. In this study we aimed at identifying genetic features associated with the development of distant metastasis in PTCs.

METHODS: A consecutive series of 2,937 thyroid malignancies, collected at our Institution over the past 40 years were searched to retrieve a large and unique cohort of PTCs that developed distant metastasis (DM-PTCs, n=50). We performed a deep profiling to explore the genomic landscape of these tumors.

RESULTS: We showed that DM-PTCs are characterized by a moderate degree of copy number alterations but display low level of microsatellite instability and a low mutational burden. We identified duplication of Chr1q, duplication of TERT genomic locus and mutations of TERT promoter as distinctive features of DM-PTCs. These three genetic variables defined a signature (THYT1) that is strongly associated with the development of distant metastasis and with other major clinical features of aggressiveness in PTCs (like age, stage, and RAI refractoriness). Furthermore, we showed that the THYT1 signature strongly correlates with reduced survival probability. THYT1 positive patients had 7-fold higher risk to die because of the tumor as compared with THYT1 negative patients. Finally, we analyzed the THYT1 signature in PTCs fine needle aspirate biopsies (FNAB) and we demonstrated the applicability of this signature as prognostic marker in the pre-operative diagnostic setting of PTCs.

CONCLUSIONS: Our analysis reveals previously unknown insights into the genetic alterations that underlie aggressiveness and metastatic progression of differentiated PTCs. Furthermore, our data lay the basis for the possible application of the THYT1 signature as prognostic marker in the early phases of diagnosis, to improve risk-based stratification and management of PTC patients.

NO CONFLICT OF INTEREST

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129 Identification of epigenetic regulators of resistance to HER2-targeted antibodies

M. Gale[1], *Z. Liu*[1], *R. Gupta*[1], *N. Wajapeyee*[1], *Q. Yan*[1]

[1] *Yale University, Pathology, New Haven- Connecticut, USA*

BACKGROUND: Drug resistance remains a major clinical problem for the treatment of HER2+ breast cancers. Based on evidence from our lab and others, we hypothesize that HER2+ breast cancer cells acquire drug resistance by epigenetic reprogramming. Therefore, epigenetic regulators are potential drug targets to combat drug resistance.

MATERIALS AND METHODS: Using the HER2+ breast cancer cell line BT474, we conducted a functional shRNA screen to identify epigenetic regulators of tolerance to the HER2-targeted antibody trastuzumab (Herceptin®) and the combination of HER2-targeted antibodies trastuzumab plus pertuzumab (Perjeta®). We knocked down ~400 epigenetic regulators with 4-5 shRNAs each, treated cells with none, one, or both drugs for ten doublings, and then used deep sequencing to identify shRNA that were enriched or dropped out. We also used Western blotting and reverse transcription quantitative PCR to characterize global epigenetic and gene expression changes that occurred after short and long term treatment with trastuzumab and the drug combination.

RESULTS: We identified candidate suppressors of drug tolerance, as well as candidate drug targets that could be utilized to combat drug resistance. Short and long term drug treatment induced epigenetic and gene expression modifications.

CONCLUSIONS: Our findings indicate that epigenetic regulation contributes to the development of resistance to trastuzumab and trastuzumab plus pertuzumab in HER2+ breast cancer cells.

NO CONFLICT OF INTEREST

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130 Low frequency variant detection and tissue-of-origin exploration using liquid biopsies

V. Kelchner[1], *A. Wood*[2], *J. RoseFigura*[3], *J. Lenhart*[3], *S. Sandhu*[3], *L. Kurihara*[3], *V. Makarov*[3], *T. Harkins*[4]

[1] *Swift Biosciences- Inc, Production, Ann Arbor, USA*
[2] *Swift Biosciences, R&D, Ann Arbor, USA*
[3] *Swift Biosciences- Inc, R&D, Ann Arbor, USA*
[4] *Swift Biosciences- Inc, Executive, Ann Arbor, USA*

INTRODUCTION: The promise of liquid biopsy assays lie in non-invasive monitoring of disease, which may assist in early-stage prognosis and further diagnosis of cancer biomarkers while monitoring treatment response through cell-free DNA (cfDNA) or circulating tumor cell DNA. As material can be limited, most liquid biopsy assays incorporate targeted sequencing to enable cost-effective deep coverage of loci of interest for detection of low frequency pathogenic variants. Critical to attaining the necessary sensitivity is an assay that produces uniform, comprehensive coverage from low DNA input. We developed a liquid biopsy workflow to enable low frequency variant detection from 10mL of blood using Swift Biosciences Accel NGS 2S DNA library preparation methodology.

MATERIALS AND METHODS: Blood samples were collected in Streck cell-free DNA BCT vials from patients with various stages of cancer. cfDNA was extracted with the Qiagen QIAamp Circulating Nucleic Acid Kit. DNA yields ranged from 10-20ng, with a size profile defined by a peak of ~170bp and a mean Alu repeat qPCR integrity score of 0.22, indicating high quality cell-free DNA lacking cellular DNA content. 20ng cfDNA was used to make Accel-NGS 2S® Hyb libraries with molecular identifiers (MIDs) followed by hybridization capture using the IDT DNA xGen® Pan-Cancer Panel and the Agilent ClearSeq Comprehensive Cancer Research Panel. MIDs were used to uniquely label each library molecule prior to PCR. Captured libraries were sequenced on the Illumina HiSeq platform to greater than 5000x depth. MIDs enabled accurate removal of PCR duplicates while preserving fragmentation and strand duplicates to maximize data recovery. Molecules containing the same MID were grouped together to generate consensus sequences, facilitating removal of false positives due to PCR and sequencing errors. Variant calling was performed with Vardict and Lofreq enabling highly sensitive and precise detection of variants down to 0.5% allele frequency.

RESULTS AND DISCUSSION: Accel-NGS 2S Hyb DNA Library Kit exhibits up to a 90% library conversion rate and provides high complexity libraries with uniform target coverage. Automation permits generation of high quality libraries simultaneously from multiple samples, enabling more efficient use of sequencing runs without detriment to data quality. This method has been validated to detect 1% mutation frequencies from 10ng of cfDNA increasing both the sensitivity and specificity of variant detection.

NO CONFLICT OF INTEREST

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131 Identifying potentially causal epigenetic markers as novel therapeutic targets for breast cancer

C. He[1]

[1] *Indiana University Richard M. Fairbanks School of Public Health, Epidemiology, Indianapolis, USA*

INTRODUCTION: Aberrant DNA methylation plays an important role in breast cancer initiation and progression. The identification of causal DNA methylation changes is critical not only for understanding breast cancer biology but also for identifying biologically relevant markers. As DNA methylation alterations can occur early in carcinogenesis and such effects are reversible, identification of causal DNA methylation markers provides a rare opportunity for a targeted and selective therapy.

MATERIAL AND METHOD: The study included 30 breast tumor tissue samples from cancer cases and 30 normal breast tissue samples from healthy women as controls. Cases and controls were matched on age and race. Genomic DNA samples were extracted from tumor and normal breast tissue and analyzed for genome-wide DNA methylation using the Illumina Methylation EPIC BeadChip. We first performed an epigenome-wide association study (EWAS) to identify breast cancer-associated DNA methylation markers. Potentially causal DNA methylation markers were then identified as those that were not only influenced by established breast cancer risk factors in normal breast tissue but also enriched for their associations with breast cancer in our EWAS. Linear regression was performed to examine the association of methylation level of each CpG site (β-value) with breast cancer or with each risk factor. We further validate our result using various publicly-available epigenetic databases.

RESULTS AND DISCUSSION: We identify novel, potentially causal DNA methylation markers in addition to those previously being reported to be associated with breast cancer development. Our result suggested these identified methylation markers might contribute to the important gene regulation process in tumorigenesis including inflammation, cell proliferation and survival. Further research is needed to identify their downstream target genes and understand the precise underlying molecular mechanisms of gene regulation.

CONCLUSION: The identified potentially causal DNA methylation markers have potential to be used as novel therapeutic target. Clinical validation is critical for translating the findings from cancer biology to the clinic.

NO CONFLICT OF INTEREST

POSTER SESSION: CARCINOGENESIS

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132 The anti-cancer effect of binding modulator targeting interaction of aurora kinase C and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha in breast cancer cells

Y.H. Chung[1], *E.H. Han*[1]

[1] *Korea Basic Science Institute, Division of Bioconvergence Analysis, Daejeon, Korea*

Aurora C kinase (AURKC) has an activity with tumorigenesis in breast cancer and may be a relevant cancer target. AURKC is an interesting target for the development of anticancer therapy, but its signaling network has not been fully characterized. Here we report the identification of IkBα as one of the AURKC binding partners and a small-molecule inhibitor targeting AURKC-IkBα complex which is having anti-tumor activity in MDA-MB-231 breast cancer cells. This AURKC-IkBα interaction was initially identified by a translocation-based cellular assays (redistribution approaches) and AURKC promotes activation of IkBα at serine 32 amino acid. Using in silico modeling and computational analyses, we have identified small-molecule inhibitor (AKCI) for inhibition of AURKC and IkBα interaction. AKCI induce G2/M cell cycle arrest. We also demonstrated that AKCI significantly inhibits MDA-MB-231 cell migration and invasion. Furthermore, AKCI showed that significant colony forming and tumor growth inhibition. The validation of the small molecule inhibitor AKCI

represents a first step towards developing targeted inhibitors of AURKC that may lead to further improvements in the treatment of breast cancer.

NO CONFLICT OF INTEREST

133 REV7 expression is associated with prognosis and cisplatin sensitivity in human malignancy

Y. Murakumo[1], *S. Okina*[2], *K. Niimi*[3]

[1] *Kitasato University School of Medicine, Pathology, Sagamihara, Japan*
[2] *Kitasato University School of Medicine, Hematology, Sagamihara, Japan*
[3] *Nagoya University Graduate School of Medicine, Obstetrics and Gynecology, Nagoya, Japan*

BACKGROUND: Human REV7 (also known as MAD2L2 and MAD2B) is involved in DNA repair, cell cycle regulation, gene transcription and carcinogenesis, and is a key protein in DNA damage tolerance system. Here, we present our study to evaluate the significance of REV7 expression in human malignancy and its possibility to be a molecular target for cancer therapy.

MATERIAL AND METHODS: REV7 expression was assessed in epithelial ovarian cancer (EOC) and diffuse large B-cell lymphoma (DLBCL) by immunohistochemical staining. REV7-depleted cells were produced using ovarian clear cell carcinoma (CCC) cell lines with the shRNA technique, and were analyzed for sensitivity to DNA damaging agents in vitro and in vivo. result REV7 expression was detected in the majority of EOCs (92.0%) with especially high levels of expression frequently observed in ovarian CCCs (73.5%) compared with that of non-CCCs (53.4%). Enhanced immunoreactivity to REV7 was associated with poor prognosis represented by reduced progression-free survival in EOC with advanced stage (stages II to IV). REV7 expression was also assessed in DLBCL, the most common type of non-Hodgkin lymphoma, in which high REV7 expression was associated with significantly shorter overall survival and progression-free survival. The effects of REV7 depletion on cell proliferation and chemosensitivity in CCC cells were also analyzed in vitro and in vivo. REV7 depletion in CCC cells decreased cell proliferation without affecting cell cycle distribution. Additionally, the number of apoptotic cells and DNA damaged cells were increased after cisplatin treatment. In a nude mouse tumor xenograft model, inoculated REV7-knockdown tumors showed significantly reduced tumor volumes after cisplatin treatment compared with those of the control tumors.

CONCLUSIONS: These findings indicate that high REV7 expression is associated with poor prognosis in EOC and DLBCL, and depletion of REV7 enhances sensitivity to cisplatin treatment in CCC, suggesting that REV7 is a candidate for molecular target in human malignancy.

NO CONFLICT OF INTEREST

134 Lysine-specific demethylase 1 (LSD1) destabilizes p62 and inhibits autophagy in gynecologic malignancies

A. Chao[1]

[1] *Chang Gung Memorial Hospital, Obstetrics and Gynecology, Taoyuan, Taiwan*

BACKGROUND: Lysine-specific demethylase 1 (LSD1) – also known as KDM1A – is the first identified histone demethylase. LSD1 is highly expressed in numerous human malignancies and has recently emerged as a target for anticancer drugs. Owing to the presence of several functional domains, we speculated that LSD1 could have additional functions other than histone demethylation. P62 – also termed sequestasome 1 (SQSTM1) – plays a key role in malignant transformation, apoptosis, and autophagy.

MATERIALS AND METHODS: Uterine serous carcinoma ARK2 cells and ovarian cancer TOV112D cells were used for the experiments of autophagy and apoptosis. Proximity ligation assay indicated the interaction between LSD1 and p62 in the nucleus of cancer cells. LSD1 inhibitor SP2509 was added to examine the suppression of LSD1. Immunohistochemistry was performed on a commercially available ovarian and endometrial cancer tissue array.

RESULTS: We show that a high LSD1 expression promotes tumorigenesis in gynecologic malignancies. Notably, LSD1 inhibition with either siRNA or pharmacological agents activates autophagy. Mechanistically, LSD1 decreases p62 protein stability in a demethylation-independent manner. Furthermore, LSD1 inhibition reduces both tumor growth and p62 protein degradation in vivo. The combination of LSD1 inhibition and p62 knockdown exerts additive anticancer effects.

CONCLUSIONS: LSD1 destabilizes p62 and inhibits autophagy in gynecologic cancers. LSD1 inhibition reduces malignant cell growth and activates autophagy. Suppression of both LSD1 and p62 displays additive inhibitory effect on cancer cell viability.

NO CONFLICT OF INTEREST

135 Long non coding RNA BCAR4 act as an oncogene in rectum adenocarcinoma

F. Aksoy[1], *S. Aksoy*[2], *B. Tunca*[2], *E. Ozturk*[1], *T. Yilmazlar*[1], *N. Ugras*[3], *U. Egeli*[2], *G. Cecener*[2], *O. Yerci*[3]

[1] *Uludag University, General Surgery, Bursa, Turkey*
[2] *Uludag University, Medical Biology, Bursa, Turkey*
[3] *Uludag University, Pathology, Bursa, Turkey*

BACKGROUND: Long noncoding RNAs (lncRNAs) are dysregulated in many cancer types and are believed to play crucial roles in regulating several hallmarks of cancer biology. LncRNA breast cancer anti-estrogen resistance 4 (BCAR4) has been identified as an oncogenic lncRNA involved in various cancers including breast cancer and osteosarcoma. However, the clinical significance of the lncRNA BCAR4 in rectum cancer is still unknown. This study aims to investigate the prognostic value of lncRNA BCAR4 in rectum cancer patients.

MATERIAL AND METHOD: In the present study, real-time quantitative reverse transcriptase-polymerase chain reaction was used to examine the relative level of lncRNA BCAR4 in 70 cases of rectum tissues and their adjacent non-tumor tissues. Patients with rectal cancer were defined as tumors located between 12 and 16 cm from the anal verge. Therefore, selected patients did not receive preoperative chemotherapy and/or radiation. SPSS and web-based Sabiosciences PCR-Data Analysis programme were used to evaluate the expression profile of BCAR4 and clinical features of these patients.

RESULTS AND DISCUSSION: The expression level of lncRNA BCAR4 was significantly higher in rectum tissues compared to their matched non-tumor tissues (P = 0.001). The BCAR4 expression was associated with the presence of mucinous component but not with the age, sex, tumor size, histological grade, and histological type (P = 0.0124). The increased expression of BCAR4 was significantly associated with poorer 5-year overall survival rate of rectum cancer patients (P = 0.0358).

CONCLUSION: In conclusion, over expression of lncRNA BCAR4 might be used as significant prognostic factors and indicators of rectum cancer patients.

NO CONFLICT OF INTEREST

136 The over expression of HULC is associated with tumorigenesis of colorectal cancer patients

S. Aksoy[1], *B. Tunca*[1], *E. Ozturk*[2], *T. Yilmazlar*[2], *N. Ugras*[3], *U. Egeli*[1], *G. Cecener*[1], *O. Yerci*[3]

[1] *Uludag University, Medical Biology, Bursa, Turkey*
[2] *Uludag University, General Surgery, Bursa, Turkey*
[3] *Uludag University, Pathology, Bursa, Turkey*

BACKGROUND: The metastatic dissemination of primary tumors is directly linked to patient's survival in many tumor entities. About 90% of the deaths caused by colorectal cancer (CRC) arise from the formation of metastasis. Long non coding RNA (lncRNA) HULC plays key role as a oncogene in several cancer cells. However, its expression and biological roles in CRC have not yet been investigated. The aims of this study were to clarify alterations of HULC expression associated with colorectal carcinogenesis and to identify specific biomarkers that could be used as new prognostic marker for patients.

MATERIALS AND METHODS: Eighty-six paraffin-embedded colorectal tumor and normal specimens were analyzed for HULC by RT-PCR. The relationship between basic histopathological characteristics such as tumor localization, invasion status, presence of mucinous component, stage and HULC expression level were analyzed using independent sample T test and SABioscience Data Analysis Software.

RESULTS: The level of HULC in malignant tissues was 6.2 fold higher than level in normal tissues (P < 0.001). The expression level of HULC in the CRC tumors of patients with stage III-IV was four as high as in the tumors of patients with stage I-II and the difference was statistically significant (P = 0.0356). 12.67 fold increase in the expression of HULC was observed in tumors which presence lymphatic invasion (P = 0.0427).

CONCLUSION: Our result suggest that high expression of HULC was involved in tumorigenesis and progression of CRC patients.

NO CONFLICT OF INTEREST

137 Elucidating the metastatic changes during ovarian cancer progression: A microdissection proteomics perspective

F. Coscia[1], *M. Eckert*[2], *M. Mann*[1], *E. Lengyel*[2]

[1] *Max Planck Institute of Biochemistry, Proteomics and Signal Transduction, München, Germany*
[2] *University of Chicago, Obstetrics and Gynecology, Chicago, USA*

Ovarian cancer (OvCa) is the deadliest gynecological cancer worldwide with a mortality rate of greater than 60% within 5 years. This poor outcome primarily result from diagnosis at a late-stage when the primary tumor has already disseminated throughout the peritoneal cavity. Understanding the complex changes that occur during disease progression in the tumor and the surrounding microenvironment is consequently of enormous clinical value and may help to identify novel treatment modalities. Here, we present a state-of-the-art discovery proteomics based approach applied to formalin-fixed, paraffin embedded (FFPE) biobank samples from 11 high-grade serous ovarian cancer patients. Our optimized workflow enabled highly sensitive quantification of a total of 7,500 proteins across pre-invasive, primary and metastatic tumor and stroma sites, obtained from only ~ 5,000 to 25,000 microdissected cells.

We found that tumor proteomes were remarkably stable across all analyzed progression sites from the same patients and no conserved metastatic protein changes were identified. In the tumor microenvironment, in contrast, we identified a novel stromal protein signature of OvCa metastasis to the omentum, the primary site of OvCa metastasis. Follow-up experiments not only revealed functional biological roles for components of the protein signature, but also linked expression of the stromal signature to differential patient outcome.

NO CONFLICT OF INTEREST

138 The down regulation of PTENP1 is associated with poor response to FOLFOX in colorectal cancer patients

B. Tunca[1], S. Aksoy[1], O. Kanat[2], A. Deligonul[2], E. Ozturk[3], T. Yilmazlar[3], N. Ugras[4], O. Yerci[4], U. Egeli[1], G. Cecener[1]

[1] Uludag University, Medical Biology, Bursa, Turkey
[2] Uludag University, Medical Oncology, Bursa, Turkey
[3] Uludag University, General Surgery, Bursa, Turkey
[4] Uludag University, Pathology, Bursa, Turkey

BACKGROUND: Colorectal cancer (CRC) is a heterogeneous disease in conditions of clinical behavior and response to therapy. Therefore, there is a need to develop beter classifiers to differentiate these cases to decide who would benefit from therapy. Long non coding RNA (LncRNA) PTENP1 plays key role as a tumor suppressor in several cancer cells. However, its expression and biological roles in CRC have not yet been investigated. We aim to investigate the prognostic role of lncRNA PTENP1 in patients received adjuvant FOLFOX for stage III CRC patients.

MATERIAL AND METHOD: Between 2006 and 2013, 75 patients (43 male, 32 female) underwent surgery and received adjuvant FOLFOX chemotherapy. After surgery, these patients received adjuvant chemotherapy with oxaliplatin plus infusional 5-FU and leucovorin (FOLFOX) at Uludag University. The presence of recurrence and short Disease Free Survival (DFS) were assessed to determine drug resistance after chemotherapy. PTENP1 expression profile was determined using Real-Time PCR in tumors and normal tissues. Relationship between data, patients clinopathological parameters and disease free and overall survival were analysed by using SPSS and web-based Sabiosciences PCR-Data Analysis progromme.

RESULTS AND DISCUSSION: The PTENP1 relative expression was 23 fold lower in tumor tissues compared with normal tissues (P = 0.0357). The relationship between each histopathological feature and PTENP1 expression status of the tumors in cases was evaluated using a Binary Logistic Regression Model. Significant difference were identified between low PTENP1 expression and presence of mucinous carcinoma (P = 0.0472). Furtheremore, lower expression of PTENP1 was associated with elevated the recurrence rate and shortening of DFS (P < 0.001, P = 0.0467; respectively).

CONCLUSION: In conclusion, in the present study has identified that PTENP1 was correlated with poor prognosis. This study indicated that down regulation of PTENP1 might serve as a potential indicator for FOLFOX resistance in CRC patients.

NO CONFLICT OF INTEREST

139 Characterization of HPV16 expression profile in cervical and in oropharyngeal squamous cell carcinoma

A. Cerasuolo[1], C. Annunziata[1], N. Starita[1], L. Buonaguro[1], S. Greggi[2], F. Ionna[3], S. Losito[4], G. Botti[4], F.M. Buonaguro[1], M.L. Tornesello[1]

[1]Istituto Nazionale Tumori IRCCS Fond Pascale, Research Department, Napoli, Italy
[2]Istituto Nazionale Tumori IRCCS Fond Pascale, Gynecology Department, Napoli, Italy
[3]Istituto Nazionale Tumori IRCCS Fond Pascale, Head and Neck Surgery Department, Napoli, Italy
[4]Istituto Nazionale Tumori IRCCS Fond Pascale, Pathology Department, Napoli, Italy

BACKGROUND: Human papillomavirus type 16 (HPV16) is the major cause of cervical cancer and of a fraction of oropharyngeal carcinoma. Few studies focused on similarities and differences in the HPV16-related transformation mechanism among the two types of tumors.

MATERIAL AND METHOD: HPV16 viral load and early (E2/E4, E5, E6, E6*I, E6*II, E7) as well as late (L1 and L2) gene expression were analyzed in 28 cervical squamous cell carcinoma (SCC) and 10 oropharyngeal SCC, along with pair-matched non-tumor tissues, as well as in four oropharynx dysplastic tissues and 10 cervical intraepithelial neoplasia (CIN) biopsies by real time PCR and nucleotide Sanger sequencing.

RESULTS AND DISCUSSION: Viral load was found higher in cervical SCC (<1 to 694 copies/cell) and CIN (<1 to 43 copies/cell) compared to oropharyngeal SCC (<1 to 4 copies/cell). HPV16 E2/E4 and E5 as well as L1 and L2 mRNA levels were low in cervical SCC and CIN and undetectable in oropharynx cases. The HPV16 E6 and E7 mRNAs were consistently high in cervical SCC and low in oropharyngeal SCC. The analysis of HPV16 E6 mRNA expression pattern showed statistically significant higher levels of E6*I versus E6*II isoform in cervical SCC (p = 0.002) and a slightly higher expression of E6*I versus E6*II in oropharyngeal cases.

CONCLUSION: Our result indicate that the HPV16 E5, E6, E6*I, E6*II and E7 mRNA levels are more abundant in cervical SCC compared to oropharyngeal SCC suggesting different carcinogenic mechanisms in the two types of HPV-related cancers.

NO CONFLICT OF INTEREST

140 Misreading Serine tRNAs contribute to tumor growth in vivo

M. Santos[1,2,3], P.M. Pereira[1,2,3], A.S. Varanda[1,2,3], J. Carvalho[2,3], N. Mendes[2,3], P. Oliveira[2,3], M. Teixeira-Pinto[2,3], F. Carneiro[2,3,4], C. Oliveira[2,3,4], M. Santos[1]

[1] University of Aveiro, Departmentment of Medical Sciences, Aveiro, Portugal
[2] Institute of Molecular Pathology and Immunology, Expression Regulation in Cancer, Porto, Portugal
[3] Instituto de Investigação e Inovação em Saúde i3 S, Expression Regulation in Cancer, Porto, Portugal
[4] University of Porto, Deptarment of Pathology and Oncology- Faculty of Medicine, Porto, Portugal

BACKGROUND: Upregulation of protein synthesis, deregulation of tRNA expression and amino acid starvation are common features of cancer. The occurrence of these events raises the hypothesis that translational fidelity is compromised in tumors (1,2). However, the relevance of tRNA misreading in cancer is still unknown. To clarify the role of tRNA misreading in cancer development, we expressed misreading tRNAs in a near-normal cell line and studied UPR and cancer-associated signaling pathways.

MATERIALS AND METHODS: NIH3T3 cell line was stably transfected with pIRES2-DsRED plasmids containing the tRNASer(WT), tRNAs that misincorporate Serine(Ser) at Alanine(Ala)-GCU or Leucine(Leu)-CUU sites and also an empty plasmid. Cell lines were injected in mice and their tumorigenic potential was evaluated. tRNA expression both in cell lines and in tumors was determined by SNaPshot sequencing. Alterations in UPR and cancer-related pathways were accessed by western blot.

RESULTS AND DISCUSSION: We report an unexpected role for misreading tRNAs in tumor growth. Our data show that expression of misreading tRNAs produce tumors with similar growth rate to K-ras-induced tumors. Remarkably, expression of misreading tRNAs increases in vivo, suggesting advantageous features of this phenotype. Our result also showed that Akt and UPR pathways are activated in a microenvironment-dependent manner. Accumulating evidence has demonstrated that UPR activation and decrease in translation fidelity are required for cancer cells to maintain malignancy and acquire therapy resistance, suggesting that compromising translation fidelity may select adaptative mutations.

CONCLUSIONS: Our result support the hypothesis that translation errors at the ribosome accelerate tumor growth by activating key pathways, and that their role in tumorigenesis is worth to explore further.

NO CONFLICT OF INTEREST

141 NR4A3, a novel target of p53, promotes apoptosis

O. Fedorova[1], A. Petukhov[1], A. Daks[1], O. Shuvalov[1], E. Vasileva[1], N. Barlev[1]

[1] Institute of Cytology RAS, Laboratory of regulation of gene expression, St-Petersburg, Russian Federation

Orphan nuclear receptors are transcription factors that modulate downstream gene expression and operate in ligand-independent manner. There is a highly evolutionary conserved group of transcription factors among these orphan receptors, which belong to the NR4A family of receptors and includes: NR4A1, NR4A2 and NR4A3. NR4A receptors are immediate- or early-response genes, which are rapidly induced by a pleiotropy of environmental cues. Early functional studies pointed to a critical role of NR4A receptors in regulation of differentiation, proliferation and apoptosis. Recently, they were shown to regulate glucose and lipid homeostasis, adipogenesis, inflammation, and vascular remodeling. We have demonstrated that expression of the orphan nuclear receptor, NR4A3 is controlled by one of the critical tumor suppressors, p53. By using ChIP and luciferase assays we have revealed that NR4A3 was a direct transcriptional target of p53. RT-PCR experiments in cancer cell lines with modulated levels of p53 expression showed that NR4A3 transcription was stimulated by various genotoxic stresses in p53-dependent manner. Progression of tumors depends on the balance between proliferation and cell death of cancer cells.

In this respect, we have shown that augmented levels of NR4A3 suppressed proliferation and survival of cancer cells. We demonstrated that expression of NR4A3 led to cleavage of PARP-1 and increased staining with Annexin-V in H1299, MDA-231 and Hek-293 cell lines, suggesting its involvement in apoptosis. In summary, the present study shows that p53 exerts its functions as a tumor suppressor in part via activation of NR4A3 expression, which result increased apoptosis of various cancer cell lines.

This work was supported by Russian Scientific Foundation (grant 14-50-00068) and RFBR (research projects No. 16-34-00869 and No. 16-34-60228 mol_a_dk).

NO CONFLICT OF INTEREST

POSTER SESSION: CELL AND TUMOUR BIOLOGY I

143 Targeting the Src/JAK/STAT3 signalling pathway: A novel and promising therapeutic strategy for pancreatic cancer

A. Morgan[1], A. Steinmann[1], D. Froio[1], R. Stark[1], A. Drury[1], K. Murphy[1], J. Samra[2], A. Gill[1], M. Pajic[1]

[1] Garvan Institute of Medical Research, The Kinghorn Cancer Centre, Sydney, Australia
[2] Royal North Shore Hospital, Department of Surgery, Sydney, Australia

BACKGROUND: Pancreatic cancer (PC) has a 5-year survival of only 6%, and persists as the 4th most common cause of cancer-related death in Western societies. A more tailored treatment approach may be beneficial as the current standard-of-care cytotoxic agent Gemcitabine, in combination with nanoparticle albumin-bound Paclitaxel, or FOLFIRINOX offers only a modest increase in overall patient survival in unselected populations. Recent large-scale genomic studies have revealed that the Src/JAK/STAT3 signalling pathway is deregulated in up to 35% of PC, and is a novel and potentially targetable subtype that has yet to be examined. Consequently, we hypothesised that targeting pancreatic tumours with activated JAK/STAT3 signalling with selective JAK1/JAK2 or JAK3 inhibitors (Ruxolitinib, Tofacitinib), and an Src inhibitor (Dasatinib) represents a promising novel therapeutic strategy for this disease.

MATERIALS AND METHODS: We utilised well-annotated patient-derived cell line models (PDCLs) isolated from sequenced pancreatic cancer patients (International Cancer Genome Initiative), along with cell lines generated from KPC (Pdx1-Cre; KrasLSL.G12D/+; p53R172H/+) mice, an aggressive, metastatic model of PC. Using these pre-clinical models we assessed the in vitro efficacy of therapeutic strategies

involving Src/JAK/STAT3 inhibition, using cell proliferation assays and 2D drug synergy screens. Effects on invasion were determined using 3D organotypic assays, which recapitulate the interaction between cancer cells, stromal cells and the extra-cellular matrix (ECM). ECM integrity post-treatment was assessed using second-harmonic generation (SHG) imaging and picrosirius staining.

RESULTS: We show that selected JAK inhibitors and Dasatinib inhibit cell proliferation in candidate PDCLs and KPC lines, characterised by activated Src/JAK/STAT3 signalling, with combination therapy being synergistic in the majority of these cell-lines. Cell invasion was significantly inhibited in organotypic matrices, and Src/JAK/STAT3 inhibition decreased collagen contractility, and reduced fibrillar collagen coverage, suggesting that these compounds may also modulate ECM integrity.

CONCLUSION: Our findings demonstrate the potential for tailored therapeutic strategies involving Src/JAK/STAT3 inhibition in pancreatic cancer, and suggest that the efficacy of this therapy may be the result of targeting both tumour cells and the tumour microenvironment.

NO CONFLICT OF INTEREST

144 RASSF6 Deters sorafenib resistance through F-actin rearragement and subsequent activation of JNK signaling pathway

Y.Y. Liang[1], *X.T. Lin*[1], *M. Zhi-Wen*[2]

[1] *cancer center of Guangzhou medical university, oncology, Guangzhou, China*
[2] *cancer center of Guangzhou medical university, Department of Radiation, Guangzhou, China*

BACKGROUND: Although the use of sorafenib increase the survival rate of Clear-cell renal cell carcinoma (ccRCC) patients, there is also a proportion of patients who exhibit a poor response to sorafenib treatment. It is therefore crucial to elucidate the mechanisms underlying sorafenib resistance and find representative biomarkers for sorafenib treatment response in ccRCC patients. Our previous findings found out RASSF6 functions as tumor suppressor in the inhibition of survival signals in development of ccRCC.

MATERIAL AND METHODS: Biopsies from 50 ccRCC patients (18 patients were resistant to sorafenib therapy and the others were sensitive) were analyzed for RASSF6 expression using real-time PCR. The growth of cells with stable over-expression of RASSF6 was determined by counting under microscope. Cell cycle and apoptosis were analyzed by flow cytometry .Changes of F-actin after treatting with sorafenib were observed by Immunofluorescence staining and laser-scanning confocal microscopy. Levels of cyclins, apoptosis-associated protein and phosphorylated JNK were detected by western immunoblotting.

RESULTS:RASSF6 is downregulated in intrinsically sorafenib-resistant ccRCC patient. Low levels of RASSF6 correlated with poor responses to sorafenib therapy in ccRCC patients. In vitro studies, over-expression of RASSF6 sensitized resistant ccRCC cells to sorafenib, whereas the silencing of RASSF6 conferred sorafenib resistance to responsive ccRCC cells. Mechanistically, RASSF6 triggers G1 cell cycle arrest and promote apoptosis upon exposure to sorafenib. Moreover, RASSF6 disrupted F-actin arrangement and subsequently, leading to the activation of JNK pathway and the development of sorafenib resistance. A JNK inhibitor restored the response to sorafenib treatment.

CONCLUSIONS: These observations indicate that RASSF6 deters sorafenib treatment response through cytoskeleton rearrangement and JNK activation. It suggests RASSF6 may serve as not only a predictive biomarker for sorafenib treatment but also as a therapeutic target to enhance response to sorafenib in ccRCC patients.

NO CONFLICT OF INTEREST

146 MiR-182, a novel target involved in sulindac anticancer activity in colon cancer

H. Zhao[1], *B. Yi*[1], *Y. Xi*[1]

[1] *LSUHSC, Genetics, New Orleans, USA*

BACKGROUND: Nonsteroidal anti-inflammatory drugs (NSAIDs) display promising antineoplastic activity in many human solid tumors including colorectal cancer. Previous studies reported that sulindac sulfide (SS) can inhibit the growth of tumor cells through cyclooxygenase-2 (COX-2) dependent or independent pathways. Obviously, COX-2 independent pathway involves a low toxic property to support the clinical potential for using sulindac as a chemoprevention drug. However, the molecular mechanisms responsible for COX-2 independent pathway have not been completely elucidated.

MATERIAL AND METHODS: In this project, we employed two human colon cancer cell lines, referred to HCT116 and HT29. HCT116 cells are characterized for low COX-2 expression, while HT29 cells show relatively high COX-2 expression. result We found that SS could unbiasedly inhibit the growth of HCT116 and HT29 cells by arresting cells in G1/G2 phases. CyclinG2 was found to be upregulated in response to SS treatment. FOXO3a has been reported to regulate CyclinG2 expression at the transcriptional level. Our result demonstrate that SS could also upregulate FOXO3a. By using the loss-of-function strategy, we found that SS could not efficiently upregulate CyclinG2 and lead to cell cycle arrest in cells with FOXO3a knockdown. In addition, we studied the mechanism that could be involved in SS regulation of FOXO3a. Our previous studies reported that a panel of miRNAs could be altered by SS treatment in both colon and breast cancer cells. In the downregulated miRNA list, we found that miR-182 could potentially target FOXO3a. By using luciferase assay, we validated the direct regulation of miR-182 on the expression of FOXO3a. When miR-182 was downregulated, SS could neither efficiently upregulate the expression of FOXO3a nor inhibit cell growth as it did in control cells. We gained highly consistent result in HT29 and HT29 cells with COX-2 knockdown.

CONCLUSIONS: Our study demonstrates a pathway consisting of miR-182/ FOXO3a/ CyclinG2 as a novel mechanism responsible for SS anticancer activity in colon cancer, which also accounts for a COX-2 independent pathway.

NO CONFLICT OF INTEREST

147 The anti-proliferative effect of Ib-7, an Ibrutinib derivative, on EGFR wild-type and T790M bearing human lung cancer cells

B. Zhang[1], *L. Wang*[1], *N. Lin*[1]

[1] *Hangzhou First People's Hospital- Nanjing Medical University, Hangzhou Translational Medicine Research Center, Hangzhou, China*

BACKGROUND: Lung cancer is one of the most malignant cancer that accounts for over millions of occurrence and death every year worldwide, and it is known to obtain heterogeneous genetic phenotypes that requires specific treatment strategies. Although targeted therapeutic medicines against lung cancer harboring epidermal growth factor receptor (EGFR) sensitive mutation have been developed rapidly, obstacles remain in obtaining successful compounds curing EGFR wild-type or T790M bearing lung cancer. In this study, we aim to explore the anti-proliferative effect of Ib-7, which is a derivative of Ibrutinib, on both EGFR wild-type and T790M bearing human lung cancer cells.

MATERIALS AND METHODS: Kinase assay was applied to analyze the kinase inhibitory effect of Ibrutinib and Ib-7. Stable isotope labeling with amino acids in cell culture (SILAC) was used to analyze the protein expression and changes in phospho-proteins. CCK-8 was used to determine the cytotoxicity of Ibrutinib and Ib-7 on PC-9, PC-9/GR, H1975, A549 and H460 cells. Flow cytometry was used to determine apoptosis. Confocal microscopy was used observe the morphology and cell nuclear. Western blot was used to detect protein expression.

RESULTS: Ib-7 displayed distinguished kinase inhibitory activity comparing with the parental compound Ibrutinib. Ib-7 showed limited effect on EGFR kinase, while Ibrutinib was reported to have strong inhibitory activity on EGFR. By using SILAC to analyze the protein expression and phospho-protein level, we surprisingly found that Ib-7 might interfere with protein synthesis by down-regulating the phosphorylation of ribosomal proteins. It was then verified by western blot that Ib-7 substantially downregulate p-mTOR, p-LARP1 and p-S6, leading to delayed proliferation in EGFR wild-type and T790M bearing lung cancer cells. Noteworthy, in A549, H460 and H1975 cells, the half maximal inhibitory effect (IC50) after 48 h exposure to Ib-7 was 5 - 10 fold lower than that of Ibrutinib.

CONCLUSION: Ib-7 could potently inhibit the proliferation of EGFR wild-type and T790M bearing lung cancer cells, possibly through mTOR-LARP1-S6 pathway and thus interfering with protein synthesis. Therefore, Ib-7 was a promising compound with specific targets that showed potential applications in EGFR wild-type and T790M harboring lung cancer.

NO CONFLICT OF INTEREST

148 Transcription factor SPZ1 promotes TWIST-mediated epithelial–mesenchymal transition and oncogenesis in human liver cancer

L.T. Wang[1], *S.H. Hsu*[1], *S.N. Wang*[2]

[1] *Kaohsiung Medical University, Graduate Institute of Medicine, Kaohsiung, Taiwan*
[2] *Kaohsiung Medical University, Division of Hepatobiliary Surgery, Kaohsiung, Taiwan*

BACKGROUND: The epithelial–mesenchymal transition (EMT) is an important process in the progression of cancer. However, its occurrence and mechanism of regulation are not fully understood. We propose a regulatory pathway in which spermatogenic leucine zipper 1 (SPZ1) promotes EMT through its transactivating ability in increasing TWIST1 expression.

MATERIAL AND METHODS: We compared the expression of SPZ1 and TWIST1 in specimens of hepatocarcinoma cells (HCCs) and non-HCCs.

RESULTS: Expression of SPZ1 exhibited a tumor-specific expression pattern and a high correlation with patient's survival time, tumor size, tumor number, and progression stage. Moreover, forced expression and knockdown of SPZ1 in hepatoma cells showed that SPZ1 was able to regulate the cellular proliferation, invasion, and tumorigenic activity in a TWIST1-dependent manner in vitro and in vivo.

CONCLUSIONS: These data demonstrate that a newly dscribed molecule, SPZ1 transactivates TWIST1 promoters, and that this SPZ1-TWIST axis mediates EMT signaling and exerts significant regulatory effects on tumor oncogenesis.

NO CONFLICT OF INTEREST

149 SNORD-X in Glioblastoma: Regulation and functional analysis

S. Gupta[1], P. Jha[1], C. Sarkar[2], R. Kulshreshtha[1]

[1] Indian Institute of Technology Delhi, Department of Biochemical Engineering and Biotechnology, New Delhi, India
[2] All India Institute of Medical Sciences, Department of Pathology, New Delhi, India

INTRODUCTION: Glioblastoma Multiforme (GBM) represents approximately 15% of all primary brain tumor and has been considered as one of the deadliest type of cancer with very poor prognosis. Thus, there is an urgent need to identify novel targets for GBM treatment. Recently, non-coding RNAs (ncRNAs) such as miRNAs and lncRNAs have emerged as promising biomarkers and as novel targets for therapy. There are recent evidences that small nucleolar RNAs (snoRNAs), a subtype of ncRNAs might also play a key role in various cancers. However, the role of snoRNAs in GBM remains little studied. Notably, we found downregulation of multiple members of SNORD-X cluster in GBM patients. Our present study provides a novel insight on the regulation and functional role of SNORD-X in GBM.

MATERIAL AND METHOD: An overexpression construct of SNORD-X was generated and used further for functional analysis in GBM cell lines (U87MG and A172). The role of SNORD-X in cell proliferation and tumor formation was studied using MTT, colony formation and soft agar assays. Effect of SNORD-X overexpression on apoptosis was confirmed using caspase 3/7 assay. Target analysis of SNORD-X was done using a combination of RNA hybrid software along with qRT-PCR and wild-type/mutated 3'UTR luciferase analysis. The effect of promoter methylation on SNORD-X downregulation was done using CpG island finder and 5'-Azacytidine treatment.

RESULTS AND DISCUSSION: We found SNORD-X downregulation in GBM tumor samples as compared to normal brain. SNORD-X overexpression brought about a significant upregulation in the proliferation capability of GBM cells as shown by MTT, colony formation and soft agar assay. SNORD-X was also found to reduce the apoptosis in a caspase dependent manner. We found EGR1, BRCA1 and DCUN1D3 to be direct targets of SNORD-X. 5'-Azacytidine treatment brought about induction in SNORD-X levels suggesting the role of methylation in downregulation of SNORD-X in GBM.

CONCLUSION: We found that SNORD-X is downregulated in GBM patients. SNORD-X was found to play oncogenic role in GBM by promoting proliferation and inhibition of apoptosis. SNORD-X was found to be epigenetically regulated by promoter methylation. Overall, our data shows for the first time correlation and functional analysis of SNORD-X in GBM.

NO CONFLICT OF INTEREST

150 P53-miR-X-SOX4 regulatory loop affects apoptosis in breast cancer

S. Sharma[1], N. Nagpal[2], P. Gosh[3], R. Kulshreshtha[1]

[1] Indian Institute of Technology, Department of biochemical engineering and biotechnology, New Delhi, India
[2] Boston Children's Hospital- Boston- MA- USA, Division of Hematology/Oncology, Boston, USA
[3] University of Delhi South Campus, Department of Biochemistry, New Delhi, India

INTRODUCTION: Breast cancer is the most common malignancy in woman. Despite tremendous advances, breast cancer treatment still remains a challenge due to development of resistance to various therapies. Therefore, there is a need to understand breast tumor biology in entirety and to identify novel mediators involved in the pathogenesis. miRNAs have been shown to play a major role in breast cancer pathogenesis and response to treatment. The work here identifies a miRNA, miR-X that functions as an anti-apoptotic miRNA in breast cancer.

MATERIAL AND METHOD: In silico analysis of miR-X upstream region bearing p53 binding sites was done using Promo 3.0 software and its direct binding was confirmed by ChIP assay. miR-X and p53 expression levels were checked by qRT-PCR. miR-X target transcripts were identified using a combination of expression profiling, qRT-PCR and 3'UTR luciferase assays and protein level of the selected target was confirmed by western blotting. miR-X mediated apoptotic effects were studied by FACS analysis, Caspase-Glo 3/7 assay and DAPI staining in two breast cancer cell lines (MCF7, ZR-75). The IC50 value of anti-miR-X alone or in combination with anti-cancer drug doxorubicin was determined by MTT cell viability assay.

RESULTS AND DISCUSSION: Here, we provide the first study demonstrating direct regulation of an oncogenic miRNA, miR-X, by p53 and existence of a regulatory feedback loop. p53 brings about direct downregulation of miR-X in breast cancer. miR-X overexpression brought about inhibition of apoptosis in breast cancer while miR-X downregulation showed the opposite. We further unveiled that SOX4 was a direct target of miR-X, which has been reported to be closely associated with p53 regulation and apoptosis. SOX4 overexpression was shown to increase p53 protein levels in MCF7 cells. miR-X overexpression brought about downregulation of SOX4 and thus p53 levels suggesting existence of a regulatory feedback loop. Interestingly, anti-miR-X treatment significantly decreased IC50 of doxorubicin drug and thus sensitized breast cancer cells to doxorubicin treatment by promoting apoptosis.

CONCLUSION: Overall, the work highlights the importance of p53-miR-X-SOX4 axis in regulation of apoptosis and drug resistance in breast cancer and offers a preclinical proof-of-concept for use of anti-miR-X and doxorubicin combination as a rational approach to pursue for better breast cancer treatment.

NO CONFLICT OF INTEREST

151 Inducing a mesenchymal-to-epithelial transition for the differentiation therapy of aggressive breast carcinomas

D. Pattabiraman[1], J. Ostendorp[1], R. Weinberg[1]

[1] Whitehead Institute for Biomedical Research, Weinberg Lab, Cambridge, USA

BACKGROUND: Cancer stem cells (CSCs) have emerged in recent years as important targets for cancer therapy owing to their elevated resistance to conventional chemotherapy as well as to their enhanced tumor-initiating ability. The epithelial-to-mesenchymal transition (EMT) confers mesenchymal traits on both normal and neoplastic epithelial cells enabling carcinoma cells to acquire malignancy-associated traits and stem-like properties. This association between the EMT program and the CSC state has presented an attractive opportunity for drug development. Here we employ a therapeutic approach that involves the differentiation of CSCs to their non-stem cell counterparts through the induction of a mesenchymal-to-epithelial transition (MET).

METHODS: We have used a combination of breast cancer cell lines, mouse models and 3D hydrogel-based organoid models of mammary epithelial development and tumorigenesis to address our hypothesis.

RESULTS: We have previously published that increases in intracellular levels of the second messenger 3'-5'-cyclic adenosine monophosphate (cAMP) and the subsequent activation of protein kinase A (PKA) induces an MET in mesenchymal human mammary epithelial cells and their neoplastic derivatives. The MET-induced differentiation is accompanied by a loss of stem-like properties and tumor-initiating ability, rendering the cells more sensitive to treatment with chemotherapeutic drugs such as doxorubicin and paclitaxel. PKA functions by activating PHF2, a histone H3K9 demethylase, which relieves H3K9me2/3-mediated repression of epithelial genes. To identify targets that are amenable to therapeutic utility in breast cancers, we have identified key G-protein coupled receptors (GPCRs) that are involved in the regulation of epithelial vs. mesenchymal state in mammary epithelial and breast cancer cells. Treatment with agonists of GPCRs that are Gs-coupled or antagonists of GPCRs that are Gi-coupled play key roles in modulation of cell state indicating that they could be active players during the process of tumor progression and metastasis.

CONCLUSIONS: This work presents an attractive strategy to induce an MET through the modulation of GPCR activity that induces activation of PKA, downstream epigenomic reprogramming and acquisition of an epithelial state. Utilizing the induction of an MET as a form of differentiation therapy may improve the response of advanced carcinomas to chemotherapy as well as prevent their progression and metastasis.

NO CONFLICT OF INTEREST

152 3'UTR polymorphisms of carbonic anhydrase IX determine the miR-34a targeting efficiency and prognosis of hepatocellular carcinoma

K.T. Hua[1]

[1] National Taiwan University- College of Medicine, institute of Toxicology, Taipei City, Taiwan

BACKGROUND: Carbonic anhydrase IX (CA9) was recently identified as a prognostic factor in hepatocellular carcinoma (HCC). However, little is known about the impacts of CA9 genetic variants and regulation on cancer progression. We aimed at evaluating the importance of CA9 polymorphism in HCC progression.

MATERIAL AND METHODS: Four single-nucleotide polymorphisms (SNPs) of CA9 in 312 healthy controls and 312 HCC patients were analyzed, and then evaluated their prognostic values. Among these selected SNPs, a CA9 rs1048638 genotype was further functionally analyzed using in vitro and in vivo assays, and its correlations with CA9 levels were also evaluated.

RESULTS: Our study found an SNP located at the 3'untranslated region (UTR) of CA9, rs1048638, which was significantly correlated with a higher risk of HCC. The CA genotype of this locus was also significantly associated with the tumor size, late stage, vascular invasion, and overall and disease-free survival of HCC patients. Moreover, we identified rs1048638 located in the target region of a previously undiscovered CA9-targeting micro (mi)RNA, miR-34a. The C-to-A substitution could increase the minimum free energy of miR-34a-3'UTR hybridization and thus lose the negative regulation of CA9 expression by miR-34a, resulting in an obvious increase in both in vitro cell migration and invasion and in vivo tumor growth and metastasis of HCC cells ($p < 0.05$).

CONCLUSION: The rs1048638 genetic variations of the CA9 3'UTR play important roles in regulating CA9 expression and cancer progression, which is a novel determinant and target for HCC metastasis and prognosis.

NO CONFLICT OF INTEREST

153 Overcoming treatment resistance in cisplatin resistant ovarian carcinoma cells with miRNA-147

M. Kleemann[1], J. Bereuther[1], S. Fischer[2], K. Marquart[1], S. Hänle[1], K. Unger[3], V. Jendrossek[4], C.U. Riedel[5], R. Handrick[1], K. Otte[1]

[1] Hochschule Biberach, Institut of Applied Biotechnology, Biberach, Germany
[2] Boehringer Ingelheim Pharma GmbH and Co.KG, BP Process Development Germany, Biberach, Germany
[3] Helmholtz Zentrum München, Research Unit Radiation Cytogenetics, München, Germany
[4] University of Duisburg-Essen, Institute of Cell Biology Cancer Research, Essen, Germany
[5] University of Ulm, Faculty of Medicine, Ulm, Germany

Ovarian cancer is a common human cancer with a high risk of death but also with a 70% 5-year survival rate of stage II patients. Novel specific biomarkers for ovarian cancer patients are necessary as the early detection of ovarian cancer is difficult. In addition, new therapeutic approaches are required due to resistances to current standard therapies with carboplatin or paclitaxel. The use of microRNAs (miRNA) as biomarker for ovarian cancer and the installation of de novo tumor suppressive function by using pro-apoptotic microRNAs might be a promising therapeutic approach. MiRNAs are conserved, small non-coding RNAs regulating gene expression by binding to their target mRNA which have been shown to regulate biological processes including apoptosis, the highly regulated form of cell death.

To extend the pool of potential miRNAs as biomarkers and anti-cancer agents, we transferred a high content miRNA screening for pro-apoptotic miRNAs to human cancer cell lines. Based on the high recovery rate of apoptosis inducing miRNAs in SKOV3 cells analyses were extended to multiple ovarian carcinoma cell lines with different genetic background: (SKOV3^{p53null}, OVCAR3^{p53R248Q}, TOV21G, TOV112D^{p53R175H}, A2780, A2780-cis^{p53K351N}). After transfection of miR-147 the apoptosis rate increased strongly in cisplatin resistant cells. In combination treatment with carboplatin the apoptotic effect increased after transfection of miR-147 in the cisplatin resistant A2780 cells. Furthermore, miR-1912-5p, miR-96-5p and miR-3073a showed significant apoptosis induction in the various ovarian cancer cell lines alone and in combined therapy with carboplatin. Western Blot analysis revealed an enhanced expression of the pro-apoptotic proteins Bak1 and Bax and a decrease in Bcl2 and Bcl-xL. We characterized the miRNAs in regard to their endogenous expression and detected an enhanced expression after apoptosis induction by chemotherapeutic drugs. Finally, analysis of The Cancer Genome Atlas data showed a positive influence on the median survival of ovarian cancer patients (> 69 years) expressing high rates of miR-147.

Our data indicate a potential benefit of tissue specific pro-apoptotic miRNAs in regard to ovarian carcinoma. Furthermore the study identified novel pro-apoptotic miRNAs with their potential use as novel therapeutic entities or prognostic biomarkers for ovarian cancer.

NO CONFLICT OF INTEREST

154 Leptin modified exosomes, from ovarian cancer cells, promote a pro-tumorigenic microenvironment

L. Abarzua-Catalan[1], S. Kato[1], V. Torres-Estay[2], M.F. Liberona[1], A.S. Godoy[2,3], M.A. Cuello[1]

[1] Pontificia Universidad Católica de Chile, Department of Obstetrics and Gynaecology, Santiago de Chile, Chile
[2] Pontificia Universidad Católica de Chile, Department of Physiology, Santiago de Chile, Chile
[3] Roswell Park Cancer Institute, Department of Urology, Buffalo, USA

BACKGROUND: Higher leptin levels have been correlated with aggressiveness and poorer outcomes among obese ovarian cancer patients. Previous studies have been focused mostly on understanding how leptin influences cancer cell behavior, but its effects on the surrounding microenvironment are not entirely clear. However, exosome-mediated cell-to-cell communication might play a critical role on these effects. We hypothesized that leptin-modified-exosomes, from ovarian cancer cells, could affect two components of the tumor microenvironment: the macrophages and the endothelial cells.

MATERIAL AND METHODS: HEY/SKOV3 ovarian cancer cells were cultured under control/leptin conditions for 24h. Upon exposure, exosomes were isolated from culture media by using a commercial kit. Exosome concentration was quantified using the Bradford Method. THP-1-derived-macrophages were treated with 100ug/ml of exosomes collected from each condition (24h and 48h). M1/M2 macrophage polarization was assessed by measuring INOS(M1) and Arginase I(M2) levels using immunoblotting. Supernatant media from treated macrophage cultures was collected and its effects on invasiveness of ovarian cancer cells was assessed using the Matrigel Boyden chamber assay. To study angiogenesis in vitro, a tube formation assay was performed using female HUVEC endothelial cells exposed to 50ug/ml of exosomes (12h) collected from each condition.

RESULTS: The exposure of THP-1-derived-macrophages to exosomes isolated from leptin treated cancer cells resulted in an increase of Arginase I and a decrease of INOS expression levels, suggesting M2 polarization. A crossover incubation using collected media from exosome-treated macrophages, resulted in an increase in invasiveness of SKOV3 and HEY cells. Lastly, incubation of HUVEC cells with exosomes collected from leptin-treated cancer cells increased pseudo-capillary structure formation compared to control conditions.

CONCLUSIONS: Our findings support a leptin effect on exosome-mediated communication among ovarian cancer cells and neighbor cells such as macrophages and endothelial cells, which may contribute to promote a pro-tumorigenic microenvironment. (Grants: FONDECYT 3140335, 1160800, 1161115)

NO CONFLICT OF INTEREST

155 Melatonin inhibits MMP-9 transactivation and renal cell carcinoma metastasis by suppressing Akt-MAPKs pathway and NF-κB DNA-binding activity

M.H. Chien[1], Y.W. Lin[2], W.J. Lee[3]

[1] Taipei Medical University, Graduate Institute of Clinical Medicine, Taipei, Taiwan
[2] Taipei Medical University, Graduate Institute of Clinical Medicine-, Taipei, Taiwan
[3] Taipei Medical University, Department of Urology- School of Medicine- College of Medicine, Taipei, Taiwan

BACKGROUND: Renal cell carcinoma (RCC) is the most lethal of all urological malignancies because of its potent metastasis potential. Melatonin exerts multiple tumor-suppressing activities through antiproliferative, proapoptotic, and anti-angiogenic actions and has been tested in clinical trials. However, the antimetastastic effect of melatonin and its underlying mechanism in RCC are unclear.

MATERIAL AND METHOD: Cell viability was examined by MTS assay, whereas cell motility was measured by migration and would healing assays. Assessment of mRNA levels, using reverse transcriptase polymerase chain reaction (PCR), and promoter assays confirmed the inhibitory effects of melatonin on matrix metalloproteinase-9 (MMP-9) expression in RCC cells.

RESULTS: In this study, we demonstrated that melatonin at the pharmacologic concentration (0.5-2 mM) considerably reduced the migration and invasion of RCC cells (Caki-1 and Achn). Furthermore, we found that melatonin suppressed metastasis of Caki-1 cells in spontaneous and experimental metastasis animal models. Mechanistic investigations revealed that melatonin transcriptionally inhibited MMP-9 by reducing p65- and p52-DNA-binding activities. Moreover, the Akt-mediated JNK1/2 and ERK1/2 signaling pathways were involved in melatonin-regulated MMP-9 transactivation and cell motility. Clinical samples revealed an inverse correlation between melatonin receptor 1A (MTNR1A) and MMP-9 expression in normal kidney and RCC tissues. In addition, a higher survival rate was found in MTNR1A (high) /MMP-9(low) patients than in MTNR1A (low) /MMP-9(high) patients.

CONCLUSION: Overall, our result provide new insights into the role of melatonin-induced molecular regulation in suppressing RCC metastasis and suggest that melatonin has potential therapeutic applications for metastastic RCC.

NO CONFLICT OF INTEREST

156 Using 3D spheroids to analyse the effects of novel estradiol analogues on breast cancer

T. Jurgens[1], A. Joubert[1], I. Van den Bout[1]

[1] University of Pretoria, Physiology, Pretoria, South Africa

BACKGROUND: Triple negative breast cancers (TNBC) form around 15% of all breast cancers. Typically, TNBC patients have a poorer prognosis due to their resistance to anti-hormone treatment. 3D cell culture methods have enabled researchers to generate an in vitro environment that closely mimics the in vivo environment of small tumours. The aim of this study was to investigate whether TNBC, specifically using the tumorigenic, non-metastatic BT-20 breast cancer cell line, grown in 3D could be used as a reliable study for drug discovery and testing. The system was tested using novel antimitotic estradiol derivatives as well as classic chemotherapeutics.

MATERIALS AND METHODS: The ability of compounds to induce cell death was compared in monolayers versus 3D spheroids, using BT-20 cells which form compact spheroids consistently yet are seldom used for 3D cell culture. The effects of the compounds were assessed in monolayer by crystal violet and in spheroids by measuring volume over time. Furthermore, we performed confocal microscopy to determine cell death using propidium iodide in the spheroids as well as phalloidan to assess any changes in the actin cytoskeleton.

RESULTS: EC50 data showed that several compounds induced some level of cell death within monolayers. High concentrations of the non-sulphamoylated compounds EE-15-one caused cytotoxicity, however this compound did not affect spheroid volume. In contrast, a sulphamoylated compound ESE-15-one induced modest inhibition in monolayers but were as effective at inhibiting cell growth in spheroids as colchicine, a classic chemotherapeutic.

CONCLUSIONS: Using the spheroid system, we have been able to determine that while EE-15-one is cytotoxic to cells in monolayers it is ineffective in killing cells when grown on 3D. On the other hand, ESE-15-one acts as an antimitotic drug both in monolayers and spheroids. ESE-15-one, a microtubule destabilising drug causes a breakdown of the actin cytoskeleton, which is not seen in taxol (e.g. paclitaxel) treated spheroids. The 3D spheroid system of the TNBC cell line BT-20 is proving to be useful in identifying drugs that may be effective in killing cancer cells in vivo as opposed to monolayers.

NO CONFLICT OF INTEREST

157 Molecular insights into metastatic breast cancer

G. Negro[1], R. Mader[1], S. Skvortsov[1], P. Lukas[1], I. Skvortsova[1]

[1] EXTRO-LAB, Therapeutic radiology and oncology, Innsbruck, Austria

INTRODUCTION: Even if the development of novel diagnostics and therapeutic approaches led to an efficient treatment of early breast cancer (BC), approximately 20-30% of patients develop distant metastasis. Moreover, these patients have a different treatment response that can be associated with specific molecular patterns of metastatic cells. Therefore, the investigation of the molecular properties

of cells with increased metastatic potential is necessary for understanding how to improve therapy response in BC patients with distant metastasis.

MATERIALS AND METHODS: Three carcinoma cell lines, MDA-MB-231, T47D and Au565, representing the most diffused molecular subtypes of breast cancer (triple negative, luminal A and HER2-positive BC) were used to obtain cells with increased migratory abilities through migratory assay with the Boyden chamber. To compare the different sensitivities of parental and migratory BC cells to ionizing radiation and chemical compounds, cell viability and clonogenic assays were used. FACS analysis to detect the presence of the tumor initiating cells (TICs) was performed, in combination with adhesion assay, scanning electron microscopy and Western Blot.

RESULTS: BC cells with increased migratory abilities demonstrated different sensitivities to ionizing radiation or chemical agents compared to their parental counterparts. All migratory cell lines were enriched for the content of TICs and showed enhanced adhesive capacities and activation of epithelial-to-mesenchymal transition. In parallel, BC migratory cells were characterized by affected cell cycle regulation that markedly contributed to their proliferation capacities and sensitivity to irradiation and chemotherapeutics.

CONCLUSION: BC cells with increased migratory abilities demonstrate their aggressive behaviour through dysregulation of cell cycle progression, changed membrane properties and activation of pro-survival mechanisms associated with affected therapy response. These observations seem, therefore, to simulate the different sensitivities to the applied treatment approaches in metastatic BC patients.

(This study was supported by FWF P29457 and Ingrid Shaker Nessmann cancer Research Foundation)

NO CONFLICT OF INTEREST

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158 Dysregulation of serum exosomes in patients with hepatitis B virus pre-S2 mutant-positive hepatocellular carcinoma

C.F. Teng[1], W.C. Shyu[2,3,4], L.B. Jeng[1,5]

[1] China Medical University Hospital, Organ Transplantation Center, Taichung City, Taiwan
[2] China Medical University, Graduate Institute of Biomedical Sciences, Taichung City, Taiwan
[3] China Medical University Hospital, Department of Neurology, Taichung City, Taiwan
[4] China Medical University Hospital, Translational Research Center, Taichung City, Taiwan
[5] China Medical University Hospital, Department of Surgery, Taichung City, Taiwan

INTRODUCTION: Chronic hepatitis B virus (HBV) infection is a major risk factor for hepatocellular carcinoma (HCC), the leading cause of cancer-related deaths worldwide. Pre-S2 mutant represents a HBV oncoprotein that can induce multiple signal pathways involved in proliferation, survival, genomic instability, and metabolism of hepatocytes, leading to HCC tumorigenesis. Plentiful evidences reveal that exosomes, which are small membranous vesicles secreted by most cell types, can promote the progression of HCC through enhancing malignant behaviors of HCC cells or modulating tumor microenvironment. However, to date the role of pre-S2 mutant in regulation of exosome biogenesis remains totally unknown.

MATERIAL AND METHOD: In this study, we collected serum samples from HBV-related HCC patients and divided the patients into pre-S2 mutant-positive or -negative groups by polymerase chain reaction-based genotyping. Serum exosomes were isolated, characterized, and subsequently quantified by enzyme-linked immunosorbent assay (ELISA) to clarify whether the amount of serum exosomes was changed in pre-S2 mutant-positive HCC patients.

RESULTS AND DISCUSSION: We analyzed 10 cases of HBV-related HCC serum samples, half of which were detected as pre-S2 mutant-positive. Serum exosomes were purified from each patients and confirmed by transmission electron microscopy for particle morphology and Western blotting for exosome markers (CD63, HSP70, CD9, and CD81) expression. The ELISA assay showed a two fold higher level of serum exosomes in the pre-S2 mutant-positive patients than the pre-S2 mutant-negative patients.

CONCLUSION: Our result suggests that elevated level of serum exosomes may serve as a biomarker for pre-S2 mutant-positive HCC patients and pre-S2 mutant may have a role in dysregulation of exosome biogenesis in HBV-related HCC patients.

NO CONFLICT OF INTEREST

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159 Amphiregulin contained in NSCLC-exosomes induces osteoclast differentiation through the activation of EGFR pathway

R. Alessandro[1,2], M. Pucci[2], M. Giallombardo[1], M.A. Di Bella[3], M.C. Santarpia[3], C. Rolfo[4], S. Taverna[1,2]

[1] University of Palermo, Biopathology and biomedical methodologies, Palermo, Italy
[2] National Research Council, Institute of Biomedicine and Molecular Immunology, Palermo, Italy
[3] University of Messina, Medical Oncology Unit- Department of Human Pathology, Messina, Italy
[4] Antwerp University, Center for Oncological Research CORE, Antwerp, Belgium

BACKGROUND: Non-small cell lung cancer (NSCLC) remains the leading cause of cancer-related deaths worldwide. Bone metastasis is the most frequent complication in NSCLC resulting in osteolytic lesions. The perfect balance between bone-resorbing osteoclasts and bone-forming osteoblasts activity is lost in bone metastasis, inducing osteoclastogenesis. In NSCLC, the epidermal growth factor receptor (EGFR) pathway is constitutively activated. EGFR binds Amphiregulin (AREG) that is overexpressed in several cancers such as colon, breast and lung. Its levels in plasma of NSCLC patients correlate with poor prognosis. AREG was recently found as a signaling molecule in exosomes derived from cancer cell lines. Exosomes have a key role in the cell-cell communication and they were indicated as important actors in metastatic niche preparation. In the present work, we hypothesize a role of AREG carried by exosomes derived from NSCLC cells and plasma of NSCLC patients, in osteoclast differentiation.

MATERIALS AND METHODS: Exosomes were collected, by CRL-2868 cells conditioned medium, by ultracentrifugation and characterized by Western Blotting (WB) and electron microscopy analysis. AREG expression was evaluated, by WB, in CRL-2868 cells and exosomes and in exosomes isolated from plasma of NSCLC patients. The osteoclasts morphology was assessed by confocal microscopy. EGFR phosphorylation was evaluated by WB. RANKL, MMP9 and TRAP mRNA expression were assessed by Real time PCR. RANKL and MMP9 secretion was evaluated by ELISA. result We observed that NSCLC cells release exosomes, containing AREG. Exosomal AREG induces EGFR pathway activation in pre-osteoclasts that in turn causes an increased expression of RANKL. RANKL is able to induce the expression of proteolytic enzymes, MMP9 and TRAP, well-known markers of osteoclastogenesis. The central role of AREG has been confirmed with gain and loss of function experiments, using recombinant AREG and AREG neutralizing antibodies. Knockdown-AREG exosomes do not induce osteoclast differentiation. Furthermore, exosomes released in plasma of NCSLC patients, contain AREG, and induce osteoclasts differentiation of human primary osteoclasts.

CONCLUSION: Exosomal AREG induces EGFR pathway activation that is able to induce RANKL expression that in turn increases the expression of MMP9 and TRAP, triggering a vicious cycle in osteolytic bone metastasis.

NO CONFLICT OF INTEREST

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160 Simvastatin impairs cell plasticity and invasive capabilities of ovarian cancer-initiating cells through regulation of E-cadherin/ß-catenin complex, Hippo/YAP pathway and small GTPase activity

S. Kato[1]

[1] Pontificia Universidad Catolica De Chile, Department of Obstetrics and Gynecology, Santiago, Chile

BACKGROUND: Ovarian cancer remains as the deadliest gynecological cancer worldwide. A key cell subset, the so-called cancer-initiating cell subpopulation (CIC), is responsible for drug resistance and later recurrence. This subpopulation exhibits stem cell-like properties and has experienced epithelial to mesenchymal transition (EMT). Cell plasticity of CIC is a major hallmark of cancer allowing metastasis, acquisition of drug resistance and cancer progression. Here, we studied whether statins could impair this characteristic affecting the metastatic potential of this subpopulation.

MATERIAL AND METHODS: Specific culture-selection conditions were used to isolate CICs from ovarian cancer cell lines (HEY and UCI101) and primary tissue cultures established from advanced high-grade serous ovarian cancers (HGS-ovC). To address the simvastatin effects (1uM, 24h) on cell plasticity and metastatic potential of CICs, stemness and EMT marker expression and different downstream signaling pathways involved in the metastatic process were analyzed either by immunoblotting or immunofluorescence. Additionally, in vitro metastatic assays were carried out using 3D-mesomimetic models.

RESULTS: Simvastatin induced a decrease in EMT and stemness markers expression (e.g. CD44). These changes correlate with decrease in activity of the RhoA, phospho-YAP translocation from nucleus to cytosol and cellular redistribution of E-cadherin and ß-catenin fragments. In terms of invasiveness, we observed a significant decrease in the number of invading cells through mesothelial/fibroblastic layers in the 3D-mesomimetic models. More importantly, mevalonate and geranyl-geranyl pyrophosphate supplementation almost completely abrogated all changes, supporting the role of mevalonate pathway in regulating cell plasticity of CICs and its metastatic potential.

CONCLUSIONS: Here we demonstrated that simvastatin, a lipophilic form of statins, is capable of modifying cell plasticity of CICs impairing its metastatic potential. This effects involved regulation of different signaling pathways including the activity of small GTPases RhoA, the signaling triggered by the uncoupling of the E-cadherin/ß-catenin complex and the activation of hippo/YAP pathway. Such a regulation will occur in a mevalonate dependent manner. From a clinical perspective, adding statins could effectively reduce cancer progression and positively impact survival of HGS-ovC patients. Fondecyt 1160800, 3140335, 3140426

NO CONFLICT OF INTEREST

161 Biomarkers in postoperative head and neck drainage fluids affect the response of cancer cells to irradiation

G. Salvatore[1], M. Mangoni[1], M. Sottili[1], T. Gualtieri[2], A. Javarone[2], M. Loi[1], P. Bonomo[1], I. Desideri[1], A. Deganello[2], L. Livi[1]

[1] University of Florence, Department of Experimental and Clinical Biomedical Sciences- Section of Radiation Oncology, florence, Italy
[2] University of Florence, Department of Surgery and Translational Medicine- Academic Clinic of Otolaryngology and Head and Neck surgery, florence, Italy

INTRODUCTION: In head and neck oncology, many efforts have been made in order to identify molecular biomarkers with potential prognostic and predictive value. The detection of significant features in the early perioperative setting could possibly lead to a refinement of current adjuvant treatments in high-risk patients. The aims of our study were to investigate the expression of molecular biomarkers in wound drainage fluids (WDF) following neck dissection in patients with head and neck squamous cell carcinoma (HNSCC), and to evaluate the effect of WDF microenvironment on the response of HNSCC cells to radiation.

MATERIALS AND METHODS: 19 consecutive surgically resected HNSCCs were studied. WDF were collected 1 day and 3 days after surgery from the cancer operative bed. EGF, CXCL-12, and osteopontin levels were measured in WDF using enzyme-linked immunosorbent assay (ELISA) kits. Clonogenic assays were performed using Cal27 HNSCC cells irradiated with 1 to 6 Gy, in presence or not of WDF and pretreated or not with cetuximab (4,4 mg/ml) 6 hours before irradiation.

RESULTS: The expression of biomarkers in WDF was correlated with pathological cancer features. We observed that CXCL-12 expression was significantly increased in WDF in presence of lymph node metastasis ($p<0,05$), extra capsular spread (ECS) ($p<0,05$), lymph node density ($p<0,05$), and close margins ($p<0,001$). Osteopontin expression was significantly increased in presence of ECS ($p<0,05$). On the contrary, TGF-β levels were significantly reduced in presence of ECS ($p<0,005$), close margins ($p<0,05$), and in patients treated for a cancer relapse ($p<0,001$).

Clonogenic assays demonstrated that WDF reduced the antineoplastic effect of irradiation on Cal27 cells: the treatment with WDF caused a significant increase in the surviving fraction of the cells compared to untreated control. Pretreatment with cetuximab decreased the surviving fraction of Cal27 to values comparable to control.

CONCLUSIONS: These preliminary data demonstrated that molecular biomarkers expressed in WDF favor residual tumor cell proliferation and affect response of HNSCC cells to radiation. Early treatment with biological therapies can increase cancer cell radiosensitivity and improve patient outcome.

NO CONFLICT OF INTEREST

162 The effect of adipose-derived stem cells from diabetic individuals on the characteristics of breast cancer cells

S.Y. Tsang[1], K.L. Yau[1]

[1] The Chinese University of Hong Kong, School of Life Sciences, Shatin- N.T., Hong Kong

BACKGROUND: Breast carcinoma is the most common invasive cancer in women, contributing to the highest percentage of cancer-related death. Previous studies have shown that diabetes and obesity are associated with breast cancer growth and progression. On the other hand, there is accumulating evidence that adipose-derived stem cells (ADSCs) may contribute to breast tumor microenvironment. Interestingly, ADSCs were shown to have different properties in normal and diabetic individuals. Here, we explored the possible role of ADSCs in breast cancer progression in diabetic condition.

MATERIALS AND METHODS: Breast cancer cells MCF-7 and MDA-MB-231 were co-cultured with ADSCs isolated from either Dock7m+/+Leprdb mice (control model) or +Leprdb/+Leprdb mice (diabetes and obesity disease model). Proliferation and migration properties were assessed by cell counting and MTT and transwell migration assay, respectively. Gene expression was determined by quantitative PCR.

RESULTS: Our result showed that ADSCs from diabetic mice had a stronger effect in enhancing breast cancer cell proliferation and migration than ADSCs from control mice. Quantitative PCR revealed that ADSCs from diabetic and control mice had differential gene expressions. Further experiments will be conducted to examine if these genes contribute to the differential effects of ADSCs from different sources to breast cancer progression.

CONCLUSIONS: These findings would provide novel insights on the roles of ADSCs in breast tumor progression in diabetic individuals.

NO CONFLICT OF INTEREST

163 Computational metabolism modeling predicts drug sensitivity in breast cancer cells

L. Trilla-Fuertes[1], M. Díaz-Almirón[2], A. Gámez-Pozo[3], G. Prado-Vázquez[3], A. Zapater-Moros[3], S. Llorente-Armijo[3], F. Gayá Moreno[2], R. Aras-López[4], E. Espìnosa[5], J.A. Fresno Vara[3]

[1] Biomedica Molecular Medicine, I+D, Madrid, Spain
[2] Hospital Universitario La Paz, Biostatistics Unit, Madrid, Spain
[3] Hospital Universitario La Paz, Molecular Oncology & Pathology Lab, Madrid, Spain
[4] Hospital Universitario La Paz, Congenital Malformations Lab, Madrid, Spain
[5] Hospital Universitario La Paz, Medical Oncology Service, Madrid, Spain

BACKGROUND: Breast cancer is one of the most prevalent cancers in the world. In previous works we observed differences in glucose metabolism between tumors from different breast cancer subtypes, suggesting the possibility to use drugs against metabolism in this disease. Flux Balance Analysis (FBA) is widely used to study biochemical networks, allowing to predict growth rates and to simulate drug response.

MATERIAL AND METHODS: Four breast cancer cell lines and different drugs against metabolic targets were evaluated with dose-response curves, and IC50 for each condition was calculated. Proteomics data from breast cancer cells lines treated with sub-lethal doses and controls were obtained applying a mass spectrometry-based approach. Differences in protein expression between treated vs. control were assessed and biological meaning was studied using gene ontology analyses. Predictions regarding cell cycle were evaluated by flow cytometry. An FBA approach using the human metabolic reconstruction Recon2 and including the protein expression values was also applied. The growth rate for each sample was estimated and differences in reactions fluxes between treated and untreated cells were evaluated.

RESULTS: Drug response was heterogeneous across different breast cancer cells. Mass spectrometry from cell samples allows identifying and quantifying 4,114 proteins. A cell cycle G2/M arrest induced by metformin and a G0/G1 arrest induced by rapamycin were predicted and confirmed by flow cytometry. FBA predicted that growth rates decrease in treated cells vs. control, as observed in cell viability assays. FBA also predicts other features related with each treatment, such as fatty acid oxidation increase and alterations in bile acid synthesis pathway induced by metformin, and CYP27A1 decrease and pyruvate kinase decrease induced by rapamycin. Furthermore, a correlation between catalase and superoxide dismutase fluxes and response to metformin was predicted. In vitro validation of these result is ongoing.

CONCLUSION: Proteomics provides insights of the mechanisms responsible for cells response to metabolism drugs. A computational model able to predict tumor growth using data from proteomics was developed. The model predicts growth rates and also dysregulation of biological processes provoked by drug treatment. Moreover, it could be used to propose new mechanisms of action and effects of metabolic drugs.

CONFLICT OF INTEREST

Ownership: JAFV, AG-P and EE are stakeholders of Biomedica Molecular Medicine S.L. and Biomedica Molecular Medicine Ltd.

Board of Directors: JAFV, AG-P and EE are part of the Board of Directors of Biomedica Molecular Medicine S.L. and Biomedica Molecular Medicine Ltd.

Other Substantive Relationships: LT-F is an employee of Biomedica Molecular Medicine S.L.

164 Decreased expression levels of RAC3 coactivator sensitizes colorectal cancer cells to the effect of chemotherapeutic drugs

M.F. Rubio[1], F.D. Rosa[2], M.C. Lira[2], L.C. Panelo[2], A.D. Sambresqui[3], M.C. Salazar Güemes[4], M.A. Costas[2]

[1] IDIM-CONICET UBA, Laboratory of Molecular Biology and Apoptosis, Cuidad Autónoma de Buenos Aires, Argentina
[2] IDIM-CONICET UBA, Laboratory of Molecular Biology and Apoptosis, Ciudad Autónoma de Buenos Aires, Argentina
[3] IDIM-UBA, Gastroenterology Department, Ciudad Autónoma de Buenos Aires, Argentina
[4] IDIM-UBA, Oncology Department, Ciudad Autónoma de Buenos Aires, Argentina

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers in the world. Despite recent advances in chemotherapies that have improved survival rates, patients with late-stage disease or elderly still have a poor prognosis.

We investigate the role of RAC3 coactivator in tumor development and we have previously demonstrated that RAC3 over expression contributes to inhibition of apoptosis and autophagy, being these two mechanisms involved in resistance to treatment with chemotherapeutic agents.

The aim of this study was to evaluate the RAC3 expression levels in three cell lines of CRC and determine their sensitivity to chemotherapeutic drugs.

Analyzing GEO data bank (GSE28702), we observed that patients who do not respond to Folic acid-5-Fluorouracil and Oxaliplatin (FOLFOX) treatment have higher RAC3 expression levels than those patients who respond to treatment, being this difference significantly greater in metastatic lesions.

To explore the potential role of RAC3 in CRC sensitivity to drugs, we determined the RAC3 expression levels in three human CRC cell lines (HT29, HCT116 and LoVo) by qPCR and Western blot and we showed RAC3 expression was higher in HT29> HCT116> LoVo (44> 9.6> 1-fold respect LoVo by qPCR). Then, we studied sensitivity

to 5-fluorouracil (FUra 0-150 µM) and oxaliplatin (Oxa 0-50 µM). Cell viability was determined by crystal violet staining and the IC50 was calculated for each cell type (Oxa: IC50 HT29 0.8±0.2 µM, HCT116 0.6±0.3 µM and LoVo 0.05±0.02 µM; FUra: IC50 HCT116 4.5±0.6 µM, LoVo 0.6±0.2 µM, HT29 did not respond to treatment with FUra in the doses used). We observed that LoVo were more sensitive to treatment with these drugs.

To study whether sensitivity observed in the different CRC lines was due to the expression levels of RAC3, the HCT116 cell line was transfected with an shRNA for RAC3 (shRAC3) and qPCR and Western blot were performed to validate the knockdown efficiency (shRAC3 0.08-fold respect HCT116 control by qPCR). Compared to the control, the shRAC3-transfected group displayed significantly decreased viability (FUra: control 4.5±0.6 µM vs shRAC3 2.4±0.2 µM and Oxa: control 0.6±0.3 µM vs shRAC3 0.17±0.1 µM).

CONCLUSION: our result show that the expression levels of RAC3 influence sensitivity to chemotherapeutic drugs. Therefore, the knowledge of the RAC3 expression levels in tumoral samples could be important in order to design new improved therapeutic strategies.

NO CONFLICT OF INTEREST

165 Loss of nephronectin promotes renal cell carcinoma progression through modulation of tight junction stability

C.W. Cheng[1], C.J. Hsiao[1], C.N. Chen[1]

[1] Taipei Medical University, Graduate Institute of Clinical Medicine- College of Medicine, Taipei City, Taiwan

INTRODUCTION: Renal cell carcinoma (RCC) is the most lethal genitourinary cancer; more than one-fourth of patients have metastasis at its presentation. It had been suggested the kidney regeneration process after acute kidney injury (AKI) presented similar features as the renal tubular cell malignancy. The extracellular matrix protein, nephronectin (NPNT) was expressed on the regenerating renal proximal tubular cells (RPTEC) after AKI. Embryos lacking a functional NPNT gene display server defects in the kidney development. However, little is known for its role in RPTEC and RCC.

MATERIAL AND METHOD: The commercialized tissue microarray arrays and kidney cancer qPCR array were applied to be examined NPNT by using immunohistochemistry and real-time PCR analysis. The shRNA-based lentiviral system technology was used to evaluate the effects and underlying mechanisms by manipulating NPNT.

RESULTS AND DISCUSSION: The mRNA and protein expression of NPNT were down-regulated in the RCC cell lines and clinical specimens. Silencing NPNT in RPTEC cell line, HK-2 cell, altered epithelial cell morphology into spindlelike shape and loss cell-cell contact junctions, these phenomena mimic to epithelial-to-mesenchymal transition process in the progression of cancer. Tight junctions (TJs) are a component of the epithelial junctional complex enables epithelial cells to create cellular sheets that separate compartments with different compositions, the formation of TJs may represent the status of epithelial cell differentiation. It is of note that tumor cells frequently show abnormal TJ function, decreased differentiation and cell polarity. Through cDNA microarray analysis, we had found NPNT was participated in pathways in cancer, cell adhesion molecules, transcriptional misregulation in cancer and ECM-receptor interaction. In addition, claudin (CLDN) 1, 2, 16 and occludin were all suppressed in NPNT silencing cell, while CLDN4 was up-regulated. These evidences supported NPNT may promote RPTEC cellular TJ formation, and by increasing intracellular junction formation, cell migration ability may be suppressed.

CONCLUSION: In all, our findings indicate the dysregulation of NPNT altered tight junction stability and promoted the progression of RCC.

NO CONFLICT OF INTEREST

166 Galectin-4 suppresses the expression of integrin β4 and reduces the migration and invasion activities of urothelial carcinoma cells

T.C. Lee[1], L. Li-Fang[1], S. Shaima'a Ahmad[1], W. Meei-Maan[2]

[1] Academia Sinica, Institute of Biomedical Sciences, Taipei, Taiwan
[2] Taipei Medical University, Department of Public Health- School of Medicine, Taipei, Taiwan

INTRODUCTION: Galectin-4 (Gal-4) is a multifunctional lectin possessing two structurally similar carbohydrate recognition domains (CDR). Recent studies have shown that Gal-4 plays certain role in cell adhesion, wound healing, intestinal inflammation, etc. However, Gal-4 functions contradictorily in tumor progression. Gal-4 acts as tumor promoter or suppressor in different cancer types. We have previously observed that Gal-4 expression is inversely associated with lymph node invasion and prognosis in patients with urothelial carcinoma (UC). In this study, we attempt to understand how Gal-4 interferes with cell migration and invasion in UC cells.

MATERIALS AND METHODS: We constructed Gal-4 expressing vector using plasmid pIRES2-EGFP. The Gal-4 expressing plasmid was transfected into UC cell lines, T24 and TSGH8301. The cell migration was analyzed by wound healing assay, while the cell invasion was assayed using gelatin-coated transwells. Anoikis assay was conducted by culturing the cells in dishes coated with poly-HEMA and flow cytometry. Protein expression was determined by western blotting. We also constructed integrin b4 (ITGb4) promoter-regulated luciferase reporter (pITGb4-Luc), which was adopted to investigate the transcriptionally inhibitory activity of Gal-4.

RESULTS AND DISCUSSION: We found that ectopic expression of Gal-4 in UC cells resulted in significant inhibition of cell proliferation, colony formation, migration, and invasion. We also demonstrated that Gal-4 overexpression led to a significant decrease in adhesion of UC cells to the basement membrane substrate laminin and triggered anoikis death. Mechanistically, we found that Gal-4 overexpression significantly reduced the expression of ITGβ4, a component of laminin receptor. In addition, we noticed that Gal-4 overexpression reduced phosphorylation of Src (pSrc) and focal adhesion kinase (pFAK). We may tentatively conclude that Gal-4 interferes with the integrin β4/Src/FAK cascade and attenuates metastasis in urothelial carcinoma. Furthermore, our preliminary result showed that Gal-4 significantly inhibited the reporter activity of pITGb4-Luc, implicating that Gal-4 may act as a transcription suppressor of ITGb4.

CONCLUSION: In UC cells, Gal-4 reduces the migration and invasion activity by interfering ITGb4/Src/FAK cascade that is an essential pathway preventing the suspended cells from anoikis. These result suggest that Gal-4 may be considered as a pivotal factor in metastasis of patients with UC.

NO CONFLICT OF INTEREST

167 Tumor microenvironment increases migration/ invasion of pheochromocytoma SDHB silenced spheroids

V. D'antongiovanni[1], S. Martinelli[1], S. Richter[2,3], L. Canu[1], D. Guasti[4], P. Romagnoli[4], K. Pacak[5], G. Eisenhofer[2,3], M. Mannelli[1], E. Rapizzi[4]

[1] University of Florence, Experimental and Clinical Biomedical Sciences, Florence, Italy
[2] Technische Universitat Dresden, Medicine III, Dresden, Germany
[3] Technische Universitat Dresden, Institute of Clinical Chemistry and Laboratory Medicine, Dresden, Germany
[4] University of Florence, Experimental and Clinical Medicine, Florence, Italy
[5] National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, USA

BACKGROUND: Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare neuroendocrine tumors. About 30-40% of PHEO/PGLs are due to a germline mutation in one of the 13 main susceptibility genes which include the genes encoding the four subunits (A, B, C and D) of the succinate dehydrogenase (SDH).

Up to 80% of patients affected by PHEO/PGL SDHB mutated develop metastatic disease, and no successful cure is at present available.

MATERIAL AND METHODS: To study the effects of SDHB silencing we used tumor spheroids (3D culture) of a PHEO cell line silenced or not (control) for the catalytic SDHB subunit. To evaluate the role of the microenvironment on migration/invasion process, SDHB silenced or wt control spheroids were cultured in co-culture with primary cancer-activated fibroblasts (CAFs).

RESULTS: We studied spheroid growth treated or not with CAF conditioned medium. Spheroid growth was rapid and very similar in both not conditioned wt and SDHB silenced spheroids. Surprisingly, if we treated the spheroids with medium conditioned by CAFs, both wt and SDHB silenced spheroid growth was significantly lower compared with that of not conditioned spheroids. We next investigated if tumor microenvironment was able to modulate spheroid migration/invasion of extracellular matrix, represented by matrigel. The migration process in single cultured SDHB silenced spheroids compared with single cultured wt spheroids was significantly increased, but migrating cells remained attached to the spheroids. When the spheroids were cocultured with fibroblasts, using the transwell inserts, we observed an evident detachment of viable cell clusters from both wt and SDHB silenced spheroids, but SDHB silenced cells show a significant increase in migratory capability than wt cells, as demonstrated by the computation of the migratory areas. Moreover, SDHB silenced cells seem to invade the surrounding space moving collectively, unlike the wt spheroids, where cells tend to move individually. Additionally, SDHB silenced spheroids develop long filamentous formations along which clusters of cells migrate far away from the spheroid, while these structures are not present in wt cells.

CONCLUSIONS: In this work we demonstrated that SDHB silencing per se increases tumor cell migration/invasion, but also that the microenvironment plays a pivotal role in enhancing collective migration/invasion in PHEO SDHB silenced tumor cells, suggesting its importance in increasing the metastasizing potential.

NO CONFLICT OF INTEREST

168 ADAM9 promotes lung cancer metastasis through suppression of microRNAs

Y.P. Sher[1,2], K.L. Chiu[3], Y.S. Lin[3], T.T. Kuo[2], W.C. Cheng[1], C.C. Lin[3], L.C. Lai[4]

[1] China Medical University, Graduate Institute of Biomedical Sciences, Taichung, Taiwan
[2] China Medical University Hospital, Center for Molecular Medicine, Taichung, Taiwan
[3] China Medical University, Graduate Institute of Clinical Medical Science, Taichung, Taiwan
[4] National Taiwan University, Graduate Institute of Physiology, Taipei, Taiwan

INTRODUCTION: Metastasis is a major cause of morbidity and mortality in lung cancer. Overexpression of a disintegrin and metalloprotease 9 (ADAM9), a member of the ADAM family of type I transmembrane proteins, is observed in many cancers and correlates with lung cancer brain metastasis. We demonstrated that ADAM9 promotes lung cancer metastasis via inducing oncogenic CUB-domain-containing protein 1 (CDCP1) expression, which plays roles in anchorage-free survival and cell migration; however, the regulated process remains unclear. Since dysregulation of

microRNAs, endogenous noncoding short RNAs, can regulate genes and promote cancer progression, we further explore whether ADAM9-associated microRNA regulation is involved in cancer metastasis.

MATERIAL AND METHOD: We have detected ADAM9-associated mRNAs and microRNAs in a genome-wide approach comparing established metastatic lung cancer sublines and their parental cancer cells. Clinical lung adenocarcinoma samples were included to investigate the clinical relevance of ADAM9-associated microRNAs. We also evaluated the effects of microRNAs in the regulation of metastases using microRNA mimics or inhibitors in lung cancer cells.

RESULTS AND DISCUSSION: We identified that the 3'UTR of CDCP1 contains the predicted binding sites of several microRNAs that were down-regulated in ADAM9-overexpressed cancer cells. Luciferase assays and western blot analysis showed that CDCP1 is a target gene of microRNAs. The up-regulation of microRNAs, in turn, reduced the expression of its target gene and further inhibited the migration ability of aggressive lung adenocarcinoma cells. Treatment of microRNA inhibitors could restore CDCP1 protein levels and enhance tumor cell mobility. Moreover, microRNAs treatment delayed tumor metastases in animals.

CONCLUSION: This study reveals that ADAM9 activates CDCP1 through the release of microRNA inhibition of CDCP1 in lung adenocarcinoma and plays a role in lung cancer metastasis.

NO CONFLICT OF INTEREST

169 Role of microenvironment on metabolic control of pheochromocytoma SDHB silenced cells

V. D'Antongiovanni[1], S. Richter[2,3], S. Martinelli[1], L. Canu[1], T. Ercolino[1], G. Eisenhofer[2,3], K. Pacak[4], M. Mannelli[1], E. Rapizzi[5]

[1] University of Florence, Experimental and Clinical Biomedical Sciences, Florence, Italy
[2] Technische Universitat Dresden, Institute of Clinical Chemistry & Laboratory Medicine, Dresden, Germany
[3] Technische Universitat Dresden, Medicine III, Dresden, Germany
[4] National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, USA
[5] University of Florence, Experimental and Clinical Medicine, Florence, Italy

BACKGROUND: Pheochromocytomas (PHEOs) and paragangliomas (PGLs) are rare neuroendocrine tumors. About 30-40% of PHEO/PGLs are due to a germline mutation in one of the 13 main susceptibility genes which include the genes encoding the four subunits (A, B, C and D) of the succinate dehydrogenase (SDH).

Up to 80% of patients affected by PHEO/PGL SDHB mutated develop metastatic disease, and no successful cure is at present available. Tumor microenvironment plays a pivotal role in modifying the metabolism and the functional characteristics of tumor cells, becoming a potential therapeutic target.

MATERIAL AND METHODS: We evaluated the effects of SDHB silencing and the role of the microenvironment on cell metabolism in mouse tumor tissue-derived cell line (MTT) silenced or not for SDHB. For the microenvironment studies, tumor cells were cultured alone or in association with primary cancer-activated fibroblasts (CAFs).

RESULTS: SDHB silenced cells showed a significant increase of both glucose and lactate uptake, and an increase of intracellular lactate compared with wt control. These increases were even more evident when SDHB silenced cells was co-cultured with CAFs. The expression levels of monocarboxilase transporter 4 (MCT4), responsible for the transport of lactate from the intracellular to the extracellular space, was found upregulated in CAFs compared with not activated primary fibroblasts. Consequently, CAFs also showed an increase in lactate production released in the extracellular medium. Surprisingly, SDHB silenced cells in co-culture showed a reduction of ATP levels compared to SDHB cultured alone and control cells in co-cultures.

CONCLUSIONS: Our data demonstrate that SDHB silencing per se affects tumor metabolism, but these changes are strongly modulated by the microenvironment. The comprehension of the mechanisms driving these changes in tumor metabolism may suggest new therapeutic targets.

NO CONFLICT OF INTEREST

170 Comparison of the response of four patient-derived NSCLC lines grown as monolayers, simple spheroids and mixed cell complex spheroids

G. Kaur[1], J. Doroshow[2], B. Teicher[2]

[1] NCI-Frederick, DCTD, Frederick, USA
[2] NCI, DCTD, Bethesda, USA

Development of new anticancer therapeutics depends upon reliable in vitro models. We investigated the growth inhibitory activity of 8 anticancer drugs in 7 patient derived NSCLC lines grown as monolayers, simple spheroids and mixed cell complex spheroids.

Drug testing was performed in standard monolayer culture, in U-bottom ultra-low adhesion (ULA) plates as 3D simple spheroids, and tumor cells co-cultured with human mesenchymal stem cells (hMSC) and human endothelial cells (HUVEC) in ULA plates as 3D complex spheroids. At 72h, cells were treated with test drugs at 9 concentrations for 72h and 96h. Cell viability in monolayer and in spheroids was measured using luminescence by CellTiter-Glo® Assay in 2D monolayer cells and CellTiter-Glo® 3D Assay in 3D simple and complex spheroids. Concentration-response curves and IC_{50}'s were calculated.

Three lung adenocarcinoma lines and 1 lung neuroendocrine line with 3 sublines were tested with DNA damaging agents (carboplatin, temozolomide), a multitargeted antifolate (pemetrexed), a PARP1 inhibitor (talazoparib), tubulin inhibitors (docetaxel, vinorelbine), EGFR inhibitor (erlotinib), and ALK inhibitor (ceritinib). All lines cultured under the 3 conditions were unresponsive to temozolomide (>100 μM) and to talazoparib (>10 μM). The LG567 line was sensitive to docetaxel and vinorelbine in monolayer culture. The large cell neuroendocrine line LG904 and sublines lines were sensitive to docetaxel (IC_{50} = 0.003-0.4 μM) and vinorelbine (IC_{50} = 0.003-0.088 μM) under all culture conditions. The 3 adenocarcinoma lines responded to docetaxel and vinorelbine in monolayer with IC_{50}'s of 0.003-0.45 μM. All 7 lines under all culture conditions were sensitive to carboplatin (IC_{50} = 1-100μM) whereas only 2D cultures were sensitive to pemetrexed (IC_{50} = 0.115-40μM). LG904 simple spheroids and complex spheroids and sublines lines were sensitive to erlotinib and ceritinib with IC_{50}'s below the clinical Cmax indicating the importance of the 3D culture format. When tested alone, hMSC were resistant to all drugs tested whereas HUVEC were sensitive to carboplatin, docetaxel, and vinorelbine.

Patient-derived NSCLC line response to anticancer drugs varied with culture conditions. The differential response to erlotinib, in 3D simple and 3D complex spheroids, versus 2D culture, suggests that tumor microenvironment may play a role in EGFR inhibitor activity and needs to be explored further with additional agents.

NO CONFLICT OF INTEREST

171 The reprogramming transcription factors sox2, c-myc and klf4 as potential therapeutic targets for resistance and metastasis in prostate cancer

E.A. Castellon[1], R.H. Valenzuela[1], F.F. Cifuentes[1], T.M. Thomson[2], H.R. Contreras[1]

[1] Faculty of Medicine. University of Chile, Department of Oncology, Santiago, Chile
[2] Institute for Molecular Biology. Science Research Council CSIC, Cell Signaling and Cancer, Barcelona, Spain

INTRODUCTION: Prostate cancer (PCa) is a leading cause of men cancer mortality in the world. Relapse, resistance and metastasis are major concerns. There is increasing evidence that cancer stem cells (CSCs) are associated to these processes. Several signaling pathways and reprogramming transcription factors are involved in maintaining the stemness phenotype, promoting CSCs survival even after therapy. Knocking down the reprogramming transcription factors (stemness genes) involved in these events may reverse CSCs features making them more sensitive to treatments. The main goal of this research is to evaluate the effect of knocking down of stemness genes on functional characteristics and metastatic capacity of CSCs in PCa.

MATERIALS AND METHODS: Tumor samples were obtained from PCa patients undergoing radical prostatectomy. All protocols were approved by institutional Ethical Committees. Cells were cultured under unattachment conditions favoring CSCs spheres formation. The reprogramming transcription factors SOX2, KLF4 and c-MYC were knocked down using specific shRNAs within lentiviral vectors. The effects of these gene knock down on CSCs apoptosis, clonogenic and invasion were assayed using an in vitro model. Moreover, the metastasis ability of SOX2-knocked down CSCs was analyzed in a orthotopic NOD/SCID mice model for human PCa.

RESULTS AND DISCUSSION: Prostate spheres were enriched in CSCs with phenotypic and genotypic stem characteristics. CSCs knocked down for SOX2, KLF4 and c-MYC showed chemotherapeutic drugs sensitization, increased apoptotic rate and decreased clonogenic and invasive abilities. SOX2-knocked down CSCs decreased the growth tumor rate and totally inhibited metastasis in a orthotopic NOD/SCID model for PCa. The reprogramming transcription factors analyzed have a relevant role in maintaining the stemness functional signature of CSCs from PCa, promoting anti-apoptotic, clonogenic, invasive, tumorigenic and metastatic properties.

CONCLUSION: The reprogramming transcription factor SOX2 seems to have a determinant role in metastatic progression, as shown in a pre-clinical model of PCa, and might be considered as a suitable therapeutic target.

Funding: Projects Fondecyt 1140417 (EAC) and 1151214 (HRC).

NO CONFLICT OF INTEREST

172 Regulation of oncogene-induced senescence by the toll-like receptors

P. Hari[1], N. Tarrats[1], I. Fernandez[1], J.C. Acosta[1]

[1] Edinburgh University IGMM, Edinburgh Cancer Research Centre, Edinburgh, United Kingdom

INTRODUCTION: Oncogene-induced senescence (OIS) is a fail-safe mechanism activated in order to halt the proliferation of cells at risk of malignant transformation. It is an irreversible programme of biological changes to the cell comprising of the activation of tumour suppressor pathways, altered cellular metabolism, extensive chromatin remodeling and the activation of secretory phenotype. The vast array of proteins that secreted by the cells not only play a role in reinforcing the senescence phenotype, but also modulate the cell's microenvironment by inducing senescence in neighbouring cells, promoting angiogenesis, and initiating an immune response through the recruitment of immune cells. Components of the innate immune system, particularly the NLRP3 subfamily of the pattern recognition receptors have been shown to play as essential role in the development of the senescence-

associated secretory phenotype through its processing and activation of caspase 1 that in turn leads to activation of IL-1B. A gene set enrichment analysis of OIS cells showed significant upregulation in the pattern recognition receptor family.

MATERIALS AND METHODS: IMR90 human diploid fibroblast cells, stably transfected with an oncogenic ER:RAS fusion protein undergo OIS upon treatment with 4-hydroxytamoxifen. A loss of function siRNA screening was conducted targeting components of the innate immune systems, including pattern cognition receptors. Potential regulators of OIS were identified through siRNA that bypassed the proliferative arrest associated with OIS.

RESULTS: Toll-like receptor 2 (TLR2) and TLR10 have been identified as potential regulators of OIS. Their overexpression in IMR90 cells induces a premature form of senescence where the cells have significantly reduced proliferative activity and display senescence-associated β galactosidase activity. Moreover, the knockdown of TLR2 and TLR10 result in suppression of tumour suppressor pathway genes, reduced signaling through the DNA damage response pathway and suppression of the SASP. TLR2 activates the non-canonical inflammasome through the activation of caspase 4.

CONCLUSION: Our result suggest that the TLR2 and TLR10 act as potential tumour suppressor genes in their essential role for the activation for OIS, particularly of the production of the SASP via activation of the canonical and non-canonical inflammasome and thereby provides a direct mechanistic link between the activation of an immune response and senescence.

NO CONFLICT OF INTEREST

174 CD44 modulation: An effective strategy to sensitize cancer stem cells to chemotherapy

B. Marengo[1], A. Speciale[1], O. Garbarino[1], M. Passalacqua[1], N. Traverso[1], M.A. Pronzato[1], C. Domenicotti[1]

[1] General Pathology section, Experimental Medicine, Genoa, Italy

INTRODUCTION: Current anticancer therapies exert cytotoxic effects by increasing reactive oxygen species (ROS) production. However, high levels of antioxidants, in particular of glutathione (GSH), allow cancer cells to defend themselves from ROS-induced damage, rendering them chemoresistant. It is known that chemoresistance characterizes cancer stem cells (CSCs) which, in having a low proliferative rate, are less affected by therapies acting on highly proliferating cells. Moreover, CSCs express CD44, a staminality marker able to indirectly modulate GSH levels by stabilizing xCT, a plasma membrane transporter promoting the influx of cystine, an aminoacid essential for GSH synthesis. Based on this evidence, we believe that GSH depletion in CSCs might be fundamental in counteracting chemoresistance and cancer relapse.

MATERIALS AND METHODS: CSCs are obtained from HTLA-230, a highly malignant human neuroblastoma cell line, and from HTLA-ER cells which are an etoposide-resistant clone selected by chronically treating HTLA-230 with etoposide. The CSCs were co-treated with etoposide plus buthionine sulfoximine (BSO), a GSH depleting agent or Gö 6976, a Protein Kinase C (PKC) inhibitor. Stem cell propagation, ROS production (cytofluorimetric analyses), GSH levels (fluorescence/HPLC analysis) and CD44/xCT cell localization (confocal microscopy) were analyzed.

RESULTS: The CSCs, originating from HTLA-ER cells, were more resistant to etoposide and not only produced lower ROS levels, but also had an higher GSH amount in comparison to the CSCs obtained from HTLA-230. Since BSO, a GSH depleting drug which sensitizes HTLA-ER-CSCs to etoposide, is toxic in vivo, it is necessary to investigate an alternative strategy in reducing GSH. In this context, the regulation of CD44 expression, which is modulated by a PKC-dependent pathway, is proposed. In fact, the co-treatment of etoposide with Gö 6976 influenced the formation and propagation of HTLA-ER-CSCs in a similar way to BSO-co-treatment.

CONCLUSION: Collectively, our result confirm that GSH plays a crucial role in chemoresistance and demonstrate that it is also essential for CSC generation. Therefore, the modulation of CD44 which indirectly regulates GSH levels might be an effective strategy for inducing CSC death, counteracting chemoresistance and cancer relapse (Grants from Genoa University).

NO CONFLICT OF INTEREST

175 Role of CD34 in Thyroid Cancer stemness and neoplastic features

F. Liotti[1], N. Prevete[2], E. Pone[3], N. Kumar[4], R.M. Melillo[3]

[1] Universita' Di Napoli Federico II, Molecular Medicine and Medical Biotechnology-, Naples, Italy
[2] Universita' Di Napoli Federico II, Translational Medical Sciences, Naples, Italy
[3] Universita' Di Napoli Federico II, Molecular Medicine and Medical Biotechnology, Naples, Italy
[4] CNR, IEOS, Naples, Italy

INTRODUCTION: Cancer cell populations contain a small proportion of cells endowed with stem-like properties, which may be responsible for overall tumor maintenance. Interleukin 8 (IL8) was identified as an autocrine/paracrine factor that could amplify thyroid cancer (TC) stemness features and cause an expansion of TC stem-like cell (TC SC) population. We exploited these IL8 properties to amplify and characterize TC SCs. We identified CD34, a widely used marker of various stem cells, as a transcriptional target of IL8. CD34 is a single-pass type-I transmembrane glycoprotein with a variety of potential functions, including enhancing proliferation and blocking differentiation of stem cells, regulating cell adhesion, morphogenesis and fate. Thus, we asked whether CD34 exerts a role in TC stemness features.

MATERIAL AND METHOD: CD34 functions in TC cells (8505c, SW1736, CAL62) were evaluated by using CD34 blocking antibodies, CD34 shRNAs and CD34-based cell immune selection. Stemness potential was evaluated by sphere-forming assays and RT-PCR. Tumorigenic potential was evaluated by xenotransplantation experiments.

RESULTS: Thyrospheres derived from various TC cell lines were significantly enriched in CD34 membrane expression compared to adherent cells. Surface CD34 levels were lower in 8505c spheres constitutively depleted of IL-8, but higher in IL-8-overexpressing 8505c spheres than in controls. Thus, CD34 is enriched in TC SCs and its expression correlates with IL8 expression in TC SCs. By selecting TC cells based on their CD34 expression, we were able to separate two populations, CD34high and CD34low. CD34high cells exhibited a significant increase in stemness marker expression and sphere-forming ability compared to CD34low cells. Accordingly, CD34 inhibition by using gene silencing or neutralizing antibodies significantly inhibited sphere-forming efficiency of 8505c cells. Moreover, CD34-silenced 8505c cells exhibited reduced tumor volume and tumor incidence compared to control cells in xenotransplation experiments.

CONCLUSION: Our data indicate that CD34 expression can be regulated by IL8 in TC cells. CD34 is enriched TC SCs and can be used as a potential marker to identify and isolate TC SCs from human tissues. Moreover, CD34 is functionally required for efficient TC cell sphere-forming activity and for TC cell tumorigenicity.

NO CONFLICT OF INTEREST

176 Role of microenvironment on proliferation and migration of an SDHB silenced pheochromocytoma cell line

S. Martinelli[1], V. D'Antongioanni[1], S. Richter[2,3], L. Canu[1], T. Ercolino[1], G. Eisenhofer[2,3], K. Pacak[4], M. Mannelli[1], E. Rapizzi[5]

[1] University of Florence, Experimental and Clinical Biomedical Sciences, Florence, Italy
[2] Technische Universitat Dresden, Institute of Clinical Chemistry & Laboratory Medicine, Dresden, Germany
[3] Technische Universitat Dresden, Medicine III, Dresden, Germany
[4] National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, USA
[5] University of Florence, Experimental and Clinical Medicine, Florence, Italy

BACKGROUND: Paragangliomas are rare neuroendocrine tumors derived from neural crest cells: if localized in the adrenal medulla they are called Pheocromocytomas (Pheo).The 30-40% of Pheo are mutated in one of the susceptibility genes among which there are genes encoding for the four subunits (A, B, C and D) of the succinate dehydrogenase (SDH). Germ line mutations of SDHB are metastatic in about 80% of the cases. Surgery is the current therapy, but in presence of metastasis there is no effective treatment.

MATERIAL AND METHODS: Using shRNA, we stably silenced for SDHB a pheo murine cell line (MTT). To study the role of microenvironment on tumor cell proliferation and migration, tumor cells were cultured alone or in association with primary cancer-activated fibroblasts (CAFs).

RESULTS: SDHB silencing induced a decrease in proliferation rate, but interestingly, when tumor cells were cocultured with activated fibroblasts this effect was reverted, with a higher increase in SDHB silenced proliferation than in wt control cells.

We observed that both control and SDHB silenced MTT cells aggregated and tended to grow in clusters. After about 10 days, aggregates are visible and with clear edges. Interestingly, when cultured in conditioned medium from CAFs, the aggregating effect disappeared, the cells assumed a longer shape and started migrating. To understand whether this change corresponded to an epithelial to mesenchimal transition (EMT), we evaluated the EMT markers and the expression of metalloproteases either secreted in the medium or expressed on membranes, but we could observed a significant difference between single cultured cells and cocultured ones. Moreover, to understand if the cross talk between fibroblasts and tumor cells was due to a signal mediated by receptors, we evaluated the phosphorylation of mTOR, Akt and ERK in SDHB silenced and control without detecting any difference.

CONCLUSIONS: These preliminary result suggest that the microenvironment, here represented by fibroblasts, affects the migration and proliferation of cancer cells. We believe that studies on this interaction are needed to identify new therapeutic targets in SDHB mutated tumors.

NO CONFLICT OF INTEREST

177 RNA sequencing approach to investigate transcripts involved in platinum resistance in high-grade serous epithelial ovarian cancer

E. Calura[1]

[1] University of Padova, Department of Biology, Padova, Italy

BACKGROUND: The most common histological subtype of epithelial ovarian cancer (EOC), the high-grade serous ovarian carcinoma (HGSOC), is generally diagnosed late, when multiple synchronous tumor lesions are localized in the ovary, as well as in other anatomical sites within the peritoneum cavity. The five-years survival rate is less than 30%, and the majority of patients, despite an initial response to platinum agents, become progressively resistant and die from the tumor that becomes incurable.

MATERIAL AND METHODS: The conventional array-based approach studies, drawn on known transcript structures, failed to identify biomarker transcripts for platinum resistance.Thus, with the aim to discover new coding and non-coding variants of known transcripts or totally novel transcripts, associated with the mechanism of resistance, we have sequenced the entire transcriptome of tumor biopsies from 14 platinum-sensitive and 14 platinum-resistant patients.

RESULTS: The transcriptome reconstruction of the 28 sequencing experiments allowed us to identify 1371 transcripts differentially expressed between pt-resistant and pt-sensitive samples. Among them, 125 transcripts showed a complete match of intron chain with known transcripts, 686 were potentially novel isoforms or showed a generic overlap with known transcripts The remaining, if validated, could be novel intergenic transcripts or anti-sense transcripts with an exonic overlap with a known transcript. Interestingly, a very small part of the collected transcriptional alterations can be ascribed to coding-genes, suggesting a prominent non-coding role in HGSEOC platinum resistance.

CONCLUSIONS: Despite interesting, these preliminary result need further validations to better define the functional and predictive/prognostic role of the detected transcriptional alterations.

NO CONFLICT OF INTEREST

178 Relevance of intra-tumor heterogeneity in tissue analysis – Colorectal cancer development from adenoma to tumor

N. Lange[1], *A. Tiedemann*[1], *M. Schoeppler*[1], *B. Baniassad*[1], *H. Juhl*[2], *K.A. David*[3]

[1] *Indivumed GmbH, Research Services Genomics, Hamburg, Germany*
[2] *Indivumed GmbH, Indivumed GmbH, Hamburg, Germany*
[3] *Indivumed GmbH, Research Services, Hamburg, Germany*

INTRODUCTION: Colorectal cancer (CRC) development underwent a step-wise transformation from normal epithelium into adenoma and can end up as an invasive and metastatic tumor. A lot of questions regarding tumor development and tumor heterogeneity are still open. Tumor heterogeneity is challenging to tumor therapy, since all subpopulations must be efficiently killed. To improve therapy, a more precise sample processing and analysis of cell populations is necessary. Here, we sequentially analyzed CRC tissue regarding histological and genetic changes occurring during the development from adenoma to tumor.

MATERIAL AND METHOD: Biospecimen of high quality were collected in a highly standardized manner. Fresh Frozen tumor and normal tissue of 1 patient was selected. The entire tumor tissue block was sequentially sectioned: 1 section for haematoxylin/ eosin (H&E) staining and 10 sections for simultaneous DNA/RNA isolation. A total of 18 fractions were created. Only 1 fraction was generated for normal tissue. DNA was analyzed on the MiSeqDx sequencer by using Illumina®´s TruSeq Amplicon Cancer Panel.

RESULTS AND DISCUSSION: Histopathological evaluation of H&E stained sections showed a continuous increase of adenoma cells with a simultaneous decrease of carcinoma cells. The first fraction revealed a carcinoma content of 70% without adenoma structures, whereas the last fraction consisted of 80% adenoma structures without carcinoma cells. Fractions in between showed a mixture of both.

Evaluation of sequencing data showed numerous genetic events. Following cross sample subtraction of normal tissue, 3 events affecting coding regions were identified. A frameshift variant in the APC gene was detected in all fractions with a frequency of ~30%. In contrast, the frequency of a KRAS missense variant increases from 26% in the adenoma to 80% in the carcinoma fraction. A stop gained variant in the FBXW7 gene showed a frequency of 30% in the adenoma fraction, decreased continuously and was not detectable in the carcinoma fraction.

The result showed significant differences in the mutation status of cell populations within a single tumor block. These data confirm intra-tumor heterogeneity and pointing out the importance to carefully analyze tissue.

CONCLUSION: Beside of high quality biospecimen, the analysis of more precise tissue samples instead of whole tissue blocks is an essential requirement to obtain reliable data and will improve our knowledge of cancer development and tumor heterogeneity.

NO CONFLICT OF INTEREST

179 CD157 contributes to the environment-mediated chemoresistance in acute myeloid leukemia

S. Augeri[1], *S. Morone*[1], *S. D'Ardia*[2], *S. Aydin*[2], *C. Dal Secco*[1], *E. Ortolan*[1], *A. Funaro*[1]

[1] *University of Torino, Department of Medical Sciences, Torino, Italy*
[2] *AO Citta` della Salute e della Scienza, SC Ematologia, Torino, Italy*

BACKGROUND: Acute myeloid leukemia (AML), the most common acute leukemia in adults, has a poor long-term prognosis due to development of chemoresistance and eventual tumor relapse. Increasing evidence suggests that the interaction between leukemic cells and bone marrow (BM) microenvironment contributes to tumor survival and exerts protective effects from chemotherapy-induced tumor cytotoxicity.

CD157 is an adhesion molecule expressed in AML blasts (especially in M4 and M5 subtypes), by BM stromal cells and by selected epithelial cancers, where it protects cells from apoptosis and chemotherapy. In this study we explored the role of CD157 in the cross-talk between myeloid blasts and BM niche.

MATERIALS AND METHODS: The functional role of CD157 was analysed in U937 and THP1cell lines engineered for the expression of CD157 and in fresh AML samples by means of specific agonist antibodies. The HS5 human BM stromal cells were used as an in vitro model to investigate the crosstalk between leukemic blasts and stromal cells. The viability of leukemic cells treated with Cytarabine (AraC) was evaluated by PrestoBlue assays and Annexin-FITC/PI staining. The CD157-mediated signalling pathway was analysed by western blot and flow cytometry.

RESULTS:CD157 ligation by an agonistic antibody both in fresh AML and cell lines, activated the phosphatidylinositol 3-kinase (PI3K)/AKT/Bcl-2 signaling pathway and inactivated BAD and GSK3β, leading to increased cell survival and adhesion, and influencing tumor cell resistance to apoptotic signals and sensitivity to AraC treatment. Furthermore, genetic loss of CD157 in AML cells i) reduced survival and increased sensitivity to AraC, ii) reduced the number of cells in G0/G1 phases, iii) enhanced sensitivity to nutrient deprivation, and iv) attenuated the protective effect exerted by BMSCs against AraC-induced cell death. On the other side, forced expression of CD157 in HS5 stromal cells strengthened the protection mediated by BMSCs on AraC-induced cell death driving leukemia cells toward quiescence.

CONCLUSION: Collectively, these result suggest that CD157 expressed both by tumor cells or by the surrounding BM stromal cells takes part to the dialogue between AML cells and microenvironment and has a role in the protective effect exerted by BMSCs against cytotoxic drugs.

NO CONFLICT OF INTEREST

180 Unraveling the potential role of autophagy in CD157-associated chemoresistance in malignant pleural mesothelioma

S. Capano[1], *A. Giacomino*[1], *Y. Yakymiv*[1], *S. Morone*[1], *E. Ortolan*[1], *A. Funaro*[1]

[1] *University of Turin, Department of Medical Sciences, Torino, Italy*

INTRODUCTION: Resistance of cancer cells to cytotoxic agents is a major challenge in malignant pleural mesothelioma (MPM) patient management. Recently, we demonstrated that the CD157 glycoprotein is expressed by ~80% of MPM surgical specimens and its expression levels correlate with poor prognosis. In vitro, high CD157 expression has been associated with enhanced cell growth, migration, invasion and activation of the PI3K/Akt/mTOR pathway leading to resistance to platinum-based chemotherapy. The inhibition of mTOR with Everolimus or of both PI3K and mTOR with BEZ-235 proved to be able to revert chemoresistance in CD157-positive cells. As increasing evidence indicates that autophagy has a key role in platinum-based chemotherapy resistance, in this study we investigated the potential role of autophagy in CD157-mediated chemotherapy resistance in MPM.

MATERIALS AND METHODS: CG98 (CD157-positive) and MSTO-211H (CD157-negative) MPM cell lines (both native and engineered for CD157 expression), were used as models, to study apoptosis and autophagy. Using Western blot and immunofluorescence, we analysed the expression of caspase-3 and PARP, as hallmarks of apoptosis, and of LC3II, a protein associated with autophagosomes. Cell proliferation in the presence or absence of Chloroquine (CQ) or Bafilomycin autophagy inhibitors was assessed by Cell Viability assay.

RESULTS AND DISCUSSION: Treatment with cisplatin (CDDP) induced a more robust caspase-3 activation and PARP cleavage in CD157-negative than in CD157-positive MPM cells, suggesting that CD157-associated resistance is at least partly related to an impaired apoptotic response. Moreover, treatment with CQ or Bafilomycin induced greater accumulation of LC3II and had a stronger growth inhibitory effect in CD157-positive than in CD157-negative cells, indicating that autophagy could act as a prosurvival mechanism contributing to CD157-associated drug resistance. Preliminary result highlighted that CDDP treatment, alone or in combination with autophagy inhibitors, promotes high levels of autophagy in CD157-positive cells, corroborating the notion that CDDP is able to elicit the autophagic flux in platinum-resistant cells.

CONCLUSION: These result support the rationale to hypothesize the implication of autophagy in CD157-associated resistance and highlight the potential clinical utility of CD157 as a marker for selecting patients with particularly aggressive MPM who might benefit from a combined tailored therapy.

NO CONFLICT OF INTEREST

181 SCD5 impairs metastatization by reversing EMT and increasing immunoreactivity through SPARC reduced secretion

R. Puglisi[1], *M. Bellenghi*[1], *G. Pontecorvi*[1], *L. Bottero*[1], *M. Petrini*[1], *M.P. Colombo*[2], *S. Sangaletti*[2], *G. Mattia*[1], *A. Carè*[1]

[1] *istituto superiore di sanità, center of gender medicine, Rome, Italy*
[2] *Istituto Nazionale Tumori, Experimental Oncology and Molecular Medicine, Milano, Italy*

INTRODUCTION: SCD5 is a Stearoyl- CoA desaturase, converting saturated fatty acids in their monounsaturated forms by the introduction of the first double bond in cis-Δ9-position of the aliphatic chain, to prevalently transform stearic and palmitic into oleic and palmitoleic acids. Few data exist on the functional role of SCD5 in cancer. We found that SCD5 is mostly silenced during melanoma progression and that its overexpression is sufficient to reduce melanoma malignancy, both in vitro and in vivo.

We have now discovered SCD5 antimetastatic properties and studied the underlying mechanisms using the murine 4T1 model of mammary spontaneous metastasis transduced to overexpress SCD5.

MATERIAL AND METHODS: SCD5 was transduced via lentiviral vector. All the expression studies and biological assays, including in vivo experiments, were performed using standard protocols. Animal experiments have been approved according to the Italian law and institutional guidelines.

RESULTS: We detected a significant reduction of SPARC secretion coupled with its cytoplasmic accumulation in SCD5-overexpressing 4T1 cells. In vivo studies showed a strong decrease of the number of lung metastases in syngeneic BALB/c mice injected with 4T1/SCD5 cells compared to controls. Morphological analysis of primary tumors showed reduced extracellular matrix deposition associated with the failed release of SPARC, eventually impairing tumor cell spreading and invasion. According to the dual role of SPARC in modulating the epithelial-mesenchymal transition (EMT) program and the immune microenvironment, we found evidence of EMT reversion at the site of 4T1/SCD5 tumor inoculation that was associated with increased immunoreactivity.

In more details, CD8+ and CD4+ T-lymphocytes were either increased and decreased, respectively, in the lung of 4T1/SCD5 injected mice and both G- and M-MDSC were significantly reduced in the spleen of the same mice in comparison to parental 4T1 injected mouse organs.

CONCLUSION: All together these data suggest the existence of a SCD5-dependent SPARC/EMT/ Immune cells interplay, reversing the malignant phenotype and replacing an effective immune response.

Supported by AIRC IG 18815

NO CONFLICT OF INTEREST

183 Nonautophagic cytoplasmic vacuolation death induction in human PC-3M prostate cancer by curcumin through reactive oxygen species-mediated endoplasmic reticulum stress

W.J. Lee[1], M.H. Chien[2], Y.M. Chow[3], J.L. Chang[4], Y.C. Wen[1], Y.W. Lin[1], C.W. Cheng[2], G.M. Lai[3], M. Hsiao[5], L.M. Lee[1]

[1] Taipei Medical University, Department of Urology, Taipei, Taiwan
[2] Taipei Medical University, Graduate Institute of Clinical Medicine, Taipei, Taiwan
[3] Taipei Medical University, Department of Internal Medicine, Taipei, Taiwan
[4] Ming Chuan University, Biomedical Engineering Department, Taoyuan, Taiwan
[5] Academia Sinica, The Genomics Research Center, Taipei, Taiwan

BACKGROUND: The antiapoptotic and antiautophagic abilities of cancer cells constitute a major challenge for anticancer drug treatment. Strategies for triggering nonapoptotic or nonautophagic cell death may improve therapeutic efficacy against cancer. We aimed at evaluating the molecular mechanisms of the curcumin in suppress prostate cancer progression by induced nonautophagic cytoplasmic vacuolation death.

MATERIAL AND METHOD: Four prostate cancer cells were evaluated the effect of curcumin on cell death. Among these analyzed, curcumin was further functionally analyzed using in vitro and in vivo assays.

RESULTS: Our study found that curcumin induced apoptosis in LNCaP, DU145, and PC-3 cells but triggered extensive cytoplasmic vacuolation in PC-3M cells. Electron microscopic images showed that the vacuoles lacked intracellular organelles and were derived from the endoplasmic reticulum (ER). Moreover, curcumin-induced vacuolation was not reversed by an apoptosis- or autophagy-related inhibitor, suggesting that vacuolation-mediated cell death differs from classical apoptotic and autophagic cell death. In addition, curcumin induced ER stress by triggering ROS generation, which was supported by the finding that treating cells with the antioxidant NAC alleviated curcumin-mediated ER stress and vacuolation-mediated death. An in vivo PC-3M orthotopic prostate cancer model revealed that curcumin reduced tumor growth by inducing ROS production followed by vacuolation-mediated cell death.

CONCLUSIONS: Our result indicated that curcumin acts as an inducer of ROS production, which leads to nonapoptotic and nonautophagic cell death via increased ER stress. These findings suggest, for the first time, that curcumin may represent a novel agent for metastatic prostate cancer treatment.

NO CONFLICT OF INTEREST

184 IL-8-induced stemness features of thyroid cancer cells

E. Pone[1], F. Liotti[2], N. Prevete[2,3], N. Kumar[3], R.M. Melillo[1,3]

[1] University of Naples "Federico II", Dipartimento di Medicina Molecolare e Biotecnologie Mediche, naples, Italy
[2] Universita' Di Napoli Federico II, Dipartimento di Scienze Mediche Traslazionali, Naples, Italy
[3] Istituto di Endocrinologia ed Oncologia Sperimentale, CNR "G. Salvatore", naples, Italy

BACKGROUND: Cancer stem-like cells (CSCs) are a rare population within tumors with stemness features, that can initiate and maintain tumors and may drive metastasis, recurrence and resistance to antineoplastic therapies. Multiple cytokine networks are crucial in regulating CSCs activity. We recently identified Interleukin-8 (IL-8/CXCL8) as a crucial paracrine/autocrine factor that sustains the epithelial-to-mesenchymal transition (EMT) and stemness features of thyroid cancer (TC) cells. Here, we analyze the molecular mechanisms that mediate IL8-induced stemness in TC.

MATERIAL AND METHODS: Stemness genes were identified by stimulating TC cells (8505c and TPC1) with IL8 and by interrogating PCR-based and promoter reporter low-density arrays focused on cancer stemness. Western blot analysis was exploited to assess the signaling pathway(s) (MAPK, PI3-K, STAT-3, NF-kB) involved in IL8-mediated stemness induction. The role of OCT4 and SOX2 in TC cells was studied by classical overexpression approaches. The features of OCT4/SOX2 overexpressing cells, including stemness and immunosuppressive activity were evaluated by RT-PCR, growth, viability and sphere formation assays, xenografts into nude mice.

RESULTS AND DISCUSSION: We found that IL-8 stimulation could significantly increase SOX2-, OCT4- and NANOG-responsive promoters, while IL-8 blockade caused the opposite effect. Consistently, 8505c thyrospheres, enriched in stem-like cells, displayed higher mRNA levels for OCT4, SOX2 and NANOG with respect to adherent cells, and IL-8 further increased their expression levels, being different signaling pathways necessary for this activity. Furthermore, IL-8 increased the immune suppressive potential of TC SCs by increasing the levels of membrane and secreted immunomodulatory molecules. Ectopical co-expression of OCT4 and SOX2 in TC cells could in part mimic IL-8 activity.

CONCLUSION: We found that IL-8 stimulation/overexpression in TC cells increased SOX2, OCT4 and NANOG levels. A combination of signaling pathways was required to fully activate IL-8-induced SOX2/OCT4 promoter activity in TC cells. The overexpression of OCT4 and SOX2 could at least in part substitute for IL-8 in inducing stemness features of 8505c and TPC1 cell lines. Our data suggest that targeting the IL-8 circuit might be a useful strategy to target TC SCs in advanced TC.

NO CONFLICT OF INTEREST

186 Cellular and molecular regulations of Pyruvate Kinase Type M2 mediated head and neck tumourigenesis

P.C. Huang[1], T.Y. Chen[1], W.C. Li[1]

[1] Institute of Oral Biology, Department of Dentistry- School of Dentistry- National Yang-Ming University- Taipei- Taiwan, Taipei, Taiwan

BACKGROUND: Unlike normal differentiated cells, cancer cells gain energy mainly through glycolytic pathway regardless of oxygen status. During glycolysis, muscle isoform of pyruvate kinase, PKM2, produces ATP in exchange for dephosphorylation of phosphoenolpyruvate (PEP) into pyruvate. The role of PKM2 in regulating carcinogenesis remained controversial; while PKM2 expression is enriched and correlated with poorer prognosis in brain, gastric and liver cancers, recent studies contradictively showed that PKM2 could be a tumour suppressor in breast cancer progression. The role of PKM2 mediated regulations during head and neck carcinogenesis, however, remained largely unknown.

MATERIAL AND METHOD: The PKM2 expression in human and mouse head and neck tumourous tissues and their normal counterparts was analysed. The role of PKM2 in regulating cellular growth and bioenergetics, both in vitro and in vivo, was further examined in shRNA mediated PKM2 deficient head and neck cancer cells.

RESULTS: Present study demonstrated that PKM2 protein expression is enriched in 4-nitroquinoline 1-oxide (4-NQO) induced mouse tongue tumour, human oral cancer tissues as well as human head and neck cancer cells indicating its potential role in promoting head and neck cancer progression. In the aspect of bioenergetics, knockdown of PKM2 in head and neck cancers resulted in an increase of mitochondrial Oxygen Consumption Rate (OCR) and decreased extracellular lactate level revealing a metabolic shift from aerobic glycolysis toward mitochondrial oxidative phosphorylation in response to PKM2 deficiency. Furthermore, PKM2 knockdown also activated mRNA expression of lipogenic and lipolytic associated genes showing a gross metabolic reprogramming in response to PKM2 deficiency. Interestingly, in cellular level, PKM2 knockdown mediated metabolic changes led to increased cell growth and greater resistance to chemotherapeutic agent cisplatin in vitro as well as greater xenografic tumour growth in vivo suggesting an alternative compensatory mechanism upon PKM2 deficiency to support head and neck cancer cell survival.

CONCLUSION: In summary, our findings demonstrated that PKM2 likely acts as a tumour suppressor during head and neck tumourigenesis.

NO CONFLICT OF INTEREST

187 Regulatory role of sterol regulatory element binding protein 1 during head and neck carcinogenesis

T.Y. Kuo[1], T.Y. Chen[1], W.C. Li[1]

[1] Institute of Oral Biology- School of Dentistry, National Yang-Ming University, Taipei, Taiwan

BACKGROUND: Cancer cells are metabolically distinct from differentiated cells by gaining energy and biomolecules mainly through glycolytic pathway and de novo lipogenesis. With intensive investigations in understanding the regulatory roles of glycolytic enzymes during cancer development, the contribution of lipogenic molecules in controlling tumorigensis, however, remained to be determined. Sterol regulatory element-binding proteins-1 (SREBP-1) is a master transcriptional regulators of fatty acid biosynthesis and known to directly target various lipogenic enzymes including ATP citrate lyase, acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase. In clinic, high level of SREBP-1 is correlated with poorer prognosis in many cancers revealing its significance in regulating tumor

progression. The importance of SREBP-1 mediated regulations during head and neck carcinogenesis is therefore defined in the present study.

MATERIAL AND METHODS: The SREBP-1 expression in human and mouse head and neck tumourous tissues and their normal counterparts was analyzed. Cellular basis of SREBP-1 in regulating cellular growth and bioenergetics, both in vitro and in vivo, was further examined in shRNA mediated SREBP-1 deficient head and neck cancer cells.

RESULTS: The result showed that nuclear SREBP-1 (active form) is abundantly expressed in 4-nitroquinoline 1-oxide (4-NQO) induced mouse tongue tumour, human oral cancer tissues as well as human head and neck cancer cell lines compared with their normal counterparts. In cellular level, SREBP-1 knockdown in head and neck cancer cells resulted in decreased cell growth in vitro, mainly through G0/G1 cell cycle arrest and deregulated apoptosis, and slower xenografic tumour growth in vivo implying an oncogenic role of SERBP-1 in controlling head and neck cancer cell growth. Surprisingly, by using Seahorse Bioanalyzer, the slower cycling SREBP-1 deficient head and neck cancer cells contained higher cellular ATP levels in comparison with control cells. Further analysis demonstrated that cellular ATP increase in response to SREBP-1 downregulation could be a result of an increased pyruvate metabolism and a greater lipolytic beta-oxidation activity implying an important role of SREBP-1 in controlling head and neck cancer cell metabolic homeostasis.

CONCLUSIONS: Our findings indicated that SREBP-1 is a key regulator for head and neck cancer cell survival and growth.

NO CONFLICT OF INTEREST

188 Characterization of Nrf2 activation in regulating cancer-initiating cell stemness properties

Y.T. Chen[1], C.W. Chang[1], J.F. Lo[1]

[1] Institute of Oral Biology- School of Dentistry, National Yang-Ming University, Taipei, Taiwan

BACKGROUND: Cancer-initiating cells (CICs) contribute to tumor initiation, progression and therapeutic resistance through critical molecular mechanisms. Redox homeostasis plays an important role in regulating cancer stemness. We recently found that enriched CICs through sphere formation lead to enhanced nuclear accumulation of Nrf2 (nuclear factor erythroid 2-related factor 2) and elevated expression of Nrf2-regulated genes. Herein, we aim to characterize the critical role of Nrf2, a master regulator of antioxidants and cellular stress responses against oxidative stress, upon the activation resulting in the translocation of Nrf2 and accumulated in nucleus to activate downstream genes. Thereafter, Nrf2 is exported out of the nucleus to become inactivated. Therefore, Nrf2 mutants intervening the shuttle in (NLS: nuclear localization signal) and shuttle out (NES: nuclear export signal) of nucleus will be generated to elucidate how activation of Nrf2 mutants to regulate CICs.

MATERIAL AND METHOD: In this study, we will use site-direct mutagenesis to establish cancer cell lines harboring Nrf2 NLS and NES mutation. The in vitro malignancy and in vivo tumorigenicity of the above cell lines will be further characterized.

RESULTS: The cancer cell lines stably expressing Nrf2 NLS and NES were established. Consequently, the intracellular localization of the mutant Nrf2 was affected.

CONCLUSIONS: Overall, these current and future result may reveal that multiple NLS and NES motifs within Nrf2 not only affect Nrf2 nuclear translocation but also regulate the induced cancer stemness in CICs. Additionally, the critical downstream genes upregulated by Nrf2 activation will be identified. At last, therapeutic targets identified through this line of research will be further characterized for future cancer treatment.

NO CONFLICT OF INTEREST

189 A novel high throughput screening system for in vitro evaluation of anticancer compounds under anchorage-independent conditions

T. Nisihino[1], A. Aihara[1], N. Fukazawa[1], K. Ostsuka[1]

[1] Nissan Chemical Industries- LTD., Biological Research Laboratories, Saitama, Japan

BACKGROUND: Traditional method for evaluating anticancer drug has generally employed single layer of cells grown on plastic surface (2-dimentional cell (2D) cultures), which is physiologically different from the natural environment of the cells. Recently, a number of approaches have been developed to generate 3D cell culture models for cancer study. However, many challenges remain, such as applying them into high throughput screening (HTS) systems and improving the efficiency of anticancer drug discovery. Previously, we screened several polymers for activity to suspend cells or cell spheroids homogeneously in liquid medium, and identified gellan gum (FP001), a bacteria-derived polysaccharide. FP001 promoted cell dispersion in the medium and improved proliferation of a wide range of cancer cell lines under low attachment conditions by inhibiting the formation of excess size of spheroids.

MATERIAL AND METHODS: Several cancer cell lines including A549 and SKOV3 cells were cultured in medium containing FP001 under low attachment conditions. The cultured spheroids were characterized in terms of cell growth, apoptosis, cell cycle, and susceptibility to anticancer drugs.

RESULTS: Cancer cells cultured with FP001-containing medium were more susceptible to inhibitors of epidermal growth factor (EGF) signaling than those cultured under attachment conditions. We also showed that ligands of EGF receptor family clearly enhance proliferation of SKOV3 cells under the anchorage-independent condition with FP001. These data suggest that FP001 promotes the proliferation of spheroid forming-cancer cells which allow practical sensitivity to anticancer compounds. We also constructed a cell proliferation assay system for anticancer drug screening using 384-well low attachment plates and FP001-containing medium. We evaluated 50,000 chemical compounds by using the system and obtained several specific growth inhibitors under low attachment conditions.

CONCLUSION: Our studies achieved a novel 3D cell culture method which is available for HTS of anticancer agents, and particularly suitable for evaluation of molecularly-targeted anticancer drugs. The approach using FP001 will be of value in the development of new and useful technologies for anticancer drug discovery.

NO CONFLICT OF INTEREST

190 Oncogene-driven sensitivity to ferroptotic cell death following deprivation of cystine

I. Poursaitidis[1], X. Wang[1], C. Thomas[1], C. Labuschagne[2], S. Cramer[3], K. Triplett[3], M. Seckl[4], S. Rowlinson[5], E. Stone[3], R. Lamb[6]

[1] Institute of Translational Medicine, Molecular and Clinical Cancer Medicine, Liverpool, United Kingdom
[2] Cancer Research UK Beatson Institute, Glasgow, United Kingdom
[3] University of Texas at Austin, Dept of Chemical Engineering, Austin, USA
[4] Imperial College London, Diuision of CRUK laboratories, London, United Kingdom
[5] Aeglea Biotherapeutics, Austin, USA
[6] Liverpool Hope University, Department of Health Sciences, Liverpool, United Kingdom

INTRODUCTION: The presence of activating oncogenic mutations in cancer cells leads to altered utilization of nutrients. Previous studies demonstrated the importance of individual amino acid nutrients such as glutamine in the context of a specific cancer/oncogenic mutation. Our initial hypothesis was that cancer cells airtight exhibit additional metabolic vulnerabilities with respect to their requirement for particular amino acids.

MATERIALS AND METHODS: In vitro requirements for individual AA nutrients were studied by selective depletion of each specific amino acid in diploid isogenic Human Mammary Epithelial (HME) cells expressing commonly activated oncogenes. Analyses included cell viability assays, FACS measurement of ROS, Western blotting for protein quantification and siRNA mediated knockdown of protein expression. The study was expanded in non-small cell lung cancer (NSCLC) cell lines and in an in vivo xenograft model of a NSCLC cell line NCI-H1650.

RESULTS AND DISCUSSION: Depletion of cystine was found to lead to cell death by ferroptosis, an iron-dependent type of death associated with accumulation of reactive oxygen species (ROS) and lipid peroxides, in HME cells expressing an activated epidermal growth factor receptor (EGFR). Inhibition of EGFR, or mitogen-activated protein kinase (MAPK) signaling, as well as addition of ROS scavengers, was found to reverse ferroptosis by blocking the production of ROS. Glutathione peroxidase 4 (GPX4), an enzyme involved in ferroptosis that can detoxify lipid peroxides, was found to be reduced in expression when MAPK was activated, and knockdown of GPX4 in wild type HME cells sensitised cells to ferroptosis. NSCLC cell lines that had the highest levels of MAPK phosphorylation were also found to be most sensitive to ferroptosis. Finally, enzymatic depletion of cystine in a xenograft model of NCI-H1650 reduced tumour growth.

CONCLUSION: Our result indicate the importance of cystine in cancer cells as an important component of their anti-oxidant defense. These findings suggest the possibility of exploiting the inability of some MAPK-driven cancer cells to overcome oxidative stress following deprivation of cystine therapeutically.

CONFLICT OF INTEREST

Ownership: "Everett Stone is an inventor on intellectual property related to part of this work and Everett Stone, and Scott Rowlinson have an equity interest in Aeglea Biotherapeutics."

191 Single cell tracking of multiple myeloma B lineage clones

L. Hansmann[1], A. Han[2], L. Penter[1], M. Liedtke[3], M. Davis[2]

[1] Charite Berlin, Hematology- Oncology- Tumor Immunology, Berlin, Germany
[2] Stanford Uniuersity, Microbiology and Immunology, Stanford, USA
[3] Stanford Uniuersity, Hematology and Oncology, Stanford, USA

BACKGROUND: Multiple myeloma is characterized by the clonal expansion of malignant plasma cells in the bone marrow. But the phenotypic heterogeneity and the contribution of less predominant clones to disease biology have been controversial. Here we used a novel combination of multi-parameter FACS single cell index-sorting and next generation sequencing of immunoglobulin light chain genes to determine whether cells bearing the dominant multiple myeloma immunoglobulin rearrangement occupy phenotypic compartments other than that of plasma cells.

METHODS: We combined 13-parameter FACS index sorting and t-Stochastic Neighbor Embedding (t-SNE) visualization with high-throughput single cell

immunoglobulin sequencing to track B lineage clones across different stages of human B cell development.

RESULTS: As expected, the predominant B lineage clones in different individuals preferentially mapped to the plasma cell compartments, albeit phenotypically altered from wild type. Interestingly, up to 1.2 % of cells of the predominant clones co-localized with B lineage cells of a normal phenotype. In addition, minor clones with distinct immunoglobulin sequences were detected in up to 9% of sequenced cells but only 2 out of 12 of these clones showed aberrant immune phenotypes. The CDR3 sequences of these minor clones were not related to the predominant one and the majority showed intra-clonal silent nucleotide differences within the CDR3s and varying frequencies of somatic mutations in the immunoglobulin genes. Therefore, the phenotypic plasticity of multiple myeloma cells in the bone marrow is not confined to aberrant-phenotype plasma cells but extends to low frequencies of normal-phenotype B cells.

CONCLUSION: We present a novel methodology that allows one to analyze the phenotypic range that a given B or plasma cell can occupy, with the immunoglobulin sequence acting as a 'bar code" to identify clonal progeny at the single cell level. The clonal B lineage expansion in multiple myeloma is not restricted to aberrant phenotype plasma cells in line with the recently reported success of B cell-targeting cellular therapies in some patients. Additional less predominant clones result from unrelated parallel malignant, or, in the majority of clones, non-malignant expansion.

NO CONFLICT OF INTEREST

192 Differential expression of MST2 and MST4 among the molecular subgroups of Medulloblastoma

M. Baroni[1], A.F. Andrade[1], G.A.V. Cruzeiro[1], C.A.P. Correa[1], C.G. Carlotti Jr[2], S. Aguiar[3], J.A. Yunes[3], S.R. Brandalise[3], C.A. Scrideli[4], L.G. Tone[1]

[1] *Ribeirao Preto Medical School - Univeersity of Sao Paulo, Genetics, Ribeirao Preto - SP, Brazil*
[2] *Ribeirao Preto Medical School - Univeersity of Sao Paulo, Surgery, Ribeirao Preto - SP, Brazil*
[3] *Centro Infantil Boldrini, Pediatrics, Campinas - SP, Brazil*
[4] *Ribeirao Preto Medical School - Univeersity of Sao Paulo, Pediatrics, Ribeirao Preto - SP, Brazil*

BACKGROUND: Medulloblastoma (MB) is the most common tumor of central nervous system in childhood. The MB molecular classification was based on several studies dividing in four molecular subgroups: WNT, SHH, Group 3 and Group 4, facilitating diagnosis and treatment. Despite the new advances, about one third of MB patients die due to recurrence and most survivors suffer from long-term side effects. The study for the understanding of tumor biology, molecular pathways and for the discovery of new therapeutic targets is necessary and urgent. In this search for potential targets, we found MST2 and MST4, members of MST kinases family (Mammalian Sterile Twenty Like). Several studies have shown the involvement of MSTs in carcinogenesis of different tumor types, and some studies show the relationship with developmental pathways associated with MB. Therefore, the aim of this study was to analyze the expression of MST kinases in MB samples, and investigated the difference of MST expression among molecular subgroups.

MATERIAL AND METHOD: We analyzed the expression of MST2 and MST4 in 48 samples of MB, 4 cell lines of MB and 5 non-neoplastic human cerebellum controls by qRT-PCR. For this analysis, it was used Taqman gene probes and the endogenous controls were GUS and HPRT. Comparisons of MST expression in tumor samples versus cerebellum non-neoplastic and molecular subgroups was performed using Mann-Whitney test.

RESULTS: MST2 is upregulated in tumors (p=0,015) and cell lines (p=0,032) when compared to non-neoplastic cerebellum, and the expression of MST4 have no difference comparing tumor and cell lines with controls. Analysis of gene expression among molecular subgroups showed difference of MST2 expression comparing Group Shh/Wnt and Group 3/4, MST2 is upregulated in the Group Shh/Wnt (p=0,033). Once MST2 is a tumor suppressor gene, down expression of MST2 may be correlated with poor prognosis of subgroup 3/4. MST4 expression is also different between the molecular subgroups. Group Wnt has MST4 downregulated compared with others groups: Wnt x Shh (p=0,001), Wnt x Group 3 (p=0,027) and Wnt x Group 4 (p=0,006). MST4 is a pro-tumorigenic effector in some tumors. So down MST4 expression may be correlated with good prognosis of Wnt subgroup.

CONCLUSION: The MST2 and MST4 expression shows relationship to molecular subgroups of MB and may be related with molecular pathways like Wnt and Shh. Studies and novel assays are being conducted to understand better these findings.

NO CONFLICT OF INTEREST

193 AKR1B10 promotes breast cancer cell migration and invasion via activation of ERK signaling

L. Dixian[1], P. Xuhong[2], L. Jia[2], H. Xinglin[3], H. Zheng[1]

[1] *the First People's Hospital of Chenzhou, Translational Medicine Institute, Chenzhou, China*
[2] *The First People's Hospital of Chenzhou, Department of ultrasound Medicine, Chenzhou, China*
[3] *The First People's Hospital of Chenzhou, Department of Dermatology, Chenzhou, China*

BACKGROUND: AKR1B10, human member of the aldo-keto reductase (AKR) superfamily, is known to be significantly induced in the cells of various cancers such as breast cancer. In the present study, we demonstrated the potential role and mechanism of AKR1B10 in the invasion and migration of breast cancer cells.

METHODS: The expression level of AKR1B10 in breast carcinoma, para-carcinoma and cancer tissues were detected by immunohistochemical evaluation and real-time polymerase chain reaction (RT-PCR), and the correlationships between AKR1B10 expression and clinicopathological features in breast cancer patientsκn=131κ were investigated.

RESULTS: We found that AKR1B10 expression was increased in malignant tissues, which was correlated positively with tumor size, lymph node metastasis (p<0.05). MCF-7/AKR1B10 cells displayed a higher ability of migration (43.57±1.04%) compared with MCF-7/vector cells (29.12±1.34%) in wound healing assay, and the migrated cell number of MCF-7/AKR1B10 was more (418.43±9.62) than that of MCF-7/vector (222.43±17.75) in transwell migration assay without matrigel. We further confirmed MCF-7/AKR1B10 cells invaded faster compared with MCF-7/vector cells by transwell matrigel invasion assay. Finally, we found AKR1B10 induced the migration and invasion of MCF-7 cells by activating EKR signaling, which promoted the expressions of MMP2 and vimentin. PD98059, an specific inhibitor of the activation of MEKERK1/2 inhibitor, blocked the migration and invasion by inhibiting the expression of MMP2 and vimentin.

CONCLUSIONS: AKR1B10 is overexpressed in breast cancer, and promotes the migration and invasion of MCF-7 by activating vimentin-ERK signaling pathway.

NO CONFLICT OF INTEREST

194 Prrx1 overexpression promotes epithelial-mesenchymal transition through control of twist and matrix metalloprotease 3 in lung cancer

J.J. Hung[1]

[1] *Taipei Veterans General Hospital, Department of Surgery, Taipei, Taiwan*

BACKGROUND: Lung cancer is the main cause of cancer-related death worldwide. Epithelial-mesenchymal transition (EMT) is considered to be one of the major molecular mechanisms inducing tumor invasion and metastasis. Prrx1, a newly found EMT-inducing transcription factor, plays a part in the invasive, migratory phenotype of breast carcinoma cells undergoing metastasis. In contrast to other EMT-activators, Prrx1 has been reported to suppress stemness trait. The relationship between Prrx1 and EMT and metastasis in lung cancer has not been well demonstrated.

MATERIAL AND METHODS: H1299-Prrx1 cell lines were generated by transfecting the pcDNA3.1-Prrx1 plasmid into H1299 cells. The H1299-Prrx1-Twisti cell lines were generated by transfecting the pSUPER-Twisti plasmid into H1299-Prrx1 cells. Western blotting was done to demonstrate expression of Snail, Twist, E-cadherin, and vimentin in H1299, H1299-Prrx1 and H1299-Prrx1-Twisti cells. Immunofluorescence staining was done to demonstrate expression of E-cadherin and vimentin in these cells. Soft agar clonogenicity assay was done in these cell lines to demonstrate anchorage-independent growth. Cell migration and invasiveness assay were also done in these cells to demonstrate their invasision and migration ability.

RESULTS: Increased Prrx1 expression correlated with the upregulation of the Twist mRNA and protein expression. Western blot analysis showed that H1299-Prrx1 cells exhibited the shift to EMT phenotypes including downregulation of the epithelial marker E-cadherin and upregulation of the mesenchymal marker vimentin. Repression of endogenous Twist expression in H1299-Prrx1 cells reversed the EMT phenotype. Immunofluorescence staining demonstrated increased cytoplasmic expression of vimentin in H1299-Prrx1 cells and decreaseded vimentin expression in cytoplasm in H1299-Prrx1-Twisti cells. Matrix Metalloprotease 3 (MMP3) expression increased in H1299-Prrx1 cells, while MMP3 were down regulated in H1299-Prrx1-Twisti cells. Soft agar clonogenicity assay showed the significantly increased colony formation activity in the H1299-Prrx1 clones and significantly decreased colony formation activity in the H1299-Prrx1-Twist1 clones. Prrx1 overexpression increased the migration and invasiveness of H1299 cells. Migration and invasion were significantly decreased in H1299-Prrx1-Twisti cells.

CONCLUSIONS: All these result demonstrate that Prrx1 overexpression promotes EMT through upregulation of Twist and MMP3 in lung cancer.

NO CONFLICT OF INTEREST

195 Loss of uPAR function and suppression of malignant cell behavior by a GPI-specific phospholipase C

M. van Veen[1], E. Matas-Rico[1], K. Van de Wetering[2], D. Leyton-Puig[1], K. Kedziora[1], V. De Lorenzi[3], K. Jalink[1], N. Sidenius[3], A. Perrakis[4], W. Moolenaar[1]

[1] *The Netherlands Cancer Institute, Cell Biology, Amsterdam, Netherlands*
[2] *The Netherlands Cancer Institute, Molecular Oncology, Amsterdam, Netherlands*
[3] *The FIRC Institute of Molecular Oncology, Cell Matrix Signaling, Milan, Italy*
[4] *The Netherlands Cancer Institute, Biochemistry, Amsterdam, Netherlands*

BACKGROUND: The urokinase receptor (uPAR) is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein that promotes tissue remodeling and tumor progression. uPAR is highly expressed in many cancers, often correlating with poor prognosis. uPAR mediates matrix degradation through protease recruitment and enhances tumor cell migration and signaling through vitronectin binding and interaction with integrins. Full-length uPAR is released from the cell surface, but the mechanism and significance of uPAR shedding are unknown.

METHODS: Cell biological and biochemical assays; super-resolution microscopy; homology modeling; knockdown/knockout studies; patient survival analysis.

RESULTS: We find that uPAR is released from the cell surface through GPI-anchor cleavage by a multi-pass membrane glycerophosphodiesterase, termed GDE3, acting in a phospholipase C (PLC)-like manner. By contrast, GDE3's closest relative GDE2 fails to cleave uPAR. We show that, by shedding uPAR, GDE3 abrogates uPAR-driven cell adhesion, spreading and lamellipodia formation on vitronectin. In breast cancer cells, high GDE3 expression depletes endogenous uPAR resulting in a less transformed phenotype, as shown by reduced matrix degradation, cell motility and colony formation. Consistent with this, high GDE3 expression correlates with higher survival probability in breast cancer patients.

CONCLUSIONS: Our findings establish GDE3 as a cell-intrinsic GPI-PLC that negatively regulates the uPAR signaling network and thereby suppresses the malignant phenotype of uPAR-positive cancer cells.

NO CONFLICT OF INTEREST

196 ETV4 over-expression in prostate cells down-regulates p21 both in vitro and in vivo

A. Pellecchia[1], I. Cosi[2], E. De Lorenzo[1], M. Sica[1], R. Notaro[1], M. De Angioletti[1,2]

[1] Istituto Toscano Tumori - AOU Careggi, Core Research Laboratory - ITT, Florence, Italy
[2] ICCOM-CNR, ICCOM, Sesto Fiorentino, Italy

BACKGROUND: The ETS transcription factors are deregulated in several tumors. In most prostate cancer ETS proteins (ERG, ETV1, ETV4, ETV5) are over-expressed because of recurrent chromosomal translocations that bring an ETS gene under the control of a promoter of a gene highly expressed in the prostate. We have previously found that, at variance with other ETS protein, ETV4 promotes cell proliferation in human prostate cell lines. Here we have investigated, in vitro and in vivo, how ETV4 expression drives an increased proliferation rate in prostate cells.

MATERIAL AND METHODS: We have assessed the proliferation rate of prostate cells in: (1) human cell lines by either over-expression (RWPE1, PNT1) or silencing (PC3) of ETV4; (2) mice with specific ETV4 prostate expression. The expression level of genes involved in cell cycle regulation has been measured by real time RT-PCR and Western blot. The regulation of the CDK1A gene (codifying for p21-WAF1/CIP1) by ETV4 has been investigated by ChIP and Dual-Luciferase assays.

RESULTS AND DISCUSSION: We confirmed that in human prostate cell lines ETV4 expression is associated with an increased proliferation rate and with variation in cell cycle. In addition, we found that the proportion of Ki67+ prostate cells was slightly but significantly increased also in 5 months-old ETV4-transgenic mice compared to wild type mice.We have quantified the expression of several cell-cycle genes; among them we found that the mRNA levels of CDK1A is reduced not only in prostate cell lines over-expressing ETV4 but also in vivo in the ETV4 mice.

To determine how ETV4 regulates the expression of CDKN1A we performed ChIP and luciferase assays. In the 2 kb upstream the transcriptional start site there are 2 putative ETV4 binding sites (BS): distal (-1409/-1403) and proximal (-704/-696) BS. ChIP assay suggested that ETV4 binds the proximal but not the distal BS of CDKN1A promoter. The Luciferase assay performed in several cell lines using a vector, in which the firefly luciferase was expressed under the control of the WT proximal ETV4 BS, showed that ETV4 over-expression induce a reduction of the relative luciferase expression, while the normal level is restored when this site is mutated.

CONCLUSION: ETV4 over-expression increases the proliferation rate of prostate cell in vitro and also in vivo. This increased proliferation rate is associated with the down regulation of p21, that seems to result from the binding of ETV4 to the p21 promoter.

NO CONFLICT OF INTEREST

197 Modeling uveal melanoma using zebrafish to predict suitable therapeutic strategies

V. Anelli[1], F. Precazzini[1], M. Mione[1]

[1] Cibio- University of Trento, Laboratory of Experimental Cancer Biology, Trento, Italy

INTRODUCTION: Uveal melanoma (UM) is the most common primary cancer of the eye and its prognosis is strongly influenced by the occurrence of metastasis, which are both rapidly developing and mostly fatal. GNAQQ209L/P and GNA11Q209L are the most frequent driver mutations, often co-occurring with mutations in the tumor suppressor BAP1, a histone H2A deubiquitinating enzyme that plays a key role in chromatin remodeling. Due to a lack of suitable animal models, the mechanisms through which mutations in these genes cause or cooperate in UM initiation and metastatic spread are still largely unknown.

MATERIAL AND METHOD: We have generated zebrafish models of UM using a binary Gal4/UAS system, where the expression of frequently mutated forms of GNAQ and GNA11 human genes in zebrafish uveal melanocytes is driven by a kita promoter. To recapitulate molecular aspects of the human pathology, we will generate somatic mutations of BAP1 in the same melanocytes expressing the mutated human oncogene(s) using cell-specific Crispr/Cas9 technology. In parallel, we are performing a high throughput chemical screen using a transgenic zebrafish model already available in our lab, where oncogenic HRAS expressed under kita promoter leads to hyperpigmented larvae that develop uveal melanocytic aggregates by 3 dpf and UM in adults.

RESULTS AND DISCUSSION: We will report on the frequency of development of UM in the new zebrafish transgenic models and on the positive hits found in the chemical screen. Strategies to combine different mutations and somatic loss of function of tumor suppressors will be reported. Key modulators will be silenced by cell-specific Crispr/Cas9 technology or by specific inhibitors. Signaling pathways and cellular responses affected by the specific mutations will be studied both in vivo and in vitro.

Finally, positive hits identified through the chemical screen will be tested, alone and in combination, in the new zebrafish UM models and in human uveal melanoma cell lines for their ability of interfering with melanocyte proliferation and migration.

CONCLUSION: The data obtained from this study will be used in the context of the UM Cure2020 project (www.umcure2020.org) and to predict suitable therapeutic strategies for human UM.

NO CONFLICT OF INTEREST

198 Environmental control of plasma cell fitness in multiple myeloma: A novel malignant co-optation of a metabolic and immune checkpoint

A. Romano[1], J.M. Garcia Manteiga[2], V. Simeon[3], V. Espina[4], E. Milan[1], M. Ghizzinardi[5], F. Cremasco[1], F. Ciceri[5], F. Di Raimondo[6], S. Cenci[1]

[1] San Raffaele Scientific Institute, Diuision of Genetics and Cell Biology- Age diseases related Unit, Milan, Italy
[2] San Raffaele Scientific Institute, Center for Translational genomics and BioInformatics, Milan, Italy
[3] IRCCS - CROB Referral Cancer Center of Basilicata, Laboratory of Pre-clinical and Translational Research, Rionero in Vulture, Italy
[4] George Mason Uniuersity, Center for Applied Proteomics and Molecular Medicine, Manassas, USA
[5] San Raffaele Scientific Institute, Department of onco-hematology, Milan, Italy
[6] Azienda Ospedaliera Policlinico e Vittorio Emanuele di Catania, Diuision of Hematology, Catania, Italy

BACKGROUND: The bone marrow (BM) environment plays a crucial role in the incurable plasma cell (PC) malignancy multiple myeloma (MM). Among putative pro-tumoral players are BM-derived high-density neutrophils (HDNs), but their role in MM is unknown. We previously disclosed that autophagy is essential for MM cell proliferation and survival. We hypothesized that in MM HDNs sustain PC fitness through autophagy.

MATERIALS AND METHODS: We integrated multiple unbiased and hypothesis-driven approaches: (1) ex vivo multiplexed phosphoprotein cell signaling analyses by Reverse Phase Protein Arrays (RPPA) on PCs from MM patients (N=35), (2) gene expression profiles (GEP) of patient-derived circulating HDNs (N=60 MM, 30 MGUS, 30 healthy controls), (3) metabolomic profiling by UHPLC/GC-MS of ad hoc-collected BM and peripheral plasma samples (N=16 MM, 17 smoldering MM, 30 MGUS, 29 controls), and (4) functional and expression in vitro studies on human MM cell lines.

RESULTS: RPPA revealed higher activity of the PI3K/AKT/mTOR pathway associated to distinctive expression of the autophagic proteins ATG5, LC3B and p62 in patients with the most aggressive clinical features (refractoriness, short time to progression, bone disease).

GEP analyses disclosed remarkable arginase-1 (Arg-1) expression in MM-HDNs, confirmed by qRT-PCR.

Arginine depletion is an established T cell-immunosuppressive mechanism. Indeed, MM-HDNs showed immunosuppressive activity in co-cultures with allogeneic T-cells, which was reverted by the selective Arg-1 inhibitor nor-NOHA. Metabolic profiling independently identified arginine significantly reduced along MM evolution. Notably, low arginine and high Arg-1 circulating levels in MM patients' sera at diagnosis predicted clinical outcome, being associated to shorter progression-free survival.

In vitro, selective arginine starvation was capable of raising both mTOR and autophagic activity in MM cells, through the sensor kinase GCN2, resulting in increased mTOR, p-S6RP, CHOP, p62, IRF-4, and Blimp-1 expression associated to an increased availability of ATP; conversely, stable lentiviral p62 silencing reduced Blimp-1, induced significant decrease of intracellular ATP and caused the in vitro extinction of MM cell lines within 10 days of culture.

CONCLUSIONS: Taken together, our findings disclose a novel micro-environmental circuit co-opted by MM evolution, whereby immunosuppressive HDNs sustain PC cell fitness and survival through selective arginine depletion.

NO CONFLICT OF INTEREST

199 Repurposing approved drugs to inhibit epithelial-mesenchymal transition in cancer; A systems pharmacology approach

F. Barneh[1], M. Salimi[2], P. Nickchi[3], F. Goshadrou[1], H. Zali[4], M. Mirzaie[5], A.R. Aref[6], M. Jafari[7]

[1] Shaheed Beheshti Uniuersity of Medical Sciences, Faculty of Paramedical Sciences, Tehran, Iran
[2] Pasteur Institute of Iran, Physiology and Pharmacology, Tehran, Iran
[3] Simon Fraser, Department of Statistics and Actuarial Sciences, British Columbia, Canada
[4] Shaheed Beheshti Uniuersity of Medical sciences, School of Aduanced Technologies in Medicine, Tehran, Iran
[5] Tarbiat Modares Uniuersity, Department of Applied Mathematics, Tehran, Iran
[6] Haruard Medical School, Dana-Farber Cancer Institute, Boston, USA
[7] Pasteur Institute of Iran, Medical Biotechnology Department, Tehran, Iran

BACKGROUND: Progressive tumors exploit Epithelial-Mesenchymal Transition (EMT) to confront with the environmental stressors during their invasion. Efficient inhibition of EMT requires optimization of multiple druggable targets. Here, we introduce a systems pharmacology approach to identify proper target combinations that potentially inhibit several pathways in EMT. We also validate anti-EMT efficacy

of a drug combination set to hit the proposed targets in a microfluidic three-dimensional (3D) device which closely simulates tumor microenvironment.

MATERIAL AND METHOD: We integrated multiple biologist-friendly bioinformatic resources which apply various statistical methods for interpretation of high-throughput data with network-based analysis and generated insights into the most important druggable targets in published EMT gene signatures. We then proposed a hypothetical drug set from the in-silico experiments that if used together can inhibit EMT. We also validated anti-EMT efficacy of drug combination by measuring cell dispersion in a microfluidic 3D device with collagen embedded A549 lung cancer cells co-cultured with HUVEC endothelial cells.

RESULTS AND DISCUSSION: Despite lack of significant overlap among EMT-associated gene signatures in various cancers, epigenetic modifications in histones were the common mechanism in mesenchymal transformation of thirteen solid cancers in our study. Kinases were other important mediators to fulfill EMT, among which, SRC kinase and IκB kinase (IKK) served as principal targets to regulate mesenchymal reprogramming. Accordingly, we selected prototypes of three Food and Drug Administration (FDA)-approved drug classes including a Histone Deacetylase Inhibitor (HDACI), a SRC kinase inhibitor and an IKK inhibitor to manipulate the identified set of targets in EMT. Interestingly, when using kinase inhibitors alone, IKK inhibitor was more potent than SRC kinase inhibitor to prevent cell dispersion. However, combination of drugs significantly inhibited proliferation and dispersion of tumor cells and their invasion toward endothelial cells in a synergistic manner.

CONCLUSION: We optimized a systems-based approach to repurpose three FDA-approved drugs that showed anti-EMT properties in a 3D in-vitro model. Owing to the safety profile of these already prescribed drugs, their entry into in-vivo and clinical trials in significantly expedited to inhibit metastasizing tumors.

NO CONFLICT OF INTEREST

200 Targeting the mitogen activated protein kinase erk5 in human melanoma

A. Tubita[1], S. Gagliardi[2], I. Tusa[1], S. Pandolfi[2], J. Wang[3], X. Deng[4], N. Gray[4], B. Stecca[2], E. Rovida[1]

[1] University of Florence, Department of experimental and clinical biomedical sciences, Florence, Italy
[2] Istituto Toscano Tumori, Core Research Laboratory, Florence, Italy
[3] Dana-Farber Cancer Institute, Department of Biological Chemistry and Molecular Pharmacology, Boston, USA
[4] Dana-Farber Cancer Institute, Department of Cancer Biology, Boston, USA

INTRODUCTION: Melanoma is the most aggressive and lethal among skin cancers, characterized by high metastatic potential and marked heterogeneity. Available treatments for melanoma, including immunotherapy or inhibitors for the BRAF-MEK1/2, have improved greatly survival for this disease although are not applicable to all patients and/or their long term benefits are still unsatisfactory. Therefore, there is urgent need to identify novel possible targets involved in melanoma growth. ERK5/BMK1 is a member of the Mitogen-Activated Protein Kinases (MAPK) family and regulates cell functions critical for tumor development. Indeed, several studies reported a direct involvement of ERK5 in several types of cancer including myeloma, prostate and breast cancer and hepatocellular carcinoma. However, no data have been reported about a possible role of ERK5 in melanoma.

MATERIAL AND METHOD: Cell lines and patient-derived primary melanoma cells (wild type B-RAF: SSM2c and M26c; BRAFV600E: A375, SK-Mel-5, SK-Mel-28, M42, 501-Mel, expressing; NRASQ61R: SK-Mel-2; MeWo) have been used for in vitro and in vivo experiments. ERK5 inhibition was achieved using ERK5 and MEK5 inhibitors or lentiviral vectors encoding shRNA specific for ERK5.

RESULTS: In silico data analysis indicated that components of the ERK5 pathway (MEKK2, MEKK3, MEK5, ERK5, MEF2D) are altered (alterations of gene copy number, mutations and mRNA upregulation) in up to 47% melanoma patients. Accordingly, we found that ERK5 is consistently expressed in primary melanocytes and in commercial and patients derived melanoma cell lines. On that basis, we investigated the role of ERK5 in melanoma cell growth. In vitro, pharmacological or genetic inhibition of ERK5 decreases 2D colony formation and the number of viable cells in several melanoma cell lines. Moreover, subcutaneous xenografts of LV-C- or LV-shERK5- transduced A375 or SSM2c cells, showed that inhibition of ERK5 strongly reduces tumor growth compared to control mice. In addition, combined treatment with ERK5 and BRAFV600E inhibitors, showed additive effects in reducing melanoma cell growth compared to single agents alone.

CONCLUSIONS: Our result identify ERK5 as a critical regulator of melanoma growth and point toward the possibility of targeting ERK5 for melanoma treatment.

NO CONFLICT OF INTEREST

201 Extracellular visfatin increases sirtuin 1 activity and cause p53 deacetylation by producing NAD

M. Nourbakhsh[1], K. Behrouzfar[2], M. Alaiee[2], A. Golestani[2]

[1] Iran University of Medical Sciences- Faculty of Medicine, Biochemistry, Tehran, Iran
[2] Tehran University of Medical Sciences- Faculty of medicine, Clinical Biochemistry, Tehran, Iran

BACKGROUND: Breast cancer is one of the most prevalent neoplasms and the second cause of cancer mortality in women. Increased visfatin levels in serum and breast tumor tissue is frequently observed in breast cancer patients and is associated with poor prognosis. Visfatin is secreted as an adipokine from visceral adipose tissue as well as immune cells such as macrophages. Intracellular form of visfatin which is called nicotinamide phosphoribosyltransferase (NAMPT), functions as the key enzyme in nicotinamide adenine dinucleotide (NAD) biosynthesis, but the enzymatic activity of its extracellular form is still under debate. NAD is needed for the activity of deacetylase enzymes like sirtuin 1 (SIRT1) which increases cell viability. p53 deacetylation by SIRT1 is one of the mechanisms by which SIRT1 protects cells from apoptosis. The aim of this study was to investigate the effects of visfatin on NAD levels, SIRT1 activity and p53 acetylation in MCF-7 breast cancer cells.

MATERIAL AND METHODS: MCF-7 cells were cultured and treated by recombinant visfatin for different time points. Total NAD levels were measured both in cell lysate and the extracellular medium by an enzymatic method using NAD/NADH assay kit. SIRT1 activity assessment was performed by measuring fluorescence intensity from a fluoro-substrate peptide in the presence of NAD. To investigate the effect of visfatin on p53 acetylation, SDS-PAGE electrophoresis followed by western blotting was performed by antibodies against p53 and its acetylated form. Beta-actin polyclonal antibody was used as normalizer. Cell proliferation was assessed by BrdU incorporation test.

RESULTS: Both intracellular and extracellular concentrations of NAD were elevated by visfatin. Additionally, visfatin significantly increased SIRT1 activity 48 h after treatment. It also reduced p53 acetylation. Cell proliferation was enhanced in the presence of visfatin, however, this effect was abolished by FK866 which is an inhibitor of enzymatic activity of visfatin.

CONCLUSION: Visfatin can enhance SIRT1 activity and p53 deacetylation by increasing NAD production. These findings can explain the relationship between visfatin and breast cancer progression.

NO CONFLICT OF INTEREST

202 LIGHT promotes osteoclast activation in non-small cell lung cancer bone metastases

I. Roato[1], D.C. Belisario[1], L. Buffoni[2], S. Colucci[3], M. Grano[3], R. Ferracini[4], G. Brunetti[3]

[1] A.O.U. Città della Salute e della Scienza, CeRMS, Torino, Italy
[2] University of Turin, Department of Oncological Sciences, Torino, Italy
[3] University of Bari, Department of Basic and Medical Sciences, Bari, Italy
[4] C.T.O Hospital, Department of Orthopaedic Oncology, Torino, Italy

INTRODUCTION: Non-small cell lung cancer (NSCLC) frequently metastasizes to bone, since NSCLC cells find a favourable soil in bone microenvironment due to the factors released by the bone matrix and the immune system. LIGHT, is a cytokine produced by monocytes, neutrophils, tumor and T cells. It regulates bone cell activity and it is known to be involved in bone loss associated to arthritis and in Multiple Myeloma osteolysis, where it stimulates osteoclastogenesis. We aim to explore the role of LIGHT in the osteolytic bone metastatic process induced by NSCLC.

MATERIALS AND METHODS: We analysed by flow citometry the expression of LIGHT on CD4 and CD8 T cells, CD14 monocytes, CD16 neutrophils from peripheral blood of NSCLC patients and controls. We dosed serum LIGHT levels in patients and controls. We studied whether LIGHT interfered with the osteoclast (OC) formation in vitro by plating PBMCs from NSCLC patients and healthy controls with and without stimulating factors (M-CSF and RANKL).

RESULTS AND DISCUSSION: CD8 showed a low expression of LIGHT, whereas CD4 expressed it at high level, but without significant differences between the groups. Interestingly, we showed that LIGHT expression was significantly higher in monocytes from bone metastatic patients than non-bone metastatic ones. PBMCs from patients with bone metastases spontaneously differentiated into OCs, but when we added an anti-LIGHT monoclonal antibody (mAb), we detected a significant dose-dependent inhibition of osteoclastogenesis. For PBMCs of patients without bone metastases and healthy controls, we induced osteoclastogenesis with M-CSF and RANKL, and when we added anti-LIGHT mAb, we again reported a significant decrease in OC formation, but only with the highest dose of the mAb, suggesting a synergic effect with RANKL. We also dosed LIGHT serum levels without detecting significant differences between patients and controls.

CONCLUSIONS: Our data demonstrated that the high expression of LIGHT sustained the formation of OCs, which are the main responsible for osteolytic lesions, suggesting a role of LIGHT in the bone metastatic process from NSCLC.

NO CONFLICT OF INTEREST

203 NRF2 activation due to acquired KEAP1 mutation in EGFR-TKI resistant lung cancer promotes invasion and metastasis

S.H. Park[1], J.H. Kim[1], J.Y. Kim[1], M.J. Park[2], M.J. Kim[3], C. Kim[4], J.Y. Lee[1]

[1] Korea university, Pathology, Seoul, Korea
[2] KIRAMS, Divisions of Radiation Cancer Research, Seoul, Korea
[3] Sookmyung Uniu, Biological Sciences, Seoul, Korea
[4] Konkuk University, Biotechnology, Chungju, Korea

BACKGROUND: Lung cancer is the leading cause of cancer-related mortality worldwide. The most generalized form of lung cancer is non-small cell lung cancer (NSCLC), comprising 80% of all lung cancer. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), gefitinib, is effective therapy against NSCLCs which with specific EGFR-activation mutation. However, such cancer eventually

develops acquired resistance by various mechanisms such as a secondary EGFR mutation, T790M, in spite of their initial response.

MATERIAL AND METHODS: In this study, we have used established EGFR-TKI, gefitinib, resistance cell lines, HCC827GR-KU to understand the acquired resistance mechanism. qRT-PCR, immunoblotting, dual-luciferase assay, invasion assay, cell cycle analysis, MTT assay etc. were used as in vitro analysis using HCC827 and HCC827GR-KU cells. In vivo experiment was performed by using Zebra fish and mouse model.

RESULTS: The HCC827GR-KU cells showed reduced expressions of EGFR, phosphorylated EGFR and downstream effectors of activated EGFR signaling along with acquired new KEAP1 mutation, p.D236H compared to HCC827 cells. KEPA1 mutation induced NRF2 transcriptional activity and NRF2 downstream target genes, such as AKR1C1, AKR1C3, NQO1, GCLC etc that modulate cell proliferation, motility and metabolism. The HCC827GR-KU cells showed highly increased growth rates and invasiveness in vitro and increased tumor formation ability and cell motility in mouse as well as zebrafish xenograft models compare to HCC827 cells, respectively.

CONCLUSIONS: Increased NRF2 transcriptional activity and its downstream target genes due to the acquired KEAP1 mutation in EGFR-TKR resistant lung cancer cell induce cell proliferation and metastasis both in vitro and in vivo. Therefore, KEAP1 mutation and NRF2 activity needs to be considered when patient treated with EGFR-TKR as EGFR-TKI resistant marker.

NO CONFLICT OF INTEREST

204 MiR-16 controls HGF production through targeting of FGFR-1 and MEK1 in lung fibroblasts and modulates their pro-migratory activity on cancer cells

L. Roz[1], F. Andriani[2], M.T. Majorini[2], M. Mano[2], F. Facchinetti[1], M. Dugo[1], M. Giacca[3], U. Pastorino[4], G. Sozzi[1], D. Lecis[1]

[1] Fondazione IRCCS Istituto Nazionale Tumori, Experimental Oncology and Molecular Medicine, Milan, Italy
[2] University of Coimbra, Center for Neuroscience and Cell Biology, Coimbra, Portugal
[3] International Centre for Genetic Engineering and Biotechnology, Molecular Medicine, Trieste, Italy
[4] Fondazione IRCCS Istituto Nazionale Tumori, Thoracic Surgery, Milan, Italy

BACKGROUND: Stromal cells play a pivotal role during tumor development by generating a supportive microenvironment for cancer progression. Fibroblasts are crucial mediators of tumor-stroma cross-talk through synthesis and remodeling of the extracellular matrix and production of multiple soluble factors which can stimulate cancer cells. MicroRNAs are potent regulators of gene expression and their alteration in stromal cells can also result in reprogramming towards a tumor supporting phenotype. Little is known however on specific determinants of pro-tumorigenic activity of fibroblasts in lung cancer

MATERIALS AND METHODS: High-throughput screening in lung fibroblasts co-cultured with lung cancer cells was used to identify specific miRNAs able to modulate their pro-tumorigenic potential both positively and negatively. Putative miRNA targets were validated through luciferase assays and by chemical or genetic inhibition. Gene and miRNA expression profiles of primary cultures of lung fibroblasts isolated from lung cancer patients were used to identify biological pathways controlled by miRNAs and correlations with clinicopathological features

RESULTS: In high-throughput screening members of the miR-17-92 family, miR-455 and miR-16 are able to modulate the pro-tumorigenic potential of lung fibroblasts. Among the most potent negative regulators, miR-16 shows remarkable activity in regulating the fibroblast secretome. Upregulation of miR-16 in fibroblasts reduces secretion of HGF and impairs their pro-migratory activity on cancer cells. This effect is partially mediated by direct targeting of HGF and depends on FGFR-1 signaling, since miR-16 upregulation reduces FGFR-1 levels and, consequently, ERK1/2 activation. Accordingly, chemical or genetic inhibition of FGFR-1 mimics the effect of miR-16 and prevents HGF production. MiR-16 also directly targets MEK1, a downstream effector of FGFR-1, thereby exerting a pleiotropic activity on this pathway. Additionally, reduced miR-16 levels were detected in fibroblasts from lungs of heavy smokers with lung cancer, compared to those from lungs not exposed to smoke. MiR-16 and HGF levels were also inversely correlated with high expression of FGFR-1 in different primary lung fibroblast cell lines.

CONCLUSIONS: Overall, these result uncover a central role for miR-16 in regulating HGF production in lung fibroblasts and may provide clues towards formation of a tumor-proficient milieu during the early phases of lung cancer development.

NO CONFLICT OF INTEREST

205 Targeting a solute carrier to reprogram tumour-promoting stromal cells in pancreatic cancer

A. Akerman[1], G. Sharbeen[1], J. Holst[2], J. Youkhana[1], S. Naim[1], J. McCarroll[3], D. Goldstein[1], M. Erkan[4], P. Phillips[1]

[1] Lowy Cancer Research Centre- UNSW Australia, Pancreatic Cancer Translational Research Group, Sydney, Australia
[2] Centenary Institute, Origins of Cancer Program, Sydney, Australia
[3] Childrens Cancer Institute- UNSW Australia, Lowy Cancer Research Centre, Sydney, Australia
[4] Koc University School of Medicine- Istanbul- Turkey, Department of Surgery, Istanbul, Turkey

INTRODUCTION: Pancreatic cancer (PC) is a lethal disease characterised by extensive fibrosis that distorts tumour vasculature creating intra-tumoural hypoxia and oxidative stress. This drives PC chemoresistance and impedes drug delivery. Cancer-associated stromal pancreatic stellate cells (CA-PSCs) orchestrate fibrosis and promote PC growth/metastases, making them potential therapeutic targets. One approach involves targeting pro-survival proteins in CA-PSCs. Solute carrier-7A11 (SLC7A11) is part of the xCT transport system that exchanges intracellular glutamate for extracellular cystine, required for synthesis of glutathione. Glutathione is a potent anti-oxidant and we therefore predicted that it is a key PSC survival factor. We previously showed SLC7A11 is upregulated in patient-derived CA-PSCs and stromal CA-PSCs in human PC sections, compared to normal human PSCs. We hypothesised that SLC7A11 inhibition will reduce CA-PSC survival and resistance to oxidative stress. Aim: To assess the effect of SLC7A11 inhibition on CA-PSC proliferation and sensitivity to oxidative stress.

MATERIAL AND METHOD: CA-PSCs (n=3-5) were transfected with control-siRNA or SLC7A11-siRNA±oxidative stress (t-butyl hydroperoxide; tBHP) and the following assessed: proliferation (trypan blue exclusion assay), senescence (β-galactosidase staining), and SLC7A11 function (14C-cystine uptake; glutathione production).

RESULTS AND DISCUSSION: SLC7A11-siRNA resulted in >80% knockdown of SLC7A11 protein in CA-PSCs, which reduced PSC proliferation (62.2±7.5% of control-siRNA; $p<0.01$). Oxidative stress enhanced this effect (control-siRNA+tBHP = 62.0±9.3% vs. SLC7A11-siRNA+tBHP = 7.6±2.6% of control-siRNA; $p<0.0001$). SLC7A11 inhibition in CA-PSCs increased senescence (201.7±28.3% of control-siRNA; $p<0.01$), mediating anti-proliferative effects. SLC7A11 inhibition reduced CA-PSC cystine uptake (51.8±8.8% of control; $p<0.05$) and intracellular glutathione (40.0±9.6% of control; $p<0.01$).

CONCLUSION: Our result provide novel evidence that SLC7A11 likely protects CA-PSCs in the oxidative stress-promoting tumour microenvironment, and may thus be a therapeutic target in PC. In addition, there is evidence that many cancer cells depend on external sources of glutamate and glutamine. Therefore, inhibition of SLC7A11 may also indirectly inhibit PC cells by disrupting this potential feeding mechanism.

NO CONFLICT OF INTEREST

206 The metabolic profile of Chronic Myeloid Leukaemia stem cell subsets as a target to suppress treatment-resistant minimal residual disease

M. Poteti[1], G. Cheloni[1], S. Bono[1], N. Mazure[2], E. Rovida[1], P. Dello Sbarba[1]

[1] University of Firenze, Scienze Biomediche Sperimentali e Cliniche, Firenze, Italy
[2] University of Nice, Institute for Research on Cancer and Ageing of Nice, Nice, France

INTRODUCTION: Chronic myeloid leukaemia (CML) is a stem cell-driven disorder caused by the oncoprotein BCR/Abl, a constitutively active Tyrosine Kinase (TK). TK inhibitors (TKi), rather than curing CML, induce a state of Minimal Residual Disease (MRD) due to the persistence of Leukaemia Stem Cells (LSC). Previous studies from our group demonstrated that BCR/Abl expression is suppressed under low oxygen/glucose, conditions resembling those of stem cell niches in vivo, so that LSC are independent of BCR/Abl signaling and refractory to TKi. We proposed a 'metabolic" model of LSC maintenance where not only oxygen and glucose, but also other players, such as lactate or glutamine or pH, are relevant.

MATERIALS AND METHODS: Cells of the K562 and KCL22 CML lines were incubated in normoxia or 0.1% O_2. Glutamine (0, 0.5, 1, 2, or 4 mM) was administered at the beginning of incubation. Cell lysates were prepared and metabolite concentrations (in a YSI Analyzer) and pH were measured daily. At day 7, cells were transferred to normoxic secondary cultures (LC2) to estimate the maintenance of their stem cell potential at the end of primary culture (LC1).

RESULTS: In day-7 glutamine-containing cultures incubated at 0.1% O_2, BCR/Abl protein and the phosphorylation of Crkl (a major BCR/Abl downstream substrate) were abolished, glucose exhausted, lactate produced with a consequent decrease of extracellular pH. On the contrary, at 0.1% O_2 in the absence of glutamine, BCR/Abl expression and Crkl phosphorylation were maintained, while glucose consumption, lactate production and medium acidification were slower. The expression at 0.1% O_2 of c-Myc, relevant to BCR/Abl-driven transformation, was suppressed in the presence of glutamine, but not in its absence. When cells from day-7 LC1 carried out at 0.1% O_2 in the presence of glutamine were transferred to normoxic LC2 for the estimate of stem cell potential, they repopulated LC2 after a lag-phase, typical of TKi-resistant LSC where BCR/Abl had been suppressed in LC1. On the contrary, cells from LC1 without glutamine repopulate LC2 immediately, a kinetics typical of TKi-sensitive LSC where BCR/Abl signaling is maintained and is therefore available since the very beginning of LC2.

CONCLUSIONS: Glutamine shortage antagonized the selection of LSC refractory to TKi at 0.1% O_2 via the reduction of glucose consumption, which is necessary for this selection. Thus, glutamine availability is crucial to the maintenance of therapy-resistant MRD.

NO CONFLICT OF INTEREST

207 Association of ozone with 5-fluorouracil and cisplatin in regulation of human colon cancer cell viability: In vitro anti-inflammatory properties of ozone in colon cancer cells exposed to lipopolysaccharides

V. Simonetti[1], V. Quagliariello[2], P. Giustetto[3], M. Franzini[4], R.V. Iaffaioli[2]

[1] Member of SIOOT, "Kaos" ONLUS Foundation and board member of SIOOT, Turin, Italy
[2] National Cancer Institute- IRCCS - Foundation G. Pascale, Abdominal Oncology, Naples, Italy
[3] University of Turin, Department of Molecular Biotechnology and Health Sciences- Molecular Imaging Center, Turin, Italy
[4] Board Member of the Oxygen- Ozone therapy National Italian Society member, Past President of KAOS non-profit Association, Turin, Italy

INTRODUCTION: Ozone therapy is an effective medical treatment for different diseases like mucositis, psoriasis, acute pain, neurovascular diseases and cancer. Emerging evidence indicates that ozone, a strong oxidant, could effectively improve organ ischemia-reperfusion, herniated disks and skin ulcers in clinical model with interesting anti-inflammatory properties through inhibition of NF-κB activation in acute and chronic disease. The aim of this study is based on the association of different ozone concentration with 5-fluorouracil and cisplatin in human colon cancer cell (HT29 cell line) in order to investigate about possible anticancer synergistic effects. Secondary endpoint of the study was based on the anti-inflammatory properties of ozone in HT29 cell line exposed to lipopolysaccharides.

MATERIAL AND METHOD: HT29 cells were incubated with ozone at different concentration ranged from 10 up to 50 µg/ml at different incubation time (2-6-12 and 24h) alone or in combination to common anticancer drugs used in clinic named cisplatin and 5- fluorouracil. Cell viability was determined looking at colon cancer mitochondrial dehydrogenase activity by means of a modified MTT method. Anti-inflammatory studies were conducted incubating HT29 with or without 20, 30 or 50 µg/ml of ozone for 30 min before exposure to lipopolysaccharides (40 ng/ml) for 12h. The culture medium without any dilution was used to assay the production of IL-6, IL-8 and IL-1β.

RESULTS AND DISCUSSION: Ozone alone has a time and concentration dependent cytotoxicity against human colon cancer cells with an IC50 value of 30 µg/ml after 24h of incubation. Association of cisplatin of 5-fluorouracil with ozone always increase of about 15-20 % cellular cytotoxicity after 24h. Pre-incubation of ozone at 50 µg/ml decrease IL-8, IL-6 and IL-1β cellular production of about 50, 56 and 70 %, respectively, compared to cells treated only with lipopolysaccharides.

CONCLUSION: Taken together, these result indicated that ozone could be useful in colon cancer management in combination to 5-fluorouracil and cisplatin with significant inhibition, under pro-inflammatory conditions, of cytokines having a central role in colon cancer cell survival and chemo-resistance. However, taking the several limitations of this study, further biological in vitro investigations (like biochemistry, gene expression and cell cycle studies), as well as in preclinical model, are being carried out in order to better understand the potential of ozone as possible adjuvant agent of colon cancer common therapies.

NO CONFLICT OF INTEREST

208 In vivo radiation resistance of brain metastasis: New models to interrogate the underlying biology

C. Monteiro[1], M. Valiente[1]

[1] CNIO, Molecular Oncology - Brain Metastasis Group, Madrid, Spain

BACKGROUND: Brain metastasis affects 10-30% of cancer patients. Standard of care is still based on palliative treatments including surgery and radiation. Chemotherapy shows limited efficiency and newly developed targeted and immunomodulatory agents only help a reduced subpopulation of patients. In order to understand why current therapies fail and whether there are ways to improve them we have developed an experimental model that incorporates whole brain radiation therapy (WBRT). With this new model we aim to identify critical mediators of radiation resistance to explore novel ways to radiosensitize brain metastasis.

MATERIAL AND METHODS: Effects of radiation in different brain metastasis models were analyzed using a variety of in vitro, ex vivo and in vivo experimental approaches. In vivo WBRT was applied to experimental mouse models harboring established brain metastases using three different hypofractionated protocols mimicking those given in the clinic. In order to identify potential mediators of resistance genomic analyses comparing experimental conditions that correlate with different sensitivities to radiation were performed by RNAseq.

RESULTS AND DISCUSSION: We found no impairment in the growth rate and thus no impact in overall survival when three different WBRT protocols were applied to mice harboring brain metastasis. However, in vitro, brain tropic cell lines from lung and breast cancer with different oncogenomic profiles were highly sensitive to radiation. Interestingly, when we used different culture methods that promote metastasis initiation capabilities or incorporates the brain microenvironment, we observed that sensitivity to radiation was significantly decreased. Genomic analysis comparing brain tropic cell lines under conditions inducing resistance versus sensitivity to radiation have allowed us to find differences among these situations. Validation of this unbiased approach has allowed us to identify new possibilities to block resistance to radiation in brain metastasis.

CONCLUSIONS: We hypothesize that dramatically different responses to radiation observed in vivo are tightly linked to interactions with different components of the brain microenvironment. Thus, the dissection of the molecular mechanisms behind this biology could offer ways to radiosensitize cancer cells in the brain.

NO CONFLICT OF INTEREST

209 Role of miRNAs in metabolic plasticity correlated with 5-FU resistance in colon cancer cells

E. Pranzini[1], P. Paoli[1], M.L. Taddei[2], P. Chiarugi[1]

[1] University of Florence, Department of Experimental and Clinical Biomedical Sciences, Florence, Italy
[2] University of Florence, Department of Experimental and Clinical Medicine, Florence, Italy

INTRODUCTION: Highly proliferative cells, including cancer cells, respond to their increased energetic and biosynthetic needs primarily through glycolysis. However, this metabolic strategy is not apt to sustain the low proliferative and quiescent state that characterize cancer cells resistant to therapy. Recent studies confirm that acquisition of therapy resistance is associated with a metabolic shift toward respiratory metabolism in several cancer models. In this scenario, metabolic plasticity acquires an important role in survival of therapy-resistant cells responsible for tumor relapse. In our model, treatment of colon cancer cells with 5-FU selects for cells with mesenchymal stem-like proprieties that undergo a metabolic reprogramming resulting in addition to OXPHOS.

Whether mechanisms underlying the role of miRNAs in cancer metabolism are as yet unknown, we postulate that miRNAs could be important in metabolic adaptations exploited by cancer resistant cells to survive and metastasize. We hypothesize that the role of miRNA and the transcriptional program they are controlling is fundamental in cancer cells that are committed to OXPHOS in response to 5-FU.

METHODS: Global gene expression profile for the entire genome on Agilent platform (miRNA) was performed. miRNAs found to be significantly and differently regulated were validated with Real Time PCR.

RESULTS: The miRNA profile indicated that several miRNAs were differentially regulated between colon cancer cell line sensible to 5-FU treatment and the corresponding resistant cells. Between these , we identified interesting miRNAs previously correlated with EMT (miR-200 a/b, miR-429, miR-96-5p, miR-183-3p), stemness (miR-21), oxidative metabolism (miR-338-3p) and 5-FU resistance (miR-215-5p). Interestingly, only nine miRNAs (miR-1183; miR-1290; miR-210-3p; miR-3944-5p; miR-4252; miR-429; miR-642b-3p; miR-6792-5p; miR-769-5p) were significantly deregulated in resistant cells under chronic treatment with 5-FU.

CONCLUSIONS: Our study demonstrate that intracellular levels of certain miRNAs are significantly deregulated in resistant cancer cells upon chronic treatment with 5-FU. The identification of these miRNAs, as well as their molecular pathways, could allow to identify molecular targets involved in metabolic plasticity, allowing resistant cancer cells to shift toward OXPHOS, thereby enhancing their survival to pharmacological treatments.

NO CONFLICT OF INTEREST

210 Sirtuin1 stimulates the proliferation and the expression of glycolysis genes in pancreatic neoplastic lesions

A. Pinho[1], A. Mawson[1], A. Gill[1], M. Arshi[2], S. Camargo[2], A. Biankin[3], J. Wu[1], I. Rooman[1,4]

[1] Garvan Institute of Medical Research, Cancer Division, Sydney- NSW, Australia
[2] University of Zurich, University of Zurich, Zurich, Switzerland
[3] Wolfson Wohl Cancer Research Centre, University of Glasgow, Glasgow- Scotland, United Kingdom
[4] Oncology Research Centre, Vrije Universiteit Brussel, Brussels, Belgium

BACKGROUND: Metabolic reprogramming is a feature of neoplasia and tumor growth. Sirtuin 1 (SIRT1) is a lysine deacetylase of multiple targets including metabolic regulators such as p53. SIRT1 regulates metaplasia in the pancreas. Nevertheless, it is unclear if SIRT1 affects the development of neoplastic lesions and whether metabolic gene expression is altered.

MATERIAL AND METHODS: To assess neoplastic lesion development, mice with a pancreas-specific loss of Sirt1 (Pdx1-Cre;Sirt1-lox) were bred into a KrasG12D mutant **BACKGROUND:** (KC) that predisposes to the development of pancreatic intra-epithelial neoplasia (PanIN) and ductal adenocarcinoma (PDAC). We validated the significance of our findings in human disease using PDAC cell lines and clinical samples and transcriptomic data from a large cohort of pancreatic cancer patients (Australian Pancreatic Cancer Genome Initiative).

RESULTS: Similar grade PanIN lesions developed in KC and KC;Sirt1-lox mice but PanINs occupied 40% less area in the KC;Sirt1-lox line, attributed to reduced proliferation. This was accompanied by reduced expression of proteins in the glycolysis pathway, such as GLUT1 and GAPDH. The stimulatory effect of SIRT1 on proliferation and glycolysis gene expression was confirmed in a human PDAC cell line. In resected PDAC samples, higher proliferation and expression of glycolysis genes correlated with poor patient survival. SIRT1 expression per se was not prognostic but low expression of Cell Cycle and Apoptosis Regulator 2 (CCAR2), a reported SIRT1 inhibitor, corresponded to poor patient survival.

CONCLUSIONS: These findings underscore a novel oncogenic function of SIRT1 during pancreatic cancer development where it stimulates the proliferation and expression of glycolytic proteins, opening perspectives for novel targeted therapies in pancreatic cancer.

NO CONFLICT OF INTEREST

211 Cell stiffness as a promising marker of epithelial–mesenchymal transition and metastatic potential in head and neck cancer-pilot study

M. Masarik[2], M. Kratochvilova[2], B. Peltanova[2], M. Svobodova[1], H. Polanska[1], M. Raudenska[2], J. Gumulec[1], J. Pribyl[3], T. Vicar[4]

[1] Faculty of Medicine- Masaryk University, Department of Pathological Physiology, Brno, Czech Republic
[2] Faculty of Medicine- Masaryk University, Department of Physiology, Brno, Czech Republic
[3] Masaryk University, Central European Institute of Technology, Brno, Czech Republic
[4] Faculty of Electrical Engineering and Communication- Brno University of Technology, Department of Biomedical Engineering, Brno, Czech Republic

BACKGROUND: Important part of cancer progression is the dissemination of individual cells from a tumour. These invasive cells emerge from phenotypic changes known as the epithelial–mesenchymal transition (EMT). During EMT, cells lose epithelial features (low motility and strong cell–cell contacts) while gaining mesenchymal traits associated with metastasis (increased motility, weak cell–cell contacts, CD90 expression). Recently, it was demonstrated that cell stiffness could serve as a biomarker of metastatic potential. A tight connection between attenuated cell stiffness and the increased metastatic potential acquired after EMT was also observed. An increased expression of CD90 was associated with enhanced EMT, presence of cancer associated fibroblasts (CAFs) and/or mesenchymal stem cells in the tumour microenvironment and consequent higher metastatic potential of tumour.

METHODS: Primary cell lines were prepared from tumour tissue obtained at surgery. For separation of CD90-positive subpopulations derived from primary cell line magnetic particles MiniMACS™ Starting Kit (CD90 MicroBeads- human, Miltenyi Biotec) were used. CD90-positive tumour cells were subjected to atomic force microscopy (AFM). AFM measurements were obtained in a humidified incubator (37°C; 5% CO2) with force measurements recorded at a pulling rate of 1 Hz. Young's modulus was calculated by HertzianSneddon model.

RESULTS: Cell stiffness of CD90-positive tumour cells derived from patients with stage of lymph node metastasis N0-1 was significantly higher than cell stiffness of CD90-positive tumour cells derived from patients with stage N2-3. This result indicate that attenuation of cell stiffness in CD90-positive tumour cells (due to supposed increase in numbers of cells arising from EMT) could be promising predictor of metastatic potential of particular tumour.

CONCLUSION: In this study, we separated CD90-positive cells from tumour tissue of head and neck cancer patients and tested the hypothesis that their cell stiffness is in association with ability of these tumour cells to form lymph node metastasis in HNSCC patients. Our result indicates, that cell stiffness could be a promising predictor for metastatic potential of head and neck cancer.

ACKNOWLEDGEMENT: This work was supported by the Ministry of Health of the Czech Republic (grant no. 16-29835A).

NO CONFLICT OF INTEREST

212 Mahonia aquifolium extracts promote antimetastatic effects of doxorubicin

A. Damjanović[1], B. Kolundžija[1], I. Matić[1], A. Krivokuća[1], G. Zdunić[2], R. Janković[1], K. Savikin[2], T. Stanojković[1]

[1] Institute of Oncology and Radiology of Serbia, Department of Experimental Oncology, Belgrade, Serbia
[2] Institute for Medicinal Plant Research- Dr. Josif Pančić, Department for Pharmaceutical Investigations and Deuelopment, Belgrade, Serbia

BACKGROUND: Doxorubicin is a first-line anticancer agent highly effective against a wide spectrum of malignancies, with dose-dependent toxicity. Recent investigations showed that Mahonia aquifolium, berberine, and similar alkaloids as plant main secondary metabolites have anticancer potential, and can effectively limit the toxicity of doxorubicin. The objectives of this study were to elucidate the effects of doxorubicin and M. aquifolium water or ethanol extracts (MAW and MAE, respectively) combination on metastatic potential of malignant cells.

MATERIAL AND METHODS: We performed in vitro scratch and colony formation assays, and detected the alterations in the expression of genes involved in the formation of cell-cell interactions and migration (MMP2, MMP9, OCLN, CTNNB1) using quantitative real-time PCR, following the treatment of lung adenocarcinoma cells (A549 cells) with subtoxic concentrations of doxorubicin (0.11 μg/mL), MAW, MAE extracts (20 μg/mL), or their combinations.

RESULTS: The migratory ability of the cells treated with extracts alone or the combination of doxorubicin and MAW/MAE extracts was shown to be considerably decreased after 48 h, compared with the control or doxorubicin-treated cells. Doxorubicin affected the ability of cells to form colonies, and doxorubicin-MAW/MAE combinations were shown to lead to an even more pronounced decrease in colony-forming ability, in comparison with that observed after doxorubicin treatment alone. The most important alterations in gene expression were observed in MMP9 and OCLN genes. MMP9 expression was largely decreased after the treatment with the extracts or combination treatment, compared with that in the control or doxorubicin-treated cells. Doxorubicin treatment was shown to inhibit the expression of OCLN, while both extracts alone, and DOX/MAE combination treatment induced the expression of OCLN, in comparison with that in the control. It was reported that the increase in MMP9 levels promotes tumorigenicity, while occludin, the component of the tight junctions, reduces cell mobility. Our result suggest that the alterations in the levels of these genes may underlie the inhibition of cell migration, colony formation, and reduction of metastatic potential of the treated cells.

CONCLUSIONS: The antimetastatic activity of doxorubicin may be significantly increased with the use of M. aquifolium extracts in combination with this chemotherapy drug.

NO CONFLICT OF INTEREST

213 Pro-tumorigenic and immunosuppressive potential of exosome-associated microRNAs in lung cancer

L. Calzolari[1], O. Fortunato[1], C. Camisaschi[2], C. Castelli[2], L. Rivoltini[2], G. Sozzi[1]

[1] Fondazione IRCCS Istituto Nazionale Tumori, Tumor Genomics Unit- Department of Experimental Oncology and Molecular Medicine, Milan, Italy
[2] Fondazione IRCCS Istituto Nazionale Tumori, Unit of Immunotherapy of Human Tumors- Department of Experimental Oncology and Molecular Medicine, Milan, Italy

BACKGROUND: Exosomes are small vesicles released by all cell types that contain specific subsets of functional biomolecules, such as microRNAs (miRNAs). It has widely been reported that exosomal miRNAs (exo-miRNAs) from cancer cells can manipulate the tumor microenvironment regulating the gene expression of recipient cells. We previously validated a three levels miRNA risk classifier (MSC) based on 24 plasma-miRNAs associated with lung cancer development and prognosis. The aim of this study was to investigate the presence of the 24 miRNAs inside exosomes and their potential role as mediators of pro-tumorigenic features in the lung microenvironment.

MATERIAL AND METHODS: Exosomes were isolated from conditioned media of A549 lung cancer cell line by differential centrifugation method. The expression of exosomal markers (CD9, CD63, CD81 and Alix) was assessed by western blot and FACS, and miRNA levels were analyzed by digital PCR. In vitro analysis were used to assess the biological effect of lung cancer-exosomes on different type of recipient cells, such as immortalized bronchial-epithelial cells (HBEC-KRASG12V), endothelial cells (HUVECs) and naκve T cells.

RESULTS: All 24 miRNAs composing the MSC were present inside A549-derived exosomes at heterogeneous levels. Lung cancer-exosomes released by A549 cells were able to increase by 50% the proliferation of HBEC-KRASG12V cell line and to enhance the ability of HUVECs to form tubular structure. In in vitro assay exosomes derived from A549 cells induced in CD4+CD25- T cells a regulatory T (Treg) cells phenotype comparable to that obtained by TGF-β treatment. We found that miR-15b and miR-320 were up-regulated during the conversion of T lymphocytes into Treg cells suggesting a potential function of these miRNAs in the induction of Treg phenotype. Moreover, depletion of miR-126 in A549-derived exosomes by transient inhibition of its expression in the donor cells impaired the angiogenesis ability of HUVECs indicating the involvement of miR-126 in determining the pro-angiogenic effect. Other analysis will follow to further investigate the role of exo-24 miRNAs in the lung microenvironment.

CONCLUSIONS: These data show that lung cancer exosomes display pro-tumorigenic features, as documented by their ability to promote proliferation of epithelial cells and to induce a pro-angiogenic and immunosuppressive microenvironment. These effects may be at least in part due to exo-miRNAs released by lung cancer cells.

NO CONFLICT OF INTEREST

214 ChIP-seq approach to investigate FUS-CHOP targets in PDX models of Myxoid Liposarcoma treated with trabectedin

L. Mannarino[1], E. Bello[1], S.A. Licandro[1], R. Frapolli[2], S. Marchini[2], M. D'Incalci[2], R. GATTA[1]

[1] Istituto di Ricerche Farmacologiche Mario Negri, Oncology, Milan, Italy

INTRODUCTION: The molecular diagnostic marker of Myxoid Liposarcoma (MLS) is the FUS-CHOP chimera, caused by t (12;16)(q13;p11) translocation. Trabectedin (ET-743, Yondelis), approved in Europe and US for the second line therapy of soft tissue sarcomas, is particularly effective in MLS by promoting adipocyte differentiation. Using Patient Derived Xenograft (PDX) models of MLS we have demonstrated that at therapeutic doses trabectedin displaces the fusion protein from some of its known targets. The aim of this work is to obtain a genome wide map of FUS-CHOP binding events in vivo by a ChIP-seq approach.

MATERIAL AND METHODS: Chromatin was obtained from formaldehyde cross-linked cells deriving from PDX at different time of trabectedin treatment (0.15mg/kg, 24 and 72 hours after 1st trabectedin dose and 15 days after the 3rd one). FUS-CHOP binding sites were investigated by using anti-FUS and anti-CHOP antibodies; anti-H3K4me3 antibody was also used to detect genomic regions transcriptionally active. Libraries were generated by the Tru-seq Library Preparation Kit (Illumina) and then run on NextSeq 500. Analysis was performed using the bcbio-nextgen pipeline for ChIP-Seq (http://github.com/chapmanb/bcbio-nextgen) on a HPC computing platform (Cloud4CARE project). Peak calling was done using MACS2 software.

RESULTS AND DISCUSSION: Sequencing deriving from control chromatin produced 933 peaks for CHOP and 427 for FUS. Peaks number were dramatically reduced after trabectedin exposure: 10 peaks for CHOP and 73 for FUS. H3K4me3 peaks slightly decreased. To initially confirm ChIP-seq data, we arbitrary chose two different genes for orthogonal validation: we indeed observed that on ROCK1 and HSPA5 genes trabectedin displaced FUS-CHOP, whose binding site is situated respectively upstream and downstream to those two genes analyzed.

CONCLUSION: Preliminary data obtained in vivo by the ChIP-seq approach in PDX of MLS showed that trabectedin displaces FUS-CHOP from its binding sites. Further analysis will be aimed to better characterize molecular pathways trascriptionally controlled by the MLS fusion protein, whose deregulation is responsible for the exquisite sensitivity to trabectedin.

NO CONFLICT OF INTEREST

215 The absence of IL-17 prolongs survival in pancreatic cancer by switching fibrosis from pro-tumoral to wound healing like

P.Cappello[1], G. Mucciolo[1], R. Curto[1], C. Roux[1], C. Curcio[1], L. Vannucci[2], F. Novelli[2]

[1] University of Torino, Dept of Molecular Biotechnology and Health Sciences, Torino, Italy
[2] Czech Academy of Sciences, Institute of Microbiology v.v.i.- Lab of Immunotherapy, Prague, Czech Republic

BACKGROUND: Pancreatic ductal adenocarcinoma (PDA) is a fatal medical condition with few advances in therapy and patient survival. The role of IL17 and Th17 in cancer progression and anti-tumour immunity is still controversial. In this study we have investigated the role of IL17 in PDA carcinogenesis.

MATERIAL AND METHOD: Genetically engineered mice (GEM) that spontaneously develop PDA were crossed with IL-17 KO mice (GEM/IL17KO) and survival was analysed by Kaplan-Meier. Pancreas and organs from GEM and GEM/IL17KO were analysed by immunohistochemistry and second harmonic generation to evaluate collagen into tumor stroma. Isolated fibroblasts from GEM and GEM/IL17KO mice were analysed by RNA expression and cytokine arrays.

RESULTS: GEM/IL17 KO mice survived significantly more than GEM mice, even if there were no changes in the tumor onset. In addition, we observed an increased fibrotic reaction in GEM/IL17 KO compared to GEM mice starting from early stage of disease with the formation of compact nets of collagen fibers. Lastly, fibroblasts isolated from GEM/IL17KO mice unveil a wound-healing profile more than a pro-tumoral phenotype.

CONCLUSION: Our data suggest that the absence of IL17 does not affect the tumor onset but the progression, likely affecting the inflammatory and immune responses, which seem to be able to better counteract tumor growth in the absence of this cytokine.

NO CONFLICT OF INTEREST

216 V-ATPase control of EV signaling in glioma stem cells

I. Bertolini[1], A. Storaci[1], A. Terrasi[2], S. Bosari[2], V. Vaira[3]

[1] Università degli Studi di Milano, Department of Pathophysiology and Transplatation, Milan, Italy
[2] Ospedale Maggiore Policlinico, Department of Pathophysiology and Transplatation, Milan, Italy
[3] Istituto Nazionale di Genetica Molecolare, Padiglione Romeo ed Enrica Invernizzi, Milan, Italy

INTRODUCTION: Recent evidences highlighted that GBM secreted microvesicles (EVs), in particular exosomes (exo) and large oncosomes (LO), play a major role in the cross-talk between tumor cell and non-neoplastic parenchyma. How GBMs manage to thrive in a highly unfavorable, acidic microenvironment is still unclear, but recent work from our group has identified the vacuolar pump H+-ATPase (V-ATPase) as an important effector of GBM growth and glioma stem cells (GSC) maintenance and we observed that V-ATPase expression correlates with MAPK/Erk pathway activation.

METHOD: Exo and LO were isolated by an Invitrogen kit and serial centrifugation, respectively, from media of patients' derived GBM neurospheres (NS), enriched in GSC (n=12). For EVs internalization studies, Exo and LO were stained using FM 1-43 FX dye and the process was followed at selected time points (30'-4h-24h), using a confocal microscopy or flow cytometry (FACS). Cultures from patients' derived brain tumor margins or primary GBM (differentiated and not) were used as EVs-recipient cells. RNA and protein content were extracted from Exo and LO and from recipient cells after co-culture. Bafilomicyn A1 (BafA1) was used for pharmacological treatment of NS producing EVs to modulate V-ATPase activity. The study was approved by the Institutional Ethical Committee.

RESULTS: NS are able to produce Exo and LO that are internalized in recipient cells after 4 hours of co-culture. Exo are able to significantly increase cell growth in recipient cells (brain tumor margins and primary GMB differentiated cells), and this effect is stronger with EVs produced by NS with higher V-ATPase expression; whereas LO are able to increase the spheres-forming ability in primary GBM cells with an higher effect compared to Exo. BafA1 treatment reduces NS clonogenicity, lysosomal acidification and MAPK/Erk pathway activation; moreover the treatment of NS producing EVs reverts their effect in recipient cells. Finally we observed that the co-culture with EVs induces increase of MAPK/Erk pathway and V-ATPase expression in recipient cells; and BafA1 treatment reverts this effect.

CONCLUSION: Altogether, these data point toward the central role of different EV types in GBM communication and suggest a role of the V-ATPase proton pump in regulating EV's contents. Furthermore these data suggest a correlation between V-ATPase expression and MAPK/Erk activation in GSC.

NO CONFLICT OF INTEREST

217 The role of apelin signaling in the malignant behavior of cutaneous melanoma

J. Berta[1], O. Drozdovszky[1], S. Török[1], J. Tóvári[2], S. Paku[3], B. Masri[4], B. Döme[1,5,6], V. László[5,6]

[1] National Korányi Institute of Pulmonology, Department of Tumor Biology, Budapest, Hungary
[2] National Institute of Oncology, Department of Experimental Pharmacology, Budapest, Hungary
[3] Semmelweis University, 1 st Department of Pathology and Experimental Cancer Research, Budapest, Hungary
[4] INSERM U1 0 4 8 -University Toulouse III, Institute of Cardiovascular and Metabolic Diseases, Toulouse, France
[5] Medical University of Vienna, Department of Surgery, Vienna, Austria
[6] Medical University of Vienna, Department of Biomedical Imaging and Image-guided Therapy, Vienna, Austria

BACKGROUND: Several studies demonstrated that apelin, a ligand of the G-protein-coupled APJ receptor plays a role in tumor angiogenesis and enhances the tumor growth in various experimental systems. Recent studies reported that apelin has also lymphangiogenic potential. Besides, apelin is overexpressed in several human cancers. Our aim was to investigate the role of apelin signaling in the malignant behavior of cutaneous melanoma.

MATERIAL AND METHODS: The murine B16 and the human A375 melanoma cell lines were stably transfected with apelin encoding or control vectors, and the impact of apelin overexpression on in vitro proliferation, spheroid formation and collagen invasion of tumor cells was investigated. APJ and apelin mRNA levels were measured by real-time PCR in two-dimensional (2D) cell cultures and spheroids. Apelin-overexpressing and control B16 cells were injected intravenously into C57BL/6J mice. The diameter of lung metastases was measured by ocular micrometer. For labelling proliferating cells in lung metastases, we performed in vivo BrdU proliferation assay.

RESULTS: Apelin overexpression had no effect on the 2D growth of B16 and A375 cell lines, but it significantly increased the diameter of spheroids formed by these cells, and, moreover, the number of B16 tumor spheroids was also elevated upon apelin transfection. Apelin overexpression resulted in significantly higher spheroid perimeter/area ratio in both cell lines in the collagen invasion assay. APJ mRNA levels were elevated in the spheroids compared to the monolayer cultures in the case of both cell lines, suggesting that the effect of apelin is enhanced in the spheroids by the elevated level of its receptor. Apelin overexpression significantly increased the size of B16 lung metastases, and, furthermore, the ratio of proliferating melanoma cells was significantly higher in these apelin-overexpressing tumors.

CONCLUSIONS: Our result suggest that apelin induces tumor growth in cutaneous melanoma and, therefore, the apelin/APJ signaling system represents a novel potential therapeutic target in this malignancy.

NO CONFLICT OF INTEREST

POSTER SESSION: CELL AND TUMOUR BIOLOGY II

218 Role of CAFs-secreted extracellular vesicles in tumor progression

A. Toti[1], P. Cirri[1], A. Caselli[1]

[1] University of Florence, Scienze Biomediche Sperimentali e Cliniche, Florence, Italy

BACKGROUND: Fibroblasts are the most abundant cells in connective tissue and in solid tumors. Within tumor microenvironment stromal and cancer cells engage a crosstalk mediated by soluble factors, cell–cell contacts and extracellular vesicles (EVs) trafficking. This crosstalk induces fibroblasts transdifferentiation into their activated form (CAFs), which, in turn, play a fundamental role in promoting tumor progression. Our study is focused in EVs-mediated proteins transfer from CAFs to cancer cells.

MATERIAL AND METHODS: Ectosomes proteins have been digested, and the resulting peptide samples were extensively fractionated on an Ultimate 3000 HPLC (Dionex) and analyzed by automated MS/MS on a LTQ Orbitrap mass spectrometer (Thermo Fisher).

RESULTS AND DISCUSSION: We have previously shown that primary fibroblasts have the ability to transfer a remarkable amount of proteins and lipids to neighboring cells, throught a particular extracellular vesicles called ectosomes. Upon fibroblasts activation to CAFs we observed an enhanced delivering of ectosomes to cancer cells compared to normal fibroblasts. As a consequence tumor cells increase their proliferation rate, indicating that ectosome-mediated trafficking could be a relevant mechanism that fuels tumor progression. In order to dissect this important CAFs – cancer cells crosstalk we have performed a proteomic analysis of ectosomes derived from both kind of cells using a shotgun approach.

After mass spectrometry analysis of CAFs and tumor's ectosomes and immune-specific detection by western blotting or cytofluorimetric analysis, we have discovered a subset of proteins that are specifically transferred from CAFs to tumor cells i.e.: Galectin-1, Thy-1, Caveolin-2 and MHC-I.

All of these proteins are broadly described to be essential for many aspect of cancer progression, and we now suggest that, at least a part of them, are of stromal origin.

CONCLUSION: Our study shows that CAFs can horizontally transfer a specific subset of their proteins to tumor cells through ectosomes cargos. We have identified Galectin-1 and other proteins that could play an important role in cancer cell physiology allowing tumor cells to gain new functionalities

NO CONFLICT OF INTEREST

220 Discovery and characterisation of two epithelial-mesenchymal transition (EMT)-promoting metastamiRs: MiR-181b-3p and miR-5003-3p

J.O. Yoo[1], S.Y. Kwak[1], H.J. An[1], Y.H. Han[1]

[1] Korea Institute of Radiological and Medical Sciences, Division of Radiation Basic Research, Seoul, Korea

INTRODUCTION: One of the initial steps in metastatic dissemination is the epithelial-mesenchymal transition (EMT), which is essential for increased cell motility and invasiveness. Several transcription factors, such as those of the Snail/Slug family, Twist, and ZEB1/ZEB2 function as molecular switches for the EMT program. Along this line, miRNAs have been shown to function as important regulators of tumor progression at various stages.

MATERIALS AND METHODS: We performed a functional screening by determining the effects of 267 synthetic miRNA mimics on the expression of EMT marker proteins. Human breast cancer-specific tissue microarray (TMA) slides containing 10 pairs of the primary infilterating ductal carcinoma and matched lymph node-metastasised tissues were used for In situ hybridisation (ISH) and immunohistochemical (IHC) analyses. The lung metastasis experiment was performed by injecting MDA-MB-231 cells transfected with miRNA mimic in the tail vein of BALB/c nu/nu mice.

RESULTS AND DISCUSSION: We performed a functional screening for EMT-regulating miRNAs and identified several candidate miRNAs. Among these, we demonstrated that miR-181b-3p and miR-5003-3p induce cellular features characteristic of EMT. miR-181b-3p and miR-5003-3p induced upregulation of Snail through protein stabilisation. YWHAG was identified as a direct target of miR-181b-3p, which is responsible for miR-181b-3p-induced Snail stabilisation and EMT phenotypes. Ectopic expression of YWHAG abrogated the effect of miR-181b-3p. MDM2 was identified as a direct target of miR-5003-3p, the downregulation of which induced Snail stabilisation. E-cadherin was also demonstrated to be a direct target of miR-5003-3p, reinforcing the EMT-promoting function of miR-5003-3p. IHC and ISH analyses indicated that the expressions of miR-181b-3p and miR-5003-3p were higher in metastatic breast carcinoma tissues than in primary ductal carcinoma tissues. YWHAG expression was inversely correlated with the expression of miR-181b-3p and Snail, and miR-5003-3p was inversely correlated with the expression of MDM2 and E-cadherin. Furthermore, transfection with miR-181b-3p or miR-5003-3p increased the frequency of metastatic nodule formation in the lungs of mice in experimental metastasis assays.

CONCLUSION: Our data suggest that both miR-181b-3p and miR-5003-3p function as a metastasis activator by promoting Snail-induced EMT, and may therefore be potential therapeutic targets in metastatic cancers.

NO CONFLICT OF INTEREST

222 Role of extracellular matrix-tumor cell interaction in breast cancer aggressiveness

M. Giussani[1], C. Fania[2], S. Pozzi[1], F. Turdo[1], M. Varricchio[1], C. Gelfi[3], T. Triulzi[1], E. Tagliabue[1]

[1] Fondazione IRCCS Istituto Nazionale Tumori, Experimental Oncology and Molecular Medicine, Milan, Italy
[2] IRCCS Policlinico San Donato, UO Proteomica Clinica, Milan, Italy
[3] Università degli Studi di Milano, Scienze Biomediche per la salute, Milan, Italy

INTRODUCTION: We recently identified an ECM gene expression pattern (ECM3) in ~40 % of breast carcinomas (BC) able to classify an independent group of BC. ECM3 was significantly associated with high risk of relapse in patients with grade III tumors, whereas no significant association was found in those with grade I-II carcinomas. The aim of this study is to understand how ECM-cancer cell interaction drives the rapid progression of this BC subgroup.

MATERIAL AND METHOD: Frozen samples of ECM3-grade III (E3G3) and ECM3-grade I-II (E3G0) BCs were analyzed at protein and RNA level by Western blot and gene expression profile, respectively. Formalin-fixed, paraffin-embedded (FFPE) tissues of the same samples were used for Masson's trichrome stain and for glycan profile by MALDI imaging after treatment with PNGase F, an enzyme for N-linked deglycosylation of glycoproteins. ECM discs were obtained by decellularization of frozen tumor-derived slices. The expression of epithelial-mesenchymal transition (EMT) genes was analyzed on MDA-MB-231-GFP cells grown on decellularized ECM discs by real-time PCR.

RESULTS AND DISCUSSION: Western blot analysis of enriched ECM fractions showed no differences in the abundance of ECM3 relevant proteins (COL6A1, COL10A1, COMP, MMP2 and SPARC) between E3G3 and E3G0 tumors as well as collagen amount and organization revealed by Masson's trichrome stain. Moreover, mRNA level of lysyl oxidase, an enzyme responsible for collagen cross linking, did not change according to grade within ECM3 tumors supporting no remarkable differences in composition and organization of ECM proteins. Glycan profile carried out on ECM-enriched areas of tumor samples by MALDI imaging, revealed significant differences in mass spectrometry features suggestive of diverse glycan chains on ECM proteins between E3G3 and E3G0 tumors. Analysis of EMT genes showed a significant increased expression of Vimentin and Snail2 ($p=0.0003$ and $p=0.018$, respectively) and a trend toward increased expression for Sox2, Zeb1 and Zeb2 ($p=0.2$, $p=0.12$ and $p=0.08$, respectively), in MDA-MB-231-GFP cells grown on E3G3 compared to E3G0 tumor-derived ECM slices.

CONCLUSION: Our result suggest that E3G3-derived ECM leads to a more aggressive phenotype than E3G0-derived likely due to a different glycosylation of ECM components. Thus the particular cross-talk between tumor cells and the surrounding microenvironment in E3G3 BC may depend on unusual ECM post-translational modifications.

NO CONFLICT OF INTEREST

224 Breast tumour kinase (Brk) modulates drug responses in breast cancer cell lines

H. Hussain[1], A. Harvey[1]

[1] Brunel University London, Biosciences, Uxbridge, United Kingdom

BACKGROUND: Breast tumour kinase (Brk) is over-expressed in 80% of all breast cancers and has shown to be involved in tumour development and progression in many different cancer types including breast cance. There is a lack of development of breast tumour kinase (Brk) inhibitors and the clinical benefits associated with Brk inhibition. Although a few have been in development, they have yet to be completely investigated in vitro and in vivo. Nonetheless, breast tumour kinase has been proposed to be a desirable therapeutic target suggesting a Brk inhibitor may yet prove to be a valuable treatment option. Our studies indicate potential for a Brk inhibitor to be used in combination with current breast cancer therapies.

MATERIALS AND METHODS: MTT cell proliferation assays were performed on breast cancer cell lines: MCF10A, T47D, GI101, BT474, SKBR3, MDA-MB 231, MDA-MB 436. These breast cancer cell lines were treated with Brk inhibitor at concentrations ranging from 0μM to 10μM and then in combination with Taxol, Doxorubicin, Lapatinib and Tamoxifen. Western blotting was carried out to determine levels of Brk and Brk substrate; STAT3.

RESULTS AND DISCUSSION: In all cell lines tested there was a proportionate decline in cell proliferation following treatment with Brk inhibitor nonetheless, there was not a significant difference in comparison with current therapies. However there was reduced cell proliferation when Brk inhibitor was used in combination therapy.

CONCLUSION: Overexpression of the Brk proto-oncogene in breast cancer cell lines may not be used as a single therapy but may offer potential to be used in combination.

NO CONFLICT OF INTEREST

225 miRNA Biomarker Candidates for the Accurate Detection of Endometrial Atypical Complex Hyperplasia: Implications for cancer development

S. Giglio[1], R. Cirombella[1], F. Omar[1], V. Stefano[2], C. De Vitis[1], E. Luciani[1], A. Vecchione[1], E. Pinchi[3]

[1] Sapienza University, Dep. Clinical and Moleculare Medicine, Rome, Italy
[2] Università degli Studi, Department of Internal Medicine- Biosystems Analysis- LTTA- Department of Morphology- Surgery and Experimental Medicine, Ferrara, Italy
[3] Sapienza University, Department of Anatomical- Histological- Forensic and Orthopaedic Sciences, Rome, Italy

INTRODUCTION: Endometrial cancer (EC) is the most common gynecologic malignancy in developed countries. Estrogen-dependent tumors (typeI) account for 80% of cases and non-estrogen-dependent (type II) account for the rest.EC typeI is generally thought to develop via precursor lesions along with accumulation of molecular genetic changes.Indeed, complex endometrial hyperplasia with atypia is the least common type of hyperplasia, but is the type most likely to progress to typeI cancer, whereas complex endometrial hyperplasia without atypia rarely progresses to cancer.MiRs are a class of small non-coding RNAs that negatively regulate gene expression mainly binding to 3'UTR region of target.

METHODS: We analyzed the expression of 1105 human miRs in the training set containing 41 speciments (22CH, 19CAH) using Affymetrix microArray.All miRs significantly differented expressed were validated in a indipendent cohort of samples (22CAH and 21CH) by RTPCR.A luciferase assay was performed to confirm a direct interaction between the 3'UTR of Smad4 and the miRNAs of interested, using wt or mutant plasmids. mRNA and proteins expression were analyzed by RTPCR and Western blot, respectively. Cell viability and migration assay after miRs transfection or Smad interfering were performed.

RESULTS: We identified a miRs signature (miR-205, miR-146a, miR-1260b) able to discriminate between atypical and typical endometrial hyperplasia.The identification of molecular markers that can differentiate between these two distinct pathological conditions is highly considered to be useful for the clinical management of the patients, owing to the fact that hyperplasia with atypical change is associated with a higher risk of develop cancer.ROC curve analysis showed that each miR has a high predictive power in discriminating the two conditions (>90%). With the aim to find a biological role for these three miRNAs we focused on a common putative target involved in endometrial carcinogenesis:SMAD4. We demonstrated that all miRs directly target SMAD4 and induced proliferation and migration of Hec1a cells.These data suggest that impairment miRs-mediated of TGF-β pathway, due to inhibition of its effector molecule SMAD4, is a relevant molecular alteration in EC development.Our findings showed a potential diagnostic role of miRs for accurate detection of complex atypical hyperplasia and improved the understanding of its pivotal role in SMAD4 regulation.

NO CONFLICT OF INTEREST

226 Lipid metabolic reprogramming in ER positive breast cancer cells following long-term oestrogen deprivation

M. Bacci[1], M. Ferracin[2], M. Ramazzotti[1], P. Chiarugi[1], A. Morandi[1]

[1] University of Firenze, department of biomedical- experimental and clinical sciences, Firenze, Italy
[2] University of Bologna, Department of Specialised- Experimental- and Diagnostic Medicine, Bologna, Italy

BACKGROUND: Aromatase inhibitors (AI) are the first-line endocrine treatment choice for postmenopausal women with oestrogen receptor positive (ER+) breast cancers. Unfortunately, the clinical benefit is still limited due to resistance. We have previously demonstrated that central carbon metabolic reprogramming is involved in response and adaptation to AI. In addition to glucose metabolic reprogramming, highly proliferative cancer cells show increased fatty acid (FA) biosynthesis and lipids storage in lipid droplets (LDs). Therefore, we analysed lipids metabolism of parental cells and compared to that of long-term oestrogen deprived (LTED) cells, a model that mimics AI resistance.

MATERIALS AND METHODS: MCF7 ER+ breast cancer cells and their LTED derivatives were subjected to Gene Expression Microarrays analysis and subsequent Gene Set Enrichment Analysis (GSEA). Lipid metabolic phenotype and lipids storage was characterised by radioactive labelled nutrients assay, confocal microscopy and Western Blotting.

RESULTS AND DISCUSSION: Unsupervised hierarchical cluster analysis divided MCF7 samples into parental and LTED groups. Statistical analysis identified ~3K genes differentially regulated between parental MCF7 group and LTED cells. GSEA revealed that gene sets related to adipocyte differentiation and FA metabolism (MSigDb M5905; MSigDb M5935) are associated with the LTED profile, thus suggesting a role for lipids metabolism in resistance to AI. Moreover, highly glucose-dependent LTED cells did not show an increase in lactate production (from glucose). Crucially, the excess of uploaded glucose in LTED cells is associated with increased lipogenesis, acetyl-CoA carboxylase activation, an established marker of de novo FA biosynthesis and increased LDs. Furthermore, enhanced acetyl-CoA reserve can be used as acetyl donor in the resistant LTED cells that indeed showed an increase of histone-3-lysine-27 acetylation, index of an increased transcription rate. Crucially, LTED cells are able to mobilise this lipid storage and use them to survive in nutrient stress condition.

CONCLUSION: The increase of de novo FA biosynthesis together with subsequent lipids accumulation could confer advantages to breast cancer cells both as energy source and as acetyl-CoA storage to contribute to epigenetic regulation, thus impacting on adaptation and resistance to AI. Further investigations are needed to identify, within the lipid metabolic pathways, potential biomarkers and therapeutic targets implicated in endocrine therapy response.

NO CONFLICT OF INTEREST

227 Exosomes secreted from human cancer cells influence the adhesion of neighboring metastatic cells: Role of microRNA-210

E. Bigagli[1], C. Luceri[1], M. D'Ambrosio[1], D. Guasti[2], L. Cinci[1]

[1] University of Florence, NEUROFARBA, florence, Italy
[2] University of Florence, Department of Experimental and Clinical Medicine – Research Unit of Histology and Embryology-, Florence, Italy

INTRODUCTION: Cancer-secreted exosomes influence tumor microenvironment and support cancer growth and metastasis. MiR-210 is frequently up-regulated in cancer tissues and correlates with metastatic disease. We investigated whether exosomes are actively released by colon and breast cancer cells, the role of exosomal miR-210 in the cross-talk between primary cancer cells and neighboring metastatic cells and its contribution in regulating epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET).

MATERIALS AND METHODS: HCT-8 and MDAMB-231cells were maintained under standard conditions and the number of viable potentially metastatic cells was assessed at different time points from seeding (48, 72, 96 h; 7d). The expression of E-cadherin and vimentin was assessed by immunocytochemistry. Cells were also fixed and embedded for transmission electron microscopy analyses. Exosomes were obtained from cell culture media by using the Exosome Precipitation Solution (Macherey-Nagel). Total RNA from exosomes and from adherent cells was extracted using the NucleoSpin miRNAs Plasma kit (Macherey-Nagel) and the miRNeasy Mini Kit (Qiagen). For exosomal miRNA, cDNAs were preamplified with MiScript Pre-AMP (Qiagen). mir-210 expression was evaluated by quantitative real-time RT-PCR (miScript SYBR Green PCR Kit, Quiagen).

RESULTS: After 7 days of culture, a subpopulation of viable cancer cells detached the monolayer and started to grow in suspension, suggesting anoikis resistance and a metastatic potential. The expression of key proteins of EMT revealed that these cells were E-cadherin negative and vimentin positive further confirming their metastatic phenotype and the acquisition of anoikis resistance. Metastatic cells, in the presence of adherently growing cells, continued to grow in suspension whereas only if seeded in cell-free wells, were able to adhere again and to form E-cadherin positive and vimentin negative new colonies, suggesting the occurrence of MET. Electron microscopy analysis demonstrated that adherently growing cells, actually secreted exosomes and that exosomes in turn were taken up by metastatic cells. When exosomes secreted by adherently growing cells were administered to metastatic cells, MET was significantly inhibited in both cell lines. miR-210 was significantly up-regulated in exosomes compared to its intracellular levels in adherently growing cancer cells and correlated to anoikis resistance and EMT markers.

CONCLUSIONS: Exosomes containing miR-210 might be considered as EMT promoting signals that preserve the local cancer-growth permissive milieu and also guide metastatic cells to free, new sites of dissemination.

NO CONFLICT OF INTEREST

228 Downregulation of Alpha-L-Fucosidase expression is related to dedifferentiation and worse prognosis in thyroid and breast cancer

A. Parascandolo[1], G. Vecchio[2], C. Ugolini[3], S. Bonin[4], F. Basolo[5], G. Stanta[4], N. Tsuchida[6], M. Laukkanen[1], M.D. Castellone[7]

[1] IRCCS SDN, Research laboratory, Napoli, Italy
[2] Università di Napoli Federico II, Medicina Molecolare e Biotecnologie Mediche, Napoli, Italy
[3] Azienda Ospedaliero-Universitaria Pisana, Dipartimento di Medicina di Laboratorio, Pisa, Italy
[4] Università degli Studi di Trieste, Dipartimento di Scienze Mediche- Chirurgiche e della Salute, Trieste, Italy
[5] University of Pisa, Dipartimento di Patologia Chirurgica- Medica- Molecolare e dell'Area Critica, Pisa, Italy
[6] Tokyo Medical and Dental University, Graduate School of Medical and Dental Sciences, Tokyo, Japan
[7] Istituto di Endocrinologia ed Oncologia Sperimentale IEOS, Consiglio Nazionale delle Ricerche, Napoli, Italy

BACKGROUND: It has been reported that α-L-fucose participates in several interaction processes between cells and extracellular matrix and that high levels of fucose are related to neoplastic progression. The lysosomal enzyme α-L-Fucosidase-1 (FUCA-1) is involved in the removal of fucose from glycans and has recently been shown to be downregulated in highly aggressive and metastatic human tumors. However, the role of FUCA-1 in tumor progression remains unclear. We have analyzed the expression of FUCA-1 gene in thyroid and breast cancers and correlated the expression of the gene with a) Degree of differentiation and aggressiveness of the different thyroid cancer histotypes; b) Recurrences and cancer specific survival in a cohort of breast cancer patients with and without lymph node involvement.

MATERIALS AND METHODS: FUCA-1 expression of various thyroid normal and cancer tissues was assayed by IHC staining and qRT-PCR. ATC-derived 8505C cells were transfected with a FUCA-1 containing plasmid to measure the ability to migrate, invade, and adhere to human E-selectin and HUVEC cells. FUCA-1 expression in breast cancer was assayed by analyzing four tissue microarrays including 305 breast cancer specimens.

RESULTS: FUCA-1 expression was high in normal thyroids and papillary thyroid carcinomas (PTC), whereas it progressively decreased in poorly differentiated, metastatic and anaplastic thyroid carcinomas (ATC). The mRNA expression from tissue samples and cell lines, protein expression levels, and enzyme activity in thyroid cancer cell lines paralleled the data observed in IHC staining. ATC-derived 8505C cells transfected with FUCA-1 gene displayed a less invasive phenotype as compared to parental 8505C cells. Similarly, absence of FUCA-1 was related to the development of later recurrences in breast cancer patients with lymph node involvement (LN+) at diagnosis: 68% of recurrent patients were negative to FUCA-1 compared to 47% of non-recurrent patients. Lastly, breast cancer patients with FUCA-1 positive staining in primary tumor and luminal B lymphnode metastasis demonstrated longer survival.

CONCLUSIONS: Our result demonstrate that FUCA-1 is downregulated in thyroid and breast cancer and that this downregulation correlates with increased aggressiveness of the cancer type as well as with a worse prognosis in women with breast cancer.

NO CONFLICT OF INTEREST

229 Investigation of the role of UBE2T in hepatocellular carcinoma

E. Lioulia[1], P. Mokos[1], G. Karayannopoulou[2], E. Parlapani[1], M. Lamprousi[1], E. Panteris[3], M. Hadzopoulou-Cladaras[1], D. Dafou[1]

[1] Aristotle University of Thessaloniki- Faculty of Sciences- School of Biology, Department of Genetics- Development and Molecular Biology, Thessaloniki, Greece
[2] Aristotle University of Thessaloniki, Department of Pathology, Thessaloniki, Greece
[3] Aristotle University of Thessaloniki- Faculty of Sciences- School of Biology, Department of Botany, Thessaloniki, Greece

BACKGROUND: Hepatocellular Carcinoma (HCC) is the fifth most common cancer type in terms of incidence and the third most common cancer type in terms of mortality. The impressive genetic heterogeneity reflects genomic variation due to variable influence of several known risk factors including alcoholism, chronic HBV/HCV infection, exposure to aflatoxin-B1 and non-alcoholic fatty liver disease (NAFLD). Hepatocarcinogenesis is a multistep process that lasts decades and involves the progressive accumulation of genetic and epigenetic alterations leading to malignant transformation.

MATERIALS AND METHODS: In order to gain insight into HCC molecular aspects, we have performed Weighted Gene Co-expression Network Analysis (WGCNA) of RNA-sequencing data (HCC=370) from 'The Cancer Genome Atlas' portal (TCGA). Correlation and survival analyses were conducted in order to identify modules associated with HCC progression and prognosis. ClueGO/CluePedia plugin of Cytoscape was applied to genes with high module membership (kME>=0.7) in order to provide insights into pathways by integrating experimental and in silico information. Functionality of the newly identified molecular target was investigated by RNA interference and overexpression studies in Huh7 and HepG2 liver cancer cells. Epithelial-to-Mesenchymal Transition (EMT) was assessed by

quantification of epithelial (E-cadherin)/mesenchymal (vimentin) marker ratio and the mRNA levels of Zeb1/2.

RESULTS: WGCNA analysis revealed 15 network modules. We focused on the module that was significantly enriched for cell cycle regulation processes and was associated with tumor grade, clinical stage and overall survival. In the respective module we have identified UBE2T as a novel potential HCC prognostic biomarker along with established ones MSH2 and TOP2A. Specifically, high UBE2T expression is significantly associated with advanced stages, grades and poor prognosis. GO enrichment analysis revealed that UBE2T is associated with DNA repair processes. UBE2T overexpression led to EMT induction in HCC. In contrast, UBE2T knockdown led to phenotype reversion.

CONCLUSIONS: The potential oncogenic role of UBE2T in HCC was verified by in vitro studies and currently being explored in vivo using models of immunocompromised mice. Furthermore, UBE2T expression is being analyzed in a human liver disease spectrum tissue array.

This work was funded by 'RESEARCH PROJECTS FOR EXCELLENCE IKY/SIEMENS".

NO CONFLICT OF INTEREST

230 Murine animal models of Ewing Sarcoma: Role of the microenvironment

L. Gonzalez[1], F. Cidre-Aranaz[1], L. García-García[1], E. Madrazo[1], C. Robledo[1], S.T. Cervera[1], S. Gambera[2], C. Rodrigez-Martín[1], J. García-Castro[2], J. Alonso[1]

[1] Instituto de Investigacion de Enfermedades Raras Instituto de Salud Carlos III, Tumores Solidos Infantiles, Majadahonda, Spain
[2] Instituto de Investigacion de Enfermedades Raras Instituto de Salud Carlos III, Biotecnología Celular, Majadahonda, Spain

BACKGROUND: Ewing Sarcoma is a childhood bone cancer characterized by chromosomal translocations that generate fusion transcription factors as EWS-FLI1, which regulate the expression of specific target genes involved in the oncogenic process. The expression profile regulated by EWS-FLI1 has been however exclusively studied in vitro. The aim of this study was study the effect of the microenvironment on the gene expression profile regulated by EWS-FLI1.

METHODS: The Ewing sarcoma cell line A673 was genetically modified to express GFP, luciferase and a doxycycline-inducible shRNA directed against EWS-FLI-1. Tumor cells were xenotransplantated in mice in two different ways: i) standard subcutaneous xenograft and ii) tumor cells embedded in a hydroxyapatite matrix to simulate bone microenvironment. In each experiment a group of animals were treated with doxycycline once the tumor had reached a volume of 1000 mm3 to study the effect of EWS-FLI1 downregulation in vivo. Tumor growth was monitored by in vivo bioluminescence imaging. At the end of the experiment tumors were removed and gene expression analyses were performed (qRT-PCR and RNAseq). Tumor samples were also analyzed by standard histology.

RESULTS AND DISCUSSION: Animals treated with doxycycline underwent a dramatic reduction in the tumor size. Standard xenograft and osteogenic implant had a similar growth behavior. EWS-FLI1 expression showed an inhibition of >70% determined by qRT-PCR in animals treated with doxycycline. Well-established EWS-FLI1 target genes, were upregulated (SPRY1, LOX) or downregulated (NR0B1) in tumors derived from doxycycline-treated animals in both models. Gene expression profiles (RNAseq) indicated that >90% of genes deregulated by EWS-FLI1 were the same in both in vivo models and in in vitro. Histological analyses showed differences between the control and the doxycycline treated groups and between models. Intratumoral adipocytes were more abundant in treated groups while an increase of collagenous fibers was observed in the hydroxyapatite implant in relation to the standard subcutaneous xenograft.

CONCLUSION: Different murine animal models could be useful to study the role of the microenvironment in the modulation of the EWS-FLI1-regulated gene expression profile. Preliminary result show differences between models and EWS-FLI1 expression at the histological level. A detailed analysis of RNAseq data is ongoing to identify the pathways and genes involved in this different behavior.

NO CONFLICT OF INTEREST

231 Deciphering the molecular architecture of triple negative breast cancer

G. Prado-Vázquez[1], A. Gámez-Pozo[1], L. Trilla-Fuertes[2], S. Llorente-Armijo[1], A. Zapater-Moros[1], R. López-Vacas[1], H. Navarro[3], J. Martín-Arevalillo[3], E. Espinosa[4], J.A. Fresno Vara[1]

[1] Hospital Universitario La Paz, INGEMM, Madrid, Spain
[2] Biomedica Molecular Medicine, I+D, Madrid, Spain
[3] Universidad Nacional de Educación a Distancia, Operational Research and Numerical Analysis, Madrid, Spain
[4] Hospital Universitario La Paz, Medical Oncology Service, Madrid, Spain

BACKGROUND: Triple negative (TN) phenotype is described by a lack of ER and PR expression and HER2 overexpression. Clinically, TN breast cancers lack from targeted therapies and its prognosis is worse than other breast cancer subtypes. Molecular subtyping of breast cancer has been pursued in the last years, but still, there is need of deep characterization of these molecular subtypes. Our objective is identifying and characterizing molecular groups of TNBC using gene expression data and probabilistic graphical models.

MATERIAL AND METHODS:

TN breast cancer gene expression data was obtained from GSE31519 (n=494) from public sources. 2000 most variable genes were selected for subsequent analysis. A functional network was built using a probabilistic graphical model approach. Functional nodes were defined, and its function was explored by Gene Ontology using DAVID. Then, a new molecular classification was generated using the functional nodes activity and a k-means strategy. Subgroups were characterized and compared with previous TN molecular classifications.

RESULTS: Probabilistic graphical models defined a functional structure comprising 27 functional nodes. We found several nodes with luminal, basal or immune-related genes. Also, a claudin-enriched node was defined. Four main subgroups were defined, following the mammary epithelial stem cell hypothesis, thus four subtypes (LAR, basal, claudin-low and claudin-high) were defined. Immune status, determined by immune functional nodes, showed prognostic value ($p>0.05$). Other features, such as angiogenesis, cell cycle, apoptosis, etc. were also studied. Finally, we compared our classification with previous ones defined by Lehmann, Burstein and the PAM50.

CONCLUSION: Functional networks can provide a relevant molecular knowledge which complements the TNs classification. Relationships between genes will be identified and associated with the different TN subtypes. Probabilistic graphical models could allow a better understanding of tumor behavior and TN breast cancer molecular uniqueness. Besides, this deep knowledge will allow a more accurate prediction of outcome and can also be used for diagnostic purposes and therapy selection.

CONFLICT OF INTEREST

Ownership: JAFV, AG-P and EE are stakeholders of Biomedica Molecular Medicine S.L. and Biomedica Molecular Medicine Ltd
Board of Directors: JAFV, AG-P and EE are part of board of directors of Biomedica Molecular Medicine S.L. and Biomedica Molecular Medicine Ltd.

Other Substantive Relationships: LT-F is an employee of Biomedica Molecular Medicine S.L.

232 Pancreatic cancer-related inflammation: In vivo screening to identify key targetable molecules

C. Roux[1,2], R. Curto[1,2], G. Mucciolo[1,2], F. Novelli[1,2], T.W. Mak[3], P. Cappello[1,2]

[1] University of Turin, Dept. Molecular Biotechnology and Health Sciences, Turin, Italy
[2] Azienda Universitaria Ospedaliera Città della Salute e della Scienza, Centre for Experimental Research and Medical Studies, Turin, Italy
[3] University Health Network, Campbell Family Institute for Breast Cancer Research, Toronto, Canada

BACKGROUND: Pancreatic ductal adenocarcinoma (PDA) is a highly aggressive malignancy characterized by resistance to chemo and radiotherapy. The few advances in treatment and the low patient survival make novel therapies an urgent need. Emerging result support the role of immune system in creating a PDA-prone environment supporting cancer progression and angiogenesis. We have demonstrated that a DNA-based vaccination strategy targeting a PDA-associated antigen, namely alpha-enolase (ENO1), significantly prolongs survival in a PDA mouse model by eliciting an integrated humoral and cellular immune response. To this end, passive immunotherapies combined with ENO1-vaccination might represent an effective strategy to target PDA-related inflammation. To identify new targetable surface molecules we used an in vivo screening of proteins strictly related to inflammation and driving PDA progression.

MATERIALS AND METHODS: We forced the expression of inflammatory-related genes in PDA cells and obtained hundred clones that were orthotopically injected in syngeneic C57BL/6 mice. We purified RNA from grown tumors and analysed by quantitative PCR which genes promoted mass formation. Those genes were individually validated in vivo. In addition, in vitro assays as proliferation, viability, migration and invasion were performed to characterize their biological role.

RESULTS: To gather which gene gave advantages in in vivo growth we used a murine PDA cell line named DT6606, with low ability to grow when orthotopically injected in the pancreas or to form metastases when injected intravenously. A first pool of ten clones injected in parallel to parental PDA cells, allowed identifying three genes, which were able to give rise tumor masses. In vitro analysis confirmed higher viability and migration rate for some of these clones that may be responsible for in vivo behaviour.

CONCLUSIONS: This project will lead to the understanding of the role of poorly studied inflammatory-related genes in promoting tumor growth, to offer novel therapeutical possibilities to treat pancreatic cancer.

NO CONFLICT OF INTEREST

233 Regulation of tissue factor by epithelial-mesenchymal transitions: Impacts for the metastatic progression

M.E. Francart[1], J. Lambert[1], A. Vanwynsberghe[1], C. Gilles[1]

[1] Universisty of Liège, GIGA Cancer- Laboratory of Tumor and Deuelopment Biology, Liège, Belgium

INTRODUCTION: During the hematogenous metastatic spread, circulating tumor cells (CTCs) are released, some of which will eventually form detectable metastases. The contribution of Epithelial-Mesenchymal Transition (EMT) to CTC biology represents today a discernible hypothesis supported by abundant literature, associating EMT with higher survival, invasive and metastatic properties. An important aspect of CTC biology relies on the evidence that tumor cells encounter

the blood, a new unusual environment in which some survive. Previous work in the laboratory revealed that EMT pathways induce the expression of Tissue Factor (TF) (a cell-associated activator of the coagulation cascade) and provide tumor cells with enhanced coagulant properties and early metastatic properties when injected intravenously in mice. We recently identified vimentin as a potential regulator of TF and we will pursue examining the implication of vimentin in this EMT/TF/coagulation axis.

MATERIAL AND METHOD: Vimentin expression was modulated by siRNA in EMT-inducible human tumor cell lines. RNA stabilization studies were performed using actinomycinD. Interactions between TF and vimentin were examined by Proximity Ligation Assays (PLA). To evaluate CTC survival/persistence in vivo, EMT-positive cells silenced or not for vimentin were injected intravenously in mice for 24 hours. The persistence of human tumor cells in the lung was analyzed by RT-qPCR for human GAPDH.

RESULTS AND DISCUSSION: We thus showed that silencing vimentin diminished TF protein expression. Vimentin siRNA transfection was further shown to decrease in vitro coagulant properties and early metastases in vivo. To decipher molecular mechanisms implicated in TF regulation by vimentin, time course experiments were performed and revealed an inhibition of TF mRNA as early as 4h after vimentin siRNA transfection. In the light of literature data that demonstrated a contribution of vimentin in RNA stabilization, we are currently exploring the possibility that vimentin could interact with the UTR regions of the TF mRNA and stabilize them. In parallel, we are also exploring whether vimentin could contribute to TF protein stabilization. Supportively PLA indeed evidenced a potential interaction between TF and vimentin.

CONCLUSION: So far, our data thus support a role of vimentin in the regulation of TF at the mRNA and/or protein level and further suggest a contribution of this regulatory axis in coagulation-dependent early metastasis.

NO CONFLICT OF INTEREST

234 Mitochondria as cafs-fuelled powerhouse for prostate carcinoma cells

L. Ippolito[1], A. Morandi[2], G. Comito[1], M.L. Taddei[3], A. Iscaro[1], G.G. Muccioli[2], P. Sonveaux[3], E. Giannoni[1], P. Chiarugi[1]

[1] University of Florence, Department of Experimental and Clinical Biomedical Sciences, Firenze, Italy
[2] Université catholique de Louvain, Louvain Drug Research Institute, Brussels, Belgium
[3] Université catholique de Louvain, Pole of Pharmacologie and Therapeutics- Institut de Recherche Expérimentale et Clinique, Brussels, Belgium

BACKGROUND: Tumor cells exhibit metabolic plasticity according to nutrients availability to meet their demands for energy supply, proliferation and metastatic advantages. The establishment of a vicious metabolic synergy between stromal and cancer cells has also been reported in our model where cancer associated fibroblasts (CAFs) exert a supporting role in prostate cancer (PCa) progression, particularly by promoting a metabolic reprogramming toward a stromal-derived lactate-based oxidative metabolism (OXPHOS). In this context, our aim was to clarify the involvement of mitochondrial reprogramming and OXPHOS exploitation in CAF-conditioned PCa cells malignancy.

MATERIALS AND METHODS: Both PC3 and DU145 cells were used as PCa cellular models and conditioned or cocultured with HPFs or CAFs - fibroblasts derived from PCa patients - respectively. TCA cycle analysis was performed through LC-MS. Mitochondria were stained by using fluorescent probes.

RESULTS: Our data demonstrate that, upon CAFs exposure, PCa cells undergo a strong improvement of their OXPHOS commitment. Indeed, we observed that CAFs induce a lactate-dependent SIRT1/PGC-1α axis activation in PCa cells, leading to an increase of mitochondrial mass and to an upgrading of mitochondrial function. The overexploitation of mitochondrial metabolism induces, in CAF-exposed PCa cells, the generation of mitoROS, closely related to EMT and pro-invasive features, as well as a deregulation of the TCA cycle, which result in the accumulation of two oncometabolites, succinate and fumarate, both responsible for PHD-mediated HIF-1α stabilization and for the improvement of HIF-dependent malignant traits. Notably, we observed that succinate is also released by CAFs and uploaded by PCa cells, further raising its intracellular content and suggesting also a plausible non-metabolic function. Furthermore, we intriguingly observed both an in vitro and in vivo ability of PCa cells to steal functional mitochondria from nearby CAFs, through tunnelling nanotubes formation. Notably, analysis of CAFs-transferred mitochondria reveals their functionality and mitoROS boosting role, allowing an improvement of PCa cells metastatic potential, upon transferring.

CONCLUSIONS: Our data reveal that CAFs promote an overexploitation of mitochondrial metabolism in PCa cells, by boosting the function of both endogenous and CAF-derived mitochondria, in order to foster tumor malignancy.

NO CONFLICT OF INTEREST

235 Implication of epithelial-mesenchymal transition (EMT) on the formation of fibrin(ogen)-rich vascular nests for circulating tumor cells (CTCs)

J. Lambert[1], M.E. Francart[1], A. Vanwynsberghe[1], M. Polette[2], C. Gilles[1]

[1] University of Liège, GIGA-Cancer- Laboratory of tumor biology and development, Liège, Belgium
[2] University of Reims, INSERM UMR-S 9 0 3 , Reims, France

BACKGROUND: The laboratory recently identified a new mechanism by which EMT induces the expression of Tissue Factor (TF, a major cell-associated initiator of coagulation), triggering an activation of the coagulation cascade that favors the early metastatic colonization of EMT-positive CTCs (Bourcy et al., Cancer Research, 2016). We examined the possibility that EMT+ CTCs display an enhanced ability to organize fibrin filaments as a pericellular network that could protect them in the circulation and facilitate the formation of pro-metastatic nests in colonized organs. We first aimed at investigating the potential role of fibrin receptors in such a process. Because of its known association with EMT/cancer stem cell phenotypes, we focused on CD44, also known as a fibrin receptor.

MATERIAL AND METHODS: CD44 expression was examined in EMT-inducible human tumor cell lines and in EMT+ MDA-MB-231 versus EMT- MCF-7 human breast cell lines. To study the interaction between CD44 and fibrin in static condition, we developed a soluble fibrin pericellular network formation assay using Platelet-Poor Plasma (PPP) and GPRP, an inhibitor of fibrin polymerization. A similar assay was also optimized under flow condition, in collaboration with Pr. Heemskerk (Maastricht, Netherlands). To analyze the role of CD44 in EMT+ CTCs early seeding in the lung, EMT+ cells, transfected or not with siRNA against CD44, were injected intravenously in Balb/C mice for 24h. The persistence of the human tumor cells in the lung was estimated by performing a nested RT-qPCR for human GAPDH.

RESULTS: We thus observed an EMT-induced overexpression of CD44 in all cell models and confirmed the higher cell surface expression of CD44 in MDA-MB-468 and PMC42LA treated with EGF and in EMT+ MDA-MB-231 cells compared to the EMT- MCF7 cells. We also showed that EMT+ cells expressing higher levels of CD44 display higher ability to interact with soluble fibrin than EMT- cells. Using the in vivo mouse model, we further observed that CD44 siRNA tend to decrease the ability of EMT+ CTCs to colonize the lung.

CONCLUSIONS: Our result demonstrated, in different human in vitro cellular models, that the expression of CD44, a fibrin(ogen) receptor, is increased during the EMT process. They further suggest a link between EMT and the formation of a fibrin-pericellular network in vitro. In vivo, preliminary result support a contribution of CD44 in the ability of EMT+ CTCs to colonize the lung.

NO CONFLICT OF INTEREST

236 Digital sorting and molecular characterization of CTCs and FFPE specimens with single-cell resolution unveils intra-tumor heterogeneity in lung adenocarcinoma

M. Terracciano[1], F. Gelsomino[2], F. Bacchi[3], F. Fontana[1], C. Forcato[1], M. Fiorentino[3], V. Del Monaco[1], C. Mangano[1], G. Medoro[1], A. Ardizzoni[2]

[1] Menarini Silicon Biosystems SpA, Biology R&D, Castel Maggiore- Bologna, Italy
[2] Policlinico Sant'Orsola – Malpighi, Unità Operativa di Oncologia Medica, bologna, Italy
[3] Policlinico Sant'Orsola-Malpighi, SSD Diagnostica Istopatologica Molecolare degli organi solidi e del relativo trapianto, Bologna, Italy

INTRODUCTION: In the era of targeted therapy, decoding intra-tumor heterogeneity is of critical importance, since hidden genetic features can guide the decision-making process. For this reason, analyzing only one type of biological specimen may provide only partial information. Here we report a multi-level approach to analyze circulating tumor cells (CTCs) and formalin-fixed paraffin-embedded (FFPE) tumor tissue-derived cells (TCs) obtained from the same patient, to investigate the underlying tumor genetic heterogeneity.

METHODS: Specimens from two advanced lung adenocarcinoma patients were analyzed. The first patient, carrying an ALK translocation, was treated with cisplatin-pemetrexed as neoadjuvant therapy and with an ALK-inhibitor; the second patient was treated with carboplatin-pemetrexed. Blood samples were enriched either with an EpCAM-based or EpCAM-independent method, and matched archival FFPE sections were obtained from pleural effusion cell blocks and primary tumor tissue respectively. The enriched blood samples were stained with Cytokeratin-PE, CD45-APC and DAPI while, after dissociation, cells from tissue were stained with Vimentin-APC, Keratin-FITC and DAPI. DEPArray™ platform detected and recovered single pure CTCs or TCs, along with WBCs or stromal cells as controls. Single-cells DNA was amplified with Ampli1™ WGA kit and used to obtain genome-wide copy-number alterations (CNA) profiles using the Ampli1™ LowPass kit. Aliquots of FFPE tumor and stromal cell lysates were used as input for the DEPArray™ OncoSeek targeted panel.

RESULTS: Low-pass copy-number profiles of both FFPE single-cells and CTCs from the first patient revealed an overabundance of gains and losses, confirming the aberrant nature of tumor cells. All single cells showed a large pattern of shared alterations, with a common amplification of a genome region comprising MYC gene, and some minor differences which indicate intra-tumor heterogeneity. A hierarchical unsupervised clustering clearly separates WBCs, described by a flat profile, from the group of FFPE single-cells and CTCs, with the latter characterized by a wider inter-cell heterogeneity. Noteworthy, copy-number analyses obtained with OncoSeek panel confirmed a >3-fold amplification of MYC gene. The analysis of the second patient resulted in a quite different situation, where clustering of low-

pass profiles highlighted an independent group formed by FFPE single-cells clearly distinct from the highly heterogeneous cluster formed by CTCs.

CONCLUSIONS: DEPArray™ technology provides a reliable approach to digitally isolate 100% pure single tumor cells from different sample types from the same cancer patient. The precision granted by this platform allows to readily dissecting genetic intra-tumor heterogeneity and the evolutionary mechanisms underlying carcinogenesis.

CONFLICT OF INTEREST

Other Substantive Relationships: I work as a biologist at Menarini Silicon Biosystems SpA

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237 A tumour suppressor role of TBX3 in fibrosarcoma

R. Omar[1]

[1] *The University of Cape Town, Human Biology, Cape Town, South Africa*

BACKGROUND: Sarcomas comprise a diverse group of malignant tumours of mesenchymal origin and remain one of the most challenging neoplasms to diagnose and treat owing to their vast heterogeneity and resistance to chemotherapy. It is therefore critical to understand the molecular mechanisms that drive progression of these cancers. TBX3, a developmentally important transcription factor, has been heavily implicated in several carcinomas and we provided novel evidence linking TBX3 to sarcomas. We showed that a diverse subset of soft tissue and bone sarcomas express high levels of TBX3 and that it is a key driver of chondro-, rhabdomyo- and lipo- sarcomagenesis. Interestingly, we also showed that TBX3 may also function as a brake to prevent tumour progression in fibrosarcomas. This implies that in different cellular contexts TBX3 must regulate different target genes which may depend, in part, on its protein partners.

METHODS: The impact of TBX3, and its isoform TBX3+2a, on fibrosarcoma cell proliferation and migration was determined using the HT1080 fibrosarcoma cell line in which these TBX3 isoforms were either knocked down or stably overexpressed. Co-factors that may mediate TBX3's tumour suppressor function were identified by mass spectrometry using HT1080 3XFLAG-TBX3 and -TBX3+2a cell lysates and further validated by immunoprecipitation. Finally, based on putative TBX3 target genes identified in a TaqMan qRT-PCR array, we performed quantitative real time PCR, western blotting and luciferase reporter assays to determine if TBX3/TBX3+2a activates fibronectin.

RESULTS AND DISCUSSION: In vitro assays show that TBX3 inhibits fibrosarcoma cell proliferation and migration. Mass spectrometry data reveal that the TBX3/TBX3+2a isoforms interact with a set of co-factors that may explain its tumour suppressor role including stratifin (14-3-3-σ), PHD finger 6 (PHF6) and replication protein A1 (RPA1). It is worth noting that these factors were not previously identified for TBX3 in sarcomas where it promotes tumourigenesis (chondro-, rhabdomyo- and lipo-sarcoma). We further provide in vitro and in vivo data that TBX3 activates the fibronectin promoter.

CONCLUSION: This study confirms a tumour suppressor role for TBX3 in fibrosarcoma and contributes to our understanding of the molecular basis for this role. Indeed we identified co-factors that TBX3 may interact with to mediate this role and we show that TBX3 upregulates the tumour suppressor fibronectin.

NO CONFLICT OF INTEREST

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238 Translational repression of pro-apoptotic mRNAs by DHX30 inhibits p53-dependent apoptosis

D. Rizzotto[1], *S. Zaccara*[1], *E. Dassi*[1], *M. Galbraith*[2], *Z. Andrysik*[2], *B. Bosco*[1], *A. Quattrone*[1], *J. Espinosa*[2], *A. Inga*[1]

[1] *Centre for Integrative Biology- CIBIO, University of Trento, Trento, Italy*
[2] *University of Colorado Anschutz Medical Campus, Department of Pharmacology, Aurora- CO, USA*

BACKGROUND: The MDM2 inhibitor Nutlin-3A is a potent activator of the p53 transcription factor. Nutlin treatment can result in p53-dependent phenotypes ranging from overt apoptosis to selective cell cycle arrest. To complement transcriptomic analyses, we examined the translatome by means of polysomal profiling for SJSA1, HCT116, MCF7, three cell lines which exemplify the different responses to Nutlin, i.e. apoptosis, cell cycle arrest or a combination of the two responses, respectively.

MATERIALS AND METHODS: Polysomal-bound mRNAs were obtained after sucrose gradient fractionation of cells treated for 12h with 10uM Nutlin. Total and polysomal RNAs were sequenced. A 3'UTR motif enriched exclusively among translationally enhanced mRNAs from SJSA1 cells was identified using Weeder. The consensus motif was inserted in a control 3'UTR downstream of the luciferase reporter gene, whose activity was evaluated after 12 or 24 hours of 10uM Nutlin. The same consensus was used for a pull-down experiment followed by mass spectrometry to identify proteins interacting with it.

RESULTS: RNA-seq data indicate that while both cell cycle and apoptotic p53 target genes are transcriptionally upregulated after Nutlin treatment in the three cell lines, there is no overlap among polysome-enriched mRNAs. In particular, mRNAs that were enhanced in translation only in Nutlin-treated SJSA1 cells are enriched for apoptotic functions. These mRNAs were found to carry a specific motif in their 3'UTR, which was sufficient to stimulate translation in reporter gene assays upon Nutlin treatment in SJSA1 but not in HCT116 cells. RNA pull-down experiments identified a set of RNA-binding proteins (RBPs) bound to the aforementioned motif. Among these, the putative RNA helicase DHX30 was specific for HCT116 cells. Silencing DHX30 in HCT116 cells enhanced i) the reporter activity in the presence of the motif in the 3'UTR, ii) the polysomal association of selected mRNA containing the motif, previously identified as translationally enhanced in apoptotic SJSA1 cells, and iii) the induction of apoptosis, all in response to Nutlin treatment.

CONCLUSIONS: We propose that next to transcriptional specificity, mRNA translational selectivity helps to shape p53-dependent cell fate choices. In this scenario, DHX30 acts as a repressor of p53-dependent apoptosis. The potential for DHX30 silencing to sensitize cancer cells to different chemotherapeutics that activate p53 is being investigated.

NO CONFLICT OF INTEREST

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239 HIF-1 inhibition and TLR3 stimulation as combined antitumor treatment of apoptosis-resistant human breast cancer cells

F. Scatozza[1], *E. Ziparo*[1], *A. Riccioli*[1]

[1] *Sapienza- University of Rome- 1 6 Via A. Scarpa, Department of Anatomy- Histology- Forensic Medicine and Orthopaedics- Unit of Histology and Medical Embryology., Rome, Italy*

INTRODUCTION: Hypoxia is a reduction in the normal level of tissue oxygen tension, and occurs during many diseases and cancer. The cells respond through hypoxia-inducible factor 1 (HIF-1) (Harris A.L., 2002 Nature Reviews Cancer). Besides hypoxia, there are other stimuli that activate HIF-1α through specific Toll-like receptors (TLR), capable to increase the formation of HIF-1 complex in normoxia and that can modulate the transcription of hypoxic genes. In our lab it was demonstrated that the TLR3 ligand, [poly(I:C)] (PIC) induces HIF-1α activation in human prostate cancer cells PC3 leading to tumor resistance (Paone A., 2010, Neoplasia). I intend to investigate whether the activation of HIF-1 could be a protective factor in PIC-induced apoptosis in breast cancer cells and whether HIF-1 inhibition through pharmacological and genetic methods could revert cancer resistance to PIC.

MATERIAL AND METHOD: MDAMB-231, MCF-7 and CAMA-1 cells were purchased from ATCC (Manassas, VA). For all the experiments, these cell lines were serum-starved and then treated with PIC (InvivoGen, San Diego, CA) in FBS-free medium for different times. The protein levels are evaluated by Western Blot on total cell lysate or on Nuclear Extracts. PI assay and Annexin V staining were performed to analyze apoptosis induced by PIC with HIF-1 inhibitors Flavopiridol (FVP) and Acriflavine (AF).

RESULTS AND DISCUSSION: PIC treatment induced HIF-1α upregulation in MDAMB-231 and MCF-7 , but not in CAMA-1 cells. Treatment with FVP, AF or in combination resulted in increased apoptosis in MDAMB-231 cells treated with PIC. Interestingly, HIF-1α was unaffected in CAMA-1 cells confirmed their apoptosis sensitivity to PIC treatment, while MDA and MCF cells are known to be PIC-insensitive. In addition, I analyzed a possible cross-talk between the TLR/NF-κB and HIF-1 signaling, which may contributes to breast cancer initiation and progression. I observed an activation of NF-κB and p-ERK proteins in MDAMB-231 cells after PIC-treatment.

CONCLUSION: In this study, I am investigating if the activation of HIF-1 complex could be a protective factor in PIC-induced apoptosis in tumors using various human breast cancer cells with different aggressiveness phenotypes as cellular models. Taken together, my result indicate that the levels of HIF-1α protein in breast cancer cells might play an important role in the resistance to TLR3 stimulation.

NO CONFLICT OF INTEREST

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240 Anticancer potential of novel steroid derivatives

I. Matic[1], *M.B. Zivkovic*[2], *N.M. Krstic*[2], *D.M. Sladic*[3]

[1] *Institute of Oncology and Radiology of Serbia, Department of Experimental Oncology, Belgrade, Serbia*
[2] *Institute of Chemistry- Technology- and Metallurgy- University of Belgrade, Center for Chemistry, Belgrade, Serbia*
[3] *Faculty of Chemistry- University of Belgrade, Department of Organic Chemistry, Belgrade, Serbia*

BACKGROUND: The aim of this study was to evaluate the anticancer properties of newly synthesized mono- and bis(1,3-thiazolidin-4-one) steroidal derivatives **4a-f** and **5a-f**.

MATERIAL AND METHODS: The cytotoxicity of a series of twelve compounds was determined against six human malignant cell lines: cervical adenocarcinoma HeLa, breast carcinoma MDA-MB-453, breast adenocarcinoma MDA-MB-361, chronic myelogenous leukemia K562, colon adenocarcinoma LS174T, lung carcinoma A549, as well as against normal human lung fibroblasts MRC-5 and human peripheral blood mononuclear cells (PBMC). Cell cycle analysis was performed by flow cytometry. The mode of cell death induced by the compounds was examined by fluorescence microscopy and flow cytometry using specific caspase inhibitors. The anti-angiogenic properties of the compounds were investigated by tube formation assay.

RESULTS: The steroid derivatives exerted selective concentration-dependent cytotoxic activities on malignant cells. HeLa and K562 cells were the most sensitive to the cytotoxic effects of the compounds. Ten out of twelve compounds exhibited strong cytotoxic effects on K562 cells with IC_{50} values from 8.47 μM to 14.95 μM, while against HeLa cells eight compounds exerted high cytotoxic actions with IC_{50} values ranging from 8.86 μM to 15.15 μM. These compounds showed high selectivity in the cytotoxic action against malignant cells when compared with normal

MRC-5 cells and PBMC. The mechanisms of the anticancer activity of the selected compounds, mono- and bis(1,3-thiazolidin-4-one) derivatives of 19-norandrost-4-ene-3,17-dione **4a** and **5a,** were further elucidated. Both compounds applied at $2IC_{50}$ concentrations (17.88 μM and 27.06 μM, respectively) for 24 h induced a significant increase of HeLa cells in the subG1, S and G2/M cell cycle phases when compared with the control cells. Fluorescence microscopy of HeLa cells revealed pro-apoptotic effects of these compounds. In addition, both compounds triggered apoptosis in HeLa cells through extrinsic and intrinsic signaling pathways. Treatment of EA.hy926 cells with sub-toxic concentrations of compounds **4a** and **5a** (30 μM and 35 μM, respectively) for 20 h, led to inhibition of cell connecting and sprouting, and tube formation.

CONCLUSIONS: Our result suggest the significant anticancer potential of novel steroid compounds due to their strong cytotoxicity against cancer cells, high selectivity in the anticancer action and anti-angiogenic properties.

NO CONFLICT OF INTEREST

241 New evidences of Metformin effectiveness on vemurafenib-treated BRAFV600E acidic melanoma cells

F. Bianchini[1], S. Peppicelli[1], J. Ruzzolini[1], E. Andreucci[1], L. Calorini[1]

[1] University of Firenze, Clinical and Experimental Biomedical Science, Firenze, Italy

BACKGROUND: Proliferating cancer cells exhibit an increased glycolysis regardless of oxygen tension (the 'Warburg effect"). Further, an abnormal vasculature can result in regional variation in oxygenation implying a dynamic change in metabolism of tumor cells from an aerobic to an anaerobic type of glycolysis. These metabolic programs lead to a change in tumor microenvironment pH, which reduces to acidosis, conferring a new adaptive aspect to cancer cells. Extracellular acidity induces in melanoma cells an EMT (epithelial-to-mesenchymal transition) program and a metabolic change to oxidative phosphorylation (Oxphos).

Metformin is a synthetic biguanide widely used as antidiabetic drug. Interestingly, recent studies have reported that metformin can block the growth of different tumor types, including melanoma.

METHODS: A375 BRAF-mutated human melanoma cells were cultivated under acidic conditions, in the presence or absence of metformin. Moreover vemurafenib, a selective inhibitor of BRAF kinase, used in the therapy of metastatic and advanced malignant melanoma, was tested alone or in combination with metformin. The activation of EMT program, cellular metabolism and cell viability were evaluated in acidic cells exposed to metformin and vemurafenib.

RESULTS: We confirmed that melanoma cells exposed to an acidic microenvironment showed an EMT profile and an Oxphos metabolism. Interestingly, we found that metformin addition to tumor cell acidic medium reduced EMT profile (N-cadherin expression and invasiveness) and reverted the Oxphos metabolism of acidic cells. We found also that metformin promoted AMPKa expression and phosphorylation in acidic melanoma cells. In addition, high doses of metformin induced a strong inhibition of acidic cells proliferation and colony formation. Vemurafenib, at different concentrations, arrested cell proliferation and induced a change in metabolism in non-acidic cells, whereas acidic cells were unresponsive. Despite vemurafenib modified the metabolic profile of non-acidic cells, leading to a metformin-sensible phenotype, the combination of two drugs induced cell death only in acidic cells, and blocked non-acid cells proliferation as vemurafenib alone.

CONCLUSIONS: Overall, these findings disclose a new potential rationale for metformin addition to therapy of advanced melanoma, highlighting the importance of a chemotherapeutic treatment planning that takes into consideration the multiple aspects of tumor microenvironment.

NO CONFLICT OF INTEREST

242 ZEB1-mediated melanoma cell plasticity enhances resistance to MAPK inhibitors

G. Richard[1], S. Dalle[1], M. Ligier[1], A. Puisieux[1], J. Caramel[1]

[1] Cancer Research Center of Lyon, Cancer Cell Plasticity, Lyon, France

BACKGROUND: Targeted therapies with MAPK inhibitors (MAPKi) are faced with severe problems of resistance in BRAF-mutant melanoma. No clear mutational mechanism is found in up to forty percent of resistant melanomas, indicating that transcriptomic or epigenetic alterations may underly acquired MAPKi resistance. In parallel to the acquisition of genetic mutations, melanoma cells may also adapt to the drugs through phenotype switching. Therefore, identification of non-genomic mechanisms by which melanoma cells reprogram their epigenome/transcriptome to evade MAPKi therapy may lead to the design of more efficient combination therapies.

RESULTS: The ZEB1 transcription factor, a known inducer of EMT and invasiveness, is now considered as a genuine oncogenic factor required for tumor initiation, cancer cell plasticity and drug resistance in carcinomas. We now uncover the role of ZEB1 as a major driver of phenotype switching in melanoma cells, providing them with a resistance to MAPKi. We show that high levels of ZEB1 expression are associated with inherent resistance to MAPKi in $BRAF^{V600}$-mutated cell lines and tumors. Moreover, ZEB1 levels are elevated in melanoma cells with acquired resistance and in biopsies from patients relapsing while under treatment. We further demonstrate that ZEB1 overexpression is sufficient to drive the emergence of resistance to MAPKi by promoting a reversible transition towards a $MITF^{low}$/$p75^{high}$ stem-like and tumorigenic phenotype. Consequently, ZEB1 inhibition promotes cell differentiation, prevents tumorigenic growth in vivo, sensitizes naive melanoma cells to MAPKi and induces cell death in resistant cells.

CONCLUSIONS: Our result demonstrate that ZEB1 is a major driver of melanoma cell plasticity, driving drug adaptation and phenotypic resistance to MAPKi. As a consequence, mutated BRAF melanoma patients with high levels of ZEB1 expression may not benefit from MAPKi treatment and ZEB1 could therefore serve as a predictive marker in order to stratify BRAF-mutated melanoma into MAPKi-sensitive and MAPKi-resistant subgroups. Finally these data should pave the way for the design of novel combination therapies targeting cell plasticity and the MAPK pathway in order to prevent the emergence of resistance in melanoma.

NO CONFLICT OF INTEREST

243 Blocking the PI3K pathway in liver metastasis from colorectal cancer reduces the local immunosuppression

R.G. Gieling[1,2], A.L. Adlard[3], B.A. Telfer[1], D. Foster[4], K.J. Williams[1,2]

[1] The University of Manchester, Hypoxia & Therapeutics Group- Division of Pharmacy and Optometry- School of Biology- Medicine and Health, Manchester, United Kingdom
[2] The University of Manchester, Cancer Imaging Centre between Cambridge and Manchester, Manchester, United Kingdom
[3] The University of Manchester, Division of Infection- Immunity & Respiratory Medicine- School of Biology- Medicine and Health, Manchester, United Kingdom
[4] The University of Manchester, Wolfson Molecular Imaging Centre, Manchester, United Kingdom

The phosphatidylinositide 3-kinase (PI3K) pathway is activated in many cancers, and increased PI3K activity enhances cellular proliferation & migration and reduces the prognosis. The PI3K pathway is a solid therapeutic target in varies cancer types including colorectal cancer (CRC). We noticed that PI3K inhibitor treated CRC livers were less oxygen-deprived (hypoxic) than untreated and wondered whether blocking PI3K signalling in CRC livers does affect the cross talk between tumour cells and microenvironment with immune cells being an obvious candidate.

Liver metastases from colorectal cancer were induced with the intrasplenic technique using murine CT26 cells (n = 12 per group). GDC-0941 was given orally twice-daily for 8 days, starting on day 4 post-operation and continued up till the end. Selective myeloid cells and lymphocytes were isolated from fresh liver tumour pieces (n = 6 per group) and processed for flow cytometry analysis. Snap frozen liver tumour pieces were kept for mouse protein cytokine arrays (32 targets), and CCL22 (C-C motif chemokine 22) Elisa assays. Pieces of CRC livers were formalin-fixed and processed for immunohistochemistry.

GDC-0941 therapy did reduce liver tumour burden effectively. Flow cytometry indicated that drug treatment reduced the number of tumour associated macrophages (TAM). Tumour-associated lymphocytes levels were also affected, in particular the level of regulatory T cells (Tregs) were reduced and invariant natural killer T cell (iNKT) numbers increased. These changes point towards an enhanced antitumour immunity in the liver of drug treated mice as iNKT cells are part of the natural host defence system and Tregs are critical for the immune evasion in liver cancer. Elisa assays revealed that CCL22 levels in the tumour livers were reduced. CCL22 is normally abundantly released by TAM, and commonly used as a marker of type M2 macrophages. CCL22 selectively recruits CCR4+ lymphocytes (including Th2 and Treg cells) to the tumour microenvironment through chemotaxis. Protein cytokine arrays revealed the up regulation of interleukin-5 (IL-5), CCL5 (RANTES) and Vascular Epithelial Growth Factor (VEGF) and the down regulation of IL-13 and IL-17.

PI3K inhibitor GDC-0941 is effective as anticancer agent in liver metastasis of colorectal cancer in part by reducing the immunosuppression.

NO CONFLICT OF INTEREST

244 Short-term inhibition of TERT induces telomere length-independent cell cycle arrest and apoptotic response in EBV-immortalized and transformed B cells

A. Celeghin[1], S. Giunco[2], R. Freguja[1], M. Zangrossi[1], S. Nalio[2], R. Dolcetti[3], A. De Rossi[1]

[1] University of Padova, Department of Surgery- Oncology and Gastroenterology, Padova, Italy
[2] Istituto Oncologico Veneto IOV-IRCCS, Immunologia e Diagnostica Molecolare, Padova, Italy
[3] CRO-IRCCS- National Cancer Institute, Cancer Bio-Immunotherapy Unit, Aviano, Italy

BACKGROUND: Besides its canonical role in stabilizing telomeres, telomerase reverse transcriptase (TERT) may promote tumorigenesis through extra-telomeric functions. The possible therapeutic effects of BIBR1532 (BIBR), a powerful TERT inhibitor, have been evaluated in different cellular backgrounds, but no data are currently available regarding Epstein–Barr virus (EBV)-driven B-cell malignancies. Our aim was to characterize the biological effects of TERT inhibition by BIBR on EBV-immortalized lymphoblastoid cell lines (LCLs) and fully transformed Burkitt's lymphoma (BL) cell lines.

MATERIAL AND METHODS: TERT-negative LCL and TERT–positive LCLs and BL cell lines were treated with BIBR. TERT levels and activity were assayed by real-time PCR and TRAP assay. Apoptosis and cell cycle profiles were evaluated by flow cytometry. The effects of BIBR on LCLs in combination with Fludarabine (FLU) or Cyclophosphamide (CY), chemotherapeutic agents employed in the treatment of B-cell malignancies, were also investigated.

RESULTS: BIBR selectively inhibited telomerase activity in TERT-positive LCLs and BL cell lines. TERT inhibition led to decreased cell proliferation, accumulation of cells in the S-phase and ultimately to increased apoptosis, compared with mock-

treated control cells. All these effects occurred within 72 h and were not observed in BIBR-treated TERT-negative cells. The cell cycle arrest and apoptosis, consequent upon short-term TERT inhibition, were associated with and likely dependent on the activation of the DNA damage response (DDR), highlighted by the increased levels of γH2AX and activation of ATM and ATR pathways. Analyses of the mean and range of telomere lengths and telomere dysfunction-induced foci indicated that DDR after short-term TERT inhibition was not related to telomere dysfunction, thus suggesting that TERT, besides stabilizing telomere, may protect DNA via telomere-independent mechanisms. TERT-positive LCLs treated with BIBR+FLU or BIBR+CY showed a significant increase of apoptotic cells, compared to cell treated with chemotherapeutic agents alone (34% vs 15% p=0.0051 and 68% vs 46% p=0.0062, respectively).

CONCLUSIONS: TERT inhibition impairs cell cycle progression and enhances the pro-apoptotic effects of FLU and CY in TERT-positive cells. These result support new therapeutic applications of TERT inhibitors in EBV-driven B-cell malignancies.

NO CONFLICT OF INTEREST

245 The bHLH transcription factors DEC1 and DEC2 promote aggressiveness of Papillary Thyroid Carcinoma

C. Gallo[1], *G. Manzotti*[1], *F. Torricelli*[1], *K. Dallaglio*[1], *V. Sancisi*[1], *A. Ciarrocchi*[1]

[1] *IRCCS-arcispedale Santa Maria Nuova, Translational Research Laboratory, Reggio Emilia, Italy*

Papillary thyroid carcinomas (PTC) are the most common type of thyroid cancer. Even if PTC are considered indolent tumors, aggressiveness and metastatic spreading is not a rare event. The characterization of molecular mechanisms driving aggressiveness is a crucial step to improve diagnosis and therapeutic approaches of PTC.

Recently, we identified Differentiated Embryonic Chondrocytes 1 and 2 (DEC1/DEC2) as strongly overexpressed in a model of highly aggressive PTC. DEC1/DEC2 are transcription factors (TF) of the bHLH family involved in the regulation of many cancer-related genes. Their role in the development and progression of PTC is still unknown. Here we explored the function of these two TF and their mechanisms of action in these tumors.

To assess the biological role of DEC1/DEC2 in PTC we altered their expression levels in two different PTC cell lines (BCPAP and TPC1) and we analyzed changes in cell phenotype and behavior. Two different approaches were used. siRNA mediated silencing was used to knock down DEC1/DEC2 in both cell lines. Inducible overexpression of DEC1/DEC2 was used to increase the level of these TF in PTC cells. Changes in cell viability and proliferation were assessed using MTT, Cell Count and Colony Forming Assay. Effects on motility and invasiveness were measured by performing Scratch, Invasion and Adhesion assay. To explore the gene expression profile associated with these TF we performed RNAseq analysis in TPC1.

We demonstrated that DEC1 silencing causes a significant reduction in cell proliferation, viability and colony forming capacity in both TPC1 and BCPAP cells. Furthermore, we demonstrated that DEC1/DEC2 are involved in promoting migration and invasiveness of both cell lines. We also observed that DEC1 expression is strongly associated with c-MYC and NOTCH1 levels suggesting an implication of these genes in the DEC1 dependent pathway in PTC.

Finally, since we previously demonstrated that the cytotoxic activity of HDAC inhibitors (HDACi) is also dependent on the repression of key cancer genes, we investigated the possibility of targeting DEC1/DEC2 expression using these drugs. We showed that HDACi treatment profoundly inhibit DEC1 expression in PTC cells lines. This was associated with a strong repression of NOTCH1, while DEC2 expression was only marginally affected. In conclusion, these data described a novel mechanism underlying thyroid cancer aggressiveness and reveal a new role of DEC proteins as drivers of PTC progression.

NO CONFLICT OF INTEREST

246 The extracellular regulated protein kinase 5 (ERK5) is a key regulator of macrophage activation in tumour

E. Giurisato[1], *B. Telfer*[1], *W. Vermi*[2], *C. Tournier*[1]

[1] *University of Manchester, Faculty of Biology- Medicine and Health, Manchester, United Kingdom*
[2] *University of Brescia, Department of Molecular and Translational Medicine, Brescia, Italy*

BACKGROUND: A number of transcription factors have been implicated in the rearrangement of the transcriptional profile that sustains the reprogramming of tumor-associated macrophages (TAMs) to facilitate carcinoma development. In contrast, we have a very limited understanding of the signaling mechanisms that governs this process in response to tumor-derived signals. The extracellular-regulated protein kinase 5 (ERK5) is a unique mitogen-activated protein kinase (MAPK) which has previously been linked to the pathophysiology of cancer.

MATERIAL AND METHODS: Mice carrying the erk5^{F} allele and the CMV-Cre^{ER} or LysM-Cre transgenes were used for in vitro and in vivo studies.

RESULTS: In this study, we have found that ERK5 is strongly expressed in pro-tumor macrophages present in various human carcinomas. Moreover, polarized erk5-deleted bone marrow-derived macrophages exhibited low level expression of anti-inflammatory mediators and trophic factors which subsequently halted tumor cell growth in vitro. Accordingly, the growth of carcinoma grafts in mice was suppressed by ablating ERK5 in the myeloid lineage. In addition, we found that pSTAT3(Y705), a master regulator of pro-tumor gene induction was impaired in erk5-deleted TAMs cells.

CONCLUSIONS: Together these result indicate that ERK5 is a critical regulator of pro-tumor macrophage activity. Owing to the prevalence of TAMs in cancer and their unique influence upon disease progression and malignancy, macrophage-targeted manipulation via anti-ERK5 therapy represents a novel promising strategy in cancer immunotherapy.

NO CONFLICT OF INTEREST

247 Inhibition of Stearoyl-Coa desaturase 1 (SCD1) enzymatic activity reverts BRAFi/ MEKi –induced selection of melanoma cancer stem cells

M.E. Pisanu[1], *A. Noto*[1], *L. Fattore*[2], *D. Malpicci*[3], *C. De Vitis*[1], *C.F. Ruggiero*[3], *Z. Jakopin*[4], *A. Tallarita*[1], *G. Ciliberto*[5], *R. Mancini*[1]

[1] *Università Sapienza, Medicina Clinica E Molecolare, Roma, Italy*
[2] *Istituto Nazionale Tumori Irccs-Fondazione Pascale, Dipartimento Melanoma- Tessuti Molli- Muscolo-Scheletrico E Testa-Collo, Napoli, Italy*
[3] *università Magna Graecia, Dipartimento Di Medicina Sperimentale E Clinica, Catanzaro, Italy*
[4] *Università Di Lubiana, Farmacia, Slovenia, Slovenia*
[5] *Istituto Nazionale Tumori - Regina Elena, Direzione Scientifica, Roma, Italy*

BACKGROUND: Therapy of melanoma has been improved by the advent of immunological checkpoint inhibitors and by targeted therapies with kinase inhibitors. The long term efficacy of targeted therapies against BRAF mutations is hampered by the development of acquired resistance. Treatment failures in melanoma patients have been attributed in part to the enrichment of cell with stem cells properties (CSCs). We previously demonstrated that lung CSCs are enriched for the expression of Stearoyl-CoA Desaturase 1 (SCD1), a key enzyme of lipid metabolism involved in the conversion of saturated into mono-unsaturated fatty acids, and that its inhibition suppresses the ability to form spheroids and selectively kills CSCs. In this study we investigated the involvement of SCD1 in melanoma stem cells survival and the interplay between BRAF/ERK inhibitors (BRAF/MEKi) and lipid metabolism.

MATERIALS AND METHODS: SCD1 gene expression data of 479 patients were downloaded from TCGA and correlated with survival. Combination of BRAF/MEKi and the selective small SCD1 inhibitor MF438 were tested by spheroid forming assays on BRAF-mutated melanoma cell lines grown in selective 3D medium.SCD1, Nanog, Oct4, CD133 expression was studied by WB and RT-PCR.

RESULTS AND DISCUSSION: Bioinformatics analysis was carried out to assess if SCD1 can act as prognostic factor in melanoma. By using online tool we found that overall survival of patients affected by melanoma was inversely correlated to SCD1 expression. This finding led us to measure SCD1 expression in CSCs obtained from melanoma two BRAF mutated melanoma cell lines. We observed that SCD1 expression is upregulated in spheroids derived from both lines. Moreover SCD1 overexpression was associated with enrichment of stem cell markers, Oct4, Nanog and CD133. Exposure to high doses of BRAF/MEKi induced increased spheroid formation. Based on these evidences we analyzed the functional role of SCD1 by inhibiting its activity using MF438. We found that while spheroids were resistant to BRAF/MEKi, combining kinase inhibitors with MF438 led to a complete inhibition of tumor spheroid formation and downregulation of stem cell markers.

CONCLUSIONS: Our result suggest that treatment of BRAF-mutated melanoma cells with BRAF/MEKi selects for cells with stem cell features and that this phenomenon is counteracted by SCD1 inhibitors. These findings have potential implication of the development of new combination therapies.

NO CONFLICT OF INTEREST

248 NSCLC depend upon YAP expression and nuclear localization after acquiring resistance to EGFR inhibitors

M. McGowan[1], *O.T. Brustugun*[1]

[1] *Radium Hospital, Cancer Genetics, Oslo, Norway*

INTRODUCTION: Adenocarcinomas overexpressing epidermal growth factor receptor (EGFR) have presented themselves as a viable target for therapeutic treatment using erlotinib, gefitinib or the new third generation inhibitor osimertinib. However, patients treated with these drugs eventually relapse and see a re-growth of the cancer. Yes-associated protein (YAP) has been found to both regulate AXL expression and drive gene expression allowing EMT to occur after binding with the transcription factor TEAD. Our report offers further evidence of YAP's involvement as a potential therapeutic target of drug-resistance in NSCLC.

MATERIALS AND METHODS: We used the NSCLC HCC827 generated to become resistant to the first generation EGFR inhibitors erlotinib and gefitinib. We also used the H1975 to be resistant to osimertinib. We assessed YAP expression using RT-qPCR and Western Blot, along with AXL expression and EMT biomarkers (e-cadherin loss and vimentin expression). To test cell viability we used resazurin sodium salt and calculated drug EC_{50}.

We used immunocytochemistry (ICC) to visualize if YAP was localized to the cytoplasm or nucleus. To assess YAP as a possible mechanism of drug-resistance we used siRNA to knock down the co-transcription factor and reintroduced their respective drugs in a cell viability assay.

RESULTS AND DISCUSSION: We found YAP was overexpressed in both drug-resistant sub-lines HCC827/ER and GR, and also in the osimertinib-resistant

H1975/OR compared to their parental cells. We confirmed this using RT-qPCR . We further evaluated as to whether YAP was active (sequested to the nucleus) or inactive (withheld in the cytoplasm). Our data visually showed that YAP was evenly distributed in both the cytoplasm and nucleus in the HCC827/ER and GR sub-lines. In the H1975/OR sub-line we observed most YAP staining in the nucleus. We confirmed that YAP was active in all sub-lines before determining if knock down had any impact on cell proliferation when re-introduced to EGFR inhibitors.

After successfully knocking YAP down using siRNA we observed a reduction of cell viability after reintroducing EGFR inhibitors. These data indicate that YAP plays a key role in cell proliferation in drug-resistant cells.

CONCLUSION: Our data have demonstrated that YAP may be a possible mechanism of drug-resistance in NSCLC and furthermore presents itself as a viable therapeutic target.

NO CONFLICT OF INTEREST

249 Loss of PI3K-C2γ, a class II PI3K, promotes KRAS-driven pancreatic tumor

M.C. De Santis[1], M. Martini[1], E. Ratto[1], F. Fan[2], M. Pregnolato[1], C.C. Campa[1], E. Hirsch[1]

[1] MBC - Molecular Biotechnology Center, Dipartimento di biotecnologie molecolari e scienze per la vita, Torino, Italy
[2] Key Laboratory of Biomedical Information Engineering of Ministry of Education- Xi'an Jiaotong University, Department of Biological Science and Bioengineering, Xi'an, China

INTRODUCTION: Pancreatic Ductal Adenocarcinoma (PDA) is the most lethal cancer across the world, with incidence equaling mortality. The lack of progress in early diagnosis and effective therapies are the main reasons why improvements in PDA death rates have been so scarce. A wealth of studies have identified that PI3K/AKT axis is uniformly activated in PDA, impacting on tumor growth and progression. Whereas the majority of efforts have so far focused on class I PI3K, increasing evidence is pointing to the importance of class II enzymes in cell proliferation and survival. In particular, PI3K-C2γ, differently from other class II members, is mainly expressed in the pancreatic tissue where it plays a pivotal role in metabolism control. In pancreatic cancer, the human PIK3C2G gene is homozygous-deleted in about 7% of patients.

MATERIAL AND METHODS: Mouse model of PDAC (K-RAS^{G12D}/Trp53^{R172H}/Cre^{Pdx1}) was crossed with mouse strain lacking PI3K-C2γ expression. Mice were weekly followed for survival and tumor growth and histopathological analyses were performed. Tumor-derived primary cells were evaluated by immunofluorescence, biological and biochemical assays.

RESULTS: We found that lack of PI3K-C2γ increases tumor development and progression, strongly reducing mice mean survival rate and driving rapid progression to PDA. Loss of PI3K-C2γ in KPC mice strongly reduces mice mean survival rate (18 weeks vs 36 weeks) and drives rapid progression of premalignant lesions to PDAC. Pik3c2g$^{-/-}$ tumors displayed a highly aggressive features and increased resistance to chemotherapeutic agents (Gemcitabine) compared to wt controls. We demonstrated that PI3K-C2γ produces a phosphatidylinositol-3,4-bisphosphate pool required for delayed and sustained AKT stimulation. Biochemical analyses of Pik3c2g-deficient tumors revealed a specific deregulation of ERK and AKT pathways, increasing cellular proliferation. Accordingly, loss of PI3K-C2γ significantly up-regulates basal autophagy, which is required for tumor cell survival and progression, through mTOR/p70 S6K pathway.

CONCLUSION: These findings define the tumor suppressor role of PI3K-C2G in PDAC mouse models and hold important implication for deeper characterization of molecular mechanisms underlying pancreatic cancer development and progression.

NO CONFLICT OF INTEREST

250 Che-1/AATF: A direct target of c-Myc drives cell proliferation in pediatric B-cell precursor acute lymphoblastic leukemia

V. Folgiero[1], C. Sorino[2], M. Pallocca[2], F. De Nicola[2], F. Goeman[3], V. Bertaina[1], L. Strocchio[1], P. Romania[1], M. Fanciulli[2], F. Locatelli[4]

[1] Bambino Gesù Children's Hospital, Onco-hematology, Rome, Italy
[2] Regina Elena National Cancer Institute, SAFU- Department of Research- Advanced Diagnostics- and Technological Innovation- Translational Research Area, Rome, Italy
[3] Regina Elena National Cancer Institute, Epigenetic- Department of Research- Advanced Diagnostics- and Technological Innovation- Translational Research Area, Rome, Italy
[4] Bambino Gesù Children's Hospital, Onco-hematology/Department of Pediatric Science- University of Pavia- Italy, Rome, Italy

BACKGROUND: Despite the overall progress in treatment of B-ALL, relapse occurs across the whole spectrum of all subtypes and has a dismal prognosis with an overall survival of 30%. The oncogene c-Myc is a master transcription factor governing many critical cell functions such as metabolism, proliferation and survival and it is involved in up to 70% of cancers. In Burkitt's Lymphoma, c-Myc gene duplications or translocations have been described leading to its constitutive transcriptional deregulation. Che-1/AATF is an important RNA polymerase II binding protein involved in the regulation of gene transcription. Che-1 is required for proliferation in early embryogenesis, and exhibits an anti-apoptotic activity in different tumour contests. Albeit it has been shown that Che-1 controls different mechanisms of tumorigenesis in several tumour contests, its role in haematological malignancies has not been deeply explored.

MATERIAL AND METHODS: Bone marrow samples were analyzed by FACS and Western blotting. RT-pcr, ChIP, RNA-seq, ChIP-seq, Co-IP assays were performed for c-Myc-dependent Che-1 regulation.

RESULTS: We found a complete correlation of Che-1 and c-Myc expression in 80 B-ALL patients compared with 15 bone marrows from healthy donors lacking the expression of both molecules. These result are in agreement with public data derived from an RNAseq experiment on non-transgenic (control) and Eμ-myc transgenic littermates (pre-tumour), and in lymphomas arising in adult Eμ-myc animals (tumour), in which our analysis demonstrated that Che-1 mRNA levels increase during lymphomagenesis. In addition we observed a total ablation of Che-1 and c-Myc in the remission status, whereas samples from relapsed patients maintained high levels of both proteins. In all these cells we observed that the down-regulation of c-Myc strongly reduces Che-1 mRNA and protein levels. Chip analysis revealed the ability of c-Myc to bind Che-1 promoter. RNA-seq analysis comparing the effect of Che-1 or c-Myc downregulation revealed a strong overlap of the controlled pathways, suggesting that Che-1 acts as a c-Myc co-factor. Finally, we show a direct interaction between these two proteins, demonstrating that Che-1 is required for c-Myc recruitment onto DNA.

CONCLUSIONS: Furthermore the modulation of Che-1 expression may represent a strategy to improve treatment outcomes particularly in high-risk patient subgroups, wherein failure of conventional chemotherapy and relapse are common.

NO CONFLICT OF INTEREST

251 The new cyclins increase the proliferation and migration of Lung Cancer cells

L. Gasa[1], A. Sanchez-Botet[1], E. Quandt[1], S. Hernández-Ortega[1], S. Simonetti[2], J. Jiménez[1], M.A. Carrasco-García[2], M.P. Ribeiro[1], S.J. Kron[3], J. Clotet[1]

[1] Universitat Internacional de Catalunya (UIC) Faculty of Medicine and Health Sciences, Sant Cugat, Spain
[2] Hospital Universitari General de Catalunya, Pathology Medicine Service, Sant Cugat, Spain
[3] University of Chicago, Department of Molecular Genetics and Cell Biology, Chicago, USA

INTRODUCTION: Lung cancer was responsible for more than 20 % of total cancer deaths in 2016 in Europe. Importantly, many patients receive a diagnosis at an advanced stage of the disease when the treatment options are very limited. The mammalian cell cycle is mainly controlled by complexes of Cyclin Dependent Kinases (CDKs) and cyclins, in a tightly regulated process; the loss of this regulation may lead to uncontrolled cell proliferation and favour the tumorigenic process. After the Human Genome Project, several new CDKs and cyclins were identified, but it is still unknown whether some of these newly discovered members might play a role in cancer as well. Therefore, the aim of this study was to characterize the function of these novel cyclins in lung cancer.

MATERIAL AND METHOD: The expression of new cyclins in lung cancer cell lines and formalin-fixed and paraffin-embedded (FFPE) human tissues was monitored by western blot analysis. The effect of new cyclins overexpression on cell viability was evaluated using the MTT assay, while cell migration was determined by transwell assay. Moreover, we assessed the prognostic value of new cyclins expression in a large set of lung cancer patients using the website-based software Kaplan-Meier Plotter.

RESULTS: AND DISCUSSION: The expression of seven new cyclins was evaluated in a cohort of FFPE tissues and compared with paired adjacent non-tumor tissues. The result revealed that some of these new cyclins are significantly overexpressed in lung tumors. Likewise, we detected that the expression of the novel cyclins is increased in lung cancer cell lines as compared with normal fibroblasts. Accordingly, the over expression of these upregulated cyclins enhanced the viability and migration of lung cancer cell lines. Moreover, we found a highly significant correlation between elevated expression levels of these cyclins and a reduced overall survival of lung cancer patients.

CONCLUSION: Our result show that some of the evaluated new cyclins play an important role in lung cancer progression and malignancy, and may represent innovative diagnostic biomarkers and therapeutic targets in lung cancer.

This study was funded by Fundació La Marató de TV3 (project number 20131010) and Ministerio de Economía y Competitividad MINECO (grant ref BFU 2013-44189-P).

NO CONFLICT OF INTEREST

252 Tumour heterogeneity and clonal evolution in a murine model of osteosarcoma

S. Gambera[1], A. Morales-Molina[1], D. Kones[1], T. Cejalvo Goyanes[1], A.J. Perisé-Barrios[1], M.A. Rodrigues Milla[1], I. Cubillo Moreno[1], A. Alfranca Gonzalez[2], J. Garcia-Castro[1]

[1] Instituto de Salud Carlos III ISCIII, Instituto de Investigacion en Enfermedades Raras IIER - Cellular Biotechnology Unit, Majadahonda, Spain
[2] Hospital Universitario La Princesa, Fundacion para la Investigacion Biomedica, Madrid, Spain

Osteosarcomas (OS) are heterogeneous tumours at radio graphical, histological, karyotypic and genetic level. Conventional OS can be further subcategorized in osteoblastic, chondroblastic and fibroblastic just depending on the main cell phenotype. This variability suggests the involvement of a common phylogenetic precursor in the disease undergoing aberrant osteogenic differentiation. A lot still need to be learnt about OS growth and clonal dynamics occurring in the disease.

In order to characterize OS heterogeneity and tumour growth dynamics, we have employed a lentiviral-based RGB tracking system. This tool consists of transducing tumour cells with a combination of three lentiviral vectors which express respectively for red, green and blue fluorescent proteins (LeGO-RGB vectors). The combination of fluorescent proteins at different expression levels confers a unique colour to each individual cell and its progeny, thus facilitate the identification of cells coming from specific clones. This technology is used to study de novo OS formation in mice.

RESULTS: show that OS cells recapitulate radio-graphical characteristics of OS. In terms of clonal composition of the tumour a great heterogeneity was detected. Clonal heterogeneity is perturbed with disease progression, where dominant clones show new characteristics in terms of proliferation and invasiveness. Those clones mostly tend to grow on the periphery of the tumour outside the primary oxification areas. Clonal areas show high cellularity and reduced matrix deposition. Population characterized by a discrete/unique colour were efficiently sorted. Sorted clonal population were further decoloured and recoloured in order to retrace them in a secondary recipient. Also this time, retraced OS cells developed multicolour tumours. Surprisingly metastatic disease is a process carried by different clones as well. Metastatic nodules can be formed by a single clone type or by the contribution of different ones.

CONCLUSION: the study shows OS heterogeneity in different aspects of the disease. Surprising dynamics occur in tumours over time like the insurgence of invasive and metastatic clones associated to disease progression.

NO CONFLICT OF INTEREST

253 A network of microRNAs potentially regulating metabolic pathways in renal cancer

J. Boguslawska[1], *P. Poplawski*[1], *B. Rybicka*[1], *K. Rodzik*[1], *H. Kedzierska*[1], *A. Piekielko-Witkowska*[1]

[1] *The Centre of Postgraduate Medical Education, Biochemistry and Molecular Biology, WARSAW, Poland*

BACKGROUND: In our recent study we found that altered metabolism of renal cancer cells is associated with severely disturbed expression of genes encoding enzymes involved in key metabolic pathways, including metabolism of glucose, amino acids, as well as TCA and urea cycles. In this study we hypothesized that altered expression of metabolic genes may result from dysregulated expression of their targeting microRNAs.

MATERIAL AND METHOD: The expression of 21 metabolic genes was validated using qPRC on independent group of 120 tissue samples (60 clear cell renal cell cancer (ccRCC) tumors and 60 paired-matched non-tumorous controls). 91 microRNAs potentially targeting 19 top altered genes were predicted using TargetScan and miRsystem, and their expression was evaluated using Pick&Mix qPCR panels. TCGA KIRC data was used data validation.

RESULTS AND DISCUSSION: Disturbed expression of 19 metabolic genes was confirmed in ccRCC tumors. Top statistically significant ($p<0.05$) changes affected the expressions of PAH (-70-fold), ALDH6A1 (-21 fold), G6PC (-11 fold). The expression of 15 microRNAs potentially regulating the expression of metabolic genes was decreased, while the expression of 13 microRNAs was increased in renal tumors when compared with non-tumorous controls (fold change by at least 1.3, $p<0.05$). Top changed microRNAs included miR-122-5p (+108 fold), miR-210-2p (+10 fold), miR-34a-5p (+3 fold). TargetScan analysis revealed in transcripts of altered metabolic genes the presence of highly conserved binding sites for microRNAs with the most significantly impaired expressions. Correlation analysis showed that the expressions of miR-34a-5p, miR-106b-5p, miR-146a-5p, miR-155-5p, miR-342-3p, negatively correlated (r Spearman < -0.5, $p<0.05$) with multiple predicted metabolic gene targets.

CONCLUSION: We found altered expressions of microRNAs and their predicted metabolic gene targets in ccRCC tumors. Strong negative correlations between the microRNAs and their predicted gene targets suggest the existence of the microRNA-gene network possibly contributing to dysregulation of key metabolic pathways in ccRCC.

The study was financially supported by National Science Centre, Poland (grant 2014/13/B/NZ5/00283).

NO CONFLICT OF INTEREST

254 Absence of neurofibromin induces an oncogenic metabolic switch via mitochondrial ERK-mediated phosphorylation of the chaperone TRAP1

A. Rasola[1], *I. Masgras*[1], *F. Ciscato*[2], *A.M. Brunati*[2], *S. Indraccolo*[3], *F. Chiara*[4], *G. Cannino*[1], *E. Papaleo*[5], *M. Pizzi*[6], *P. Bernardi*[2]

[1] *Università di Padova, Dipartimento di Scienze Biomediche, Padova, Italy*
[2] *Università di Padova, Dipartimento di Medicina Molecolare, Padova, Italy*
[3] *IRCCS, Istituto Oncologico Veneto, Padova, Italy*
[4] *Università di Padova, Dipartimento di Scienze Cardiologiche Toraciche e Vascolari, Padova, Italy*
[5] *Danish Cancer Society Research Center, Computational Biology Laboratory- Unit of Statistics-Bioinformatics and Registry, Copenhagen, Denmark*
[6] *Università di Padova, Dipartimento di Medicina, Padova, Italy*

BACKGROUND: Mutations in the NF1 gene cause the tumor-predisposing syndrome neurofibromatosis type 1 by targeting the Ras GTPase-activating protein neurofibromin, whose loss leads to the oncogenic activation of the Ras/ERK transduction pathway, which also contributes to the metabolic reprogramming of neoplastic cells. Mitochondria take part in this bioenergetic rewiring in several ways, including accumulation of oncometabolites that orchestrate oncogenic programs through transcriptional regulation. One such oncometabolite is succinate, and an increase in intracellular succinate levels prompted by the inhibitory interaction between succinate dehydrogenase (SDH) and the mitochondrial chaperone TRAP1 leads to the pro-neoplastic activation of the hypoxia-inducible transcription factor 1 (HIF-1). Here we connect deregulated activation of Ras/ERK signaling in cells lacking neurofibromin and the metabolic switch mastered by TRAP1.

MATERIALS AND METHODS: Experiments were performed on cell types with or without neurofibromin in which TRAP1 expression was either knocked down by RNAi or knocked out with a CRISPR/Cas9 approach. Cells were used for: i) biochemical assays aimed at investigating interactors and post-translational modifications of TRAP1; ii) bioenergetic measurements; iii) cytofluorimetric inspections to assess mitochondrial potential and cell viability; v) tumorigenicity assays. Immunohistochemical stainings were performed on tumor samples, and molecular dynamics analyses on the TRAP1 structure.

RESULTS AND DISCUSSION: We observe that cells lacking neurofibromin exhibit enhanced glycolysis and decreased respiration in a Ras/ERK-dependent way. Active ERK1/2 associates with SDH and TRAP1 in mitochondria of these cells. ERK1/2 kinase activity is enhanced by the interaction with TRAP1, and induces both formation of this multimeric complex and SDH inhibition. In turn, TRAP1 is phosphorylated in an ERK1/2-dependent way. TRAP1 silencing or mutagenesis at the serine residues targeted by ERK1/2 abrogates tumorigenicity, a phenotype that is reverted by a cell-permeable succinate analog.

CONCLUSION: Our findings reveal that, in cells lacking neurofibromin, Ras/ERK signaling controls SDH inhibition and the ensuing pro-neoplastic succinate accumulation mastered by TRAP1. These result pave the way for identifying selective TRAP1 inhibitors to be tested as antineoplastic compounds.

NO CONFLICT OF INTEREST

255 BET inhibitors repress the expression of RUNX2 through the disruption of the interplay between the promoter and the enhancers

V. Sancisi[1], *G. Manzotti*[1], *G. Gobbi*[1], *M. Gugnoni*[1], *F. Catellani*[1], *G. Gandolfi*[1], *A. Ciarrocchi*[1]

[1] *Arcispedale Santa Maria Nuova IRCCS, Scientific Direction, Reggio Emilia, Italy*

INTRODUCTION: BET inhibitors (BETi) are a class of anti-cancer epigenetic drugs that are currently under evaluation in clinical trials. A number of oncogenes are under the control of BET proteins and are repressed by BETi, leading to inhibition of cancer cells proliferation and to apoptosis. RUNX2 is a transcription factor involved in embryonic development and increasingly recognized in cancer biology for its oncogenic properties. High levels of RUNX2 expression have been linked to progression and metastasization of different types of tumors. Here we investigated the role of BET proteins in regulating RUNX2 expression in cancer and we characterized a novel mechanism of action of BETi in the context of solid tumors.

MATERIALS AND METHODS: We used the ENCODE annotation data to predict potential regulatory elements within the RUNX2 locus. We used molecular biology approaches to investigate the function of these elements in 2 thryoid cancer cell lines (TPC1 and BCPAP) and two breast cancer cell lines (MDA-MB231 and MCF7). Chromatin-immuno precipitation (ChIP) was performed to explore occupancy of BRD4 on RUNX2 regulatory regions. To block the BET proteins activity we used the pan-BET inhibitor JQ1. Following BETi treatment, we evaluated: cell proliferation, gene expression by qPCR, chromatin structure by ChIP and Chromosome Conformation Capture (3C).

RESULTS AND DISCUSSION: Using 3C and CRISPR/CAS9 genome editing approaches, we demonstrated that the expression of RUNX2 in thyroid and breast cancer cells relies on the functional cooperation of three previously uncharacterized enhancers (ENHs) with the RUNX2 P2 promoter. As already shown for other pro-oncogenic genes, we showed a significant accumulation of BRD4 on RUNX2 distal ENHs. We characterized a multi-protein complex, comprising BRD4 and MED1, that mediates the physical interaction between the ENHs and the promoter. Pharmacological inhibition of BRD4 determines a strong repression of RUNX2 expression that was associated with a profound reorganization of the chromatin structure in the RUNX2 locus, impairing both RNA-PolII recruitment and progression on RUNX2 gene.

CONCLUSION: This work shows that the expression of RUNX2 in cancer cells relies on the BRD4-dependent functional cooperation of a complex network of regulatory regions. Furthermore, we identified for the first time RUNX2 as new target of BETi in cancer and characterized a novel MYC-independent mechanism mediating the cytotoxic effect of these drugs.

NO CONFLICT OF INTEREST

256 Glutamine deprivation in breast cancer: An additional tool for targeted therapy

A. Chinnici[1], *A. Turdo*[1], *M. Gaggianesi*[1], *E. Lipari*[1], *T. Apuzzo*[1], *G. Stassi*[1], *M. Todaro*[1]

[1] *University of Palermo, Department of Surgical and Oncological Sciences, Palermo, Italy*

Introduction

Cancer cells are endowed with an atypical metabolism, which relies on glutamine (GLN) addiction and depends on cancer molecular landscape.

Since the expression of CD44 variants is strongly related to the activity of a glutamate-cystine antiporter, xCT, and cysteine is a substrate for the synthesis of an antioxidant tripeptide, glutathione (GSH), CD44 variants allow cancer cells to survive with high extracellular ROS levels.

Starting from the premise that the depletion of a 'conditionally essential" amino acid from the diet could be a valuable strategy to target cancer cells reducing side effects, we explored whether glutamine starvation could affect breast CSCs growth, acting as a combinatorial tool for targeted therapy.

MATERIAL AND METHOD: Breast cancer cell lines were cultured in presence or in absence of GLN. Proliferation assays, Flow cytometry analyses and Western blot assays were performed in order to investigate the effects of GLN deprivation. Multiple combinations of chemotherapeutic agents were used and the response to therapy was analyzed through ATP quantitation.

RESULTS AND DISCUSSION: We observed that luminal or basal subtypes of breast cancer cells, bearing mutations in PTEN-PI3K signaling pathway, are strikingly dependent on the presence of glutamine in culture medium. Interestingly, we noticed that mutated cells arrest their growth in G2/M phase. The chemotherapeutic compound Paclitaxel was used to target cells in G2/M and was combined with PDK1 and PI3K-MAPK pathway inhibitors. The cells starved from GLN showed to be more sensitive to anti-cancer therapy, lowering the effective doses of drugs and avoiding the use of highly toxic combinatorial therapies.

CONCLUSION: The lack of effective therapeutic regimens, especially for metastatic breast cancer, points to the need of studying alternative strategies able to reduce side effects and to increase patient response. In this context, we propose starvation from GLN as an additional weapon against breast cancer.

NO CONFLICT OF INTEREST

257 Mitochondria as cisplatin targets: New approach to counteract resistance mechanism

V. Cocetta[1], *C. Vianello*[1], *D. Catanzaro*[1], *E. Ragazzi*[1], *M. Montopoli*[1]

[1] *Università degli Studi di Padova, Department of Pharmaceutical and Pharmacological Sciences, Padova, Italy*

BACKGROUND: Cisplatin is a first-line chemotherapeutic agent used in treatment of several types of tumors but, unfortunately, the onset of resistance is a main problem of this therapy. Although several studies regarding cisplatin resistance has been performed, the molecular mechanisms are not completely understood. Previous studies of our laboratory has demonstrated that cisplatin-resistant ovarian cancer cells (C13) when compared to the sensitive counterpart (2008), are characterized by a reduced respiratory chain activity correlated to a lower mitochondrial mass as well as a higher glucose dependency for their survival. In this study we worked on the phenotyping of different human cancer cell lines sensitive and resistant to cisplatin, exploring mitochondrial function and morphology in tumor cells. The aim was to clarify the molecular mechanism involved in platinum-drugs resistance and identify new molecular targets for a combination therapy.

MATERIAL AND METHODS: In order to identify alternative pathways exploited by cancer cells to escape cisplatin cytotoxicity different metabolic stresses like glucose free/galactose medium and incubation with rotenone were used. Moreover we used flow cytometry to detect possibly differences in mitochondrial membrane potential ($\Delta\psi$) and mass between CDDP-resistant and sensitive cells, immunocytochemistry to investigate the mitochondrial network phenotype and immunoblot assay to evaluate the expression of proteins that play a role in the coordination of mitochondrial dynamic.

RESULTS: result shown different susceptibility to the metabolic stresses between sensitive and resistant cells; data revealed no significant differences in mitochondrial membrane potential and mass but they shown a different mitochondrial phenotype, in particular alterated mitochondrial network in resistant clones.

CONCLUSIONS: Acquiring knowledge in energy metabolism and in mitochondrial remodelling in particular in cisplatin-resistant cancer, helps to identify new targets useful to innovative pharmacological approaches. Moreover, these data could be useful to clarify the mechanism of action of cisplatin, which is not completely clear.

NO CONFLICT OF INTEREST

258 Differentiation affects the release of exosomes from colon cancer cells and their ability to modulate the behavior of recipient cells

D. Lucchetti[1], *F. Calapà*[1], *V. Palmieri*[2], *C. Fanali*[1], *F. Carbone*[1], *A. Papa*[3], *R. De Maria*[1], *M. De Spirito*[2], *A. Sgambato*[1]

[1] *Institute of General Pathology, Catholic University of the Sacred Heart, Rome, Italy*
[2] *Institute of Physics, Catholic University of the Sacred Heart, Rome, Italy*
[3] *Gastroenterology Unit, Fondazione Policlinico Universitario Gemelli, Rome, Italy*

BACKGROUND: Differentiation of normal and cancer stem cells is one of the most important issue in biology. Exosomes are involved in inter-cellular communication. We previously reported that sodium butyrate-induced differentiation of HT29 colon cancer cells is associated with a reduced CD133 expression. This study aimed to analyze the role of exosomes in the differentiation of HT29 and Caco2 cells.

MATERIAL AND METHODS: Exosomes were prepared using ultracentrifugation. The correct isolation of exosomes was confirmed by Dynamic Light Scattering , Electron microscopy, and Western-Blot analysis. The Bradford assay was used for the quantitative evaluation of isolated exosomes. Protein, microRNA and mRNA expression levels were evaluated by western blot and RT-PCR analyses, respectively. The uptake of exosomes in recipient cells was analyzed by confocal microscopy. The proliferation rate was assessed by MTT assay and confirmed with the Electric Cell-substrate Impedance Sensing. Cell motility was assessed using the scratch test and confirmed by Confocal Microscopy.

RESULTS: Sodium butyrate-induced differentiation of HT29 and Caco-2 cells increased the levels of released exosomes and their expression of CD133. Cells differentiation and the decrease of cellular CD133 expression levels were prevented by blocking multivescicular body maturation. Exosomes released by HT29 differentiating cells carried increased levels of microRNAs, induced an increased proliferation and motility of both colon cancer cells and normal fibroblasts, increased the colony forming efficiency of cancer cells and reduced the sodium butyrate-induced differentiation of HT29 cells. Such effects were associated with an increased phosphorylation level of both Src and Erk proteins and with an increased expression of EMT-related genes. These data were confirmed by confluent-induced differentiation in both colon cancer cancer cell lines.

CONCLUSIONS: Exosomes' cargoes could increase the phosphorylation of key molecules involved in adhesion, motility, proliferation and EMT pathway, which might promote migration, and proliferation of tumor and stromal cells. This could be a mechanism allowing surrounding tumor cells to escape the effects of differentiation. Release of exosomes is affected by differentiation of colon cancer cells; exosomes might be used by differentiating cells to get rid of components that are no longer necessary but might continue to exert their effects on recipient cells.

NO CONFLICT OF INTEREST

259 The anti-inflammatory protein MCPIP1 regulates the malignant phenotype and metastatic progression of ccRCC cells

K. Miekus[1], *P. Marona*[1], *J. Górka*[1], *J. Jura*[1]

[1] *Jagiellonian University, Faculty of Biochemistry- Biophisics and Biotechnology- Department of General Biochemistry, Krakow, Poland*

INTRODUCTION: Monocyte chemotactic protein 1-induced protein 1 (MCPIP1), suppresses chronic inflammation by promoting the mRNA decay of proinflammatory cytokines, such as IL-6, IL-1 and IL-12 and regulating NFκB and AP1 activity. Last findings shows that MCPIP1 also regulates viability and proliferation of tumor cells and degrades the mRNA of antiapoptotic gene transcripts in breast cancer cells. Moreover, MCPIP1 overexpression induces apoptosis in vitro and reduces tumor growth and metastatic disease in vivo.

The main objective of our study was to determine the role of MCPIP1 in the process of clear cell renal cell carcinoma progression and metastasis.

MATERIALS AND METHODS: MCPIP! was down or upregulated using lentiviral vectors, the doxycycline-dependent TetON system or retroviral vectors. The level of genes and proteins were studied by real-time PCR, western blot and flow cytometry. Chemotaxis, invasion and motility assays were performed to check migration and invasion. Foxn1nu/Foxn1nu and NOD-SCID mice were used in in vivo experiments.

RESULTS: Our studies show that mice injected i.sc. with ccRCC shMCPIP1 had considerably more lung metastases than mice injected with control cells. Time-lapse recording revealed that motile activity was strongly increased in ccRCC with MCPIP1 downregulation and parameters for distance and speed were significantly higher for shMCPIP1 cells. Western blot analysis revealed that MCPIP1 silencing decreases the level of various epithelial to mesenchymal transition (EMT) markers. Downregulation of MCPIP1 suppressed Ecadherin and upregulated β-catenin and vimentin. MCPIP1 silencing induced the expression of E-cadherin repressors, Snail and ZEB2. We also demonstrate that stable upregulation of MCPIP1 slowed tumor growth and resulted in a decrease in the number of tumor cells metastasizing to the lungs. MCPIP1 overexpression in ccRCC cells caused a significant decrease in motile activity, a strong increase in E-cadherin and a decrease in vimentin, β-catenin and ZEB2 expression.

CONCLUSIONS: We showed that a lack of MCPIP1 increased ccRCC cell motility, induced metastasis to the lungs and enhanced the mesenchymal phenotype. Moreover, MCPIP1 overexpression in ccRCC cells impairs the malignant phenotype of ccRCC cells.

ACKNOWLEDGEMENT: This study was supported by research grant from the National Science Centre 2013/09/D/NZ/00249, 2015/19/B/NZ5/01405 and KNOW 35p/3/2015.

NO CONFLICT OF INTEREST

260 Long non-coding RNAs: A new class of diagnostic biomarkers in human parathyroid tumors

A. Morotti[1], I. Forno[1], A. Terrasi[1], V. André[2], C. Verdelli[2], V. Guarnieri[3], A. Scillitani[4], L. Vicentini[5], V. Vaira[1], S. Corbetta[6]

[1] University of Milan, Department of Pathophysiology and Transplantation-Division of Pathology Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[2] IRCCS Istituto Ortopedico Galeazzi, Laboratory of Experimental Endocrinology, Milan, Italy
[3] IRCCS Casa Sollievo della Sofferenza, Genetic Medicine, San Giovanni Rotondo FG, Italy
[4] IRCCS Casa Sollievo della Sofferenza, Endocrinology Unit, San Giovanni Rotondo FG, Italy
[5] IRCCS Fondazione Ca' Granda Ospedale Maggiore Policlinico, Endocrine Surgery, Milan, Italy
[6] IRCCS Istituto Ortopedico Galeazzi, Department of Biomedical Sciences for Health- Endocrinology Service-University of Milan, Milan, Italy

INTRODUCTION: Parathyroid tumors are characterized by genetic and epigenetic alterations resulting in aberrant expression of protein-coding genes and in many classes of non-coding RNAs, including long non-coding RNAs (LncRNAs) and microRNAs (miRNAs).

Here, we investigate the LncRNAs expression profiles in a series of parathyroid tumors derived from patients with non-hereditary primary hyperparathyroidism, in order to discover new molecular diagnostic markers.

MATERIALS AND METHODS: LncRNAs profile was investigated in 4 parathyroid carcinomas (PCas), 12 parathyroid adenomas (PAds) compared with 2 normal parathyroid glands (PaNs) by LncProfilers™ qPCR Array kit with SYBR® green detection. Variations in LncRNAs expression profiles among samples were analyzed by different statistic tools.

In order to validate statistically significant data, we performed a qRT-PCR with TaqMan® Assay in a second samples set including 4 atypical adenomas (aPAds), 2 PaNs, 22 PAds and 3 PaCs. A set of miRNAs deregulated in human PCas from the first set of patients was validated in PCas from the second set. To investigate the correlation between significant LncRNAs and miRNAs previously identified, we performed prediction analysis using DIANA tools.

RESULTS AND DISCUSSION: Unsupervised clustering distributed samples in two major groups clearly distinguishing PCas from PaNs. Significance Analysis of Microarray (SAM) identified 9 LncRNAs significantly deregulated between PCas and PaNs (BC200, HOXA6as and snaR upregulated in PCas), 12 LncRNAs between PAds and PaNs and 23 LncRNAs between PAds and PCas. Five of the significantly deregulated LncRNAs were validated by TaqMan® qRT-PCR: MEG3, SNHG6 and KCNQ1OT1 downregulated in PCas, NEAT1 and HAR1B upregulated in PAds. The 5 LncRNAs identified 3 different classes of tumors in a distinct validation samples set: cluster 1 included 2 PaNs and 10 PAds; cluster 2 included 8 PAds; cluster 3 included 4 PAds, all the aPAds and PCas. Prediction tool showed several interactions between significant LncRNAs and miRNAs differentially expressed in PCas patients comparing to PaNs, although the molecular mechanism needs to be elucidated.

CONCLUSIONS: The study identified a set of differentially expressed LncRNAs, most of which downregulated (MEG3, SNHG6 and KCNQ1OT1), able to distinguish different human parathyroid histotypes, suggesting LncRNAs as new genes involved in tumorigenesis and as potential diagnostic markers.

NO CONFLICT OF INTEREST

261 High frequency and prevalence of Yellow Fever virus-specific CD8 T cells can be inherited

A. Bovay[1], V. Zoete[2], G. Dolton[3], D.K. Cole[3], P. Rizkallah[3], K. Beck[4], O. Michielin[2], D.E. Speiser[1], A.K. Sewell[3], S. Fuertes-Marraco[1]

[1] LICR / CHUV, DOF, Epalinges, Switzerland
[2] Swiss Institute of Bioinformatics, Molecular Modeling Group, Lausanne, Switzerland
[3] Cardiff University School of Medicine, Division of Infection and Immunity and Systems Immunity Research Institute, Cardiff, United Kingdom
[4] Cardiff University School of Dentistry, Biophysics, Cardiff, United Kingdom

INTRODUCTION: Immune-based therapies against infectious disease and cancer have made significant progress during recent years. However, cancer-specific CD8 T cell responses are still often insufficient for protection, even after immunotherapy. Therefore, there is a high need for further optimization. Interestingly, data suggest that the principles governing CD8 T cell-mediated protection are similar in infection and cancer. Immune responses following vaccination against the Yellow Fever virus (YFV) can be used as a reference model, providing essential information on mechanisms of induction and maintenance of protective CD8 T cell responses in humans.

Material and Methods

We studied the HLA-A*0201-restricted epitope LLWNGPMAV (A2/LLW), which generates an immunodominant and prevalent CD8 T cell response. The T cell receptor (TCR) repertoire was characterized at molecular and functional levels using various techniques.

RESULTS: We discovered that A2/LLW-specific CD8 T cells are highly biased for usage of the TCR a chain TRAV12-2. This bias is already present in A2/LLW-specific naïve T cells before vaccination, which are surprisingly very frequent. Interestingly, TRAV12-2 does not confer a functional advantage. Instead, molecular modeling indicated that the germline-encoded complementarity determining region (CDR) 1α loop of TRAV12-2 critically contributes to A2/LLW binding, in contrast to the conventional dominant dependence on somatically rearranged CDR3 loops. A well-known model antigen for which T cells are biased for TRAV12-2 usage is the melanoma A2/ELA epitope. A2/ELA-specific T cells are indeed highly frequent and prevalent amongst HLA-A*0201 healthy individuals as well as melanoma patients. Intriguingly, the binding between the MEL5 TCR expressing TRAV12-2 and A2/ELA occurs via dominant contacts with the CDR1 loop of TRAV12-2.

CONCLUSIONS: The germline TCR component TRAV12-2 confers an advantage during thymic positive selection, explaining the unusually high precursor frequency, immunodominance, and prevalence of TRAV12-2+ T cells in the human population.

NO CONFLICT OF INTEREST

264 BET bromodomain proteins regulate immune checkpoints in triple negative breast cancer

G. Denis[1], G. Andrieu[1]

[1] Boston University School of Medicine, Cancer Center, Boston- MA, USA

INTRODUCTION: Antibodies targeting the PD-1/PD-L1/PD-L2 pathway are being evaluated clinically for several cancers and show early success. However the realization that not all patients respond well to immunotherapy suggests other modalities could be combined to improve efficacy. The BET bromodomain proteins BRD2, BRD3 and BRD4 are epigenetically acting co-regulators of transcription, and are critical for cancer proliferation and metastasis. They are newly 'druggable' with small molecule inhibitors (BETi) such as JQ1, IBET151 and IBET762. BRD4 is known to bind directly to the PD-L1 promoter; its targeting suppresses PD-L1 expression while increasing CD8+ T cell activity to limit progression in ovarian tumor models.

HYPOTHESES: BET proteins regulate PD-L1 and PD-L2 expression in breast tumors and PD-1 in CD8+ T cells; BETi could combine well with checkpoint inhibitors. BET proteins are effectors of pro-inflammatory cytokine signaling, regulating PD-L1/2 expression in the microenvironment.

METHODS: No available small molecule BETi drug discriminates sufficiently among family members. Each BET protein plays an independent role in different cell types; they sometimes oppose each other. Furthermore, there are wide individual-level differences in expression of each BET family member among non-tumor cells in the microenvironment. Therefore, we used sequence-specific siRNA to knockdown individual BET proteins in human breast cancer cell lines, including triple negative breast cancer (TNBC), and compared functional and transcriptional outcomes to control siRNA and BETi drugs.

RESULTS: BRD2 regulates PD-L1, but BRD4 regulates PD-L2 in TNBC. Individual BET proteins control PD-1 expression in primary CD8+ T cells. Individual BETs differentially regulate genes important for epithelial-mesenchymal transition in TNBC, in ways that BETi drugs alone obscure. BET expression correlates with cytokine signaling and PD-1 expression in human T cells from diverse patients. BET inhibition in activated T cells in the microenvironment prevents cytokine-driven PD-1 expression.

CONCLUSION: Next-generation BETi drugs may combine well with PD-1 or PD-L1/2 -targeted immunotherapies in difficult-to-treat cancers**.** Personalized BET profiles could inform individual patient responses to BETi and checkpoint inhibitors. Therapeutic approaches that treat the microenvironment should be leveraged to maximize efficacy of checkpoint inhibitor approaches for specific cancers.

NO CONFLICT OF INTEREST

266 Proline Dehydrogenase expression and regulation in Non Small Cell Lung Carcinoma

S. Grossi[1], P. D'Antona[1], E. Gini[1], R. Cinquetti[1], G.P.M. Binelli[1], A.M. Chiaravalli[2], F. Sessa[3], P. Campomenosi[1]

[1] University of Insubria, Department of Biotechnology and Life Sciences DBSV, Varese, Italy
[2] Ospedale di Circolo e Fondazione Macchi, Pathology Unit, Varese, Italy
[3] University of Insubria, Department of Medicine and Surgery DMC, Varese, Italy

INTRODUCTION: Non-Small Cell Lung Cancer (NSCLC) is one of the most frequent and deadly cancers and comprises two main histotypes, adenocarcinoma (ADC) and squamocellular carcinoma (SCC). Identification of markers to better define the diagnosis, prognosis and therapeutic options of NSCLC is needed. Proline dehydrogenase (PRODH) is a mitochondrial flavoenzyme that catalyzes the key step in proline degradation and is involved in the regulation of cell survival, autophagy and apoptosis.

MATERIAL AND METHOD: We characterized expression of PRODH in a panel of ADCs and SCCs by immunohistochemistry and qPCR and tested if there is correlation between expression of PRODH and clinical features of the tumors or expression of other markers. By means of transfection experiments and expression analyses, we also tested the role of TTF-1 as a possible transactivator of the PRODH gene in two NSCLC cell lines. Moreover, four putative TTF-1 response elements, bioinformatically predicted in the PRODH promoter, were cloned and tested in luciferase reporter assays.

RESULTS AND DISCUSSION: We found PRODH immunostaining in the majority (70%) of lung ADCs. Patients with PRODH positive tumors showed a better survival than patients with negative tumors. Protein staining in tumors was paralleled by high transcript levels. TTF-1, a homeodomain-containing transcription factor essential for thyroid and lung development and physiology, was found to have a similar expression pattern in normal lung tissues and in NSCLCs. Based on their similar expression, their involvement in the same tumors or genetic pathologies, and the presence of putative TTF-1 response elements in PRODH promoter, we hypothesized that PRODH and TTF-1 may act in the same pathway and that TTF-

1 could directly transactivate the PRODH gene. Indeed, transfection of a TTF-1 expression construct into A549 and NCI-H1299 ADC cell lines led to an increase in PRODH transcript in both cell lines.

Luciferase reporter assays performed in the same cell lines suggested that one of the four REs is indeed responsible for the observed PRODH transactivation by the TTF-1 transcription factor.

CONCLUSION: Our data support a possible application of PRODH immunostaining as a prognostic marker and opens up new research perspectives aimed to investigate PRODH transactivation by TTF-1 and the role of PRODH in the biology ofNSCLC.

NO CONFLICT OF INTEREST

267 Characterization of metabolic reprogramming in melanoma by inhibition of OXPHOS

K. Dallaglio[1], G. Tondelli[1], M.E. Pistoni[1], C. Gallo[1], A. Maresca[2], V. Carelli[2], A. Ciarrocchi[1]

[1] Laboratory of Translational Research- Arcispedale Santa Maria Nuova IRCCS Reggio Emilia, Scientific Direction, Reggio Emilia, Italy
[2] University of Bologna, Department of Biomedical Sciences, Bologna, Italy

Melanoma (MM), the deadliest skin cancer, is an aggressive and treatment-resistant malignancy. Although recently-approved therapies for advanced MM have improved overall survival, they often display lack of response and drug resistance. New evidence points at metabolic plasticity as a hallmark of cancer associated with therapeutic failure: to escape drug killing, MM cells reversibly change their metabolic profile by switching the ATP-production strategy from oxidative phosphorylation (OXPHOS) to glycolysis, and viceversa. We have recently shown that alteration of MM cell metabolism with the OXPHOS-blocking agent phenformin (phe) affects both cancer stem cells (CSC) and non-CSC viability and growth. However, molecular mechanisms behind metabolic changes and how they contribute to therapeutic failures are still largely unexplored. Here we analysed the effects of short and long-term OXPHOS blockade on gene expression and MM cell behaviour in order to find new players in MM metabolic reprogramming. To this aim, A375 MM cells were treated with or without 0.5mM phe for 48h and analysed for gene expression by microarray analysis. Differentially expressed genes were subjected to a comprehensive search to identify interaction-networks and molecular/cellular function categories by Ingenuity Pathway Analysis (IPA). Gene known to partake to energetic metabolism were significantly de-regulated upon phe treatment. Genes involved in cell-cell/cell-matrix interaction were also overrepresented in phe-treated MM cells. To investigate effects of long-term OXPHOS blockade, A375 and MNT-1 MM cells were exposed to phe for up to 3 months leading to derivation of resistant cells (R). As compared to parental (S) cells, R-cells are slow cycling, display decreased colony formation, increased apoptosis and senescence as well as decreased cell migration and invasion. Interestingly, CSCs content is similar in S- and R-cells. R-cells display also lower ATP-production than S-cells and decreased oxygen consumption by Seahorse analysis, suggesting a low-energy metabolic profile. Interestingly, upon phe withdrawal, R-phenotype resembled that of S-cells. Finally, gene expression analysis confirmed a different metabolic asset of R-cells which is accompanied by an interesting overexpression of immunological players. All together our result suggest that changes in MM energetic metabolism induce a phenotypic switch which may have considerable implications in therapeutic responses.

NO CONFLICT OF INTEREST

268 PD1 and GITR combination immunotherapy drives durable anti-tumor responses

D. Skokos[1]

[1] Regeneron Pharmaceuticals- Inc, Immuno-Oncology, Tarrytown- New York, USA

Single or combination therapy targeting immune checkpoints PD-1 and CTLA-4 shows significant clinical benefit in certain cancer patient populations. However, the majority of patients either are resistant or only responds transiently, raising fundamental questions about the selection of the optimal immune-modulatory targets to address patient-specific tumor sensitivity. To promote durable responses, combination treatments, targeting specific coinhibitory and costimulatory pathways inducing a stronger T cell activation, may provide the answer. Here, we used PD1 and GITR combination therapy a pre-clinically validated model, currently in highly anticipated phase one clinical trials. To gain a deeper molecular understanding behind durable anti-tumor responses and identify biomarkers additional to PD-1/PDL-1 to improve patient stratification and tumor sensitivity we sequenced single cell RNA-seq libraries prepared from over 2,000 tumor infiltrating $CD8^+$ T cells. We found that combination of GITR and PD-1 antibodies synergistically enhances $CD8^+$ T cell effector function by restoring the balance of key homeostatic regulators, resulting in substantial tumor rejection and long-term responses. Indeed, combination therapy synergistically regulated the strength of $CD8^+$ T cell response, eliciting potent adaptive immunity. Furthermore, we identified that high levels of a key immune checkpoint receptor are correlated with better prognosis in patients with certain types of cancer. Systematic approaches unmasking the molecular pathways driving durable anti-tumor responses by rebalancing T cell homeostatic regulators will be important to optimize existing and/or design future combination therapies.

NO CONFLICT OF INTEREST

269 Acyl-CoA thioesterase 7 is involved in cell cycle progression

S.H. Jung[1], H.A. Park[1], J.S. Lee[1]

[1] Inha University College of Medicine, Molecular Medicine, Incheon, Korea

INTRODUCTION: Acyl-CoA thioesterase 7 (ACOT7) is a major isoform of the ACOT family that catalyzes hydrolysis of fatty acyl-CoAs to free fatty acids and CoA-SH. ACOT7 has high specificity for arachidonoyl-CoA, which regulates inflammation through the production of arachidonic acid and prostagladin. It was recently demonstrated that ACOT7 also regulates fatty acid metabolism in neurons and protects against neurotoxicity. Although ACOT7 has a potential to regulate the intracellular levels of acyl-CoA, free acid, and CoA, their cellular functions are not fully understand.

MATERIAL AND METHODS: We examined changes in the gene expression profile of irradiated cancer cells, we conducted gene expression analysis of an IR-exposed MCF human breast carcinoma. Gene expression analysis showed that ACOT7 expression markedly decreased in IR-exposed MCF7 cells. To functional analysis of ACOT7 in cancer cell proliferation, we transfected siRNA against ACOT7 into MCF7 cells and observed the effect of ACOT7 depletion on cell proliferation and survival.

RESULTS: ACOT7 was shown to be responsive to genotoxic stresses such as ionizing radiation (IR) and the anti-cancer drug doxorubicin in time- and dose-dependent manners. ACOT7 knockdown induced cytostasis via activation of the p53-p21 signaling pathway in MCF7 human breast carcinoma and A549 human lung carcinoma cells. PKCz was specifically involved in ACOT7 depletion-mediated cell cycle arrest as an upstream molecule of the p53-p21 signaling pathway. However, ACOT1 or ACOT11, other members of the ACOT family, was not responsive to genotoxic stresses and did not play a role in activation of the PCKζ-p53-p21 signaling pathway. Analysis of the ACOT7 prognostic value revealed that low ACOT7 levels also prolonged overall survival periods in breast and lung cancer patients. Furthermore, ACOT7 mRNA levels were higher in lung cancer patient tissues compared to normal tissues. We also observed a synergistic effect of ACOT7 depletion in combination with either IR or doxorubicin on cell proliferation in breast and lung cancer cells.

CONCLUSION: We demonstrated the synergistic effect of ACOT7 depletion and treatment with either IR or doxorubicin on cancer cell proliferation, suggesting ACOT7 might be a novel target for anticancer therapy.

NO CONFLICT OF INTEREST

271 Targeting the metabolic addiction of metastatic cancer-initiating cells in TNBC

L. Cardone[1], R. Dattilo[2], M. Muscolini[3], F. Papaccio[1], A. Lamolinara[4], I. Manni[3], D. Trisciuoglio[1], M. Ciocce[1], R. De Maria[5]

[1] IFO, Dept. of Research- Advanced Diagnostic- and Technological Innovation, Roma, Italy
[2] ISS, Biobank Unit- Department of Hematology- Oncology and Molecular Medicine, Rome, Italy
[3] Pasteur Institute, Pasteur Institute, Rome, Italy
[4] Aging research center- G. D'Annunzio- University of Chieti, Aging research center- G. D'Annunzio- University of Chieti, Pescara, Italy
[5] Cattolica University, Cattolica University, Rome, Italy

The aggressiveness of Triple-Negative Breast Cancer (TNBC) is mediated by a subpopulation of cancer cells with stem-like properties, referred as Cancer Initiating Cells (CICs) that have the ability to self-renew, invade and support tumour growth and metastases. These cells are particularly resistant to chemotherapy, increasing the risk of disease relapse and metastases growth. Thus, the efficient treatment of metastatic TNBC is limited by the ineffective killing CICs.

Metabolic phenotypes supporting the growth of tumour cells and metastases have been identified and do support cancer cells colonization to distinct sites and metastases. Although CICs have been implicated in advanced and metastatic TNBC, it is unknown the metabolic phenotypes of these aggressive cells and whether the targeting of their metabolic phenotypes might be therapeutically exploited to control metastatic disease. However, the metabolic plasticity of cells, e.g. the ability to shift metabolic fluxes by minimizing metabolic stress, limits the therapeutic success of antimetabolites strategies, suggesting that the tailored understanding of cancer cells metabolic plasticity represent a key strategy.

By means of metabolomics and genes expression profile studies, we are investigating the major metabolic features of CICs-derived from breast tumor and related metastatic lesions, obtained from an orthotopic mouse model of TNBC metastatic disease. We have monitored major fuel sources supporting the survival and growth of CICs in vitro and the addiction to major metabolic pathways, by using selective inhibitors. Along these studies, we have identified a FDA-approved drug targeting mitochondria electron transport chain activity that restricted the metabolic plasticity and energetic adaptive strategies of CICs. This drug, by inducing a metabolic crisis, had a potent and selective cytotoxic activity, reduced stem cells survival, mammospheres formation, self-renewal potential in vitro and tumor growth in vivo. We are investigating the keys metabolic targets able to kill metastagenic CICs. Our result identified some metabolic strategies and major metabolic routes supporting survival and growth of stem-like cells of TNBC. Our future goal is to confirm the similarity among the metabolic addictions observed in vitro and the key metabolic features of CICs in vivo and in tumor microenvironment, for a tailored and effective antimetabolites strategies against TNBC.

NO CONFLICT OF INTEREST

273 ETV7 drives doxorubicin-resistance in breast cancer cells via modulation of DNAJC15 expression

L. Pezzè[1], F. Alessandrini[1], Y. Ciribilli[1]

[1] Laboratory of Molecular Cancer Genetics- CIBIO, University of Trento, Trento, Italy

INTRODUCTION: Doxorubicin is commonly used as adjuvant and neoadjuvant chemotherapy for breast cancer treatment. However, despite its high cytotoxic potential on most cancer cells, Doxorubicin-treated cells can develop mechanisms of drug resistance. ETV7 is a poorly characterized ETS transcription factor known to act as transcriptional repressor that has been implicated in different types of cancer. In this work, we investigated the effects of ETV7 expression on Doxorubicin-resistance in breast cancer cells and the regulation of its novel identified target, DNAJC15.

MATERIAL AND METHODS: We treated MCF7 and MDA-MB-231 breast cancer cell lines with several chemotherapeutic drugs and analyzed mRNA expression by RT-qPCR. We assessed doxorubicin-resistance by cell viability assay (MTT) and expression of drug efflux pumps. Direct binding of ETV7 was tested by site directed mutagenesis of ETV7 putative binding sites within the DNAJC15 promoter, gene reporter assay, and chromatin immunoprecipitation. Methylation analysis was performed by bisulfite PCR followed by sequencing and by 5-Aza-2'-deoxycytidine treatment.

RESULTS: Treatment with doxorubicin and other chemotherapeutic drugs induced the expression of ETV7 in cancer cell lines of different origin. We then observed that ETV7 expression could significantly reduce sensitivity to doxorubicin in breast cancer cell lines. Moreover, we demonstrated that ETV7 could transcriptionally downregulate the mRNA levels of DNAJC15, a co-chaperone protein whose low expression has been previously associated with drug resistance. We pinpointed the binding site for ETV7 within the promoter of DNAJC15 and verified the direct binding of ETV7 to this region. Furthermore, we identified DNA methylation as a putative mechanism of transcriptional repression exerted by ETV7 on the DNAJC15 promoter. Finally, ETV7-mediated Doxorubicin resistance could be partially rescued by DNAJC15 overexpression.

CONCLUSION: We propose a novel role for ETV7 in breast cancer and we identified DNAJC15 as a new target gene partially responsible for ETV7-mediated Doxorubicin-resistance. A better understanding of this mechanism could improve the design of combinatorial regimens including Doxorubicin with the aim of avoiding resistance and relapse.

NO CONFLICT OF INTEREST

274 MCPIP1 regulates vascularity of clear cell renal cell carcinoma via IL-6 and IL-8

P. Marona[1], Z. Mazurek[1], J. Jura[1], K. Miękus[1]

[1] Faculty of Biochemistry- Biophysics and Biotechnology, Department of General Biochemistry, Krakow, Poland

INTRODUCTION: Formation of new blood vessels is a critical step during tumorigenesis and metastatic spread. Recent reports indicate an important role of inflammation and angiogenesis in clear cell renal cell carcinoma (ccRCC) development. Monocyte Chemotactic Protein-1 Induced Protein (MCPIP-1) mediates inflammatory processes by regulating the stability of transcripts coding for proinflammatory cytokines such as IL-6 or IL-8, which are also engaged in controling angiogenesis. Our result suggest that the MCPIP-1 protein may be involved in the process of cancer growth and affects the formation of tumor vasculature.

MATERIALS AND METHODS: Patients ccRCC tissue samples were analysed with western blots and microarrays. ccRCC cell lines (Caki-1 and Caki-2) were transduced shRNAs and TET-ON lentiviral vectors to down- or upregulate MCPIP1. The level of genes and proteins involved in proliferation and angiogenesis were studied by real-time PCR, western blot and ELISA assays. Chemotaxis assay was performed to check migration of endothelial cells. NOD-SCID and Nude mice were used in in vivo experiments. Ultrasonography of high resolution (VEVO) and IHC stainings were used to show vessel formation in emerging tumors.

RESULTS: Our result show, that MCPIP1 protein level decreases during the process of renal cancer progression. Moreover, proangiogenic transcripts vary depending on the stage of the disease. We identify that low MCPIP1 level correlates strongly with increased proliferation in vitro, tumor growth in mice and enhanced vascularity of emerging tumors. We found that MCPIP1 silencing increases chemotaxis of microvascular endothelial cells due to increased levels of VEGF, IL-8 and IL-6. VEVO and IHC stainings, demonstrate a significant increase in the volume of functional blood vessels in tumors formed by cells with downregulation of MCPIP1. In addition, we have observed that the transcript level of IL-6 and IL-8 in cells with D141N point mutation, which abolishes ZC3H12A RNase activity, is similar to control cells whereas MCPIP1 upregulation led to a decrease in the expression of these cytokines. Moreover, tumors formed by cells with D141N mutation are significantly bigger than with MCPIP1 upregulation.

CONCLUSIONS: We showed that reduction of MCPIP1 protein level in ccRCC cells is crucial for tumor growth and blood vessel formation. These observations make MCPIP1 a plausible target in ccRCC therapy.

Supported by grants: NSC 2015/19/B/NZ5/01405, KNOW BMN 09/2016.

NO CONFLICT OF INTEREST

275 PATZ1 is a new diagnostic and prognostic marker of glioblastoma enriched in the proneural subtype and involved in counteracting the proneural-to-mesenchymal transdifferentiation through downregulation of CXCR4

E. Guadagno[1], M. Vitiello[2], P. Francesca[2], G. Cal[2], F. Caponnetto[3], D. Cesselli[3], E. Crescenzi[2], L. Cerchia[2], M.L. Del Basso De Caro[1], M. Fedele[2]

[1] University of Naples "Federico II", Department of Advanced Biomedical Sciences- Pathology Section, Naples, Italy
[2] Institute of Experimental Endocrinology and Oncology "Gaetano Salvatore" IEOS, Department of Biomedical Sciences- National Council of Research, Naples, Italy
[3] University of Udine, Department of Medical and Biological Sciences, Udine, Italy

INTRODUCTION: Glioblastoma (GBM), the most malignant of the brain tumors, has been classified on the basis of molecular signature into four subtypes: classical, mesenchymal, proneural and neural, among which the mesenchymal and proneural subtypes have the shortest and longest survival, respectively.

PATZ1 is a member of the POK (POZ and kruppel-like zinc finger) family, which we recently showed playing a critical role in counteracting the epithelial-to-mesenchymal transition in thyroid cancer.

CXCR4 is a chemokine receptor, which has been shown to induce and assist in the maintenance of a mesenchymal expression profile in GBM cells

MATERIAL AND METHOD: We analyzed the expression of PATZ1 gene and protein on public datasets and a local cohort of 45 GBM, respectively, correlating it to GBM subtype and patients' overall (OS) and progression-free (PFS) survival. We also analyzed PATZ1 expression in patients-derived GBM stem cells (GSC) and studied the expression of CXCR4 in a primary GBM cell line expressing or not PATZ1.

RESULTS AND DISCUSSION: We show that PATZ1 gene is highly expressed in a significant number of GBMs, whereas it was undetectable in normal glia. Moreover, we showed that PATZ1 is expressed in GSCs both in vitro (in GBM-derived stem cells) and in vivo (human tissue specimens), is downregulated in differentiated cells from the same tumor as compared to GSCs, and its expression is higher in GSCs growing as spheres (likely proneural) than in GSCs growing as adherent cells (likely mesenchymal). These observations were validated and completed in independent glioma datasets, finding a significant overlap of high PATZ1 expression with the proneural subtype and conversely of low PATZ1 expression with the mesenchymal subtype in both GBMs and GSCs. Interestingly, survival analysis demonstrated that PATZ1 lower levels informed poor prognosis in GBM and, specifically, in the proneural subgroup, suggesting it may serve a role as diagnostic and prognostic biomarker for intra-subtype heterogeneity of proneural GBM. We also show that PATZ1 suppresses the expression of the mesenchyme-inducer CXCR4, and that PATZ1 and CXCR4 are inversely correlated in GSC and proneural GBM. Therefore, we propose a role for PATZ1 in the negative regulation of CXCR4, through that it counteracts the proneural-to-mesenchymal transdifferentiation of GBM.

CONCLUSION: Overall these findings support a central role of PATZ1 in regulating malignancy of GBM.

NO CONFLICT OF INTEREST

276 The shelterin protein TRF2 can alter the secretoma of colon cancer cells with an impact on tumor angiogenesis

P. Zizza[1], R. Dinami[1], M. Porru[2], C. D'Angelo[1], C.A. Amoreo[3], M. Mottolese[3], I. Sperduti[4], C. Leonetti[2], A. Biroccio[1]

[1] Regina Elena National Cancer Institute, Oncogenomic and Epigenetic Unit, Roma, Italy
[2] Regina Elena National Cancer Institute, SAFU Unit, Roma, Italy
[3] Regina Elena National Cancer Institute, Pathology Unit, Roma, Italy
[4] Regina Elena National Cancer Institute, Biostatistics Units, Roma, Italy

INTRODUCTION: TRF2, which is at the heart of the molecular events that maintain telomere integrity, has been found overexpressed in several human malignancies and in the vasculature of many cancers cell types. However very little is known about its role in tumor angiogenesis. Here we questioned if TRF2 can alter the secretome of cells, stimulating the formation of new vessels around the tumor.

METHODS AND RESULT: By using a high-throughput approach based on multiplexed Luminex X-MAP technology, we demonstrated here that TRF2 dramatically affects the levels of VEGF-A, which in turn stimulates endothelial cell differentiation and angiogenesis. In order to give insight into the molecular mechanism/s through which the shelterin protein modulates VEGF-A, we found that TRF2affectsthe expression of the gene encoding for SULF-2, a sulfotransferase that induce a post-synthetic modification of heparan sulfate proteoglycans (HSPGs),modulating the binding of growth factors with an heparin-binding domain. Finally, we found a direct correlation between TRF2 and VEGF protein levels in a cohort of colon rectal cancers, and more relevant the simultaneous high expression of TRF2 and VEGF is an independent predictor of worse prognosis in a multivariate analysis.

CONCLUSION: We identify a new mechanism through which TRF2, acting on the tumor microenvironment, regulates tumor angiogenesis with an obvious impact on tumor progression, addressing the relevance of our findings in tumor patients.

NO CONFLICT OF INTEREST

277 The in vitro anticancer activity of novel phenothiazines in brain cancer cells

S. Omoruyi[1,2], O. Ekpo[1], S. Prince[2], A. Jardine[3]

[1] University of the Western Cape, Medical Bioscience, Cape Town, South Africa
[2] University of Cape Town, Division of Cell Biology- Department of Human Biology, Cape Town, South Africa
[3] University of Cape Town, Department of Chemistry, Cape Town, South Africa

BACKGROUND: Glioblastoma is an aggressive and the most common type of brain tumour with limited treatment options. Despite recent advances in treatment, patient survival remains poor hence new treatment options are needed. Our collaborating laboratory has succeeded in remodelling phenothiazines (PTZ) which are important heterocyclic compounds not only widely recognized for their antipsychotic effects, but also known to have a wide variety of biological activities. In this study, the anti-cancer activity of PTZ was evaluated in U87 and U251 glioblastoma (GBM).

METHODS: Thirty two (32) novel phenothiazine derivatives were screened and two lead compounds were identified. MTT assay was used to determine the short-term cytotoxicity of phenothiazines while the effect on the long-term survival of cells was determined by clonogenic assay. Furthermore, the impact of PTZ on the cell cycle profile was analysed using FACS while the mode of cell death and the signalling pathways were determined using western blotting.

RESULTS: Novel PTZ potently suppressed the growth of malignant glioblastoma cells, possibly due to a G1 phase arrest of the cell cycle thus leading to cell death. This arrest could be as result of the activation of the p-p38 and pERK1/2 Mitogen-activated protein kinases (MAPKs) pathways thus leading to a significant increase in expression levels of the cell cycle regulatory proteins p21 and p53. Furthermore PTZ also lead to the inhibition of p-AKT, a serine-threonine kinase activated by the phosphatidylinositol 3-kinase (PI 3-kinase) signalling pathway.

CONCLUSION: Taken together, this study suggests that PTZ could be a promising anti-cancer drug against brain cancers.

NO CONFLICT OF INTEREST

278 miR-214 and miR-148b coordinate breast cancer and melanoma progression and are putative targets for therapy

D. Taverna[1], F. Orso[1], D. Dettori[1], E. Penna[1], R. Coppo[1], F. Virga[1], L. Quirico[1], M.B. Stadler[2], C.L. Esposito[3], V. De Franciscis[3]

[1] University of Torino/MBC, Molecular Biotechnology and Health Sciences, Torino, Italy
[2] Friederich Miescher Institute, Swiss Institute of Bioinformatics, Basel, Switzerland
[3] Istituto di Endocrinologia ed Oncologia Sperimentale, Consiglio Nazionale delle Ricerche, Napoli, Italy

MicroRNAs are small non-coding RNAs that negatively regulate gene expression and play a central role in tumor progression. We demonstrated that miR-214 is upregulated in melanoma and breast cancer and coordinates metastatic dissemination via a novel pathway including the downmodulation of miR-148b. More recently, we showed that the inhibition of miR-214 and the simultaneous overexpression of miR-148b lead to a strong reduction of metastasis formation due to a additive effect on tumor cell extravasation coordinated by ALCAM and ITGA5, two direct miR-148b targets. Our investigations underline the relevance of the network including miR-214 and miR-148b in the control of metastasis formation, therefore, modulation of these miRNAs offers considerable therapeutic potential. To this aim, we are exploring the consequences of single or simultaneous miR-214 depletion and/or miR-148b overexpression in tumors by using sponges/anti-miRs and pre-miRs, expressed within tumor cells or delivered systemically in tumor/metastasis-bearing mice and positive preliminary effects are observed. Alternatively, we are preparing and delivering chimeric nucleic acid aptamers (Axl-miR/anti-miR) in mice to attempt miR/anti-miR cell specific delivery to tumor cells. Therapeutic tools will be tested in mice carrying xenotransplants and in miR-214 overexpressing (miR-214OVER) and knock out (miR-214KO) murine models that develop endogenous tumors. The therapeutic potential of these tools as well as the phenotype of the genetically modified mice will be presented and discussed. In conclusion, our data demonstrate that the cascade of events involving miR-214 and miR-148b is controlling melanoma and breast cancer progression and it can be exploited for miR/anti-miR-based therapeutic interventions.

NO CONFLICT OF INTEREST

279 p140Cap protein counteracts ERBB2-dependent tumor progression through inhibition of Rac dependent pathways

J. Chapelle[1], S. Grasso[1], V. Salemme[1], M. Gai[1], C. Riganti[1], E. Turco[1], P. Defilippi[1]

[1] University of Torino, Molecular Biotechnology and Health Sciences, Torino, Italy
[2] University of Torino, Oncology, Torino, Italy

BACKGROUND: Breast cancer is the most common malignancy in women worldwide. ERBB2 gene amplification and receptor over-expression occurs in 20-30% of breast cancer and defines a distinct molecular breast cancer subtype. p140Cap is an adaptor protein that negatively controls tumor cell features. In breast cancer, we have recently shown that p140Cap limits ERBB2-mediated breast cancer progression interfering with Rac GTPase-controlled circuitries (Grasso et al., 2017).

MATERIAL AND METHODS: We set up two human (SKBR3 and BT474 cells) and a mouse (NeuT-TUBO) models of ERBB2 breast cancer, over-expressing or down-regulating p140Cap, to investigate tumor features with both in vitro and in vivo experiments.

RESULTS: We show that p140Cap limits ERBB2-dependent Rac activation, by binding and inactivating Tiam1, one specific Rac GEF. Rac activity was also strongly decreased in HEK293T cells transfected with Tiam1 and p140Cap. We dissect the molecular interaction between p140Cap and Tiam1, both in cancer cells in the HEK293 cell model. We found that p140Cap associates to Tiam1 through its aminoterminal region. We are currently investigating their reciprocal localization in tumor cells, in terms of cell polarity and cell-cell adhesion proteins.

CONCLUSIONS: Overall, these data highlight p140Cap as a negative regulator of Tiam1, which in turn affects ERBB2-dependent Rac signaling in breast cancer cells.

NO CONFLICT OF INTEREST

280 Targeting oncogene-dependent replication stress with PARP inhibitors and cell cycle modulators for the treatment of MYCN-overexpressing tumors

V. Colicchia[1], M. Petroni[2], G. Guarguaglini[3], M. Sahun Roncero[4], C. Capalbo[4], F. Belardinilli[4], A. Coppa[5], G. Peruzzi[2], P. Lavia[3], G. Giannini[4]

[1] University of Rome "La Sapienza", Molecular Medicine, Roma, Italy
[2] Istituto Italiano di Tecnologia, Center for Life Nano Science@Sapienza, Rome, Italy
[3] Institute of Molecular Biology and Pathology, CNR National Research Council- c/o University La Sapienza, Rome, Italy
[4] University of Rome "La Sapienza", Molecular Medicine, Rome, Italy
[5] University of Rome "La Sapienza", Experimental Medicine, Rome, Italy

INTRODUCTION: MYCN amplification (MNA) is associated with poor outcome in several tumors, including neuroblastoma and medulloblastoma. In the case of MNA neuroblastoma, the five-years survival is very limited, making the search for new therapeutic approaches for this tumor subset an absolute priority. PARP inhibitors-based combination schemes are being used in clinical trials and have been also tested in neuroblastoma preclinical models with encouraging results. However, the expression of PARPs and the biochemical consequences of their inhibition on the DNA damage response (DDR) were not characterized in these models.

MATERIALS AND METHODS: We performed in silico analysis of the expression of PARP family members in primary human neuroblastoma datasets and we have used MYCN-dependent tumor models to characterize the effects of PARP inhibitors, with or without cell cycle modulators, on cell proliferation, death, replication stress and DNA damage, by using multiple assays.

RESULTS: Analysis of R2-datasets indicates that PARP1 and PARP2 are highly expressed in high-risk and MNA primary human neuroblastomas. Their expression is significantly associated with poor survival, indicating their potential and new prognostic value. In this context, inhibition of PARP activity via olaparib® induces cell death by mitotic catastrophe, anticipated by the accumulation of DNA damage. Indeed, olaparib® treated MYCN-overexpressing cells show DDR signs including H2AX and p53 phosphorylation, 53BP1 foci, micronuclei and anaphase bridges. Furthermore, at earlier time, olaparib® treatment yields activation of a typical replication stress-checkpoint causing a transient delay in the S-phase of the cell cycle. Nonetheless, PARP-inhibited MYCN amplified and overexpressing cells fail to sustain a prolonged checkpoint and progress through mitosis in the presence of damaged DNA, eventually undergoing mitotic catastrophe. PARP silencing or treatment with PARP inhibitors with different trapping potency, indicate that PARP trapping is required for the enhancement of replication stress, DNA damage and cell death in MYCN-overexpressing cells. Inhibition of the S-phase checkpoint anticipates and increases the occurrence of mitotic catastrophe induced by olaparib®.

CONCLUSION: These data highlight a novel route for cell death induction by PARP inhibitors and support their introduction, together with cell cycle modulators, in therapeutic approaches for MYCN-dependent tumors.

NO CONFLICT OF INTEREST

281 Bcl-2 regulates miR-378a-5p expression in melanoma

M.G. Tupone[1], D. Trisciuoglio[1], S. Donzelli[2], A. Sacconi[2], M. Spagnuolo[3], M. Desideri[2], M. Di Martile[1], G. Blandino[2], M.G. Rizzo[3], D. Del Bufalo[1]

[1] Regina Elena National Cancer Institute, Translational Research Functional Area- Research- Advanced Diagnostics and Technological Innovation Department, Roma, Italy
[2] Regina Elena National Cancer Institute, Translational Oncogenomic Unit, Roma, Italy
[3] Regina Elena National Cancer Institute, Department of Research- Advanced Diagnostics and Technological Innovation- Genomic and Epigenetic Unit, Roma, Italy

INTRODUCTION: Melanoma, the most aggressive form of skin cancer, is frequently associated with alterations in many genes, among which the Bcl-2 oncogene plays an important role in survival, progression, chemosensitivity and angiogenesis. Also microRNAs (miRNAs), play an important role in melanoma development and progression affecting tumor proliferation, migration and invasion. Among these, miR-378a-5p has been found differentially expressed in some types of cancer and has oncogenic properties. However, effects and potential mechanism of miR-378a-5p in melanoma have not been explored.

MATERIAL AND METHOD: To identify novel putative miRNAs, whose expression could be modulated by Bcl-2, human melanoma M14 parental cells have been transfected with a smart-pool RNA targeting human Bcl-2 mRNA, and a Human miRNA Microarray has been performed. QRT-PCR has been carried out to validate and confirm downregulation of miRNAs after Bcl-2 silencing. MiR-378a-5p mimic

and inhibitor were transfected to melanoma cells and verified by qRT-PCR. Target prediction analysis was administrated using MirWalk 2.0 and TargetScanHuman 7.2 softwares.

RESULTS: and Discussion

Four independent experiments indicate that, in cells with reduced Bcl-2 expression compared to control ones, 13 miRNAs were significantly downregulated. QRT-PCR confirmed downregulation of miR-301b-3p, miR-378a-5p, miR-501-3p, miR-502-5p and miR-4660 after Bcl-2 downregulation. Among these, miR-378a-5p has been identified as significantly deregulated in both M14 and JR8 human melanoma cell lines, after Bcl-2 silencing. Based on the target prediction analysis, we identified several miR-378a-5p target genes, such as SUFU, TUSC2, TOB2, VEGF, GABPA and ESRRg, that may explain the mechanism by which miR-378a-5p modulation by Bcl-2 protein can affect tumorigenesis and tumor maintenance in melanoma.

CONCLUSION: Although a deeper understanding of the molecular mechanism involved in Bcl-2 modulation of microRNA and in particular of miR-378a-5p is needed, our work indicate Bcl-2 involvement in non-canonical antiapoptotic functions.

NO CONFLICT OF INTEREST

282 Aberrant choline metabolism in epithelial ovarian cancer: At the cross-road of chemoresistance and immune evasion

A. Rizzo[1], R. Nicoletti[1], P.Alberti[1], A. Satta[1], M. Figini[1], F. Raspagliesi[2], A. Ricci[3], E. Iorio[3], M. Bagnoli[1], D. Mezzanzanica[1]

[1] Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Experimental Oncology and Molecular Medicine, Milan, Italy
[2] Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Gynecologic Oncology, Milan, Italy
[3] Istituto Superiore di Sanità, Department of Cell Biology and Neurosciences, Roma, Italy

BACKGROUND: an increased phosphocholine intracellular content sustained by choline kinase-alpha (CHKA) over-expression and hyper-activity contributes to define in EOC a 'cholinic phenotype" that reflects the interactions between oncogenic signaling and cell metabolism. Aim of our study is the valuation of the CHKA biological relevance, focusing the attention on its possible druggability in EOC.

MATERIALS AND METHODS: human EOC cell lines, non-tumoral immortalized ovarian cells (IOSE h-TERT) and ex-vivo EOC patient-derived ascitic tumor cells (PDAT) were used. By in-vitro and ex-vivo studies we evaluated the biological role of CHKA on EOC growth and behavior in order to define the molecular and metabolic pathways in which it is involved.

RESULTS: CHKA silencing in different in-vitro and ex-vivo EOC cells, affects their aggressive phenotype by reducing cell proliferation and migration capabilities. It also alters oxidative stress redox status, decreasing glutathione (GSH) content and raising reactive oxygen species (ROS) levels. Interestingly, the increase in intracellular ROS species improved EOC cells sensitivity to standard chemotherapeutic drug treatments, clearly contributing to cell death triggering. We are currently dissecting the apoptotic pathway and other death mechanisms related to ROS accumulation. Moreover, upon CHKA silencing both in-vitro and in PDAT cells, we observed an enhanced expression of several TNF receptor superfamily members, such as TRAIL-R2. Our preliminary data indicated that increased TRAIL-R2 expression sensitized cancer cells to TRAIL-mediated citotoxicity, thus further increasing tumor cells weakness. The role of CHKA in sustaining EOC aggressiveness was confirmed by its over-expression by transient transfection in non-tumoral immortalized ovarian cells. CHKA transfected IOSE-hTERT showed increased cell proliferation and migration while CHKA silencing did not affect their growth capability, GSH metabolism or drug sensitivity, thus opening an interesting therapeutic window for EOC. Our in-silico analysis on EOC public datasets has shown that CHKA expression is correlated with tumor aggressiveness, sustaining its possible druggability.

CONCLUSION: based on our observations, we hypothesize that the 'cholinic phenotype", being able to properly sustain and characterize EOC aggressive features, represents an appropriate target for new therapeutic approaches to be used alone or in combination with conventional drugs for EOC treatments.

NO CONFLICT OF INTEREST

283 Deciphering the role of ChrXq27.3 miRNA cluster in regulating drug response in epithelial ovarian cancer

R. Nicoletti[1], M. Bagnoli[1], L. De Cecco[2], P. Alberti[1], W. Zhang[3], F. Raspagliesi[4], S. Canevari[2], D. Califano[5], S. Pignata[6], D. Mezzanzanica[1]

[1] Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Molecular Therapies Unit- Department of Experimental Oncology and Molecular Medicine, Milan, Italy
[2] Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Functional Genomics- Department of Experimental Oncology and Molecular Medicine, Milan, Italy
[3] M.D. Anderson Cancer Center, Department of Pathology- The University of Texas MD Anderson Cancer Center, Houston- TX, USA
[4] Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Unit of Gynaecological Oncology, Milan, Italy
[5] Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G Pascale"- IRCCS, Functional Genomic Unit, naples, Italy
[6] Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G Pascale"- IRCCS, Department of Urology and Gynecology, naples, Italy

INTRODUCTION: In spite of optimal surgery and chemotherapy, a major challenge in Epithelial Ovarian Cancer (EOC) remains prediction of chemoresistant relapse. We previously identified the ChrXq27.3 miRNA cluster independently associated with early relapse in advanced stage EOC patients. Deciphering its role in regulating networks related to drug resistance and disease progression could improve the management of the disease.

MATERIAL AND METHOD: The involvement of miR-506 in regulating DNA repair machinery was investigated in in-vitro EOC models, assessing the potential of miR-506 expression to phenocopy/support the effects of BRCA1/2 mutation on platinum (Pt) and PARP inhibitor sensitivity. The potential synthetic lethality of mir-506 forced expression/RAD17 silencing with these drugs as well as with small molecule inhibitors of cell-cycle checkpoint kinases was evaluated. A set of 11 EOC patients' matched primary tumor (P), omental secondary localization (M) and tumor at relapse(R) from INT-Pascale and INT-Milan, was selected for analysis of miRNA/gene expression profiles, considering time to relapse as clinical endpoint.

RESULTS AND DISCUSSION: We confirmed miR-506, belonging to the ChrXq27.3 cluster, to directly target RAD17 and RAD51 genes, which actively drive DNA strand-break repair guiding BRCA1/2 to DNA and stabilizing active signalling. In EOC, inactivation of DNA repair genes (BRCA1/2) is associated with better prognosis, due to sensitization to DNA damaging drugs. Interestingly, we observed that miR-506 forced expression/RAD17 knockdown sensitize EOC cells to Pt and PARP inhibitors and show a synthetic lethal effect with CHK1 and WEE1 inhibitors. We also observed that miR-506 expression is strongly associated to patient's response to Pt treatment in a case material of 45 matched samples, in which miR-506 resulted downregulated in M versus the corresponding P. These result prompted us to define the role of ChrXq27.3 miRNA cluster in tumor relapse and preliminary qRT-PCR data confirm the downregulation of these miRNAs also in R samples. Also a significant number of genes differentially expressed in M and R samples compared to P tumors were identified. The integrated analysis of miRNAs and gene expression profiles is ongoing.

CONCLUSION: Mir-506 is involved in regulating the response to chemotherapy and its expression may help in selecting patients who may most benefit from chemotherapeutic treatment.

Partially supported by AIRC and CARIPLO Foundation.

NO CONFLICT OF INTEREST

284 Functional significance of the interaction of ubiquitin ligase Pirh2 with RNA-binding protein HuR

A. Daks[1], O. Fedorova[1], A. Petukhov[1], E. Vasileva[1], O. Shuvalov[1], N. Barlev[1]

[1] Institute of cytology RAS, Laboratory of Gene Expression Regulation, St Petersburg, Russian Federation

Pirh2 (product of the RCHY1 human gene) is a RING-finger containing E3 ligase that modifies the p53 family of proteins with ubiquitin residues leading to their subsequent degradation in proteasomes. As in the case of Mdm2, another E3 ligase, Pirh2 is the transcriptional target of the p53 protein and its homologs forming a regulatory negative feedback loop. Despite its obvious oncogenic function in regards to p53, p63 and p73, Pirh2 shows ambiguous role in different p53-negative types of cancer, which warrants further investigation. Besides p53, there are several known Pirh2 targets playing key roles in apoptosis, cell cycle progression and DNA repair, such as Chk2, p27Kip and PolH. However, there is still very limited information available on targets of Pirh2 and their roles in tumorigenesis.

To fill these gaps we applied a proteomic approach in which we identified over a hundred novel Pirh2-interacting proteins. Among those, we revealed the RNA-binding protein, HuR, as an interacting partner of Pirh2. HuR is one of the most widely studied critical regulators of eukaryotic posttranscriptional gene expression. By binding to 3' UTR of target mRNAs it modulates their stability. HuR plays physiological role in mediating cellular response to various stimuli, including apoptosis, proliferation, and survival thereby altering expression of many crucial factors such as c-Myc, cyclins A, B1, D1 and E, p53, and TNFα. There are many reports about the oncogenic role of HuR due to its ability to promote proliferation and drug resistance of cancer cells. Thus, we confirmed a physical interaction between Pirh2 and HuR via reciprocal GST-pulldown and co-immunoprecipitation. We also showed that Pirh2 functions as E3 ligase for HuR and hence affects mRNA levels of HuR targets under heat shock conditions. Collectively, we uncovered a novel potential mechanism by which Pirh2 regulates expression of many gene products via controlling the function of HuR in cancer cells. These result also shed light on the controversial role of Pirh2 in different types of cancers.

This work was supported, in part, by RFBR research projects No.16-34-00869 mol_a and No.16-34-60228 mol_a_dk, and RSF research project No.14-50-00068.

NO CONFLICT OF INTEREST

286 MT1 melatonin receptor as a prognostic marker in breast tumors

D.Aparecida Pires de Campos Zuccari[1], B.D.C. Tialfi[2], A. De Lima Mota[2]

[1] FAMERP, Bioquímica, São José do Rio Preto, Brazil
[2] FAMERP, Biociências, São José do Rio Preto, Brazil

BACKGROUND: Breast cancer is the tumor with the highest prevalence and mortality inwomen worldwide. Is a heterogeneous dicease with multiple phenotypic and biologicalchanges. The current prognostic considers, in addition to histological types, molecularsubtypes characterized as Luminal A and Luminal B (both positive for estrogenreceptor (ER)); HER2-positive (oncogene HER-2

overexpression) and Triple negative(absence of ER, PR (progesterone receptor) and HER-2 expression), the latter havingthe worst prognosis. Approximately 80% of breast tumors are classified as ER positiveand is correlated with better prognosis. Melatonin and estrogens are coregulators inproliferation and differentiation in breast cancer. Melatonin and estrogensare coregulators in proliferation and differentiation in breast cancer. The melatonin hormone appears to have an oncostatic effect in different types of cancers withemphasis to breast cancer. In mammals, there are two main subtypes of melatoninreceptors: MT1 and MT2. They are expressed in human cells of various organs, inneoplastic cells and breast cancer cells, and it facilitates all the melatonin'sactions. The present study aimed investigate the MT1 receptor as a possibleprognostic marker in breast cancer through its gene and protein expression in thedifferent molecular subtypes of breast cancer.

METHODS: It was used 50 paraffin blockswith tumor of each patient. Immunohistochemical (IHC) technique for MT1 receptorwas used. The method uses the enzyme horseradish peroxidase in the presence ofprimary and secondary antibody, and peroxidase-biotin- streptavidin revealed with DABchromogen. For genetic expression, 20 paraffin-embedded blocks were used to extractRNA with specific KIT and validate the expression of the MT1 receptor. result andDiscussion: Both gene and protein MT1 expression levels were higher in the tumorswith good prognosis and decreased gradually as this condition worsened. The ratio ofMT1 expression may be related to serum melatonin levels that can act directly on cellsthrough these receptors. This shows the importance of an adequate blood level ofmelatonin and also the need for MT1 expression in tumor samples.

CONCLUSION: According to our results, we conclude that the expression of the melatonin receptor(MT1) can be used as a prognostic marker because its protein and gene expressions inbreast cancer are inversely proportional to the aggressiveness of the disease.

NO CONFLICT OF INTEREST

287 Mechanisms of acquired resistance of HER-2 overexpressing breast cancer cells to small molecule tyrosine kinase inhibitors

A. Stanley[1], *H. Modjtahedi*[1], *H. Ashrafi*[1], *A. Seddon*[1]

[1] *Kingston University London, School of Life Science, London, United Kingdom*

INTRODUCTION: Breast cancer remains one of the leading causes of mortality worldwide, with acquired resistance being the major cause of treatment failure. In our previous study, we demonstrated the improved efficacy of irreversible pan-HER tyrosine kinase inhibitors (TKIs) (e.g. afatinib) over the reversible EGFR/HER2 inhibitor lapatinib in HER2 over-expressing (HER2+) breast cancer cells, as well as the potential therapeutic benefit of combining HER-family inhibitors with inhibitors of other kinases. Here, we investigated the mechanisms of acquired-resistance to various HER-family inhibitors.

MATERIAL AND METHOD: SKBr3 TKI-resistant variants were established following treatment with chronic doses of the HER TKIs. Next, we examined the sensitivity of parental cells and their resistant variants to treatment with various inhibitors and chemotherapeutic drugs using colorimetric assay, and the possible mechanisms of drug-resistance using various techniques including flow cytometry and Western blot analysis.

RESULTS AND DISCUSSION: We found that SKBr3 cells made resistant to one HER-family inhibitor also developed resistance to other HER-family inhibitors. Interestingly, the resistant variants became more sensitive to dasatinib, supporting a role for Src in conferring resistance, and also to the chemo agent gemcitabine. We found that acquired resistance to afatinib was accompanied by a decrease in the expression of HER2, pEGFR, pHER-2 and pHER-4, upregulation of pSrc, but no changes in the level of pMAPK and pAKT. In contrast, acquired resistance to lapatinib was accompanied by a decrease in the phosphorylation of Akt, MAPK and Src. Finally, we found that treatment with irreversible HER-family inhibitors was not sufficient to overcome resistance to first generation reversible inhibitors.

CONCLUSION: Our result suggest that mechanisms of acquired resistance to various types of HER inhibitors are not identical and that Src may play an important role in acquired resistance to the irreversible pan-HER-TKI. Further research is warranted to investigate the interactions between these proteins and their co-targeting as a therapeutic intervention.

NO CONFLICT OF INTEREST

288 Pim kinases promote metastatic growth of prostate cancer cells

N.M. Santio[1], *S. Eerola*[1,2], *S.K.J. Landor*[3], *M. Salmela*[4], *J. Tuomela*[5], *P. Härkönen*[5], *C. Sahlgren*[3], *P.J. Koskinen*[1]

[1] *Department of Biology, University of Turku, Finland*
[2] *Prostate Cancer Research Center, University of Tampere, Finland*
[3] *Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Finland*
[4] *Department of Biochemistry, University of Turku, Finland*
[5] *Institute of Biomedicine and Anatomy, University of Turku, Finland*

INTRODUCTION: The oncogenic Pim family kinases are often overexpressed in hematological malignancies as well as in solid cancers like prostate cancer. We have previously shown that these serine/threonine-specific kinases promote cancer cell survival as well as radioresistance. In hematopoietic cells, we have noticed that Pim kinases stimulate activities of several transcription factors such as Myb, NFATc as well as RUNX family members that regulate production of survival cytokines. Pim kinases also promote cytokine-independent survival and inhibit apoptosis by several mechanisms, including phosphorylation-induced inactivation of the pro-apoptotic Bad protein.

MATERIALS AND METHODS: We have used PC-3-derived lines of prostate cancer cells that have been grown in cell culture, on top of chorioallantoic membranes of chick embryos or as orthotopic xenografts in the prostates of nude mice to study the effects of Pim overexpression on cell motility and metastatic growth, to identify relevant Pim substrates mediating these effects and to analyse the ability of selective inhibitors to prevent them.

RESULTS AND DISCUSSION: We have shown that overexpression of Pim kinases promotes growth and migration of prostate cancer cells both in cell culture and in our *in vivo* models, where we have observed Pim-dependent enhancement of tumor growth, angiogenesis and lymphangiogenesis. Most interestingly, the Pim-overexpressing cells have been able to migrate from the prostate not only to nearest lymph nodes, but all the way to the lungs. Furthermore, we have shown that Pim-selective inhibitors can efficiently reduce both growth and metastatic behaviour of the cancer cells. We have also identified several Pim substrates as mediators of the pro-migratory effects, including the transcription factors NFATc1 and FoxP3, the GSK3B kinase as well as the chemokine receptor CXCR4, whose activities are either stimulated or inhibited by Pim-dependent phosphorylation. In addition, we have shown that Pim kinases and their most recently recognized substrate Notch1 synergistically enhance metastatic growth of prostate cancer cells.

CONCLUSIONS: Altogether, our data indicate that Pim kinases can support metastatic growth of prostate cancer cells by multiple mechanisms and that Pim-selective inhibitors provide a promising therapeutic approach to restrict formation of fatal metastases associated with aggressive forms of prostate cancer.

References: Santio NM *et al*., *Mol Cancer* 2010, *PloS ONE* 2015, *Exp Cell Res* 2016, *Oncotarget* 2016

NO CONFLICT OF INTEREST

289 MDM2 ubiquitin-ligase affects cancer-related metabolism

O. Shuvalov[1], *A. Shakirova*[1], *A. Petukhov*[1,2], *E. Vasileva*[1], *O. Fedorova*[1], *A. Daks*[1], *A. Kizenko*[1], *N. Barlev*[1]

[1] *Institute of Cytology RAS, , Saint-Petersburg, Russia,*
[2] *Institute of Hematology, Almazov Federal North-West Medical Research Centre, Saint-Petersburg, Russia*

MDM2 is an E3 ubiquitin ligase, which targets p53, one of the most important tumor suppressor, for degradation. This mechanism, which protects normal cells from excessive p53-induced death, is frequently deregulated in different types of tumors.

MDM2 also has p53-independent oncogenic functions. Besides p53, MDM2 targets with ubiquitin a number of proteins, including the catalytic subunit of telomerase (hTERT), transcription factors Snail and Slug, etc. Thus, MDM2 possesses both tumor promoting and tumor suppressing functions, depending on a particular cellular context.

To uncover additional targets of MDM2 we carried out a proteomic study in which GST-MDM2 was used as a bait to pull-down interacting proteins, which were then identified by mass-spectrometry (LC-MS/MS). Whole cell extracts derived from U2OS (human osteosarcoma) cells, MCF7 (breast carcinoma, luminal subtype), and MDA-MB-231 (breast carcinoma, basal subtype) were used as a source of proteins that potentially bind MDM2.

According to the data obtained, we consistently observed a significant number of various metabolic enzymes among the proteins interacting with MDM2. Importantly, they are the key enzymes for different metabolic pathways that are frequently deregulated in different cancers. We have verified interactions of MDM2 with several enzymes identified and studied the influence of MDM2 on their expression, ubiquitination and stability. Moreover, we have shown that MDM2 affects the metabolic state of cancer cells, as well as their susceptibility to several pharmacological inhibitors of corresponding metabolic pathways. Taken together, these data revealed a novel role for MDM2 ubiquitin ligase in the regulation of cancer-related metabolic pathways.

The common set of metabolic alterations is considered now as one of the "hallmarks of cancer" and the corresponding enzymes are promising targets for anticancer therapeutics. These notions emphasize the importance of MDM2 for cancer metabolism and warrants further investigations.

This work was supported, in part, by RFBR research projects No.16-34-00869 mol_a and No.16-34-60228 mol_a_dk, and RSF research project No.14-50-00068.

NO CONFLICT OF INTEREST

290 Combination therapy of oncolytic herpes virus and anti-angiogenesis agent (bevacizumab) against human gastric cancer xenograft

H. Kasuya[1], G. Tan[1], C. Luo[1], T. Ichinose[1], I. Bustos[1], Y. Naoe[1]

[1] Cancer Immune Research Center, Nagoya University Graduate School of Medicine, Nagoya, Japan

BACKGROUND: The high prevalence and poor prognosis of gastric cancer provides a strong rationale for developing new treatment strategies. Oncolytic replication-competent herpes viruses has a promising prospect because of its selectively infecting, replicating and killing tumor cells. Bevacizumab is a monoclonal antibody which blocks vascular endothelial growth factor subtype A (VEGFA) thus inhibits angiogenesis. In this study, the antitumor effect of oncolytic herpes virus hrR3, a ribosomal reductase deficient herpes simplex subtype 1 derived virus combined with Bevacizumab was evaluated both in vitro and Experimental Model of Human Gastric cancer Xenografts.

METHODS: The VEGFA gene and protein expression was measured in a variety of human cancer cell line by RT-PCR and western blot. The cell line with highest VEGFA gene and protein expression was selected as candidate. MTT assay was performed to evaluate efficiency of combination therapy in vitro. The effect of Bevacizumab on viral replication was evaluated by PCR and tittering of the virus replicating under various doses of Bevacizumab. The in vivo study involved 24 BALB/c nude mice which were injected 106 cells into the right flank region. Control group received no treatment. hrR3 group received single dose of 107 pfu virus intratumorally. Bevacizumab group received 5mg/kg intraperitoneally twice a week. Combination group received both intratumoral hrR3 and intraperitoneally bevacizumab at the same dose. Tumor diameters were measured twice a week. 2 days following the last dose of the treatment tumors were collected. Microvascular density, apoptosis and the viral replication in tissue were evaluated by CD31, apoptag and β–Galactosidase (LacZ) staining respectively.

RESULTS: Human gastric cancer cell line MKN45 had the highest VEGFA gene and protein expression. In the in vivo study, combination group had the smallest tumor volume comparing with other groups($P<0.05$). The Microvascular density was highest in the hrR3 group ($P<0.05$). Combination group had higher angiogenesis when compared to Bevacizumab and control groups. LacZ induction was highest in the combination group when compared to hrR3 group ($P<0.05$). Apoptosis were increased in the combination group ($P<0.05$).

CONCLUSION: The penetration of genetically engineered viruses in tumor remains a obstacle. Thus virus-associated agent is needed to enhance the antitumor effect of the oncolytic virus. Our study shows that Bevacizumab increased the viral replication、apoptosis and potent oncolysis resulted in reduction of the tumor. It can be a ideal virus-associated agent in the antitumor therapy. Oncolytic herpes viruses hrR3 seems to induce angiogenesis.

NO CONFLICT OF INTEREST

291 Mitochondrial genome defects as identifier of the "at risk" metastatic subtype among triple negative breast tumors

M. Guha[1], S. Srinivasan[1], P. Raman[2], Y. Jiang[3], B.A. Kaufman[3], D. Taylor[2], N.G. Avadhani[2]

[1] Department of Biomedical Sciences, School of Veterinary Medicine, University of Pennsylvania
[2] Department of Biomedical and Health Informatics, Center for Mitochondrial and Epigenomic Medicine, The Children's Hospital of Philadelphia, USA
[3] Center for Metabolism and Mitochondrial Medicine, Division of Cardiology, Department of Medicine

INTRODUCTION: Tumor metastasis accounts for the high mortalities in breast cancer. Various genetic factors contribute to the breast tumor etiology and metastasis. Breast tumors are highly heterogeneous (intra and inter tumor heterogeneity) based on their cell of origin and molecular gene expression signatures which makes it clinically challenging to identify and target "at-risk" metastatic tumors. Based on their molecular signatures, breast tumors are broadly classified as Luminal A and Luminal B (ER, PR receptor positive), ERBB2/HER2 overexpressing (Her2+) and receptor triple negative (TNBC). While targeted therapies for the receptor positive and Her2+ tumors are clinically promising, currently there are no targetable therapies for the highly aggressive TNBCs. We reported that mitochondrial (mt) DNA copy number depletion in mammary cell lines result in cellular reprograming similar to an epithelial-mesenchymal transition, suggesting that low mtDNA copy number tumors might be more metastatic. We also reported the epithelial splicing regulatory protein (ESRP1) is a molecular intermediate in mtDNA loss induced cellular plasticity indicative of the role of alternative splicing. While it is known that breast tumors contain more mtDNA defects than normal tissue, it is unknown whether the four major breast tumor subtypes differ in the amount and nature of mtDNA defects. Here we tested the variability of mtDNA defects among the four tumor subtypes classified based on their molecular signatures.

RESULTS AND DISCUSSION: By analyzing human tumors, cell lines and the TCGA dataset, we found that the most aggressive Triple Negative (TNBC, negative for estrogen, progesterone, EGF2 receptors) subset of breast cancers have the lowest mtDNA content and highest level of mtDNA sequence imbalance in the mtRNR1 region. Additionally Receptor triple negative basal-like cells when compared to Luminal and HER2+ cells had the lowest cellular respiratory capacity indicative of impaired mitochondrial functions. In agreement, we observed differential expression of metabolic genes in TNBCs compared to the less aggressive luminal (A and B) or HER2 expressing tumors. Interestingly we also observed lower expression of epithelial splicing regulatory protein (ESRP)-1 in triple negative tumors and receptor negative basal-like cells, a marker of metastasizing cells.

CONCLUSION: Our study identifies unique differences in mtDNA content, mitochondrial genome imbalance, and mitochondrial metabolic gene expressions among TNBCs, suggesting that these combinatorial markers can potentially be used to identify individuals "at-risk" for metastasis and dictate therapeutic regimen targeting the mitochondrial genome defects in a subset of highly aggressive TNBCs.

292 Neogenin-1 participates in metastasis through Integrin ß1activation in neuroblastoma

A.A. Villanueva[1], C. Cubo[2], V.A. Torres[3], P. Sanchez-Gomez[2], V. Palma[1]

[1] Laboratory of Stem Cells and Deuelopmental Biology, Faculty of Sciences, Universidad de Chile
[2] UFIEC, Instituto de Salud Carlos III, Majadahonda
[3] Faculty of Dentistry, Universidad de Chile, Santiago, Chile

INTRODUCTION: Neogenin-1 (NEO1) is a Dependence-Receptor involved in axonal guidance, neuronal cell migration, and cell death. NEO1 binds to the Netrin and RGM ligand families, however, little is known about the specific function of these proteins and their downstream signaling throughout neuroblastoma metastasis progression. Our analysis of public databases of neuroblastoma tumors revealed that high expression of NEO1 and Netrin ligands correlates with poor patient survival. Here, we evaluate the function of the Netrin/NEO1 signaling complex in neuroblastoma cell migration and metastasis process, and the mechanism associated with this function.

MATERIAL AND METHODS: Stable *in vitro* knock-down SK-N-SH and LAN1 cell lines for Netrins and NEO1 or transiently NEO1 overexpressing cells were evaluated for cell death, proliferation and cell migration. Co-immunoprecipitation of NEO1/NETRIN and NEO1/Integrin ß1 was evaluated with specific antibodies against NEO1 and revealing against either NETRIN1, 4 or Integrin ß1. Presence of Netrin ligands and NEO1 in patient samples was determined through immunohistochemistry. *In vivo* metastasis was analyzed through NSG mouse models and by chick chorioallantoic membrane (CAM) assay.

RESULTS AND DISCUSSION: Knockdown of NEO1 reduces cell migration promoted by Netrin-1 and Netrin-4, the main ligands of the Netrin family. Knock-down of NEO1 also induces an increase in adhesion to fibronectin. Co-immunoprecipitation assays show that NEO1 interacts with Integrin ß1 and immunofluorescence in a spreading assay indicates different integrin activation levels in NEO1 knock-down cells. Analysis of patient samples show that both NEO1 and its ligands are expressed in tumor cells. *In vivo* analysis of metastasis implicates NEO1 in tumor metastasis.

CONCLUSIONS: Our result show a possible mechanism involving Netrins acting as novel dependence factors for NEO1 in neuroblastoma modulating the motility and subsequently metastasis of these cells via Integrin ß1.

Funding: FONDECYT-1140697(VP), CONICYT-21130521(AV), FONDECYT-1140907(VT).

NO CONFLICT OF INTEREST

POSTER SESSION: EXPERIMENTAL/MOLECULAR THERAPEUTICS, PHARMACOGENESIS I

293 Design, synthesis and in vitro anticancer evaluation of novel Rad6 ubiquitin conjugating enzyme inhibitors

H. Kothayer[1], S. Spencer[2], K. Tripathi[2], A. Westwell[3], K. Palle[2]

[1] Faculty of Pharmacy- Zagazig University, Medicinal Chemistry Depatment, Zagazig, Egypt
[2] USA Mitchell Cancer Institute, Department of Oncologic Sciences, Mobile- Alabama 3 6 6 0 4 ., USA
[3] School of Pharmacy and Pharmaceutical Sciences- Cardiff University, Medicinal Chemistry, Cardiff- CF1 0 3 NB- Wales., United Kingdom

INTRODUCTION: The ubiquitin-proteasome system consists of a cascade of enzymes (E1, E2 and E3) and its alterations contribute to cancer progression. The success of targeting this system was emphasized after the development of bortezomib (Velcade®) in multiple myeloma therapy**.** The requirement for specific inhibiting targets like the design of E2 or E3 inhibitors, has appeared to reduce the side effects of bortezomib. We chose Rad6B (an E2 ubiquitin conjugating enzyme) to be our target as it plays a fundamental role in post replication repair of DNA and found to be over-expressed in breast cancer, and associated with β -catenin stabilization.

We have reported the diamino-triazines TZ8 and TZ9 as the first selective inhibitors of Rad6B [1].We have also used the Rad6B crystal structure and the lead compounds TZ8 and TZ9 to guide further design modifications and we succeeded to report triazines with IC_{50} values superior to those of TZ8 and TZ9 [2]. Most recently, we designed and report some new analogues of the lead compounds via making small modifications to their structures and we succeeded to synthesize novel triazines [3].

MATERIAL AND METHODS: The triazines were synthesized from either arylbiguanides or bis-arylbiguanides. Evaluation of the synthesized triazines was carried out in various human cancer cell lines using MTS assay to assess the cell viability. To evaluate their Rad6 inhibitory activities both in its conjugation to ubiquitin and to the substrate H2A, in vitro ubiquitin conjugation assays were performed in comparison with TZ9 [3].

We are currently carrying out further work to synthesize more triazines to expand the SAR of the Rad6B inhibitor triazines which will help for future discovery of more efficient inhibitors.

RESULTS AND DISCUSSION: The synthesized triazines showed superior Rad6B inhibitory activities in comparison to selective Rad6 inhibitor TZ9. Similarly, the synthesized triazines showed better IC50 values in survival assays.

CONCLUSION: Series of triazines were synthesized**.** Compared to the reported Rad6B-inhibitor TZ9, new triazines showed more potent IC50 values in survival assays and superior Rad6B inhibitory properties, in in vitro ubiquitin conjugation assays. Further trials are currently being performed to synthesize more triazines to find a more active analogue**.**

REFERENCE:

[1] Sanders MA etal. Mol. Cancer Therap.**2013**, 12.

[2] Kothayer H etal. Bioorg. Med. Chem. Lett. **2013**, 23.

[3] Kothayer H etal. Bioorg. Med. Chem. Lett. **2016**, 26.

NO CONFLICT OF INTEREST

294 The design and development of novel biologics for cancer immunotherapies

J. Gu[1]

[1] *Shanghai Hengrui Pharmaceutical Co.- Ltd., Biologics Discovery, Shanghai, China*

BACKGROUND: Cancer immunotherapies have brought hope to many cancer patients world wide, who have failed traditional chemo- and/or targeted therapies. While showing promise in the clinics, the current limitation of anti-CTLA-4 or anti-PD-1 therapies is low response rate and high toxicity. Next generation cancer immunotherapies will focus on combination therapies. Bispecific antibodies offer a number of exciting opportunities. This presentation will cover a few late stage pre-clinical bispecific programs for cancer immunotherapies.

MATERIALS AND METHODS: Bispecific antibody generation and characterization in vitro and in vivo.

RESULTS: In vitro and in vivo data will be disclosed at the conference.

CONCLUSIONS: BsAb offers a unique opportunity in Cancer Immunotherapies. Tumor targeting T cell regulators via bsAb may enhance anti-tumor activity with reduced systemic toxicity. PD1/PD-L1 + X Combination/bsAb need further evaluation in the clinics.

NO CONFLICT OF INTEREST

295 Identification of dual inhibitors of glutaminase and glutamate dehydrogenase that disrupt mitochondrial function and prevent growth of cancer cells

B.H. Ruan[1], *M. Zhu*[1], *J. Fang*[1], *J. Zhang*[1], *Z. Zhang*[1], *J. Xie*[1], *Y. Yu*[1], *J.J. Ruan*[1]

[1] *Zhejiang University of Technology, School of Pharmacy, Hangzhou, China*

Glutaminase (KGA/ isoenzyme GAC) is an emerging and important drug target for cancer. Traditional methods for assaying glutaminase activity are coupled with several other enzymes, which do not provide stringent characterization of glutaminase inhibitors. Ebselen was reported as a potent 9 nM KGA inhibitor in the KGA/Glutamate Oxidase (GO)/Horse Radish Peroxidase (HRP) coupled assay, but showed very weak activity in inhibiting the growth of glutamine - dependent cancer cells. For rigorous characterization, we developed a direct kinetic binding assay for KGA using Bio-Layer Interferometry (BLI) as the detection method; Ebselen was identified as a GDH inhibitor but not a KGA inhibitor. Furthermore, we designed and synthesized several benzo[d][1,2]selenazol -3(2H)-one dimers which were subjected to SAR analysis by several glutaminolysis specific biochemical and cell based assays. Novel glutamate dehydrogenase (GDH) or dual KGA/GDH inhibitors were discovered from the synthetic compounds; the dual inhibitors completely disrupt mitochondrial function and demonstrate potent anticancer activity with a minimum level of toxicity.

NO CONFLICT OF INTEREST

296 The discovery of Max-40279, a dual FLT3/FGFR inhibitor for the treatment of AML

Y. Wang[1]

[1] *Maxinovel Pharmaceuticals, Preclinical Research, Shanghai, China*

BACKGROUND: Inhibition of FLT3-ITD has been the mainstream of drug discovery for AML treatment. Compounds such as Novartis' FLT3 inhibitor PKC-412 is awaiting FDA approval after successful clinical trials. However, studies of drug resistance of FLT3 inhibitors have revealed that inhibition of FLT3 will activate FGFR to produce cross-talk resistance (E. Traer, etc. Cancer Research 2016, 76: 6471). Thus, it is highly essential to inhibit both FLT3 and FGFR for the new generation AML drugs.

MATERIAL AND METHOD: The compounds were prepared with commercial starting materials. The biology assays as well as DMPK studies were contracted to Shanghai Chempartner, an internationally known preclinical CRO in China with ALACC certification. All of the studies with animals were in accordance with international standards and government regulations at Shanghai Chempartner.

RESULTS: With extensive SAR studies, Max-40279 was found to be potent inhibitor for FLT3, FLT3-ITD and FGFR1&2 in Kinase assay, cellular assay, signal introduction assay and xenograft assays. In the head to head testing with PKC-412, Max-40279 demonstrated superior activity for the FGFR 1&2 inhibition while maintaining similar activity for the FLT3 and FLT3-ITD inhibition.

CONCLUSION: Max-40279, a potent inhibitor of FLT3 and FGFR, offers better solution than PKC-412 for the treatment of AML because it can overcome the drug resistance by the FLT3 inhibition. Details of data will be presented in the meeting as a poster.

NO CONFLICT OF INTEREST

297 From bench to clinic: DBPR114 as a potent multi-kinase inhibitor in the treatment of various cancers

H.P. Hsieh[1], *W.H. Lin*[1], *J.T.A. Hsu*[1], *C.T. Chen*[1], *T.K. Yeh*[1], *H.C. Wu*[2]

[1] *National Health Research Institutes, Institute of Biotechnology and Pharmaceutical Research, Maioli County, Taiwan*
[2] *Academia Sinica, Institute of Cellular and Organismic Biology, Taipei, Taiwan*

BACKGROUND: Newer generation of anticancer drugs that target protein kinases play a pivotal role in cancer therapy. However, clinical resistance toward drugs that primarily target single kinase/pathway can develop rapidly. It is thus desirable to design drugs that can inhibit multiple kinases in different pathways. Several drugs that target multiple kinases of signal transduction pathway have been successfully developed in the past decade, including sorafenib and sunitinib for example. It is noteworthy that these multi-kinase inhibitors were very effective in some malignant diseases which were difficult to treat previously.

METHOD: We established a knowledge-based screening, followed by high throughput parallel synthesis to facilitate new drug discovery of kinase inhibitors targeting Aurora kinase. Thus, more than 1300 compounds were synthesized, 55 compounds were conducted for pharmacokinetics (PK) study, 60 compounds were evaluated for acute toxicity test, and 22 potent compounds were further examined for pharmacological evaluations to identify a potential multiple-targeted kinase inhibitor.

RESULTS: A series of quinazoline analogues with a thiazole urea side chain were synthesized via scaffold-hopping and hybridization. After detailed SAR exploration, several compounds were undertaken enzymatic and cellular assays then **DBPR114** was identified as a novel small molecule multi-kinase inhibitor with potent activities against more than 57 oncogenic kinases, including Aurora-A, FLT3, CSF1R, MET, etc. **DBPR114** was classified as a multi-kinase inhibitor among 458 human kinases, and 24 kinases inhibited by 99% particularly. Furthermore, the PK study showed that **DBPR114** exhibited favorable PK profiles: a long half-life ($t_{1/2}$ = 23.5 h), moderate clearance and high volume of distribution. Also **DBPR114** can effectively inhibit the growth of human acute myeloid leukemia MOLM-13 and MV4-11, MIA PaCa-2, Hep3B, MKN45, Colo205, and NTUB1 solid tumor xenografts in vivo without causing significant body weight loss.

CONCLUSION: Overall, the in vitro and in vivo result suggest that **DBPR114** is a multi-kinase inhibitor which may provide therapeutic benefit over existing treatment and warrants further preclinical investigations. The NOAEL values in the 28-day toxicity study in rats and dogs have been determined. Hence, preclinical studies have been completed and investigational new drug application is currently preparing for submission.

NO CONFLICT OF INTEREST

298 Systemically delivered human telomerase reverse transcriptase (hTERT)-targeting p53-laden adenovirus (ad5CMV- hTERT-p53): Efficacy and toxicity test

W.S. Lee[1], *H.L. Kang*[2], *A. Nagappan*[1], *J.W. Yun*[1], *G. Kim*[3], *S.C. Hong*[4], *S.J. Lee*[5], *I.H. Kim*[2]

[1] *Gyrong sang national university hospital, Internal medicine, Jin-Ju, Korea*
[2] *National Cancer Center, 2 Molecular Imaging and Therapy Branch, Goyang, Korea*
[3] *Gyrong sang national university hospital, School of Veterinary and Research Institute of Life Science, Jin-Ju, Korea*
[4] *Gyrong sang national university hospital, Surgery, Jin-Ju, Korea*
[5] *National Cancer Center, Molecular Imaging and Therapy Branch, Goyang, Korea*

BACKGROUND: Human telomerase reverse transcriptase (hTERT) RNA and p53 tumour suppressor were previously suggested as useful targets for cancer gene therapy. In this study, we made a systemically delivered adenoviruse harboring trans-splicing ribozyme that could target hTERT and induce p53 transcript (ad5CMV- hTERT-p53, ATP-53) in targeted cancer cells. We assessed in vivo therapeutic efficacy of ATP-53, and determine toxicity threshold without causing liver toxicities.

MATERIAL AND METHODS: We constructed ATP-53 driven by a cytomegalovirus promoter. The tumor-specific trans-splicing reaction and tumor-killing effects of ATP 53 were tested in vivo using liver metastasis model of human hepatocellular carcinoma Hep3B cells in male BALB/c-nude mice, and toxicity test was performed with ICR mice. For the determination of toxicity threshold of ATP-53, we tested toxicities at 4 doses (0, $5.x10^{10}$, $1x10^{11}$, $2x10^{11}$, $5x10^{11}$) in non–tumor-bearing mice.

RESULTS: The splenic injection of human hepatocellular carcinoma cells (5×10^6) in 100 µl PBS produced multiple large protruding tumor nodules on the surface of livers in control group. ATP-53 (0.5 $X10^{11}$ Virus) intravenous injection produced dramatically decreased tumor burdens in terms of number and size of residual

tumor nests, compared to the control Microscopic examination revealed that only small residual tumor nests were observed in the ATP-53 treatment group, while large tumor nodules were both in surface and central area of the liver. Next we performed toxicity test with 4 doses ($0.5 X10^{11}$, $1 X10^{11}$, $2 X10^{11}$, and $5 X10^{11}$ of ATP-53), which revealed that there were no toxic death, but 2-4 fold increased in AST and ALT with no changes in bilirubin, BUN, Creatinine levels). The treatment with ATP-53 cause no abnormal behavior occurred at any doses which was delivered.

CONCLUSIONS: This study demonstrates that ATP-53 could be a safe and promising cancer treatment for multiple nodular or metastatic hepatocellular carcinoma in the liver. [This study was supported by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare & Family Affairs, Republic of Korea. (0820050).]

NO CONFLICT OF INTEREST

299 A novel palladacycle complex with anti-cancer activity against breast cancer and melanomas also exhibits potent cytotoxicity in a range of sarcomas

J. Bleloch[1], R. Ballim[1], S. Aliwaini[1], A. Blanckenberg[2], S. Mapolie[2], S. Kimani[1], S. Prince[1]

[1] University of Cape Town, Cape Town, Western Cape, South Africa
[2] Stellenbosch University, Stellenbosch, Western Cape, South Africa

Cisplatin and second-generation alternatives to cisplatin, represent some of the most active and clinically useful agents in the treatment of cancer. However, their application in the clinic is marred by side-effects and drug resistance. We have identified the binuclear palladacycle, AJ-5, as a lead anticancer compound with potent activity against advanced melanomas and estrogen receptor -positive and -negative breast cancers. AJ-5 displayed an IC50 of 0.2μM in melanoma and breast cancer cell lines which was 50 fold less than that for cisplatin and our in vivo result show that it efficiently reduces melanomas in nude mice without any obvious side effects. AJ-5 also showed activity against breast cancer stem cells which are associated with drug-resistance and often not targeted by traditional chemotherapeutic agents.

This study investigates the potential of AJ-5 as an anti-cancer agent in sarcomas, which are a heterogeneous group of neoplasms that account for approximately 21% of paediatric malignancies and treatment outcomes for patients with metastatic disease are dismal. Cytotoxicity assays were performed and sub-micromolar IC50 concentrations were obtained for rhabdomyosarcoma, chondrosarcoma, liposarcoma, synovial sarcoma and osteosarcoma. Clonogenic assays show that AJ-5 greatly compromises the long-term ability of the sarcoma cells to survive and proliferate. To determine the underlying molecular mechanisms by which AJ-5 exerts its cytotoxicity, western blot analyses were performed with antibodies to key proteins involved in the DNA damage response in rhabdomyosarcoma cell lines. The result show that AJ-5 induces high levels of γH2AX, a marker of double-stranded DNA breaks, and that it may exert it's cytotoxicity through the p38 MAP kinase stress pathway. Furthermore, Annexin V–FITC/propidium iodide staining, Caspase Glo assays and western blotting demonstrated that AJ-5 induces intrinsic and extrinsic apoptosis more effectively than doxorubicin, a drug currently used in the treatment of rhabdomyosarcomas. AJ-5 treatment also led to autophagy as confirmed by the formation of autophagosomes, increased levels of LC3-II and the presence of LC3 puncta. Finally, pharmacokinetic studies show that AJ-5 has a promising half-life of 11.2 hours in mice and in addition its volume of distribution is high and its clearance is low while its intraperitoneal absorption is good. Thus the PK data correlates well with our observed efficacy of the drug in our mouse model. Together these findings suggest that AJ-5 may be an effective chemotherapeutic for treating a range of drug-resistant and advanced cancers.

NO CONFLICT OF INTEREST

300 Nanomedicine changing glioma microenvironment by targeting laminin-411–β1 integrin–Notch system provides effective preclinical treatment

J. Ljubimova[1], A. Galstyan[1], R. Patil[1], T. Sun[1], W. Cavenee[2], F. Furnari[2], M. Penichet[3], E. Holler[1], L. Alexander[1], K. Black[1]

[1] Cedars-Sinai Medical Center, Neurosurgery, Los Angeles, USA
[2] University of California San Diego, Department of Cellular & Molecular Medicine, San Diego, USA
[3] University of California Los Angeles, Department of Microbiology- Immunology and Molecular Genetics, Los Angeles, USA

INTRODUCTION: Tumor microenvironment is important for malignant growth, invasion and avoidance of immune system attack. It also serves as a niche for cancer stem cells (CSCs) that are largely responsible for tumor resistance to therapy and development of recurrence.

MATERIAL AND METHODS: Clinical material from 236 patients of different glioma grades was analyzed. Nanodrug syntheses. To examine the interaction mechanisms between GBM cells and their extracellular matrix microenvironment, blood-brain barrier (BBB) passing nanobioconjugates based on poly(β-L-malic acid) (PMLA) were synthesized that specifically inhibit α4 and β1 chains of trimeric laminin-411. In vitro and in vivo studies to confirm the interactions of glioma microenvironment and tumor growth were performed on two human LN229 and U87MG GBMs cells using xenogenic mouse models.

RESULTS AND DISCUSSION: Overexpression of laminin-411 (α4β1γ1) in tumor vascular basement membranes positively correlated with higher recurrence rate and shorter survival of glioblastoma multiforme (GBM) patients. In patient tumors, higher tumor grade was associated with increased expression of laminin-411, β1 integrin, Notch family members, and cancer stem cell (CSC) markers CD133, nestin, and c-Myc. In vitro. Normal brain endothelial cells and astrocytes had higher expression of β1 integrin, Notch-1, and Notch ligands when seeded on 'malignant" laminin-411 as compared with 'normal" laminin-421. All these markers were downregulated in two GBM cell lines treated with antisense oligonucleotides against laminin a4 and b1 chains, suggesting the regulation of Notch pathway by laminin-411 through integrin β1. In vivo. In GBM xenograft mouse models increased expression of laminin-411 correlated with overexpression of integrin β1 and Notch signaling pathway members found in human high grade gliomas. In two mouse models with intracranial human LN229 and U87MG GBMs, treatment with PMLA-based nanobioconjugate against tumor microenvironment protein laminin-411 led to significantly increased animal survival, associated with marked suppression of laminin-411–β1 integrin–Dll4–Notch axis and CSC markers CD133, Nestin, and c-Myc.

CONCLUSION: BBB crossing and tumor-targeted nanodrug therapy using laminin-411 suppression provided a promising tool to study mechanistic interactions between tumor microenvironment and signaling pathways for efficient glioma treatment.

Support: NIH grants R01 CA136841, U01 CA151815, R01 CA188743, R01 CA209921

CONFLICT OF INTEREST

Board of Directors: Drs. Ljubimova and Black are the Board Directors of Arrogene Inc

301 Evaluation of a FLT3 inhibitor LDD1937 as an anti-leukemic agent for acute myeloid leukemia

H.J. Lee[1], J. Baek[1], Y.W. Chin[2], Y.H. Choi[2], S.Y. Han[1]

[1] Gyeongsang National University, College of Pharmacy, Jin-Ju, Korea
[2] Dongguk University-Seoul, College of Pharmacy and BK21 PLUS R-FIND team, Goyang, Korea

FMS-like receptor tyrosine kinase-3 (FLT3) belongs to the family of receptor tyrosine kinase (RTK), FLT3 mutation is observed in 1/3 of acute myeloid leukemia (AML) patients. We have identified potent FLT3 inhibitor containing indirubin skeleton, LDD1937. Potent inhibitory activity of LDD1937 against FLT3 was shown in in vitro kinase assay (IC_{50} = 3 nM) The LDD1937 compound selectively inhibited the growth of MV4;11 cells (GI_{50} = 1 nM) and induced apoptotic cell death. LDD1937 caused cell cycle arrest at G_2/M phase and increased cell population at sub-G_1 phase. Phosphorylation of STAT5, which is downstream signaling of FLT3, was significantly reduced by LDD1937 dose-dependently. Pharmacokinetic properties of LDD1937 in mice were investigated. And in vivo anti-tumor effect was carried out using MV4;11 xenograft. With 5 mg/kg and 10 mg/kg of intravenous administration to nu/nu mice, tumor volume and weight was significantly reduced compared to control.

NO CONFLICT OF INTEREST

302 MiR-148a increases the sensitivity to cisplatin by targeting Rab14 in renal cancer cells

T.J. Lee[1], J.Y. Kim[1], E.A. Kim[1]

[1] Yeungnam University College of Medicine, Department of Anatomy, Daegu, South Korea

BACKGROUND: MicroRNA (miR) can exert various biological functions by targeting oncogenes or tumor suppressor genes in numerous human malignancies. Recent evidence has shown that miR-148a increases the drug sensitivity of various cancer cells.

RESULTS: Here, we show that ectopic expression of miR-148a induces apoptosis, reduces the clonogenicity, and increases the sensitivity to TRAIL and cisplatin in renal cancer cells. The luciferase reporter assay showed that miR-148a negatively regulated ras-related protein 14 (Rab14) expression by binding to the miR-148a binding site in the 3' untranslated region (3'UTR) of Rab14. Rab14-specific siRNA-induced downregulation of Rab14 increases the sensitivity to cisplatin, while forced expression of Rab14 lacking 3′-UTR abrogated the pro-apoptotic function of miR-148a in renal cancer cells.

CONCLUSIONS: These findings suggest that miR-148a acts as a tumor suppressor and holds great potential for renal cancer therapy by directly targeting Rab14.

NO CONFLICT OF INTEREST

303 Gellan gum microsphere encapsulate the nanocarrier of doxorubicin as the chemoembolization agent of hepatoma therapy

M.W. LEE[1], Y.S. Tyan[2], M.F. Hsu[3]

[1] Chung Shan Medical University, Department of Clinical Laboratory, Taichung, Taiwan
[2] Chung Shan Medical University, Department of Medical Imaging and Radiological Sciences, Taichung, Taiwan
[3] Chung Shan Medical University, Department of Medical Laboratory and Biotechnology, Taichung, Taiwan

BACKGROUND: Hepatocellular carcinoma (HCC) represents the sixth most common cancer in the world and the second in the mortality of cancer in Taiwan. Transcatheter Arterial Embolization (TAE) is the most commonly used locoregional therapy worldwide for patients with unresectable liver cancer and has been shown to provide substantial survival benefit in a subset of patients with unresectable hepatocellular carcinoma. In this study we arm to design novel chemoembolization

agent for hepatoma therapy. The chemoembolization agent is based on gellan gum microsphere which contain hyaluronan/polyethylenimine-doxorubicin (HH/PH-DOX) nanoparticles.

MATERIAL AND METHODS: Hyaluronan conjugated with histidine (HH) and Polyethylenimine conjugated with histidine (PH) were synthesized. The anticancer drugs DOX were physically encapsulated into HH/PH nanoparticles by dialysis. The gellan gum microsphere (GG) composed of HH/PH-DOX nanoparticles was fabricated by the emulsification process. The gellan gum base chemoembolization agent and HH/PH-DOX nanoparticles was characterized. HH/PH-DOX nanoparticle uptake by liver cancer cell (HepG2) was performed.

RESULTS: ^{1}H NMR spectrum showed that the peaks of HH were at 8.064 ppm (-N=CH-) and 6.979 ppm (-N-CH=C-) which were ascribed to the methenyl group located in imidazole group of Histidine. The newly appeared peaks at 7.714 ppm (-N=CH-) and 6.959 ppm (-N-CH=C-) revealed that PH was successfully obtained. From the change of zeta potential, we found the doxorubicin can encapsulate into HH/PH matrix successfully. The gellan gum microsphere contain HH/PH-DOX were fabricated using the W/O emulsion method, and the diameter of these microspheres was 300± 100 um. The delta-potential of HH and PH were -42.7 and 43.4 mV, respectively. The delta-potential of doxorubicin solution was -10 mV, when PH reacted with doxorubicin can form PH-DOX by covalent bonding formation and the delta-potential of PH-DOX was 33.6 mV. And then, PH-DOX mixed with HH at the w/w ratio of 1:4 to form HH/PH-DOX polyion complexes (delta-potential was -25.5mv). From the zeta potential analysis, we found the doxorubicin can encapsulate into HH/PH matrix successfully. The cellular uptake test, result showed that HH/PH-DOX could be into HepG2 cells by endocytosis after co-incubation for 24h.

CONCLUSION: Our result supported that the gellan gum microsphere with HH/PH-DOX nano particles have the potential as the chemoembolization agent in the future.

NO CONFLICT OF INTEREST

304 Hexavalent CD27 agonists show single agent anti-tumor activity in mouse syngeneic tumor models which is augmented by combination with anti-PD-1

M. Thiemann[1], C. Gieffers[1], D. Richards[1], J. Sykora[1], M. Redondo-Müller[2], K. Heinonen[3], C. Merz[1], V. Marschall[3], H. Fricke[4], O. Hill[3]

[1] Apogenix AG, Protein Analytics, Heidelberg, Germany
[2] Apogenix AG, Preclinical Development, Heidelberg, Germany
[3] Apogenix AG, Molecular Biology, Heidelberg, Germany
[4] Apogenix AG, Clinical Development, Heidelberg, Germany

Apogenix has developed a proprietary technology platform for novel hexavalent TNFRSF agonists (HERA) for the treatment of cancer. HERA proteins are capable of clustering six receptors in a spatially well-defined manner and - in contrast to agonistic anti-TNFRSF antibodies - signaling is entirely independent of secondary crosslinking through FcγRs. The HERA engineering concept has been successfully translated to TRAIL, GITRL, CD40L, OX40L, 4-1BBL, LIGHT and CD27L. Here we focus on CD27L, a potent co-stimulatory molecule driving T cell activation and survival through interaction with its receptor CD27.

HERA-CD27L was manufactured in CHO suspension cells resulting in homogenous, aggregate-free protein lots. Immune cells isolated from healthy-donor blood were cultured in media containing αCD3, αCD28 and HERA-CD27L. Changes in activation and memory markers on T cells and proliferation rate (CFSE assay) was assessed by multicolor flow cytometry. The effect of HERA-CD27L on the antigen-specific T cell response was assessed in an OT-1 adoptive transfer mouse model. Two syngeneic mouse models – MC38-CEA and CT26 – were used for demonstrating anti-tumor efficacy.

HERA-CD27L binds CD27 expressed on primary human CD4+ and CD8+ T cells. Binding significantly increased T cell expansion following αCD3/αCD28 stimulation and led to increased expression of OX40 on CD4+ T cells and 41BB on CD8+ T cells. A single dose of 1mg/kg HERA-CD27L increased clonal expansion of antigen-specific CD8+ T cells upon immunization with Ovalbumin (Ova) in the mouse OT-1 model with a kinetics leading to peak levels of >25% Ova-specific CD8+ T cells. In both syngeneic murine tumor models treatment with HERACD27L resulted in a dose dependent inhibition of tumor growth. CT26 tumor bearing BALB/c mice treated with 1mg/kg HERA-CD27L, twice weekly showed a 43% tumor-growth inhibition (TGI). Additional anti-tumor efficacy was observed with 10 mg/kg αPD-1: 29% TGI alone, 66% TGI in combination with HERA-CD27L. A significant TGI of 48% was seen in the MC38-CEA C57Bl/6 mouse model twice weekly treated with 10mg/kg HERA-CD27L. Analysis of peripheral lymphoid tissues in these mice showed that HERA-CD27L treatment led to enhanced memory formation in both CD4+ and CD8+ T cells.

HERA-CD27L shows potent immune cell-driven anti-tumor efficacy, both alone and combined with αPD-1. Hence, HERA-CD27L could be clinically applied for treatment of cancer as single agent or in combination with PD-1/PD-L1 inhibitors.

CONFLICT OF INTEREST

Corporate-sponsored Research: All authors are employees of Apogenix AG.

305 Cell cytotoxic effects of clioquinol and prepare of a gellan gum/glucosamine hydrogel carrier for oral cancer therapy

S.J. Chang[1], H.Y. Chen[2], M.W. Lee[2]

[1] I-Shou University, Department of Biomedical Engineering, Kaohsiung City, Taiwan
[2] Chung Shan Medical University, Department of Medical Laboratory and Biotechnology, Taichung City, Taiwan

BACKGROUND: Clioquinol (CQ) is an antifungal drug and antiprotozoal drug. It is a member of a family of drugs called hydroxyquinolines which inhibit certain enzymes related to DNA replication. In this study, we focued on the anti-cancer effects of CQ on human oral squamous cell carcinoma (OSCC). In order to improve clioquinol bioavailability, a gellam gum/glucosamine hydrogelate membrane containing CQ was prepared and characterized. The in vitro dynamic release behavior of CQ from the gellam gum/glucosamine membranes was analyzed.

MATERIAL AND METHOD: Cell cytotoxic effects of clioquinol was determined via MTT assay. Cell apoptosis, caspase-3 activation, mitochondria membrane potential change and ROS production were analyzed by flow cytometer. Various kinds of gellam gum/glucosamine/CQ membrands with different weight ratio was fabricated. The membrands were then crosslinked by immersing into the DDW solution containing 15mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), for 24 h at room temperature. Gellam gum/glucosamine/CQ membranes and drug release studies were characterized.

RESULTS AND DISCUSSION: We found that CQ with copper could enhance the cytotoxicity of CQ in two kinds of oral cancer cells, OC-2 and HSC-3 cells. Further, CQ with copper would induce cell apoptosis, decrease mitochondria membrane potential and increase ROS production in OSCC cells. Moreover, we also found the effect of CQ with copper caused aberrant expression of apoptosis related protein in intrinsic cell apoptosis pathway. The gel content of gellam gum/glucosamine/CQ membrands was found to be 85±2.0%. The tensile strength result indicated that gellan gum membrane without glucosamine or CQ had high tensile strengths. The release kinetics curve of CQ had a steep slope between 0 and 12 h, which contributes to the initial burst of elution. The experiment lasted 24 h, and the amount of CQ released was 40%. After 24 h, the release rate of CQ stabilized, and the percentage of CQ released increased by approximately 5% every other day. After the release experiment had lasted 120 h, the amount of CQ released was 60%.

CONCLUSION: Our result supported that clioquinol would act as a potential selective anti-cancer drug due to the accumulation of copper in tumor part but not normal tissue, and induce OSCC cells apoptosis by intrinsic apoptosis pathway. These findings should provide insights into the properties of gellam gum/glucosamine/CQ therapeutic patche for oral cancer therapy.

NO CONFLICT OF INTEREST

306 Preclinical models of patient derived ovarian cancer xenograft (OC-PDX) to study the response of the PARP inhibitor olaparib

F. Bizzaro[1], A. Decio[1], L. Mannarino[1], F. Ricci[1], S.T. Barry[2], M.J. O'Connor[2], M.R. Bani[1], R. Giavazzi[1]

[1] IRCCS-Mario Negri Institute for Pharmacological Research, Oncology, Milan, Italy
[2] Astrazeneca, Bioscience-IMED Oncology, Cambridge, United Kingdom

BACKGROUND: Up to 50% of HGS ovarian cancer patients exhibit homologous recombination deficiency (HRD) through mechanisms including germline BRCA mutations, somatic BRCA mutations and BRCA promoter methylation. The significant activity of olaparib, a poly(ADP-ribose)polymerase (PARP) inhibitor, in the treatment of germline BRCA-mutated-advanced ovarian cancer has recently led to its approval. The aim of our study was to evaluate the responses to olaparib mono-therapy in patient derived ovarian cancer xenografts (OC-PDX) and the association to their somatic BRCA1/2 mutational status.

MATERIALS AND METHODS: Targeted resequencing (MiSeq, Illumina) was performed on HGS-OC-PDX (n=15) to identify somatic mutations in BRCA1/2. Five OC-PDX were selected for future testing. To confirm the NGS results, Sanger sequencing was performed on tumors DNA and RNA. Olaparib was administered by oral gavage, at the dose of 100mg/kg, for 4 weeks and in maintenance. Efficacy was evaluated as the best T/C%.

RESULTS: OC-PDX3 and OC-PDX4, respectively BRCA1 and BRCA2 mutated (homozygous frameshift mutations, resulting in non-functional/truncated proteins), showed sustained inhibition (T/C = 3% and 22%) of tumor growth following a short and maintenance regimen. To the contrary, OC-PDX2-C and OC-PDX6 (BRCA1 and BRCA2 wild type) were resistant to olaparib single therapy (T/C = 70% and 66%). OC-PDX5, identified as BRCA2 mutated by NGS (mutated fraction <50%), carried the mutations in heterozygosis. The Sanger sequencing of RNA confirmed the presence of both the wild type and the mutated transcript forms. This OC- PDX5 was poorly responsive to olaparib single therapy (T/C = 57%).

CONCLUSIONS: A cohort of OC-PDX has been molecularly characterized to support in vivo pharmacological studies with the PARP inhibitor olaparib. Our data showed that only tumors with BRCA1/2 mutation in homozygosis responded to olaparib; the drug was not active on those with mutation in heterozygosis ($BRCA2^{+/-}$) or wild type. These findings suggest that tumor somatic mutations and transcripts play a role in the response to olaparib. Models of OC-PDX moderately responsive to olaparib offer the opportunity to assess the potential of combination treatments.

CONFLICT OF INTEREST

Other Substantive Relationships: ST Barry and MJ O'Connor AstraZeneca employees

307 SG3400, a novel pyrrolobenzodiapine (PBD) antibody-drug conjugate payload with an extended therapeutic window

J. Hartley[1], L. Masterson[1], E. Dunny[1], L. Adams[1], R. Pardo[1], N. Patel[1], B. Vijayakrishnan[1], M.J. Hinrichs[2], S. Afif-Rider[2], P. Howard[1]

[1] Medimmune, Spirogen, London, United Kingdom
[2] Medimmune, Toxicology, Gaithersburg, USA

BACKGROUND: The pyrrolobenzodiazepine (PBD) dimer payload tesirine (SG3249) has been successfully conjugated to anti-DLL3, CD19 and CD25 antibodies, and the resulting antibody-drug conjugates (ADCs) are undergoing phase I and II clinical trials. In order to further extend the therapeutic window of the ADCs we synthesised the novel payload SG3400. This incorporates a warhead with a shorter three carbon tether than tesirine. The shorter tether was anticipated, based on the known SAR of PBD dimers, to reduce the potency of the warhead. It was envisaged that the less potent payload would limit toxicity arising from non-specific uptake of the ADC whilst still delivering a sufficiently potent warhead to the target tumour.

MATERIALS AND METHODS: SG3400 was synthesised in 15 steps and conjugated to anti-HER2, CD22, 1C1 and CD79b antibodies. The resulting ADCs (DAR2) underwent in vitro evaluation in relevant human tumour cells lines followed by in vivo evaluation in corresponding xenograft models. To investigate safety and tolerability, SG3400 was conjugated to the non-targeting antibody R347 for toxicity studies in rat and cynomolgus monkey.

RESULTS: As expected, the SG3400 ADCs exhibited good activity in human tumour cells lines in the 10 ng/ml range (e.g. 11.5 ng/ml in NCI N87) but were less potent than their tesirine counterparts (1.5 ng/mL in NCI-N87). The SG3400 ADCs were evaluated in several xenograft models including NCI-N87 (HER2 +++) gastric carcinoma model. The minimum effective dose (MED, 28 days tumour stasis) in NCI-N87 for anti-HER2 ADC was 1 mg/kg and 10/10 tumour free survivors were achieved at a single dose of 2 mg/kg. The MED in this solid tumour model was three times higher than for the equivalent tesirine ADC (0.3 mg/kg). As expected a SG3400 ADC targeting CD22 was more potent in the haematological Daudi model, with an MED of 0.1 mg/kg. The R347 conjugate of SG3400 was well tolerated in rat with a maximum tolerated dose (MTD) of 7 mg/kg compared to 1.5 mg/kg for the R347 conjugate of tesirine. An MTD of 4.5 mg/kg was obtained in cynomolgus monkey.

CONCLUSIONS: Switching to a less potent payload/warhead combination improved the tolerability of the ADC and as expected, the less potent payload/warhead combination was less efficacious in xenograft models. However, the therapeutic index (TI, Rat MTD/Mouse MED) was increased. Indeed, a TI of 70 was recorded in a haematological context, making SG3400 an attractive choice in a liquid tumour context.

CONFLICT OF INTEREST

Other Substantive Relationships: All the authors are employees of Medimmune.

308 Molecular modelling, molecular dynamics simulations and in vitro evaluation of Berberine as an inhibitor of MAP kinase and PI3K pathway

P. Jabbarzadeh Kaboli[1], P. Ismail[1], K.H. Ling[1]

[1] Universiti Putra Malaysia UPM, Biomedical Science, Serdang, Malaysia

INTRODUCTION: Berberine, a plant-based alkaloid, has been shown to inhibit PI3K and MAP kinase pathways in various cancer cell lines. However, the immediate targets of berberine are not well-understood. The objective of this study is to perform in silico modelling followed by in vitro analyses to describe the effects of berberine against EGFR, p38 MAPK, ERK1/2, and AKT.

MATERIAL AND METHODS: Using molecular docking, thermodynamics parameters such as the binding energy, inhibition constant (Ki), and pKi have been computed for 13 protein crystals and several berberine derivatives. To validate the results, molecular dynamics (MD) simulations were performed based on 10 nanosecond simulations. Target-specific binding efficiency index for each berberine derivative were calculated to compare the effectiveness of berberine as compared with other derivatives. The interactions have been modelled. In vitro studies were performed by using of MCF-7 and MDA-MB231 breast cancer cell line. Cytotoxicity assays was done, IC50 was calculated, and apoptosis assay was performed. To evaluate the effects of berberine on EGFR, p38 MAPK, ERK1/2, and AKT, ELISA was used to detect both phosphorylated kinases and total enzymes in different concentrations. We also used lapatinib as a positive control for both in vitro and in silico studies.

RESULTS: EGFR and AKT were targeted by berberine derivatives especially via LYS745 and LYS179, respectively. The most important interactions were H-bonding and cation-π interactions within which aromatic rings of berberine and positive charged amino group of lysine are involved. Average Ki calculated of berberine for EGFR and AKT were 6.3 and 2.5 μM, respectively. BBR-9, a newly generated ligand, seems to be the more effective than original berberine with less Ki for both targets. According to ELISA quantitative experiments, the activity of four enzymes (EGFR, AKT, P38, and ERK1/2) were affected by berberine with the most effect on EGFR and AKT which the total concentration was highly affected as well as the phosphorylated enzymes. These result suggested that berberine might be act as a moderate inhibitor for MAP kinase pathway; however, other berberine derivatives were analyzed to suggest the most effective inhibitor.

CONCLUSION: Berberine derivatives that can be docked to different targets were selected, and three novel berberine derivatives were generated against EGFR and AKT.

NO CONFLICT OF INTEREST

309 Core-shell lipo-polymersomes stabilized by iron oxide nanoparticles for synergistically targeted and magnetically-guided gene delivery to enhance tumor therapy

S.Y. Chen[1]

[1] National Chiao Tung University, Materials Science and Engineering, Hsinchu, Taiwan

BACKGROUND: Gene therapy holds promise to suppress carcinomas requires a safe and effective delivery system. However, it is necessary to mitigate the interactions between cationic gene vehicles and blood components in order to avoid clearance by the reticuloendothelial system (RES) and enhance transfection efficiency. Herein, we report on the rational design of surfactant-free lipo-polymersomes (LPPs) to overcome both problems simultaneously using a lipid-stabilized double emulsion approach.

MATERIAL AND METHODS: The LPPs were designed as a compartmentalized internal structure with an inner reservoir core to conceal the cationic lipids/plasmid DNA and an outer iron oxide nanoparticles/neutral lipid shell using a water-in-oil-in-water (W/O/W) double emulsion.

RESULTS: Guided by an external magnetic field and the folic acid (FA), that target integrins on the cell surface, the vectors demonstrated a 10–20-fold increase in cell uptake and a 3–4-fold increase with respect to in vitro gene expression. Furthermore, this synergistic tumor-targeted approach can enhance a 10-fold increase in transfection efficacy by promoting the delivery of LPPs to cancer cells and facilitating the endosomal escape and nuclear accumulation of gene molecules in vivo.

CONCLUSIONS: The new insights and capabilities represent a major step in nanovector engineering for safe and efficient gene delivery and, potentially, for widespread use as a high-performance gene delivery system for enhancing tumor therapy.

NO CONFLICT OF INTEREST

310 Molecular modelling of berberine derivatives as inhibitors of smoothened receptor using a new drug discovery script for a large numbers of compounds

P. Jabbarzadeh Kaboli[1], P. Ismail[1], M. Bazrafkan[2], K.H. Ling[1]

[1] Universiti Putra Malaysia UPM, Biomedical Science, Serdang, Malaysia
[2] University of Padova, Department of Pharmaceutical and Pharmacological Sciences, Padua, Italy

INTRODUCTION: Modern drug discovery involves the identification of screening hits, medicinal chemistry and optimization of those hits to increase the affinity, selectivity, efficacy/potency, metabolic stability, and oral bioavailability. Once a compound that fulfills all of these requirements has been identified, it will begin the process of drug development prior to clinical trials. The objective of this study is to design a one-click drug discovery tool which is able to do the whole procedure of drug discovery for a large number of ligand that can be understandable and usable by pharmaceutical industries.

METHODS: To run this algorithm, only a bash command under Linux platform is needed and depends on the number of compounds of interest, the running time would be different. While finished, a CSV-formatted Microsoft Excel file containing a sorted list of hundreds or thousands of compounds is saved. For the current project, four crystals of Smoothened receptors have been selected and the number of run set to 30 (N=30). Therefore, algorithm start running of 120 dockings for all 485 ligands, and made the averages and other properties in the result file.

RESULTS: This algorithm, automatically, processes four steps. (1) At first step, downloading of structure files of each compound based on the PubChem CIDs provided in the reference folder will be started and then each sdf-formatted file will be automatically converted to pdb-formatted file and will receive a number and saved in a different folder. (2) At second step, AutoGrid executive file will be run. (3) Then, AutoDock executive file will be run. (4) The druglikeness properties such as topological polar area surface (TPSA), molecular weight (MW), LogP and so on will be predicted based on the online database, and then the binding efficiency index will be calculated.

CONCLUSION: As the traditional drug discovery procedures do not consider the ADME/tox properties of each ligand, this algorithm finalizes the result based on the binding efficiency index which are calculated based on the both thermodynamics and physico-chemical properties of each ligand. This algorithm not only increases the number of ligands and crystals at a one-click process, but it also finalizes the result so that can be easily depicted for further discussion.

NO CONFLICT OF INTEREST

311 Optimization and pharmacological evaluation of inhibitors targeting DYRK kinases in glioblastoma

L. Munoz[1]

[1] University of Sydney, Sydney Medical School, Sydney NSW, Australia

The DYRK family contains kinases that are up-regulated in malignancy and control several cancer hallmarks. The most studied member of the family, DYRK1A is up-regulated in glioblastomas and has been shown to prevent endocytotic degradation of EGFR. DYRK1A activity result in enhanced EGFR signalling and tumour growth. Therefore, inhibition of DYRK1A is a potential therapeutic intervention for EGFR-dependent glioblastomas.

To assess the anti-cancer potential of inhibitors targeting DYRK kinases, we developed a series of novel DYRK inhibitors based on the 7-azaindole scaffold. All compounds were tested for their ability to inhibit DYRK1A, DYRK1B, DYRK2 and the structurally related CLK1. The library was screened for anti-cancer efficacy in established and stem cell-like glioblastoma cell lines. The most potent inhibitors ($IC_{50} \leq 50$ nM) significantly decreased viability, clonogenic survival, migration and invasion of glioblastoma cells. Target engagement was confirmed with genetic knockdown and the cellular thermal shift assay. We demonstrate that DYRK1A's thermal stability in cells is increased upon compound treatment, confirming binding in cells. Furthermore, we will present data demonstrating that DYRK inhibition with small molecules effectively increased EGFR degradation, leading to tumour regression.

In summary, we present synthesis, structure-activity relationship, mechanism of action and efficacy in glioblastoma-relevant models for a library of novel DYRK inhibitors.

NO CONFLICT OF INTEREST

312 Thermosensitive pluronic lecithin organogel for intratumoral injection to co-deliver Docetaxel and Cisplatin: A synergistic combination therapy for ovarian cancer

C.E. Chang[1], H.J. Jhan[1], C.Y. Su[1], M.T. Sheu[1], H.O. Ho[1]

[1] Pharmacy, Taipei Medical University, Taipei, Taiwan

INTRODUCTION: Combination therapy for cancer treatment is becoming more popular because it generates synergistic anticancer effects. Increasing attention has also been paid to localized drug delivery systems for cancer chemotherapy, which can facilitate drug release at target sites with relatively high drug concentration and less nonspecific drug distribution in normal organs. Docetaxel (DOC) and cisplatin (CIS) are considered the most crucial antitumor drugs used clinically for cancer treatment. However, the poor water solubility of DOC is a major limitation and a major drawback of CIS is its indiscriminate distribution in normal tissues causing adverse side effects. In this study, a novel microemulsion system using Capryol 90 as the oil phase was developed to carry DOC, which was then incorporated into an pluronic lecithin organogel (PLO) containing CIS to formulate a dual-drug injectable PLO for intratumoral (IT) delivery.

MATERIAL AND METHOD: The various attributes of PLO starting from screening of various ratio of gelator, organic and aqueous phase was studied and an optimal hydrogel composite, 13P1.5C0.15S, composed of PF127:Capryol 90:Lecithin at a 13:1.5:0.15 weight ratio was obtained. The thermal-sensitive property of the 13P1.5C0.15S was investigated by rheological analysis. The cytotoxicity were assessed by measuring cell viability using MTT assay. The SKOV-3 tumor-bearing mice were IT injected 3 times with PBS, placebo gel, free DOC, free CIS, free DOC+free CIS and 13P1.5C0.15S on days 0, 4 and 8 to evaluate its tumor inhibition rate.

RESULTS AND DISCUSSION: The solution–gel transition temperature of 13P1.5C0.15S PLO was found to be 33°C. It also demonstrated that the co-loaded 13P1.5C0.15S at a 1:1 ratio of DOC:CIS exhibited synergistic effects on cytotoxicity against SKOV-3 ovarian cancer in vitro. The xenograft study showed that tumor inhibitory rate of 13P1.5C0.15S (DOC & CIS 4 mg/kg each) after IT injection was 69.4%, whereas it was 62.2%, 38.9%, 32.26%, 29.7% and 4.2% for free DOC+free CIS (4 mg/kg each), 13P1.5C0.15S (DOC & CIS 2 mg/kg each), free DOC+ free CIS (2 mg/kg each), free CIS (4 mg/kg) and free DOC (4 mg/kg), respectively. This demonstrates that dual-drug 13P1.5C0.15S (DOC & CIS 4 mg/kg each) were significantly more efficacious than all the other formulations.

CONCLUSION: The local co-delivery of DOC and CIS by 13P1.5C0.15S PLO may be a promising approach for enhancing ovarian cancer treatment with minimal systemic toxicities.

NO CONFLICT OF INTEREST

313 Oxadiazolopyrazine derivatives: Synthesis and anticancer mechanism investigation

W.S. Li[1], W.C. Chen[2]

[1] Academia Sinica, Institute of Chemistry, Taipei, Taiwan
[2] National Taiwan Normal University, Department of Chemistry, Taipei, Taiwan

BACKGROUND:A novel series of oxadiazolopyrazine derivatives, a new class of non-camptothecin topoisomerase I inhibitors, were designed, synthesized and evaluated for their antiproliferative activities in various human cancer cell lines.

MATERIAL AND METHOD: The effects of oxadiazolopyrazine derivatives on cell viabilities were determined using the MTT assay. Furthermore, the gel mobility assay was conducted to compare the Top1-mediated DNA cleavages at different concentrations of oxadiazolopyrazine derivative, Camptothecin, and Doxorubicin. To examine whether oxadiazolopyrazine derivative suppresses cell growth through cell cycle disruption, BT549 cancer cells were incubated for 24 h in the presence of 0.2, 0.3, 0.4, and 0.5 μM **FC-10b** or vehicle DMSO by flow cytometry. Oxadiazolopyrazine derivative induces apoptosis, necrosis, and autophagy which were measured by flow cytometry, confocal microscopy analysis and immunoblotting western blot analysis.

RESULTS AND DISCUSSION: In vitro studies showed that most of the molecules exhibited significant efficacy to different cancer cells, breast cancer (MDA-MB-231, MCF-7 and BT549), colon cancer (SW620), ovarian cancer (OVCAR-3), and pancreatic cancer (PANC-1), with IC_{50} values falling in the nanomolar range. In this effort, oxadiazolopyrazine derivatives, having an either fluoride or methoxy substitution at the benzene ring, were identified as potential strong lead compounds based on their effectively therapeutic action. For anticancer mechanism study in topoisomerase I-mediated DNA cleavage pathway, oxadiazolopyrazine derivatives display the same pattern as that of camptothecin (a well-known topoisomerase I inhibitor). On the other hand, observations suggest that the oxadiazolopyrazine derivatives mediate DNA damage against cancer cell line involving G2/M phase arrest of the cell cycle and induce apoptosis (major pathway), necrosis (minor pathway), and autophagy (minor pathway) which were supported by flow cytometry, fluorescence microscopy analysis and immunoblotting western blot analysis.

CONCLUSIONS: The findings arising from the studies described above open a possible approach to the design and development of new and potent non-camptothecin topoisomerase I inhibitors, such as oxadiazolopyrazine derivatives, that serve as anticancer agents for the treatment of human cancer.

NO CONFLICT OF INTEREST

314 CellAccumulator (ACCUM): A novel technology that enhances trastuzumab-emtansine cellular accumulation and cytotoxic effectiveness

S. Beaudoin[1], M. Barok[2], M. Charbonneau[3], C. Dubois[3], H. Joensuu[4], L.H. Tai[3], J. Leyton[1]

[1] University of Sherbrooke, Nuclear Medicine and Radiobiology, Sherbrooke, Canada
[2] University of Helsinki, Laboratory of Molecular Oncology, Helsinki, Finland
[3] University of Sherbrooke, Pediatrics, Sherbrooke, Canada
[4] Helsinki University Central Hospital, Oncology, Helsinki, Finland

BACKGROUND: The many resistant mechanisms by cancer cells to antibody-drug conjugates (ADCs) are effective at ultimately reducing the cellular accumulation of the delivered drug and hence, reducing cytotoxic effectiveness. The current mechanistic center point for delivering drugs is reliant on ADC receptor-mediated internalization followed by ADC entrapment in endosomes. The endosomes are routed to the lysosome where ADC degradation result in drug release. Kadcyla® (trastuzumab-emtansine [T-DM1]) is a benchmark as it is the first solid-tumor approved ADC. Specifically, T-DM1 is effective at killing Her2+ breast cancer cells resistant to chemotherapy and Herceptin®. Despite this new therapeutic option, most patients eventually progress due to resistance mechanisms that reduce the cellular concentration of DM1 below the threshold to evoke cell death.

CellAccumulator (**ACCUM**) is a natural composite compound that conjugates to surface lysines and enables antibodies to escape endosomal entrapment followed by active routing to the nucleus. ACCUM-modified antibodies retain high tumor cell selectivity and improve tumor delivery of molecular payloads in vivo. We asked whether the modification of T-DM1 with ACCUM would enhance Her2+ tumor cell accumulation and whether increased accumulation result in enhanced cytotoxic effectiveness relative to T-DM1.

METHODS: T-DM1 surface lysines were reacted with the covalent crosslinker SM-PEG_2 at increasing linker-to-T-DM1 ratios followed by purification and reaction with excess ACCUM. Cancer cell lines SKBR3 and OE-19 (Herceptin-sensitive), MCF7 (Her2-negative), and JIMT1 (Herceptin-resistant) were treated with increasing concentrations of ACCUM-T-DM1 and T-DM1 for 72 h. Cytotoxicity dose response was quantified with the alamarBlue® assay. The chicken chorioallantoic membrane assay was used to determine relative ACCUM-T-DM1 (1 and 10 mg doses) toxicity.

RESULTS: ACCUM modification did not perturb T-DM1 affinity, specificity or cause aggregation. The IC_{50} of ACCUM-T-DM1 was increased 100-, 50-, and 10-fold relative to T-DM1 in SKBR3, OE19, and JIMT1 cells, respectively. Cytotoxicity in MCF7 cells was equivalent for ACCUM-T-DM1 and T-DM1 (5% at 1 mg/mL). In vivo toxicity was not observed.

CONCLUSION: The favourable effectiveness of ACCUM-T-DM1 suggests our strategy of empowered intracellular drug delivery for ADCs offers an innovative and effective approach to drug accumulation and cytotoxicity, while maintaining low toxicity.

NO CONFLICT OF INTEREST

315 Cell-free circulating tumor DNA as a surrogate marker for detecting GIST recurrence or progression

C.Y. Jeff[1], C.N. Alex[2], Y.S. Yan-Shen[3]

[1] Cathay General Hospital, Department of Pathology and Laboratory Medicine, Taipei 106, Taiwan
[2] Chang Gung Memorial Hospital, Department of Surgery- GIST team, Taoyuan 333, Taiwan
[3] National Cheng Kung University Hospital, Department of Clinical Medicine Research and Surgery, Tainan, Taiwan

INTRODUCTION: As GISTs with exon 9 mutation are relatively resistant to standard dose of imatinib and even imatinib escalation, second line therapy should be used as soon as possible for those with poor responsiveness. Unfortunately, many patients are reluctant to the periodic CT scanning for follow-up, leading to a significant number of patients of this kind being delayed for second line therapy. Although growing evidence has shown that liquid biopsy, a method of detecting circulating free DNA in blood, has a potential to be used for monitoring tumor recurrence, liquid biopsy has not been routinely employed because of its cost and requiring sophisticated equipment. Here we test if a low-cost, simplified liquid biopsy can be used to monitor the progression of GIST with exon 9 mutation.

MATERIAL AND METHOD: Locked nucleic acid primers were used in this study. The primer sequences were as follows: 5'-ACT TCT GCC TAT GCC TAT T-3' for mutant forward primer, and 5'-GAT ATG GTA GAC AGA GCC-3' for mutant reverse primer. The PCR product length was 104 bp. After gel electrophoresis following ethidium bromide staining, the PCR products were visually examined under UV light to detect band pattern and size. As long as an easily noticeable band with ~104 bp in size was seen by both of two lab staff, the result was reported as 'presence" of KIT exon 9 mutant. Each sample was subjected to two PCR runs on separated days by different lab staff. The result was classified as '2+" if both PCRs showed 'presence"; '1+" if only one of the PCR showed 'presence"; and '0" if both runs failed to show the 'presence" of KIT exon 9 mutant.

RESULTS AND DISCUSSION: Among 92 samples subjected to this analysis, 42 were obtained from patients with progressive disease and 50 from those with stable disease. If "1+" was classified as 'negative", the positive predictive rate was 90.9% while the negative predictive rate was 60.5%. If "1+" was classified as 'positive", the positive predictive rate was 75.0% while the negative predictive rate was 67.2%. Therefore, a positive result of liquid biopsy suggests that approximately 75% to 90% of patients taking imatinib treatment are suffering from disease progression, suggesting a need to carefully consider switching to second line therapy at this point of time.

CONCLUSION: The demonstrated cost-effective test has a potential to be used for a frequent monitoring of the disease progression in cancer patients as exemplified by GIST patients with exon 9 mutation.

NO CONFLICT OF INTEREST

316 Mechanisms of resistance to FGFR-targeted therapy in bladder cancer

G. Pettitt[1], H. McPherson[1], C. Hurst[1], J. Burns[1], O. Alder[1], M. Dunning[1], M. Knowles[1]

[1] Leeds Institute of Cancer and Pathology, Faculty of Medicine and Health, Leeds, United Kingdom

INTRODUCTION: Fibroblast growth factor receptor 3 (FGFR3) signalling is altered in ~80% of non muscle-invasive and ~54% of muscle-invasive bladder cancers via activating mutations (point mutations or gene fusions), overexpression or both. FGFR inhibitors have entered clinical trials in advanced bladder cancer. As with other targeted therapies, intrinsic and acquired resistance are expected to limit treatment efficacy. We have used an in vitro model to explore possible mechanisms of resistance.

METHODS: The urothelial cancer cell line RT112 expresses an FGFR3-TACC3 fusion protein and is sensitive to FGFR inhibition. Isogenic resistant cell lines, termed R1, R2 and R3, were derived by long-term culture of RT112 in the presence of the FGFR inhibitor PD173074. These were characterised by proliferation and cell viability assays and morphology. Exome sequencing was conducted in the parental and resistant derivatives. RNA microarray and phospho-receptor tyrosine kinase array analyses were conducted on parental cells cultured in normal medium or acutely treated with PD173074, and resistant derivatives cultured with drug or without drug for four passages.

RESULTS: The resistant derivatives R1 and R2 had a spindle-like morphology and had undergone gene expression changes associated with an epithelial-mesenchymal transition. R1 and R2 had a reduced proliferation rate compared to parental RT112. These changes were reversed when R1 and R2 were cultured without PD173074 for four passages. Despite this, the cells retained their resistance when re-exposed to drug. The mechanism of resistance in R1 and R2 appears to be epigenetic. The resistant derivative R3 retained a more epithelial phenotype and a faster growth rate than R1 and R2. Exome sequencing uncovered a mutation that is the likely cause of resistance in R3.

CONCLUSION: Investigation of RT112 PD173074-resistant derivatives has uncovered genetic and epigenetic alterations. Our data suggests that diverse mechanisms of resistance occur following prolonged FGFR inhibition.

NO CONFLICT OF INTEREST

318 NMS-P088 is a small molecule potent CSF1R kinase inhibitor with macrophages immunomodulatory activity

M. Ciomei[1], M. Modugno[1], G. Texido[1], W. Veronelli[1], D. Ballinari[1], R. Alzani[1], W. Pastori[1], F. Gasparri[1], A. Lombardi Borgia[1]

[1] Nerviano Medical Sciences Srl, Oncology / Biology, Nerviano Milano, Italy

BACKGROUND: Tumor-associated macrophages (TAMs) are an attractive therapeutic target as they represent key orchestrators of various tumor-promoting processes, such as escape of immune surveillance. CSF-1R signaling is a vital component in TAMs and its blockade represents an attractive approach to modulate TAM survival and/or activation.

NMS-P088 is a potent small molecule kinase inhibitor of FLT3, KIT and CSF1R kinases that has demonstrated excellent preclinical in vivo activity against FLT3 and KIT driven cancers. Its potent in vitro inhibitory activity against CSF1R suggests the use of this molecule to target TAMs in a therapeutic setting.

MATERIALS AND METHODS: The capability of NMS-P088 to inhibit CSF1R was evaluated in a number of cellular in vitro and in vivo models. Anti-proliferative activity was measured on murine Ba/F3 cells engineered to be dependent on CSF1R and in bone marrow-derived macrophages. Autophosphorylation of CSF1R was evaluated in different cellular models treated with compounds to confirm CSF1R signaling inhibition. In vivo, CSF1R-positive macrophages (Kupffer cells) were quantitatively evaluated in liver of mice treated with different doses of NMS-P088.

RESULTS: NMS-P088 inhibited proliferation of CSF1R-dependent Ba/F3 cells with an IC50 of 55 nM. Potent inhibition of CSF1R-dependent signaling was observed in the Ba/F3 CSF-1R driven model cells, as well as in human THP1 monocytic and NKM-1 myeloid leukemia cell lines, comparing favorably with the reference compounds Pexidartinib and BLZ945. Furthermore, NMS-P088 inhibited CSF1R-dependent signaling and proliferation and induced apoptosis in isolated murine macrophage preparations. In vivo, NMS-P088 efficiently and dose-dependently decreased tissue infiltration of CSF1R expressing macrophages, with no damage to surrounding tissue, in livers of all treated mice, consistent with potent inhibition of CSF1R.

Together, these data demonstrate that NMS-P088 can potently inhibit CSF1R in cellular and in vivo models and that this activity has the potential to induce a phenotypic effect on macrophages.

CONCLUSIONS: NMS-088 confirmed to be a potent inhibitor of CSF-1/CSF1R in cellular contexts. These features, together with its excellent tolerability profile, suggest that NMS-P088 is a promising new agent for combination with immune checkpoint inhibitors in settings where relief of macrophage–dependent immune suppression would yield clinical benefit

CONFLICT OF INTEREST

Other Substantive Relationships: All authors are employed by Nerviano Medical Sciences Srl

319 Antitumor and antimetastatic activity of trabectedin in preclinical models of melanoma

L. Carminati[1], D. Pinessi[1], P. Borsotti[1], R. Giavazzi[1], M. D'Incalci[2], G. Taraboletti[1]

[1] Mario Negri Institute for Pharmacological Research, Oncology, Bergamo, Italy
[2] Mario Negri Institute for Pharmacological Research, Oncology, Milano, Italy

BACKGROUND: Trabectedin is a marine-derived compound, approved for the treatment of soft tissue sarcoma and relapsed ovarian cancer. With a complex mechanism of action, the drug affects proliferation, differentiation, apoptosis, and the production of cytokines and chemokines in both cancer cells and the tumor microenvironment, finally affecting tumor growth, inflammation and angiogenesis. Melanoma is one of the most aggressive skin cancers, characterized by a marked invasive and metastatic potential, and sustained by a tolerant and favorable microenvironment. Aim of this study was to investigate the antitumor and antimetastatic activity of trabectedin on melanoma.

MATERIAL AND METHODS: The activity of trabectedin was studied on the murine melanoma B16-BL6 and K1735-M2, implanted in syngeneic or immunodepressed nude mice. The cytotoxic and anti-invasive effects were studied in vitro on both cell lines. The antimetastatic activity of trabectedin was investigated using a lung colonization assay, following i.v. injection of tumor cells, and in a spontaneous metastasis model, after primary tumor surgical removal (adjuvant setting).

RESULTS AND DISCUSSION: Trabectedin significantly inhibited the subcutaneous growth of both melanoma models, with K1735-M2 showing the highest response. The two lines had a similar sensitivity to trabectedin in vitro, suggesting that the different response in vivo was driven by an affect on the tumor microenvironment. In agreement, when implanted in immunodeficient mice, B16-BL6 tumors showed a reduced response to trabectedin, indicating a possible role of adaptive immunity in drug activity. Trabectedin had a strong antimetastatic activity, reducing the number of lung colonies in vivo and inhibiting cell invasion in vitro. The drug affected metastasis also when given as an adjuvant treatment after surgical removal of the primary tumor, in a clinically relevant experimental setting.

CONCLUSIONS: In two preclinical models of melanoma, trabectedin showed antitumor activity and antimetastatic effects, by targeting the tumor cells and through a host-mediated response. Studies are ongoing to investigate the effects of trabectedin on melanoma microenvironment. These findings open a new prospect

for the use of trabectedin for the treatment of melanoma, as a single agent or in combination with other stroma-modifying agents, including immunotherapy.

Supported by Comitato EmmeRouge in ricordo di Mara Nahum

NO CONFLICT OF INTEREST

320 Using bispecific antibody non-covalently bound to docetaxel-loaded mPEGylated nanoparticle to enhance therapeutic efficacy in the MCF-7 HER2 tumor bearing mice

C.Y. Su[1], M. Chen[2], K.H. Chuang[2], M.T. Sheu[1]

[1] Taipei Medical University, School of Pharmacy, Taipei, Taiwan
[2] Taipei Medical University, Graduate Institute of Pharmacognosy, Taipei, Taiwan

INTRODUCTION: Poly(ethylene glycol) to nanoparticles (PEG-NPs) can accumulate in tumors due to the enhanced permeability and retention (EPR). However, often nanoparticle accumulation is only near tumor site but not to penetrate into tumor. Bifunctional proteins have been developed to non-covalently modify NPs to increase tumor retention and intracellular uptake. Herein bispecific antibodies (BsAbs), which designed the Fab fragment to bind to the methoxy ends of mPEG molecules on the surface of mPEG-NPs and single chain antibodies (scFv) with specificity to the HER2 tumor antigens, was established to modify mPEGylated nanoparticles for improving therapeutic effectiveness.

MATERIAL AND METHOD: mPEGylated Nanoparticle were formulated as a modification of the solvent evaporation technique. One mL of the lecithin nanosuspension was used to hydrate the thin film containing docetaxel and hydrophilic polymer and then the mixture was subjected to ultrasonication at least 5 min. BsAbs (mPEGxDNS and mPEGxHER2) were mixed with mPEG-NPs in BSA/PBS buffer (0.05% (wt/vol) for 1 h to form the BsAbs modified mPEG-NPs referred to as, αDNS-NPs or αHER2-NPs, respectively.

RESULTS AND DISCUSSION: The physical characteristics of BsAbs modified mPEG-NPs are similar to mPEG-NPs. The mean particle sizes are around 150 nm; The zeta potential of BsAbs modified mPEG-NPs were more negative than mPEG-NPs. In cell cytotoxicity studies showed that αHER2-NPs could enhance the cytotoxicity of mPEG-NPs and the antibody control group of αDNS-NPs to antigen-positive cancer cells (MCF-7 HER2). To examine whether mPEGxHER2 can increase the therapeutic efficacy of mPEG-NPs to $HER2^+$ tumors, mPEG-NPs, αDNS-NPs and αHER2-NPs were given to tumor bearing mice. result showed that αHER2-NPs suppressed the growth of tumors significantly more than mPEG-NPs. To investigate tumor targeting of BsAbs-NPs in vivo, the biodistribution and imaging of the mice on an IVIS Spectrum optical imaging system were studied. The fluorescent intensity of αHER2-NPs was 2-fold higher than that for mPEG-NPs leading to high drug accumulation in tumor.

CONCLUSIONS: In conclusion, BsAbs that can confer target-specificity to mPEG-NPs and minimal changes in the physical properties of BsAbs modified mPEG-NPs were established. BsAbs non-Covalent to mPEG-NPs can target to the tumor with drug cargo to enhance the drug accumulation in tumor leading to greater antitumor activity against antigen-positive tumors.

NO CONFLICT OF INTEREST

321 Prevention of Adriamycin-induced Cardiac Damage by NAD-Modulation

S.H. Yang[1], K.B. Kwon[2], D. Khadka[3], G.S. Oh[4], H.J. Kim[3], S.B. Lee[3], A. Pandit[3], S. Lee[3], H.S. So[3]

[1] Wonkwang University School of Medicine, Internal Medicine, Iksan-city- Jeonbuk, South Korea
[2] Center for Metabolic Function Regulation, Korean Physiology- Wonkwang University School of Korean Medicine, Iksan, South Korea
[3] Center for Metabolic Function Regulation, Microbiology, Iksan, South Korea
[4] Center for Metabolic Function Regulation, Micorbiology, Iksan, South Korea

BACKGROUND: Adriamycin (ADR), a potent anticancer chemotherapeutic agent, is used to treat a variety of human neoplasms. However, its clinical use is hampered by severe side effects including cardiotoxicity. It has been reported that ADR-induced cardiotoxicity is related to myocardial oxidative stress, disruption of cellular and mitochondrial Ca^{2+} homeostasis and DNA damage. Nevertheless, the remedy for ADR cardiotoxicity is still not developed. Here we describe the effect of NAD^+/NADH modulation by NQO1 enzymatic action on ADR-induced cardiotoxicity in mice.

MATERIALS AND METHODS: C57BL/6 male mice were intraperitoneally injected with ADR. Before and after exposure to ADR, the mice were orally administrated with WK0202, a substrate of NQO1, (20 mg/kg body weight of mice). Cardiac biomarkers (CPK, Trop I, LDH and SGOT) in plasma levels, oxidative biomarkers and mRNA levels of pro-inflammatory cytokines were determined to compare cardiac toxicity of each experimental group.

RESULTS: Cardiac biomarkers in sera, oxidative biomarkers, and mRNA levels of pro-inflammatory cytokines were significantly increased in ADR-treated mice. However, these increases were significantly alleviated by WK0202. We also demonstrated that the downfall in SIRT1 and SIRT3 activities is critically involved in ADR-induced cardiotoxicity through acetylation of NF-κB p65 and p53. However, increase of NAD^+/NADH by WK0202 through NQO1 enzymatic action attenuated ADR-induced cardiotoxicity through recovery of SIRT1 and SIRT3 activities and subsequent deacetylation of NF-κB p65 and p53.

CONCLUSIONS: WK0202 has a protective effect against ADR-induced acute cardiotoxicity through NQO1 enzymatic action. Therefore, WK0202 might be a new therapeutic option for preventing chemotherapy-associated side effects.

NO CONFLICT OF INTEREST

322 Trichodermin exhibits the potent and selective antitumor activity against human pancreatic cancer

S.W. Wang[1], T.H. Lee[2], M.H. Chien[3], K.T. Hua[4]

[1] Department of Medicine, Mackay Medical College, New Taipei City, Taiwan
[2] Institute of Fisheries Science, National Taiwan University, Taipei, Taiwan
[3] Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan
[4] Graduate Institute of Toxicology, National Taiwan University, Taipei, Taiwan

BACKGROUND: Pancreatic cancer is an aggressive malignancy, which generally responds poorly to chemotherapy. Herein, we investigated whether trichodermin (trichothec-9-en-4-ol,12,13-epoxy-acetate), an endophytic fungal metabolite from Nalanthamala psidii, has any efficacy against human pancreatic cancer.

MATERIAL AND METHODS: The anti-cancer effects of trichodermin and its mechanism of action were determined by in vitro cell-based models and an orthotopic xenograft model.

RESULTS: Trichodermin showed selective antiproliferative effects against pancreatic cancer cells, especially p53-mutated cells (MIA PaCa-2 and BxPC-3) rather than normal pancreatic epithelial cells, by inducing G_2/M cell-cycle arrest with an increase in cells at the sub-G_1 phase. Trichodermin induced MPM-2 immunoreactivity, p-Bcl-2 and cyclin B1 upregulation, mitochondrial membrane depolarization, caspase-9/3 activation, and PARP cleavage in MIA PaCa-2 cells, indicating that trichodermininduced mitochondrial-mediated apoptotic death due to mitotic arrest. Moreover, trichodermin promoted the activation of c-Jun N-terminal kinase (JNK), and inhibition of JNK by its inhibitor, shRNA, or siRNA significantly reversed trichodermin-mediated caspase-dependent apoptosis. Furthermore, the DNA damage and phosphorylation of H2AX and p53 were observed in trichodermin-treated MIA PaCa-2 cells. In vivo experiments showed that trichodermin with efficacy similar to gemcitabine, significantly suppressed tumor growth through inducing intratumoral DNA damage and subsequent cell apoptosis.

CONCLUSIONS: Trichodermin has potential for the management of various pancreatic cancer cells regardless of the expression of mutant p53. Importantly, this study provides new insights into trichodermin's molecular mechanism of action and therapeutic potential in the treatment of pancreatic cancer.

NO CONFLICT OF INTEREST

323 Hyperthermia induces the response of endoplasmic reticulum and promotes apoptosis-based therapeutic effectiveness in malignant melanoma

T. Mantso[1], S. Vasileiadis[2], E. Lampri[3], I. Anestopoulos[2], S. Botaitis[4], C. Simopoulos[4], K. Chlichlia[2], A. Pappa[2], M. Panagiotidis[1]

[1] Northumbria University, Applied Sciences, Newcastle uopn Tyne, United Kingdom
[2] Democritus University of Thrace, Department of Molecular Biology & Genetics, Alexandroupolis, Greece
[3] University of Ioannina, Medical School, Ioannina, Greece
[4] Democritus University of Thrace, Second Department of Surgery, Alexandroupolis, Greece

INTRODUCTION: Malignant melanoma is a very aggressive and one of the deadliest malignancies. Although early detection and increased surveillance have contributed to increased survival, its mortality rates still remain high as the disease does not respond to current therapeutic strategies. Thus, there is a great need for improved and more effective therapeutic regimens. To this end, hyperthermia is defined as the application of exogenous heat induction and is one of the most common therapeutic modalities. It is known to act by directly killing tumor cells and/or sensitizing them against other therapeutic regimens.

MATERIALS AND METHODS: We have established a hyperthermia-based experimental exposure platform in order to assess its therapeutic efficacy in an in vitro human melanoma model [consisting of normal immortalized keratinocytes (HaCaT), malignant melanoma (A375) and non-melanoma epidermoid carcinoma (A431) cells] as well as an in vivo mouse model [consisting of female C57BL/6 mice injected with mouse malignant melanoma (B16-F10) cells]. Commercially available kits were used for the determination of caspase activities and levels of apoptosis, necrosis, cytotoxicity, etc. Finally, gene and protein expression levels were evaluated by means of RT-PCR and western immunoblotting respectively.

RESULTS AND DISCUSSION: Our in vitro data revealed that when utilized a RT-PCR-based microarray gene expression system (in order to profile the apoptotic response), a number of critical gene targets were identified to be involved at various stages of both the intrinsic and extrinsic apoptotic cascades suggesting a differential regulation of several pro- and anti-apoptotic molecules depending on the hyperthermic exposure conditions. Moreover, we have been able to uncover the response of the endoplasmic reticulum pathway by means of identifying mechanisms triggering its activation as well as molecules mediating its linkage to apoptosis when exposed to hyperthermia. Our in vitro findings were further validated when utilizing an in vivo mouse model by means of demonstrating considerable inhibition of tumor growth (under hyperthermic conditions) accompanied by an altered expression profile of several target apoptotic genes and associated proteins.

CONCLUSION: Our result further support the therapeutic efficacy of hyperthermia in treating malignant melanoma by identifying underlying molecular mechanisms capable of mediating its beneficial effects on tumor growth inhibition.

NO CONFLICT OF INTEREST

324 Rice protein prolamin promotes anti-tumor immune reaction and inhibits leukemia growth in vivo

Y.J. Chen[1], H.F. Liao[2], Y.Y. Chen[3], C.W. Chi[4]

[1] Mackay Memorial Hospital, Department of Radiation Oncology, Taipei, Taiwan
[2] National Chiayi University, Department of Biochemical Science and Technology, Chiayi, Taiwan
[3] National Taiwan College of Physical Education, Department and Graduate School of Physical Education, Taichung, Taiwan
[4] Mackay Memorial Hospital, Department of Medical Research, Taipei, Taiwan

BACKGROUND: Rice (Oryza sativa) is an important cereal for staple food worldwide. Prolamin is a heat-stable storage protein of rice with immunomodulatory activity. This study aimed to examine the effect of prolamin on anti-tumor immune response in vitro and leukemia growth in vivo.

MATERIALS AND METHODS: The prolamin-enriched rice fractions were prepared for in vitro test to stimulate the isolated peripheral blood mononuclear cells (MNC). The MNC-conditioned medium (MNC-CM) was collected to treat leukemia U937, hepatoma ML-1 and HepG2 cells followed by viability assessment. Purified prolamin was orally administered to syngeneic L1210-bearing DBA/2 mice with various dosages for consecutive 5 days. Weights of body, tumor, liver and spleen as well as peripheral blood neutrophil count were assessed. Cytokine levels in MNC-CM and mice serum and biochemistry profile in mice were measured.

RESULTS: Prolamin-prepared MNC-CM, but not prolamin per se, inhibited the viability of leukemia and hepatoma cells, indicating an immune modulation effect. In leukemia U937 cells, the growth inhibition was accompanied by differentiation toward macrophages which expressed surface CD14 and CD68 and obtained phagocytosis activity. The growth inhibition activity of proplamin-prepared MNC-CM was partially blocked by neutralization of prolamin polyclonal antibody. In syngeneic L1210-bearing DBA/2 mice, oral administration of purified prolamin dose-dependently suppressed the tumor weight and attenuated the leukemia-induced reduction of liver and spleen weights. Prolamin inhibited the increase of peripheral blood leukocyte count in the L1210-bearing mice. The levels of tumor necrosis factor-α and interferon-γ in MNC-CM and mice serum were significantly increased by prolamin treatment. No significant change in body weight, serum alanine aminotransferase and creatinine levels was noted by prolamin treatment.

CONCLUSIONS: Rice protein prolamin could effectively promote anti-tumor immunity and inhibit leukemia growth without significant toxicity.

NO CONFLICT OF INTEREST

325 Effects of norcantharidin on inhibiting distant metastasis of the colorectal cancers

H.F. Liao[1], Y.F. Lin[1], Y.J. Chen[2]

[1] National Chiayi University, Department of Biochemical Science and Technology, Chiayi, Taiwan
[2] MacKay Memorial Hospital, Department of Radiation Oncology, Taipei, Taiwan

INTRODUCTION: Colorectal cancer (CRC) is a malignant neoplasm. Metastasis of CRC patients has become the leading cause of death in Taiwan. Our previous study demonstrated that norcantharidin (NCTD) is a potential compound for CRC treatment.

MATERIAL AND METHOD: The present study designed NCTD-conjugated nanoparticles and developed drug release system by high frequency magnetic field (HFMF). BALB/c mice were injected with murine CRC CT26 cells and then treated with NCTD-conjugated nanoparticles and HFMF. The circulating miRNA expression were screened by miRNA array.

RESULTS AND DISCUSSION: NCTD release via HFMF system decreased the cell viability, induced sub-G1 phase and cell cycle arrest at G2/M phase, as well as down-regulated b-catenin expression in CT26 cells. The levels of circulating miR-720 were increased in both CT26-implanted mice and mice with metastasis. Further investigation demonstrated that CT26 cells transfected with miR-720 inhibitor decreased cell viability. Additionally, real-time PCR assay of CT26 cells showed that the miR-720 expression was down-regulated by NCTD treatment.

CONCLUSION: This study demonstrated that miR-720 may be a potential biomarker for CRC. The release of NCTD by drug carrier and release system inhibited CRC growth and accompanied with miR-720 down-regulation. Development of new inhibitors against miR-720 may be a new strategy for CRC treatment in the future.

NO CONFLICT OF INTEREST

326 Low molecular weight phosphotyrosine protein phosphatase (LMW-PTP) targeting increases sensitivity of melanoma cancer cells toward chemo- and radio-therapy

P. Paoli[1], G. Lori[1], A. Caselli[1], R. Marzocchini[1], P. Cirri[1], M. Mangoni[1], C. Talamonti[1], G. Rauge[1]

[1] University of Firenze, Scienze Biomediche Sperimentali e Cliniche, Firenze, Italy

BACKGROUND: Melanoma is an aggressive and lethal skin cancer. Treatment options include surgical resection of primary melanoma, treatment with kinase inhibitors or with cytotoxic drugs, radiotherapy and immunotherapy. Unfortunately, only a little fraction of patients with advanced stages of disease responds to these treatments. For this reason, development of new therapeutic approaches is a priority.

MATERIALS AND METHODS: Cell viability and apoptosis were evaluated by MTT and Annexin V/Pi assay, respectively. Long-term effects of drug combination were evaluated by colony formation assay. The synergism between LMW-PTP inhibitor and dacarbazine or 5-FU was evaluated using CompuSyn software. Data of LMW-PTP mRNA expression levels in patients were obtained from Oncomine database.

RESULTS: Our result indicate that LMW-PTP protein is a new pharmacological target to enhance sensitivity of melanoma cancer cells toward therapy-induced apoptosis. First, we demonstrated that LMW-PTP is overexpressed in melanoma cells and that its down-regulation strongly contributes to enhance cytotoxic activity of dacarbazine and 5-FU, or the effectiveness of radiotherapy. Moreover, we found that silencing of LMW-PTP impairs the ability of melanoma cancer cells to form new colonies. Similar result were obtained by pre-treating melanoma cancer cells with a soluble low toxic LMW-PTP inhibitor before chemo- or radio-therapy. In particular, we demonstrated that the combined therapy (LMW-PTP inhibitor plus regular chemo-therapy), does not impairs viability of non-cancerous cells, while it strongly impairs the viability and the colony-forming ability of melanoma cancer cells. All together, our result demonstrated that LMW-PTP targeting could be a useful strategy to selectively reprogram cancer cells toward a phenotype more sensitive to cytotoxic treatments.

CONCLUSIONS: We showed that LMW-PTP contributes to enhance resistance of melanoma cancer cells toward cytotoxic drugs and radiotherapy. Moreover, we identified a molecule with low general toxicity that is able to trigger transient down-regulation of LMW-PTP: we demonstrated that this compound could be used to potentiate the effectiveness of classical anticancer drugs or radiotherapy. We think that this molecule may be used as a low toxic adjuvant to enhance the effectiveness of classical therapeutic protocols used for treatment of patients affected by advanced melanoma.

NO CONFLICT OF INTEREST

327 Targeting of breast cancer stem-like cells by the duocarmycin-based HER2-targeting antibody-drug conjugate SYD985

T. Tomar[1], R.A.M. Meijering[1], M.M.C. Van der Lee[2], P.G. Groothuis[2], W.H.A. Dokter[2], S. De Jong[1], F.A.E. Kruyt[1]

[1] Cancer Research Center Groningen- University of Groningen- University Medical Center Groningen, Department of Medical Oncology, Groningen, Netherlands
[2] Synthon Biopharmaceuticals, Department of Preclinical R&D, Nijmegen, Netherlands

INTRODUCTION: The HER2-targeting antibody-drug conjugate (ADC) SYD985 is based on trastuzumab conjugated to a cytotoxic duocarmycin analogue through a cleavable linker. The potential clinical benefit of SYD985 is currently evaluated in several expanded cohort studies in patients with different HER2 high- and low- expressing tumors, including HER2 positive metastatic breast cancer (BC). In BC, the most common form of cancer in woman, BC stem-like cells (BCSC) have been identified that are hypothesized to drive tumor formation and progression, metastasis, therapy resistance and relapse of disease. Thus, effective therapy should eradicate BCSCs. Here, we explored whether SYD985 is able to target BCSCs using mammosphere cultures derived from BC cell lines with variable HER2 expression. Data obtained with SYD985 is compared to the effect of T-DM1 (Kadcyla).

MATERIALS AND METHODS: A panel of BC cell lines (n=8) with variable HER2 expression (HER2-3+, 2+, 1+ and 0) were treated with ADCs SYD985 and T-DM1. Cells were tested for their mammosphere forming potential under long-term (12 days) 3D stem cells-promoting conditions (M1) and subsequent passaging of cells from M1 cultures (M2). result were validated on PKH-26 fluorescent dye-labeled cells as a marker-independent method for isolating slow-dividing BCSCs.

RESULTS AND DISCUSSION: Treatment with SYD985 and T-DM1 during M1 mammosphere formation resulted in dose-dependent and significant inhibition ($p<0.01$) in number and size of mammospheres in BT474, HCC1954 (HER2-3+); MDA-MB361 (HER2-2+) and ZR175, MDA-MB175-VII (HER-1+) cells. No activity was seen in HER2 negative cell lines (MCF7, HCC38 and BT549). SYD985 displayed significantly more activity in low HER2 expressing cell lines (HER2-1+) than T-DM1. The effective depletion of BCSCs by SYD985 treatment was also illustrated by the fact that M2 mammosphere formation from SYD985-treated M1 mammosphere cells was significantly reduced ($p<0.001$) in all HER2 expressing cell lines. In contrast, growth was not inhibited when cells from ADC-treated M1 mammospheres were passaged as 2D monolayers. Furthermore, SYD985 showed significant dose-dependent inhibition ($p<0.05$) of mammosphere forming potential of PKH-26-retaining BC cells in comparison to the unselected counterpart.

CONCLUSION: Taken together, our study shows an unanticipated long-term effective targeting of BCSC by SYD985 that may contribute to the anticipated clinical benefit for patients with either high, moderate, or low HER2 expressing BC.

NO CONFLICT OF INTEREST

328 Human Recombinant Arginase I (Co)-PEG5000 [HuArgI (Co)-PEG5000]-Induced arginine deprivation leads to autophagy-mediated cell death in Pancreatic Cancer Cells

R.Abi-Habib[1], *N. Khalil*[1], *A. Bekdash*[1], *A. Frankel*[2]

[1] *Lebanese American University, Department of Natural Sciences, BEIRUT, Lebanon*
[2] *University of Texas southwestern, Hematology/Oncology, Dallas- Texas., USA*

INTRODUCTION: Arginine auxotrophy, secondary to a lack of argininosuccinate synthetase-1 (ASS-1) expression, is a hallmark of a number of tumors, including AML, ALL and GBM, allowing for selective targeting of these tumors using arginine deprivation. HuArgI (Co)-PEG5000, a recombinant PEGylated human arginase I in which the two active site Mn^{++} ions have been replaced by Co^{++} ions, degrades extracellular arginine leading to arginine deprivation. We investigate the level of arginine auxotrophy in pancreatic cancer cells and the mechanisms of arginine deprivation-induced cell death.

MATERIALS AND METHODS: 5 pancreatic cancer cell lines were used to determine extent of arginine auxotrophy, sensitivity to [HuArgI (Co)-PEG5000], expression of ASS-1, mechanisms of cell death, activation of autophagy and contribution of autophagy to arginine deprivation-induced cell death.

RESULTS: All 5 cell lines proved to be auxotrophic for arginine and sensitive to [HuArgI (Co)-PEG5000]-induced arginine deprivation. The extent of arginine auxotrophy varied between cell lines with 2 being completely auxotrophic (not rescued by excess L-citrulline) and the remaining 3 being partially auxotrophic to arginine (rescued by excess L-citrulline). The extent of arginine auxotrophy matched ASS-1 expression with a total lack of expression seen only in completely auxotrophic cell lines. Arginine deprivation-induced cell death was non-apoptotic with negative annexinV staining and no caspase activation. Autophagy was significantly activated with a net increase in autophagic flux indicated by the increase in the number of autophagosomes. Inhibition of autophagy, using its downstream inhibitor chloroquine, significantly decreased or completely prevented cell death following arginine deprivation indicating that autophagy plays a deleterious role in pancreatic cancer cells exposed to arginine deprivation.

CONCLUSION: This study demonstrates that pancreatic cancer cells are either partially or completely auxotrophic to arginine and can be selectively targeted using [HuArgI (Co)-PEG5000]. Furthermore, our data indicates that arginine deprivation activates autophagy in these cells and that the observed cytotoxicity may be a form of autophagy-induced cell death, particularly following prolonged deprivation.

NO CONFLICT OF INTEREST

329 Development of a novel antibody-drug conjugated targeting endosialin/TEM1: Potent antitumor activity in sarcoma

S. Iacobelli[1], *G. Sala*[1], *E. Capone*[1]

[1] *University G. D'Annunzio, Dipartimento di Scienze Mediche- Orali e Biotecnologiche, Chieti, Italy*

BACKGROUND: The TEM-1/Endosialin/CD248 receptor is expressed in the cell surface of tumor-associated stroma cells, as well as in sarcoma and neuroblastoma cells. This receptor is emerging as an attractive molecule in diagnostics and therapeutics because of its expression across the stroma of many human tumors, the low to absent expression in normal tissues and accessibility from the vascular circulation.

MATERIAL AND METHODS: In this study, we present evidence of the preclinical efficacy of a novel Antibody-Drug Conjugate (ADC). It consists of a humanized TEM-1 monoclonal antibody (E.8-3) conjugated to a highly potent payload (TEM-1-ADC).

RESULTS. In TEM-1 expressing cancer cell lines, this TEM-1-ADC demonstrated a powerful, specific and target-dependent killing activity. High expression levels of TEM-1 in cells correlated with efficient internalization, efficacy, and cytotoxic effects in vitro. Efficacy studies demonstrated that TEM-1-ADC treatment leads to a long lasting tumor growth inhibition of cell line-based models of human sarcoma.

CONCLUSIONS.Taken together, our result demonstrated that TEM-1 is an attractive target in sarcoma and suggest that TEM-1-ADC has the potential to be developed into a biotherapeutic agent in these malignancies.

CONFLICT OF INTEREST

Ownership: MediaPharma Srl. Corporate-sponsored Research: MediaPharma Srl is the main sponsor of the research whose result are presented at the Congress. Other Substantive Relationships: None

330 An Antibody Drug Conjugate targeting HER-3 demonstrates promising antitumor efficacy in a wide range of human cancer

G. Sala[1], *E. Capone*[1], *R. Ippoliti*[2], *S. Ponziani*[2], *R. Gentile*[2], *F. Giansanti*[2]

[1] *University G. D'Annunzio, Dipartimento di Scienze Mediche- Orali e Biotecnologiche, Chieti, Italy*
[2] *University of L'Aquila, MESVA, L'Aquila, Italy*

The HER-3 receptor is emerging as an attractive molecule in therapeutics because of its overexpression across many human cancers and because of its role in in several compensatory processes that underlay emergence of resistance to certain cancer drugs. In this study, we present evidence of the preclinical efficacy of a novel Antibody-Drug Conjugate (ADC) targeting HER-3. It consists of a humanized HER-3 monoclonal antibody (mAb EV20), which recognizes the HER-3 extracellular domain, conjugated to three different payloads (HER-3-ADC)s. In HER-3 expressing cancer cell lines, these HER-3-ADCs demonstrated a powerful, specific and target-dependent killing activity. High expression levels of HER-3 in tumor cells correlated with efficient internalization, efficacy, and cytotoxic effects in vitro. Efficacy studies demonstrated that HER-3-ADCs treatment leads to a long lasting tumor growth inhibition of cell line-based models of human head and neck, breast, pancreatic, prostatic, lung, stomach cancers and melanoma. Overall, these findings validate HER-3 as an attractive therapeutic target in multiple solid tumors and support further clinical development and application of HER-3 targeting ADCs.

CONFLICT OF INTEREST

Ownership: MediaPharma Srl. Corporate-sponsored Research: MediaPharma Srl is the main sponsor of the research whose result will be presented at the Congress

331 Chemoresistance of lung cancer is curable through a CD44 targeted drug delivery system

Y.H. Quan[1], *J.Y. Lim*[2], *B.H. Choi*[2], *Y. Choi*[3], *J.H. Park*[2], *H.K. Kim*[1]

[1] *Korea University Guro Hospital- College of Medicine- Korea University, Department of Thoracic and Cardiovascular Surgery, Seoul, Korea*
[2] *Korea Advanced Institute of Science and Technology, Department of Bio and Brain Engineering, Daejeon, Korea*
[3] *Korea University, Department of Bio-Convergence, Seoul, Korea*

Effective chemotherapy for non-small cell lung cancer (NSCLC) remains a major challenge with chemoresistance. Here, we demonstrated the importance of cluster determinant 44 (CD44) in NSCLC chemoresistance, and report our efforts towards the development and evaluation of a hyaluronic acid (HA)-liposome as a drug delivery system to overcome chemoresistance by efficiently delivering siRNA to CD44-overexpressing NSCLC cells.

We investigated the relationship between CD44 expression and sensitivity of the chemotherapeutic response in various NSCLC cell lines. We found that CD44 expression was inversely proportional to the degree of chemotherapeutic response in NSCLC cells. Furthermore, to determine whether there was a correlation between CD44 expression and chemoresistance, we generated and characterized cisplatin-resistant NSCLC cell lines. We found significantly increased CD44 expression in chemoresistant cells compare to that found in wild type cells. Additionally, we confirmed by CD44 knockdown that chemosensitivity of resistant cells was directly associated with CD44 expression. To overcome chemoresistance in NSCLC, we developed a HA-liposome drug delivery system that specifically targets CD44, and effectively delivers CD44 siRNA to chemoresistant cells that overexpress CD44. We found that the HA-liposome (CD44 siRNA) successfully inhibited CD44 expression in resistant cells and improved chemosensitivity.

We found the corelation between chemoresistance and CD44 expression in NSCLC, and successfully developed the drug delivery system that significantly inhibits CD44 expression. This study supports the need for future investigation of the HA-liposome (CD44 siRNA) as a possible chemotherapy carrier for targeting CD44, and to assess its effectiveness to inhibit chemoresistance via downregulation of CD44 expression in NSCLC.

NO CONFLICT OF INTEREST

332 Suppression of breast carcinogenesis and metastasis by targeting glucose metabolism with HJC0152

J. Dong[1], *H. Kim*[1], *D. Li*[1], *J. Xu*[2], *H. Wang*[1], *Z. Zheng*[1], *H. Chen*[2], *Z. Zhang*[1], *J. Zhou*[2], *Q. Shen*[1]

[1] *University of Texas MD Anderson Cancer Center, Clinical Cancer Prevention, Houston, USA*
[2] *University of Texas Medical Branch, Department of Pharmacology and Toxicology, Galveston, USA*

INTRODUCTION: Lack of targeted strategies for preventing and treating estrogen receptor (ER)-negative breast cancer (ENBC) is an unmet clinical challenge. ENBCs including triple-negative BCs (TNBC) constitute 30-40% of BC cases and are prone to develop remote metastasis and local recurrence, leading to the majority of deaths in BC patients. Dysregulated glucose and energy metabolism is critically involved in the development and progression of various cancers via promoting aberrant cell growth, malignant transformation and metastasis. Nevertheless, the potential role of glucose/energy metabolism in ENBC carcinogenesis has sparsely been explored, thus representing a key knowledge gap and a potential avenue for effective targeted therapies. Despite a substantial amount of effort has been made towards anticancer metabolic and biogenetic medications, none has progressed into clinical use, due to their limited potency, specificity or drug properties such as toxicity and poor bioavailability.

MATERIAL AND METHOD: HJC0152, a novel small molecule, was developed using structure- and fragment-based drug design strategies and molecular modeling techniques, in our initial attempt to develop non-peptide STAT3 inhibitors for anticancer use. HJC0152 was further tested in BC cells, xenograft tumor models and transgenic mouse models that develop spontaneous ENBC and TNBC.

RESULTS AND DISCUSSION: HJC0152 significantly inhibits proliferation of BC cells, induces apoptosis, reduces ER-negative mammary tumor development, suppresses ENBC xenograft tumor growth, and blocks lung metastasis in vivo. Intriguingly, HJC0152 differentially modulates expression of glycolytic enzymes including HK1, PFK-L, PFKFB2, ENO2, PDH, PDK1, PGAM1 and ALDOA in a time-dependent manner. HJC0152 also regulates the transcription of genes involved in glucose and mitochondrial energy metabolism, including the subunits of

mitochondrial respiratory chain complexes. Functional assessments further demonstrate that HJC0152 significantly modulates respiratory chain complex function. Via in silico and a Unique Polymer Technology (UPT) strategy, we identified a list of potential HJC0152 interacting targets for validation studies.

CONCLUSION: Our findings suggest that HJC0152 is capable of reprogramming/restoring the dysregulated glucose metabolism by inducing specific glycolytic enzyme expression and mitochondrial respiratory chain function, likely via targeting upstream key signal molecule(s) that regulates glucose and energy metabolism, thereby suppressing breast cancer development and progression to metastasis.

This work was supported by Grants P50 CA097007, and P30DA028821 (JZ) from the NIH, CPRIT (JZ), John Sealy Memorial Endowment Fund (JZ), DFI Grants from MD Anderson Cancer Center (QS), Holden Family Research Grant in BC Prevention (QS), and NCI PREVENT Program HHSN26100002 (QS).

NO CONFLICT OF INTEREST

333 Novel small-molecule MDM2 inhibitors: A potent anti-cancer therapeutics

S. Jelen[1], M. Mazur[1], J. Jaszczewska[1], W. Lewandowski[1], I. Kalinowska[1], J. Witkowski[1], K. Woś-Latosi[1], P. Rózga[1], M. Teska-Kamińska[1], M. Feder[1]

[1] Adamed Group, Research and Deuelopment, Czosnów, Poland

INTRODUCTION: A growing number of preclinical and clinical investigations has been recently presented concerning small molecule inhibitors targeting the MDM2-p53 interactions dedicated as potent therapeutic agents against various cancer types. In almost half of the cancer patients TP53 gene is mutated or deleted leading to defective p53 protein expression and subsequent deregulation of cell cycle checkpoints promoting malignant cell growth. The remaining patients with wild-type TP53 gene have functional p53 protein, which is however, rapidly degraded by aberrantly expressed regulatory protein such as MDM2. Thus, molecules interrupting MDM2-p53 interactions are an attractive clinical approach to restore anti-tumor activity of p53 in patients bearing wild-type TP53 gene. Encouraged by the therapeutic potential, we have developed a series of self-designed highly potent MDM2 inhibitors.

MATERIAL AND METHOD: We have generated an extensive library of derivative molecules sharing a common core structure. The molecules have been tested according to the following pattern of consecutive studies: in vitro efficacy (MTT assay), receptor binding (fluorescence polarization test), in vitro ADME studies including microsomal stability (MS), drug permeability in Caco-2 cells monolayer, CYP450 inhibition, LogD, plasma protein binding, in vivo mouse pharmacokinetic (PK) study including exposure (AUC), determination of bioavailability (F), drug clearance (CL), half-life (T1/2), and in vivo efficacy in mice models.

RESULTS AND DISCUSSION: Based on the aforementioned parameters, 15 precandidates have been selected and submitted to the ongoing mouse in vivo efficacy studies. Additionally, the basic mechanism of action of the selected molecules has been verified. Upon selection of the lead compound toxicological studies on relevant species will be performed.

CONCLUSION: We have generated meticulously designed MDM2 inhibitors with very well understood structure-activity relationship (SAR) characterized by high in vitro and in vivo efficacy, lower toxicity as well as favorable ADME and PK profile. We believe that our lead compounds represent a new generation of highly effective anti-cancer therapeutics.

NO CONFLICT OF INTEREST

334 Development of novel anti-breast cancer agents based on omega-3 epoxy fatty acid analogues

Y.Al-Zubaidi[1], T. Rawling[2], K. Bourget[1], N. Koolaji[1], C. Pazderka[2], M. Murray[1]

[1] Univuersity of Sydney, Discipline of Pharmacology, Sydney- NSW 2006, Australia
[2] Univuersity of Technology, School of Mathematical and Physical Sciences, Sydney- NSW 2007, Australia

INTRODUCTION: Many experimental and epidemiological studies have shown that omega-3 polyunsaturated fatty acids (ω-3-PUFAs) reduce the risk of certain cancers. We recently synthesised a metabolically stable analogue of ω-3-PUFA epoxide termed CTU that inhibits proliferation and activates apoptosis in highly metastatic MDA-MB-231 breast cancer cells. In this study, further CTU analogues were synthesised and tested for their anti-proliferative activity.

MATERIALS AND METHODS: New CTU analogues termed MM16, CP19, CP21 and CP22 were produced by modifying the nature of the aromatic system in CTU. The viability of MDA-MB-231 cells was evaluated by ATP formation, cell cycle distribution was determined by flow cytometry and immunoblotting was used to evaluate the expression of cyclin regulatory proteins.

RESULTS: CP22 and CP21 were more effective than CTU and CP19 in decreasing ATP production in MDA-MB-231 cells compared to control (43±5.2%, 56±8.8%, 65±13% and 76±12.7%, respectively; 10 μM, 24h). However, MM16 was inactive.

Flow cytometry analysis showed an increment in the cell proportion in sub-G1 phase with CP22 treatment relative to control (7.2-fold) followed by CP19 (6.1-fold), CP21 (5.4-fold) and CTU (4.2-fold). On the other hand, a decrement of the cell population in G0/G1 phase was also noted with CP22 treatment compared to the control followed by CP21, CP19 and CTU.

Consistent with findings from flow cytometry, treatment of MDA-MB-231 cells with CP22, CP21, CP19 and CTU (10 μM, 24h) produced decreases in cyclin D1 (6-fold, 5.9-fold, 3.2-fold and 3.7-fold, respectively) and CDK4 (6.4-fold, 2.9-fold, 2-fold and 1.9-fold, respectively) immunoreactive protein expression. In contrast, cyclin D1 and CDK4 expression were unaffected by MM16.

CONCLUSION: CP22, CP21 and CP19 were more effective than CTU in impairing energy metabolism in MDA-MB-231 breast cancer cells and disrupting the cell cycle in sub-G1 phase by down-regulating the cyclin D1/CDK4 complex. These properties are promising for the development of novel therapeutic agents.

NO CONFLICT OF INTEREST

335 Inhibition of βIII-tubulin in mouse pancreatic tumors in vivo using RNAi-based nanomedicines reduces tumor growth and metastases

J. McCarroll[1], G. Sharbeen[2], J. Youkhana[2], J. Liu[2], D. Goldstein[2], C. Boyer[3], T. Davis[4], M. Kavallaris[5], P. Phillips[2]

[1] Children's Cancer Institute- Lowy Cancer Research Centre- UNSW Australia, Children's Cancer Institute, Sydney, Australia
[2] Lowy Cancer Research Centre- UNSW Australia- Sydney- Australia, Pancreatic Cancer Translational Research Group, Sydney, Australia
[3] Australian Centre for NanoMedicine- ARC Centre of Excellence in Conuergent Bio-Nano Science and Technology- UNSW Australia, Chemistry Department, Sydney, Australia
[4] Monash Institute of Pharmaceutical Sciences- Monash University- Australia., ARC Centre of Excellence in Conuergent Bio-Nano Science and Technology, Melbourne, Australia
[5] Australian Centre for NanoMedicine- ARC Centre of Excellence in Conuergent Bio-Nano Science and Technology- UNSW Australia, Childrens Cancer Institute, Sydney, Australia

INTRODUCTION: Pancreatic ductal adenocarcinoma (PC) is a highly lethal disease that has seen little improvement in patient survival for the last 30 years. This is due to metastases and chemoresistance. Current treatments often fail as they are sequestered by the extensive tumor fibrosis or cannot pass through the distorted tumor vasculature. We have previously identified that βIII-tubulin is a key player in promoting pancreatic cancer growth and survival, and silencing its expression may be a potential therapeutic strategy to increase the long-term survival of pancreatic cancer patients (Oncotarget, 2015, 10;6:2235-49). However, there are no pharmacological inhibitors for βIII-tubulin. Our lab, in collaboration with the Australian Centre for Nanomedicine, has developed a novel nanoparticle (STAR-PEG) that can overcome the physical barrier to drug delivery in PC and that can deliver siRNA to specifically inhibit βIII-tubulin in PC tumors in vivo (Biomacromolecules, 2016, 17: 2337–2351). We hypothesized that STAR-PEG+βIII-tubulin siRNA could reduce PC tumor growth and metastatic spread. **Aim:** To assess the effect of systemic delivery of STAR-PEG+βIII-tubulin siRNA on PC tumor growth and metastatic spread in vivo.

MATERIALS AND METHODS: MiaPaCa-2 PC cells were injected into the pancreas of mice (n=10/group). 5 weeks later, mice were injected twice weekly with STAR-PEG+βIII-tubulin siRNA or control siRNA for a further 3.5 weeks. βIII-tubulin levels were assessed by western blot and immunohistochemistry. Tumor volume was calculated from ex vivo caliper measurements using the formula (Length x Width x Height /2). Macroscopic metastases were counted and confirmed by histology.

RESULTS AND DISCUSSION: STAR-PEG+βIII-tubulin siRNA inhibited βIII-tubulin expression in PC tumors, relative to controls. STAR-PEG+βIII-tubulin reduced PC tumor growth (103 ± 17.5 mm^3; $p<0.05$) compared to control (194.9 ± 31.2 mm^3). Macroscopic metastases were also significantly reduced by STAR-PEG+βIII-tubulin siRNA treatment (94.7 ± 5.3 % decrease, relative to control siRNA; $p<0.0001$).

CONCLUSIONS: Systemic delivery of STAR-PEG+βIII-tubulin siRNA reduces PC tumor growth and metastatic spread. STAR-PEG+βIII-tubulin siRNA represents a new class of therapeutic that has the potential to improve PC patient outcome.

NO CONFLICT OF INTEREST

336 Involvement of cellular stress response in hypoglycemia-induced reversible resistance to different modalities of cancer therapy

M. Jeong[1], M.H. Kim[1], M. Lee[1], S. Park[1], J. Sohn[1], Y.G. Park[1]

[1] Korea Univuersity College of Medicine, Department of Biochemistry and Molecular Biology- Korean Institute of Molecular Medicine and Nutrition, Seoul, Korea

BACKGROUND: Acquired resistance to anti-cancer therapies including chemotherapy and anti-cancer immunotherapy is a serious obstacle to treating cancer and preventing recurrence. Cancer cells are under the harsh conditions of low pH, hypoxia, and nutrient deficiency. Many studies revealed that hypoxia enhances resistance of cancer cells to various anticancer therapies. However, it is still unclear whether and how nutrient deficiency induces resistance to anticancer therapies.

MATERIAL AND METHODS: In this study, A549 human lung adenocarcinoma cells were cultured in the medium containing different low concentrations of glucose, FBS or both for long-term periods. IC50 against cisplatin was measured by CCK-8 every two weeks and regulation of cellular stress response pathway was detected by western blot. CTL assay was performed using MALT-pulsed A549 cells and MALT-specific human T cells.

RESULTS: In A549 cells cultured under hypoglycemic condition for 2 and 28 weeks, cisplatin resistance was observed. Interestingly, the cisplatin-resistant cancer cells are observed to be resistant to lysis by cytotoxic T lymphocytes. Apoptotic cell death

by cisplatin was definitely inhibited in the cell maintained under hypoglycemic condition. However, the reversion to normal culture condition only for short term periods makes the cells susceptible to cisplatin-induced cell death. In hypoglycemic condition, cellular stress responses including ER stress responses were increased and maintained for relatively long-term periods.

CONCLUSIONS: This study demonstrated that hypoglycemia-induced cisplatin resistance is reversible and transient, and suggests that hypoglycemia-induced resistance to different modalities of cancer therapy may be dependent upon cellular stress response.

This research was supported by the National Research Foundation of Korea (2016R1D1A1A0293 and 2013M3A9D3045881).

NO CONFLICT OF INTEREST

337 HDAC4 and HDAC6 sustain DNA double strand break repair and stem-like phenotype thus promoting radioresistance in glioblastoma cells

C. Festuccia[1], A. Vetuschi[2], R. Sferra[2], S. Pompili[2], F. Megiorni[2], S. Simona Camero[2], H.P. McDowell[3], L. Ventura[4], F. De Felice[5], V. Tombolini[5], C. Dominici[2], R. Maggio[1], G.L. Gravina[1], F. Marampon[1]

[1] University of L'Aquila, Applied clinical sciences and biotechnologies, L'Aquila, Italy
[2] University La Sapienza rome, Department of Paediatrics and Infantile Neuropsychiatry, rome, Italy
[3] Alder Hey Children's NHS Foundation Trust, Department of Oncolog, Liverpool, United Kingdom
[4] San Saluatore Hospital, Diuision of Pathology, L'Aquila, Italy
[5] Policlinico Umberto I- La Sapienza Uniuersity, Department of Radiological- Oncological and Pathological Sciences, Rome, Italy

BACKGROUND: Glioblastoma (GBM) radioresistance restricts the curative potential of radiotherapy (RT). Despite histone deacetylases (HDACs) mRNA levels have been shown inversely related to GBM malignancy, HDACs inhibitors affect GBM radioresistance trough unknown molecular mechanisms. result We found that tumor samples from 31 GBM patients who underwent temozolomide and radiotherapy (RT) combined treatment showed HDAC4 and HDAC6 expression in 93.5% and 96.7% of cases, respectively. Retrospective clinical data analysis showed that high intensity of HDAC4 and/or HDAC6 immunostaining was predictive of poor outcome. In vitro experiments demonstrated that silencing HDAC4 or HDAC6 by specific shRNAs radiosensitized U87MG and U251MG GBM cells by promoting DNA double-strand break (DSBs) accumulation and preventing RT-induced DNA-PKcs or ATM protein nuclear translocation. Furthermore, we found that silencing HDAC6 induced U251MG apoptosis- or U87MG autophagy-mediated RT-induced cell death, while silencing HDAC4 predisposed GBM cells to RT-induced p21 WAF1/CIP1-mediated cellular senescence in a p53 WT dependent manner. Finally, HDAC4 or HDAC6 silencing reduced GBM stemness potential.

CONCLUSIONS: Altogether, these observations suggest that HDAC4 and HDAC6 are guardians of RT-induced DNA damages and stemness, thus promoting radioresistance, and represent potential prognostic markers and therapeutic targets in GBM.

NO CONFLICT OF INTEREST

338 Anti-αVβ3 integrin peptidomimetic-Sunitinib dual conjugates as therapeutic tool for the inhibition of integrin/growth factor crosstalk in human melanoma cells

F. Bianchini[1], S. Peppicelli[1], E. Andreucci[1], M. Lulli[1], F. Vacondio[2], L. Battistini[2], A. Sartori[2], F. Zanardi[2], L. Calorini[1]

[1] University of Firenze, Clinical and Experimental Biomedical Science, Firenze, Italy
[2] University of Parma, Pharmacy, Parma, Italy

BACKGROUND: Metastasis is the dramatic final step of progression of neoplastic disease exerting the leading cause of death in the world after heart disease and infection disease. In the recent years, increasing knowledge of the molecular causes of tumor progression and metastatic dissemination had significantly improved chemotherapeutic treatment approaches, that, together with surgery, increased patient progression free survival and time to progression. Despite this, chemotherapeutic drug delivery systems still have to deal with many challenges as the lack of selectivity or the achievement of sufficient drug accumulation at the tumor site. In this view we have developed anti-αVβ3 Arginine Glycine Aspartic acid (RGD) integrin peptidomimetic-Sunitinib dual conjugates to improve tumor cell targeting and to synergistically dampen the strict crosstalk between receptor tyrosine kinase and integrin-dependent activated pathways.

METHODS: M21 human melanoma cells and B16F10 murine melanoma cells, overexpressing αVβ3 integrin receptor, were used. Immunofluorescence assays were used for the evaluation of sunitinib internalization. We evaluated the ability of the different compounds to inhibit tumor cell adhesion to vitronectin (an RGD containing substrata), and to inhibit tumor cell growth and invasiveness. Moreover, we evaluated the effect of the compounds to inhibit tyrosine phosphorylation of the Vascular Endothelial Growth Factor Receptor (VEGFR) and Platelet Derived Growth Factor Receptor (PDGFR) two target of Sunitinib.

RESULTS: Compounds were designed wherein the two moieties (RGD antagonist and Sunitinib) are positioned 11-to-22 bonds away and are connected via robust triazole/ether/amide linkages to do not reciprocally compromise each moiety activity and to exclude premature detachment. We found that the conjugation of the RGD peptidomimetic ligand with the sunitinib moiety didn't compromise their αVβ3-integrin binding ability. Sunitinib tumor cell internalization was dependent by the RGD-αVβ3 integrin interaction. Finally, a synergistically effect, by the use of the conjugates, was found in the inhibition of tyrosine kinase receptor phosphorylation.

CONCLUSIONS: The synthesis of anti-αVβ3 integrin peptidomimetic-Sunitinib dual conjugates may represent a valuable strategy to improve chemotherapeutic drug delivery.

NO CONFLICT OF INTEREST

339 Pan-RAF and MEK vertical inhibition enhances therapeutical response in nonV600E BRAF mutant cell lines

E. Molnár[1], T. Garay[2], D. Rittler[1], M. Grusch[3], W. Berger[3], B. Döme[4], J. Tímár[2], B. Hegedűs[5]

[1] Semmelweis University, 2 nd Department of Pathology, Budapest, Hungary
[2] HAS-SE, Molecular Oncology Research Group, Budapest, Hungary
[3] Medical University of Vienna, Institute of Cancer Research, Vienna, Austria
[4] Medical University of Vienna, Department of Thoracic Surgery, Vienna, Austria
[5] University Duisburg-Essen, Department of Thoracic Surgery, Essen, Germany

BACKGROUND: Oncogenic mutations of BRAF can be found in a variety of tumors. While there are already BRAF V600E mutation specific inhibitors in clinical practice, there is no effective targeted therapy against nonV600E BRAF mutant tumors. Accordingly, in the current study we investigated the possibility of blocking oncogenic signaling of nonV600E BRAF mutation by inhibiting two signaling elements in the MAPK pathway.

MATERIAL AND METHODS: Six BRAF mutated human tumor cell lines A375 (V600E), CRL5885 (G466V), WM3629 (D594G), WM3670 (G469E), MDAMB231 (G464V), CRL5922 (L597V) were treated with a pan-RAF inhibitor (PRi, sorafenib), a MEK inhibitor (MEKi, selumetinib) or their combination and the effects on proliferation, signaling pathways activation, apoptosis induction and migration were investigated.

RESULTS: All cell lines showed a significant growth inhibition with synergism of the PRi and MEKi combination. Combination treatment resulted in higher Erk activation inhibition and in increased induction of apoptosis when compared to single agent treatments. PRi+MEKi combination treatment could also decrease migratory capacity in all the cell lines.

CONCLUSIONS: The vertical combination inhibition of RAF and MEK activity can be an effective approach for blocking proliferation and migration and for induction of apoptosis in nonV600E BRAF mutant tumors.

NO CONFLICT OF INTEREST

340 Indocyanine green and doxorubicin-loaded fucoidan/protamine nanoparticles as a multifunctional drug carrier for combination cancer therapy

M. Fwu-Long[1], J. Pei-Ru[2], L. Kun-Ying[2], W. Sin-Yu[2]

[1] Taipei Medical Uniuersity, Department of Biochemistry and Molecular Cell Biology, Taipei, Taiwan
[2] Taipei Medical Uniuersity, Graduate Institute of Medical Sciences, Taipei, Taiwan

BACKGROUND: Indocyanine green (ICG) is a near-infrared (NIR) dye used in medical diagnostic and photothermal therapy while doxorubicin (DOX) is a widely used chemotherapeutic drug. Co-delivery of ICG and DOX in a single dosage form can be a promising strategy to integrate chemotherapy and photothermal therapy for cancer treatment and NIR fluorescence imaging.

MATERIAL AND METHOD: Mutlifunctional nanoparticles were prepared by self-assembly of fucoidan and protamine. Their pH-/enzyme-responsive properties were characterized by circular dichroism (CD) spectroscopy, dynamic light scattering (DLS), and zeta potential analysis. ICG and DOX were able to be loaded in the fucoidan/protamine nanoparticles. Photostability and photothermal effect of the ICG-loaded fucoidan/protamine nanoparticles were investigated in phosphate-buffered saline (PBS) under 808 nm NIR light irradiation. NIR fluorescence cell imaging and synergistic effect of chemo-photothermal treatment of DOX-resistant breast cancer cells (MCF-7/ADR cells) were also examined.

RESULTS AND DISCUSSION: Fucoidan/protamine nanoparticles increased the stability of ICG and prevented ICG from aggregation, consequently improved their dispersity and photostability in physiological buffer solutions. The ICG-loaded fucoidan/protamine nanoparticles showed clear NIR fluorescent image in cells and remarkable photothermal effect under NIR laser irradiation. The ICG-loaded fucoidan/protamine nanoparticles exhibited significant inhibitory effect against MCF-7/ADR cells upon NIR laser irradiation. The ICG-loaded fucoidan/protamine nanoparticles promoted the DOX loading efficiency and enhanced cytotoxicity against MCF-7/ADR cells through combination of the anticancer drug with local hyperthermia due to the synergistic effect of dual chemo-photothermal therapy.

CONCLUSION: The result suggest that fucoidan/protamine nanoparticles are promising drug carriers for effective delivery of ICG and DOX to cancer cells. The combined photothermal-chemotherapy can improve therapeutic efficacy and overcome DOX resistance in MCF-7/ADR breast cancer cells.

NO CONFLICT OF INTEREST

341 FOXO1 contribution to ovarian carcinoma cell response to the XPO1 inhibitor KPT-330 in combination with cisplatin

C. Corno[1], S. Stucchi[1], M. De Cesare[1], N. Carenini[1], E. Ciusani[2], N. Zaffaroni[1], L. Gatti[1], P. Perego[1]

[1] Fondazione IRCCS Istituto Nazionale dei Tumori, Experimental Oncology and Molecular Medicine Department, Milano, Italy
[2] Fondazione IRCCS Istituto Neurologico C. Besta, Laboratory of Clinical Pathology and Medical Genetics, Milano, Italy

BACKGROUND: Ovarian carcinoma is often a lethal disease due to late diagnosis and treatment failure. Platinum drug-based therapy efficacy is limited by drug resistance. Because the karyopherin XPO1 contributes to the regulation of the cell localization of the transcription factor FOXO1 (forkhead box O1) which participates in apoptosis regulation, the aim of this study was to examine if increase of FOXO1 nuclear localization by XPO1 (exportin 1) inhibition may be exploited to improve cisplatin efficacy.

MATERIAL AND METHODS: Preclinical pharmacology approaches including growth inhibition assays, western blot analyses, gene knockdown by small interfering RNAs, quantitative Real-time PCR, immunofluorescence analyses and tests in in vivo models were employed. The drug interaction was analyzed using the Chou and Talalay method.

RESULTS: A marked sensitivity to the XPO1 inhibitor KPT330 was found in a panel of ovarian carcinoma cell lines. The effect of the combination of cisplatin and KPT-330 was investigated in the IGROV-1 cells, using different schedules. When KPT-330 followed cisplatin exposure the most favourable drug interaction was observed, as shown by the combination index values. KPT-330 and the cisplatin/KPT-330 combination produced a modulation of proteins involved in apoptosis (p53, Bax) and in cell cycle progression (p21), besides G1 and G2/M accumulation in IGROV-1 cells. KPT-330-treated cells exhibited FOXO1 nuclear staining, in keeping with the capability of the compound to inhibit FOXO1 nuclear export. FOXO1-silenced cells displayed a reduced sensitivity to KPT-330, but no significant changes in cisplatin sensitivity, as shown by knock-down experiments by RNA interference. Moreover, FOXO1 silencing tended to reduce the efficacy of the drug combination at selected cisplatin concentrations. KPT-330 was capable to significantly inhibit tumor growth when IGROV-1 cells were subcutaneously injected in immunodeficient mice or growth intraperitoneally.

CONCLUSIONS: The XPO1 inhibitor KPT-330 appears to be a promising agent for ovarian carcinoma treatment. Since a synergistic interaction occurs when cells are treated with cisplatin followed by KPT-330, the combination efficacy is dependent on the treatment schedule. Such an interaction can be modulated by silencing of FOXO1. Our result suggest that therapies selected on the basis of the molecular tumor features should be used to tailor treatments to the specific patient characteristics.

NO CONFLICT OF INTEREST

POSTER SESSION: EXPERIMENTAL/MOLECULAR THERAPEUTICS, PHARMACOGENESIS II

342 Conjugates of PAMAM dendrimers with doxorubicin and vector protein decrease survival of cancer cells demonstrating resistance to traditional anticancer agents in vitro

E. Selivanova[1], O. Matchuk[2], K. Churyukina[2], V. Kudryavtzev[2], N. Yabbarov[2], E. Nikolskaya[2], I. Kondrasheva[2], E. Severin[2], I. Zamulaeva[1]

[1] National Medical Research Radiological Centre of the Ministry of Health of the Russian Federation, Department of Radiation Biochemistry, Obninsk, Russian Federation
[2] Institute of Molecular Diagnostics, Department Biochemistry, Moscow, Russian Federation

Development of new systems for selective transport of anticancer agents in tumor cells on the base of dendritic polymers (dendrimers) is appeared to be one of the most effective approaches to overcome cancer chemoresistance associated with the existence of cancer stem cells (CSCs) and multidrug resistance (MDR). The aim of this work was to study cytotoxic effects of polyamideamine dendrimers of the second generation (G2) covalently conjugated to doxorubicin (Dox) and a vector protein (the third recombinant domain of the alpha-fetoprotein - 3D AFP) on two breast cancer cell lines (MCF-7 and MCF-7/MDR1) differing in sensitivity to anticancer drugs and to compare sensitivity of CSCs and non-CSCs to these conjugates.

As the components of targeted delivery system synthesized we used ndPAMAM dendrimers (G2), recombinant receptor binding AFP domain fused with additional sequence consisted of 21 glutamic acid residues (rAFP3DpGlu21) and doxorubicin (Dox). Firstly, terminal amino groups of rAFP3DpGlu21 and G2 were partially modified to introduce terminal alkyne and azide moieties respectively. This modification affords us to couple the protein and dendrimer at last stage of conjugate synthesis by specific Cu^{1+}cycloaddition reaction. Cis-aconitic anhydride was used as an acid labile linker between G2 and Dox . The cytotoxic effects were assessed after incubation with free Dox, G2-Dox and 3D-G2-Dox at concentration of 2.5 μm for Dox using clonogenic test. CSCs were identified in MCF-7 line by flow cytometry as side population of Hoescht33342-excluding cells 24 hours after incubation with these agents.

Free Dox reduced survival of MCF-7 / MDR1 cells to 78% and the cell line MCF-7 to 1% ($p<0.001$). While after incubation of both cell lines with G2- Dox significant cytotoxic effect was not found. Conjugate 3D-G2- Dox had a pronounced cytotoxic effect on the cell line MCF-7 / MDR1 (19% survival), in contrast to the MCF-7 cells (70% survival). After 24 hours of incubation MCF-7 cells without substances intracellular content of Dox was significantly higher in the case of free Dox than G2-Dox or 3D-G2-Dox. Accumulation of free Dox in MCF-7/MDR1 cells was 4 times lower than that in the MCF-7 cells and was 4-5 times lower than that of G2-Dox and 3D-G2-Dox .

The result show promising use of dendrimers as nanocarriers of anticancer drugs for overcome tumor cell chemoresistance. This work was supported by grant of RSF # 15-15-10013.

NO CONFLICT OF INTEREST

343 Idiotype-specific peptides as tool for multiple mieloma monitoring by tumor-derived exosomes targeting in 5T33MM mouse model

E. Iaccino[1], S. Mimmi[2], F. Albano[1], A. Lupia[1], A. Pisano[1], S. Ceglia[1], T. Golino[1], E. Vecchio[1], G. Fiume[1], C. Palmieri[1], I. Quinto[1], G. Scala[1]

[1] University "Magna Graecia" Medical School, Department of Experimental and Clinical Medicine, Catanzaro, Italy

BACKGROUND: Multiple Myeloma (MM) is a clonal B-cell malignancy characterized by the aberrant expansion of plasma cells within both bone marrow and extra medullary sites. Drug resistance is a frequent event and the outcome is still incurable. Exosomes are small membrane vesicles secreted by different cell types containing a wide range of RNA molecules and proteins involved with immune response, antigen presentation, tumor survival, cell migration, tumor invasion, cell differentiation and angiogenesis. Such characteristics qualify circulating serum exosomes as potential biomarkers to predict cancer burden at an early stage and impact personalized cancer care. Although several methods have been developed to purify exosomes, none of these can clearly discriminate between normal and tumor derived exosomes (TDEs) and to completely avoid contaminations by other shed membranes. Recently we reported that small peptide ligands (Id-peptides) targeting the immunoglobulin B-cell receptor are a unique tool to target both transformed and clonogenic precursors of B-cell malignancies. As shown in recently published works, MM released exosomes constitutively express the Ig-BCR making MM secreted exosomes a reliable target for id-peptides binding.

METHODS: Tumor-specific Id-peptides for multiple myeloma cell line 5T33MM has been selected by screening phage-displayed random peptide libraries (RPLs) using the secreted 5T33MM-IgG as bait. ELISA binding analysis and plasmon resonance spectroscopy evaluated the specificity and the relative affinity of each 5T33MM Id-peptides. The specific binding of identified Id-peptides to target 5T33MM cells and TDEs was evaluated by flow cytometer.

RESULTS AND DISCUSSION: TDEs were first purified using standard procedures and then characterized by scanning electron microscope (SEM), nanosizer and Western Blotting (WB) analysis. Id-peptides decorated streptavidin nanoparticles were evaluated for TDEs identification both in vitro and in vivo resulting in a specific detection of significant proportion of TDEs in the peripheral blood of diseased animals.

CONCLUSIONS: According to the above-mentioned results, this work provides an innovative approach for the diagnosis and the clinical evaluation of the selected disease. Indeed, taking advantage of a validated approach for MM bioactive binders selection based on phage display technology, we validated a novel strategy to target TDEs.

NO CONFLICT OF INTEREST

344 A plant-derived potential new drug 'G' targets TYMS and GOT1 genes to kill pancreatic ductal adenocarcinoma cells at low doses

P. Chivers[1], J. Moulin[2], B. Martin[2], C. Sousa da Silva[2], Y. Robichon[2], M. Rayman[1], M. Ajaz[1], F. Green[1]

[1] University of Surrey, School of Biosciences & Medicine- Faculty of Health & Medical Sciences, Guildford-Surrey, United Kingdom
[2] University de Poitiers, UFR Sciences Fondamentals et Appliques, Poitiers, France

BACKGROUND: Pancreatic ductal adenocarcinoma (PDAC) remains one of the most refractory and lethal human malignancies, with a 20% five-year survival in rare operable cases. Despite intense efforts in targeted therapy, outcome improvements have been largely confined to small changes in standard chemotherapy with 5-fluorouracil (5FU)/capecitabine, gemcitabine (Gem), paclitaxel and radiotherapy with a clear need for augmentatory strategies. In a colon cancer genome-wide study, we found that a plant extract used in other studies, coded here as G, significantly reduced expression of thymidylate synthase (TS; a key 5FU metabolism gene) and glutamic-oxaloacetic transaminase 1 (GOT1), reported to be essential for PDAC growth. Animal studies have shown no significant toxicity of G in high doses. In this study, we investigated the impact of G, with/without some established PDAC treatment regimens.

MATERIALS AND METHODS: Three PDAC cell lines, differing in TS 28-bp tandem repeat genotype, Panc1 (3R/3R), AsPC1 (2R/3R), BxPC3 (2R/2R), and of variable sensitivity (IC_{50}) to 5FU and Gem, were treated with G with/without Gem, 5FU and

ionising radiation (IR). The tests carried out were for: Cell viability (MTT assay), gene expression (qPCR and Western Blot-WB), cytotoxicity and clonogenic assays. GraphPad was used for statistical analysis.

RESULTS AND DISCUSSION: G at 5μg/ml enhanced the cytotoxicity of 5FU and Gem ($p < 0.0001$); at 10μg/ml with 5FU caused complete cell death in all cell lines. G was highly effective in inhibiting cell growth compared to 5FU in AsPC1 and Panc1 ($p<0.0001$), with complete loss of clonogenicity at 10μg/ml. Panc1 and BxPC3 were relatively IR sensitive but at the clinically typical fractionated dose of 2Gy, G at 2.5μg/ml converted sublethal effects to complete cell death. AsPC1 was relatively IR resistant but up to the 'stereotactic' doses of 18–24Gy, G significantly and dose-dependently potentiated the effect, with complete cell death at 5μg/ml. TS qPCR showed a 50-80 fold expression decrease on treatment with G + 5FU ($p < 0.001$); TS WB gave similar results. GOT1 expression showed a significant decrease in BxPC3 with G at 5μg/ml.

CONCLUSIONS: G is a novel, effective, in vitro cytotoxic agent against three PDAC cell-lines, active alone and in enhancing current clinical therapies with 5FU, Gem and IR. Given the favourable toxicity profile in rodents, we propose in vivo therapeutic evaluation with a view to phase-1 clinical studies.

NO CONFLICT OF INTEREST

345 Multifunctional nanocapsules with multistage size-shifting ability for enhancing permeation in deep tumor tissue

Y.H. Lai[1]

[1] *National Chiao Tung University, Department Of Materials Science And Engineering, Hsinchu, Taiwan*

INTRODUCTION: Chemotherapy is one of the most common ways to treat cancer in clinical treatment because vast majority of the nanomedicine are trapped within extracellular matrix (ECM) attached on the tumor vasculature or between tumor cells, indicating carrier design and particle size of nanomedicines play important roles in penetrating tumor microenvironment to enhance therapeutic efficacy.

MATERIAL AND METHOD: A novel THNCs integrated sub-nanocarrier system (Greece soldiers, GSs) in amphiphilic gelatin (AmG) matrix-carrier system with hydrophobic paclitaxel (PTX) and hydrophilic losartan (LST) encapsulated, respectively, using emulsion process. The GSs were composed of porous hollow superparamagnetic magnetite nanoparticles (PHMNPs) which using Fe/Fe3O4 core-shell to form it by Kirkendall effect and being coated with Pluronic® F127.

RESULTS AND DISCUSSION: The THNCs with AmG matrix digested by matrix metalloproteinase-2 (MMP-2) can release LST drug in the tumor microenvironment and deliver a group of GSs into tumor tissue seated in deep region simultaneously for achieving different-stage drug release. The in vitro cytotoxicity and penetration of GSs and THNCs in a 3D tumor spheroid model constructed by HeLa cell line indicate that the small-size drug-loaded GSs nanocapsules released from the THNCs exhibit a higher cytotoxicity and deeper penetration.

CONCLUSION: The result demonstrate that THNCs with dual-drug co-delivery and controlled release in various tumor tissue show great potentials for enhanced tumor therapy.

NO CONFLICT OF INTEREST

346 Development and characterization of small interfering RNA (siRNA) loaded zein-sodiume caseinate nanocomplexes for treating pancreatic cancer

C.M. Hsieh[1], *W.C. Hong*[2], *H.O. Ho*[2], *M.T. Sheu*[2]

[1] *Providence University, Department of Cosmetic Science, Taichung City, Taiwan*
[2] *Taipei Medical University, School of Pharmacy- College of Pharmacy, Taipei City, Taiwan*

INTRODUCTION: The use of RNA interference (RNAi), and specifically small interfering RNA (siRNA) have shown much promise for the treatment of cancer and a broad spectrum of other diseases, however some limitations make their clinical application difficult, such as delivery problems and side effects due to off-target actions. New methods and vectors are needed to effectively deliver RNAi to target tissue. Thus, the objective of this study is to design a simple, effective, and safe biopolymer nanoparticles siRNA delivery system to overcome one or more of the barriers encountered after administration.

MATERIALS AND METHODS: Biocompatible, biodegradable, and nontoxic zein was selected to from a complex with siRNA in low pH environment by liquid-liquid dispersion method. Sodium caseinate was used a stabilizer in optimum concentration (forming zein/caseinate nanoparticles; ZNs) to maintain the structure and prevent zein particles form aggregation under stronger ion strength environment. Characterization of ZNs included measuring size and stability by dynamic light scattering. The binding efficiency of siRNA to ZNs was determined by gel electrophoresis and SYBR Green staining. The cell uptake was investigated by encapsulation of 3,3-Dioctadecyloxacarbocyanine perchlorate (DIO) as a fluorescent marker incubated with human pancreatic carcinoma cell line (MIA PaCa-2) and examined by fluorescence microscopy. A MTT assay was designed to measure the inhibition of cell proliferation by using ELISA reader at the absorbance wavelength of 570 nm. In vitro gene silencing efficiencies of ZNs/siRNA complexes with various weight ratios were compared through evaluating the quantification of integrin-linked kinase (ILK) mRNA expression analyzed by RT-PCR.

RESULTS: ZNs were in a size range of 100-250 nm and were stable upon dilution with Hank's balanced salt solution (HBSS) after 24 hours (174.0±1.93 nm). Gel retardation assay confirmed the complexation of the ZNs loaded with siRNA. Cell uptake of ZNs was enhanced by increasing the content of sodium caseinate. The MTT assay showed a good inhibition of cell proliferation after transfection of siRNA loaded ZNs. The in vitro silencing effect achieved >80% target protein inhibition when administered ZNs loading with siRNA at nanomolar concentrations.

CONCLUSION: The biocompatible and biodegradable properties represent zein as potential candidate for in vivo siRNA delivery. Sodium caseinate can stabilize ZNs and increases the siRNA uptake in human pancreatic carcinoma cell line. Silencing ILK and proliferation inhibition of pancreatic carcinoma cell indicate that siRNA loaded ZNs could be a potentially useful therapeutic approach for treating pancreatic cancer.

NO CONFLICT OF INTEREST

349 Tumor heterogeneity affects the activity of EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer (NSCLC) patients

A.M. Rachiglio[1], *F. Fenizia*[1], *A. Morabito*[2], *M.C. Piccirillo*[3], *M. Lambiase*[1], *A. De Luca*[4], *G. Rocco*[5], *G. Botti*[6], *F. Perrone*[3], *N. Normanno*[4]

[1] *Istituto Nazionale Tumori "Fondazione G. Pascale"-IRCCS, Laboratory of Pharmacogenomics-CROM, Naples, Italy*
[2] *Istituto Nazionale Tumori "Fondazione G. Pascale"-IRCCS, Medical Oncology- Thoraco-Pulmonary Department, Naples, Italy*
[3] *Istituto Nazionale Tumori "Fondazione G. Pascale"-IRCCS, Clinical Trials Unit, Naples, Italy*
[4] *Istituto Nazionale Tumori "Fondazione G. Pascale"-IRCCS, Cell Biology and Biotherapy Unit, Naples, Italy*
[5] *Istituto Nazionale Tumori "Fondazione G. Pascale"-IRCCS, Thoracic Surgery- Thoraco-Pulmonary Department, Naples, Italy*
[6] *Istituto Nazionale Tumori "Fondazione G. Pascale"-IRCCS, Surgical Pathology Unit, Naples, Italy*

BACKGROUND: Recent findings suggest that a fraction of EGFR mutant NSCLC carries additional driver mutations which could potentially affect the activity of EGFR tyrosine kinase inhibitors (TKIs). We investigated the role of KRAS, NRAS, BRAF, PIK3CA, MET and ERBB2 mutations (other mutations) on the outcome of NSCLC patients treated with EGFR TKIs.

MATERIAL AND METHODS: EGFR mutant NSCLC patients who received first line therapy with EGFR TKIs were eligible. Genomic DNA from EGFR mutant NSCLC samples as assessed with routine diagnostic methods, was retrospectively analyzed with a targeted resequencing panel by next generation sequencing (NGS).

RESULTS: The study included 133 EGFR mutant patients, treated between June 2008 and December 2014 in 7 centers. Median age was 71 (range 41-92), 69.2% were women, 61.4% never smokers. Analysis of tumor samples with NGS revealed the presence of hotspot mutations in genes other than the EGFR, including KRAS, NRAS, BRAF, ERBB2, PIK3CA or MET, in 29/133 cases (21.8%). A p.T790M mutation was also found in 9/133 tumor samples (6.8%). The progression free survival (PFS) of patients without other mutations was 11.3 months versus 7 months in patients with other mutations (Log-rank test univariate: p=0.047). In a multivariate Cox regression model including the presence of other mutations, age, performance status, smoking status and the presence of p.T790M mutations, the presence of other mutations was the only factor significantly associated with PFS (Hazard Ratio 1.63, 95% CI 1.04-2.58; p=0.035). In contrast, no correlation was found between TP53 mutations and patients' outcome.

CONCLUSIONS: These data suggest that a subgroup of EGFR mutant tumors have intra-tumor heterogeneity and that this phenomenon might affect the activity of first line EGFR TKIs.

NO CONFLICT OF INTEREST

351 Development of a new orally absorbable lactoferrin-heparin conjugate with antiangiogenic activity to cure brain cancer

H.H. Hwang[1], *D.Y. Lee*[1]

[1] *Hanyang University, Bioengineering, SEOUL, Korea*

BACKGROUND: Glioblastoma multiforme (GBM), also known as Grade IV astrocytoma, is most aggressive malignant tumor in human beings. To cure GBM clinically, treatments are involved in chemotherapy, radiotherapy and surgical pathway. Specially, in the case of chemotherapy, temozolomide plus radiation regime is a standard for most cases of glioblastoma. Recently, antiangiogenic therapy with medications such as bevacizumab (Avastain) is tried to slow the GBM growth. However, it is reported that this drug can induce several side effects such as stroke, kidney problem and fertility for women. To overcome limitations, we newly developed an orally absorbable heparin that can attenuate angiogenic activity through binding to growth factors around the tumor tissue. For this purpose, lactoferrin (Lf) was conjugated with heparin because lactoferrin can be orally absorbed by lactoferrin receptor (Lf-R) that is expressed on the intestine, blood-brain barrier (BBB) and glioma tumor mass.

MATERIAL AND METHODS: Heparin, typically enoxaparin, was gifted by Prof. YR Byun (Seoul National Univ., Korea). Lactoferrin was chemically conjugated with heparin via amide bond formation (Lf-heparin). Anticancer and antiangiogenic activity was evaluated by using U87MG, HUVEC and rat aorta ring assay. Oral absorption was evaluated with Caco-2 monolayer system through measurement of transepithelial electrical resistance and immunohistochemistry. Also, therapeutic

effect of lf-heparin was evaluated through IHC and nissl staining in the GBM mouse model.

RESULTS: Through this study, we confirmed that conjugation between lactoferrin and heparin successfully through amide bond formation with more advanced physicochemical properties such as pharmacokinetics and stability in the acidic condition. And this new material inhibited angiogenesis in vitro experiments without toxicity. In addition, when lf-heparin was orally administered to the GBM orthotopic mouse model, it was absorbed in the small intestine and delivered specifically to the brain tumor by receptor transcytosis (Lf-R). Also, Lf-heparin attenuated angiogenesis progression in the GBM mouse model.

CONCLUSIONS: This study demonstrated that lf-heparin conjugate could act as an anti-angiogenic drug to the glioblastoma multiform effectively and systemically. And it could be absorbed orally and transported to the brain via lactoferrin receptor. Collectively, lf-heparin could be used to treat glioblastoma as a new oral medication.

CONFLICT OF INTEREST

Ownership: This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number : HI14C2099).

352 Development and characterization of lecithin stabilized micellar drug delivery system for improving efficacy and safety of chemotherapy in CT-26 colon carcinoma

L.C. Chen[1], *C.Y. Su*[1], *M.T. Sheu*[1], *H.O. Ho*[2], *H. Tseng*[2]

[1] *Taipei Medical University, School of Pharmacy- College of Pharmacy, Taipei, Taiwan*
[2] *Taipei Medical University, School of Medicine- College of Medicine, Taipei, Taiwan*

INTRODUCTION: Micelles traditionally are composed of amphiphilic molecules caused low drug loading and less drug stability. To improve the limitation, additionally lipid shell as stabilized to encapsulate micelle can improve high drug leakage and instability resulted from the drug only entrapped in the matrix of amphiphilic polymer. In this study, it was attempted to develop a lecithin stabilized micellar drug delivery system (LSMD) for enhancing therapeutic efficacy and minimizing systemic toxicity of docetaxel (DTX).

MATERIAL AND METHOD: LSMD was prepared by firstly forming a thin film of self assembling micelles containing DTX and subsequently hydrated with lecithin nanosuspension.

RESULTS: The physical characteristics of optimized LSMD were obtained with a mean size<200nm, encapsulation efficiency (E.E.) >90%, drug loading (D.L.)>5%, stability after reconstitution at RT>8 hrs. The drug release profiles demonstrated that DTX released from LSMD was slower than that of Tynen® (DTX solvent-based formulation) in phosphate-buffered saline (PBS). LSMD further shows better cytotoxicity than Tynen® for CT-26 in cell viability assay. The immunofluorescence staining assay indicated that the alpha-tubulin in DU145 treated with LSMD obviously was polymerized and the nucleus was fragmented. To compare the effect of Tynen® with LSMD formulations on cell cycle arresting in different cell lines, cell cycle were profiled using flow cytometry to analyze after PI staining of DNA. result showed that all treatments significantly increased G2/M phase and decreased in G0/G1 phase compared with control in DU145ĸPC-3 and CT-26 cell lines. The percentage of G2/M phase was similar to Tynen® in DU-145 and PC-3 cell line, but not in the CT-26 cell line, and LSMD formulations were more effective on cell cycle arresting in comparison with that for Tynen®. This data suggest that our formulations could cause multiple drug resistance cancer cell line death by arrest cell cycle at G2/M phase. The in vivo antitumor efficacy of LSMD was evaluated in the C26 tumor-bearing mice mode. The result demonstrated that LSMD were more efficacious than Tynen®. In vivo biodistribution and MTD studies, treatment with LSMD led to high drug accumulation in tumor and maximal tolerance dose was two folds higher than Tynen®.

CONCLUSION: LSMD could be a potential carrier for delivering hydrophobic chemotherapeutic agent that could enhance the efficacy of cancer chemotherapy and reduced toxicity.

NO CONFLICT OF INTEREST

353 Resveratrol represses Hsp27 expression in human glioma cells

E. Onay Uçar[1], *A. Sengelen*[1]

[1] *Istanbul University, Molecular Biology and Genetics, Istanbul, Turkey*

BACKGROUND: Glioblastoma (GBM) is the most malignant of all astrocytic tumors worldwide. Prognosis for GBM is very poor, the mortality rate is over 50%, and average survival rates are less than 15 months. Despite surgical, radiological, and chemotherapeutic interventions, these rates have not changed much. Therefore, finding additional effective and safe treatment options are required. There are many studies showing that Hsp27 is overexpressed in gliomas. These studies have shown that small interfering (si)RNAs-mediated gene silencing is a powerful tool for suppressing Hsp expression. Also several antioxidant agents are used for repressing of Hsps in cancer cells, such as resveratrol, quercetin etc. In this study, we aimed to determine the effects of resveratrol, siRNA-mediated silencing and combined therapy on the expression of Hsp27 in U87MG human glioma cells. We also aim to find out to assess whether they have higher efficacy when administered alone or in combination.

MATERIAL AND METHODS: The cytotoxic effects of siRNAs and resveratrol were measured using MTT assay. Glioma cell line (U87MG) was transfected with Hsp27-spesific siRNAs at concentrations of 25 and 50 nM. Also different concentrations of resveratrol were evaluated for their potential inhibition effects on Hsps in glioma cells. After these applications, Hsp27 protein expression levels were determined by Western blot assay.

RESULTS: We found that all treatments caused to alteration in Hsp27 expression levels. Treatment of glioma cells with resveratrol reduced Hsp27 levels by 20-45%. As a result of Hsp27 gene silencing with siRNA, Hsp27 protein levels decreased with ratio of ~50%. In U87MG cells, 15 µM resveratrol and co-administered with 25 nM Hsp27 siRNA silenced the expression of Hsp27 with the ratio of ~93%. These result demonstrate that resveratrol may be an effective adjuvant in gliomas, and combinations of Hsp27 siRNA may be an effective therapeutic option in the treatment of brain cancer.

CONCLUSION: The findings from this study will contribute to the building blocks for a new anticancer therapy approach; and the development of new anticancer drugs and related treatments of brain tumors with observed aggressive growth, poor prognosis and high mortality rate.

NO CONFLICT OF INTEREST

354 The effects of vitamin E on molecular damages induced by indomethacin in glioma cells

N. Arda[1], *M. Pekmez*[1], *E. Onay UÇAR*[1]

[1] *Istanbul University, Dept. of Molecular Biology & Genetics, Istanbul, Turkey*

BACKGROUND: Indomethacin is one of the non-steroidal anti-inflammatory drugs (NSAID), which has a high potential to be used for the treatment of glioma tumors. However, it induces generation of ROS and causes molecular damages while exhibiting its effects. Vitamin E is used as an adjuvant for the cancer treatment due to its ROS scavenging ability. The main purpose of this study is to investigate the effects of α-tocopherol succinate (α-TOS), as a vitamin E derivative against the molecular damages caused by indomethacin in C6 glioma cells and to get informative data on the molecular mechanisms of possible interactions.

MATERIAL AND METHODS: Four experimental groups were established by evaluation of the cytotoxic doses of different binary (α-TOS+ indomethacin) combinations and the analyses were carried out on the following groups of cells. Control group cells were grown in the medium alone, experimental group cells were grown in the medium containing 10 µM α-TOS (TOC), and 200 µM indomethacin (Ind), and 10 µM α-TOS+200 µM indomethacin (TOC+Ind). The ROS and the molecular damages, such as protein carbonyls, apurinic/apyrimidinic DNA sites and lipid hydroperoxides generated in C6 glioma cells under the experimental conditions were determined. COX and Src-kinase activities were measured to determine the positive or negative effects of indomethacin on cellular metabolism with addition of α-TOS. Non-receptor tyrosine kinase (c-Src) analyses were run on the basis of gene expression by RT-PCR , as well as on protein level by western blot.

RESULTS: The findings concerning oxidative stress parameters revealed that ROS generation and protein damages were induced in all groups. The significantly high level of DNA oxidation detected in TOC and Ind groups, while it was very close to control level in TOC+Ind group. Lipid peroxidation levels were below the control for all experimental groups. RT-PCR findings indicated that Src gene expression level were higher in all groups, except TOC than that of control. According to the result of Western blot analysis, the amount of the protein product of this gene seemed to be over the control in all experimental groups. COX enzyme activity in all groups was lower than that of control.

CONCLUSION: According to data obtained in this study, it was concluded that 10 µM vitamin E along with 200 µM indomethacin, which causes 50% inhibition of cell proliferation reduced the DNA and lipid damages and induced protein damages by increased generation of ROS in C6 glioma cultures.

NO CONFLICT OF INTEREST

355 Effects of rosmarinic acid and siRNA combined therapy on heat shock protein 27 expression in human glioma cells

A. Sengelen[1], *E. Onay UÇAR*[1]

[1] *Istanbul University, Molecular Biology and Genetics, istanbul, Turkey*

BACKGROUND: Gliomas are one of the cancer types with fatal consequences. Glioblastoma multiforme (GBM, grade IV) is the most common form of high grade malignant gliomas. For patients, median survival times are <15 months and mortality rate is over 50%. Therefore, scientists and clinicians worldwide are still searching for better therapies for malignant gliomas. Recently, researchers have found a relation between cancer cells and heat shock proteins (Hsps), and reducing Hsps levels in the treatment of brain tumors has become a target. Several experimental studies have shown that for suppressing Hsp expression, small interfering (si)RNAs-mediated gene silencing is a powerful tool and has a potential therapeutic strategy for treating cancer. Also, various studies have shown that some antioxidant agents have a suppressive effect on Hsp expression. Antitumoral and antioxidant effects of rosmarinic acid (RA) have been shown in different studies.

We aimed to find out to assess the effects of RA, siRNA and combined therapy on the expression of Hsp27 in U87MG cells. In addition, the effects of RA and RA-siRNA combined application on Hsp27 expression in glioma cells was first examined in this study.

MATERIAL AND METHODS: MTT assay was used to measure cytotoxic effects of RA and siRNAs. 80 and 215 μM RA were applied to U87MG human glioma cells. The cells were transfected siRNA targeting on Hsp27. Hsp27-spesific siRNAs were added to the medium at concentrations of 25 and 50 nM. Besides RA and siRNA combined therapy was performed to the cells. After these applications, protein expression levels of Hsp27 were determined by Western blot assay. Mouse monoclonal HRP-conjugate GAPDH antibody was used for optimizing data.

RESULTS: We found that all treatments caused to alteration in Hsp27 expression levels. Treatment of glioma cells with RA reduced Hsp27 levels by 30-45%. As a result of Hsp27 gene silencing with siRNA, Hsp27 protein levels decreased with ratio of ~50%. In U87MG cells, 215 μM RA and co-administered with 25 nM Hsp27 siRNA silenced the expression of Hsp27 with the ratio of ~90%. These result demonstrate that combinations of Hsp27 siRNA and RA applied to the glioma cells may be an effective therapeutic option in the treatment of brain cancer.

CONCLUSIONS: The data obtained from this study will contribute to the development of new anticancer drugs and related treatments of brain tumors with observed aggressive growth, poor prognosis and high mortality rate.

NO CONFLICT OF INTEREST

356 Identification and characterization of SSE15206, a microtubule depolymerizing agent that overcomes multidrug resistance

S. Manzoor[1], S. Iftikhar[1], A. Bilal[2], S. Khan[1], R. Ullah[2], R.S.Z. Saleem[1], A. Faisal[2]

[1] Lahore University of Management Sciences, Department of Chemistry- Syed Babar Ali School of Science and Engineering, Lahore, Pakistan
[2] Lahore Uniuersity of Management Sciences, Department of Biology- Syed Babar Ali School of Science and Engineering, Lahore, Pakistan

INTRODUCTION: Microtubules are the major cytoskeletal components that provide structural integrity to the cells and are crucial for key cellular processes such as vesicular trafficking, intracellular signaling, cell motility and cell division. Drugs that interfere with microtubule dynamics have been successfully used in the clinic for decades. Despite their success in a broad range of cancers, patients undergoing chemotherapy with such drugs often suffer severe toxicities and can acquire resistance, thereby limiting their efficacy. Development of microtubule-targeting drugs with limited toxicity and ability to overcome resistance are therefore actively pursued.

MATERIALS AND METHODS: The antiproliferative activity of SSE15206 was determined through a 3 day SRB proliferation assay in various cell lines. Effect of SSE15206 on cell cycle and mitotic index was examined by flow cytometry and immunofluorescence, respectively. Evaluation of the apoptotic effect of SSE15206 was carried out through Annexin V/PI staining and PARP-cleavage. Effect of SSE15206 on tubulin polymerization was evaluated in vitro using purified tubulin and in cells using immunofluorescence. SSE15206 was also evaluated for its ability to overcome drug resistance, including multidrug resistance in cell line overexpressing MDR-1.

RESULTS: Screening of HCT116 cells with a library of 17 in-house compounds resulted in the identification of SSE15206, a pyrazoline derivative with potent antiproliferative activities in cancer cell lines of different origins. Treatment of cells with SSE15206 caused aberrant mitosis and therefore cell cycle arrest at G2/M phase due to incomplete spindle formation, indicating that it might interfere with microtubule polymerization. SSE15206 indeed inhibited microtubule polymerization both in vitro and in cells and bound to colchicine binding site in tubulin as shown by docking studies. Longer treatment of cell with compound resulted in apoptotic cell death (increased PARP cleavage and Annexin V/PI staining) accompanied by p53 induction. Importantly, SSE15206 was able to overcome resistance to chemotherapeutic drugs in different cell lines including multidrug resistant A2780-Pac-Res cell line overexpressing MDR-1.

CONCLUSIONS: We have identified SSE15206 as a microtubule polymerizing agent that potently inhibits proliferation of cancer cells and overcomes multidrug resistance. SSE15206 therefore can be used as a lead compound to develop drugs that can overcome resistance to microtubule-targeting agents.

NO CONFLICT OF INTEREST

357 Gellan gum/ grapheme/doxorubicin preparation and performance assessment multifunctional arterial embolization microsphere for hepatoma therapy

S.Y. Chen[1], M.W. Lee[2]

[1] I-Shou University, Biomedical Engineering, Kaohsiung, Taiwan
[2] Chung Shan Medical University, Medical Laboratory and Biotechnology, Taichung City, Taiwan

BACKGROUND: Transarterial chemoembolization (TACE) is the first-line treatment at present when liver cancer can't be cut, but no have the ideal embolic agent currently. In this study we prepared the chemoembolization microspheres (GG/Gra/DOX) composed of gellan gum (GG), graphene (Gra) and doxorubicin (DOX) by the emulsification. In this system, gellan gum with good biocompatibility and suitable as the embolic agent for TACE, grapheme with the function to combinate with DOX and physically penetrate the cell membrane. The nano grapheme is made by the Hummer's method.

MATERIAL AND METHOD: The nano grapheme (Gra) conjugated with anticancer drugs doxorubicin (DOX) were synthesized. The gellan gum microspheres (GG) composed of Gra/DOX nanoparticles was fabricated by the emulsification process. The gellan gum microsphere base chemoembolization agent and Gra/DOX nanoparticles was characterized. Gra/DOX nanoparticle uptake by liver cancer cell (HepG2) was performed. The in vitro dynamic release behavior of DOX from the GG/Gra/DOX microsphere was analyzed.

RESULTS: The size of nano graphene was 1564nm by the Laser Diffraction Particle Size Analyzer. The size of the microspheres GG/Gra/DOX were ranged from 200-400μm by the Laser Diffraction Particle Size Analyzer. The size of microspheres were affected by various gellan gum concentration. The scanning electron microscope found the surface of microsphere changed from smooth to reticular after adding the nano graphene. The delta-potential of Gra and DOX were -11.5 mV and -10 mV, respectively. The delta-potential of Gra/DOX nanoparticle was 36.2 mv. The result evidenced Gra/DOX was stable in the positive state. The cellular uptake test, found that Gra/DOX could be uptake into HepG2 cells by endocytosis after co-incubation for 1hr, 3hr, 6hr and 9hr. In vitro dynamic release behavior of DOX from the GG/Gra/DOX microsphere, the Huguchi model fit the drug release data with high correlation coefficients (R^2= 0.97704, respectively).

CONCLUSION: Our result supported that the gellan gum microsphere with Gra/DOX nanoparticles have the potential as the chemoembolization agent in the future.

NO CONFLICT OF INTEREST

358 Detaching Hexokinase 2 from mitochondria elicits a Ca2+-dependent apoptosis in cancer cells and reduces tumor growth

F. Ciscato[1], R. Filadi[1], I. Masgras[1], O. Marin[1], M. Pizzi[2], V. Guzzardo[2], P. Pizzo[1], P. Bernardi[1], A. Rasola[1]

[1] Uniuersita degli Studi di Padoua, Bioimedical Sciences, Padoua, Italy
[2] Uniuersita degli Studi di Padoua, Medicine, Padoua, Italy

INTRODUCTION: Enhanced glucose utilization is a key feature of many tumor cells, and its first step is glucose phosphorylation by hexokinases. The most active hexokinase isozyme is hexokinase 2 (HK2), which is highly expressed in several neoplastic cells, where it binds to the outer mitochondrial membrane. Mitochondrial HK2 is crucial for the metabolic rewiring of tumor cells towards an aerobic glycolysis phenotype, and it also displays an important and poorly characterized anti-apoptotic activity. Therefore, HK2 is a good target for the development of antineoplastic strategies.

Here we have designed and tested a HK2-directed molecule that induces tumor cell death both in vitro and in vivo, and we have characterized its mode of action.

Materials and methods
HK2-directed peptides were synthesized using an automatized peptide synthesizer and tested on a variety of human and mouse cancer cell lines, both wild-type and silenced through RNAi for HK2. Cell assays included in vitro HK2 enzymatic activity, mitochondrial membrane potential, intracellular Ca^{2+} fluxes, cell viability and in vitro tumorigenesis. Peptides were also injected in mice undergoing allografic tumor growth. Immunohistochemical and biochemical analyses were performed on tumor samples.

RESULTS AND DISCUSSION: We demonstrate that a peptide able to detach HK2 from mitochondria induces apoptosis in human and rodent cancer cell types. In all tested cells, HK2 is located at contact sites between mitochondria and endoplasmic reticulum (ER). Peptide treatment does not perturb HK2 enzymatic activity and detaches HK2 from these contact sites, leading to a rapid Ca^{2+} release from ER and to a subsequent mitochondrial Ca^{2+} overload. In mitochondria Ca^{2+} induces opening of the permeability transition pore and inner membrane depolarization, eventually prompting cell death. Peptide-mediated detachment of HK2 from mitochondria also elicits both cell death in in vitro tumorigenic assays and reduction of growth in allografic cancer models, without noxious effects in healthy tissues.

CONCLUSION: Here we have demonstrated that HK2 detachment from mitochondria is an efficient way to prompt death in tumor cells via a rapid induction of Ca^{2+} dyshomeostasis. These data suggest that our HK2-targeting peptide might be a promising anti-neoplastic strategy.

NO CONFLICT OF INTEREST

359 Lactoferrin-conjugated gold nanoparticle for targeting glioblastoma multiforme photothermal therapy via oral delivery

H. Kim[1], D.Y. Lee[1]

[1] Hanyang University, Department of Bioengineering- College of Engineering, Seoul, South Korea

BACKGROUND: Gold nanoparticle as a photothermal therapy agent has been reported in various application due to its physical properties. If gold nanoparticle can be orally absorbed, its application may be extended. Unfortunately, it has low absorption efficiency from the gastrointestinal (GI) tract to blood stream. In general,

lactoferrin receptor is highly expressed on the small intestinal epithelial cell. Therefore, to overcome its limitation orally, here we newly synthesized lactoferrin-conjugated gold nanoparticles (Lf-PEG-AuNP). The nano-scale of gold nanoparticle were expected to be able to pass through the Blood-Brain Barrier(BBB) due to its EPR effect of GBM. Interestingly, glioblastoma in brain cancer highly express lactoferrin receptor, thus, we adapted nano-sized AuNP modified with the lactoferrin (Lf), glutathione (GSH) and polyethylene glycols (PEG) for preparation of not only for GBM targeting efficacy but also long-circulating AuNP with improved half-life.

MATERIAL AND METHODS: Physicochemical properties of Lf-PEG-AuNP were evaluated with ICP-MS, HR-TEM, SDS-PAGE, UV-Vis spectrophotometer, and so on. Moreover, we confirmed the ratio of each compartment in our definitive synthesized Lf-PEG-AuNP particle by UV-visible spectrometry and BCA assay. Oral absorption was evaluated with Caco-2 monolayer system through measurement of transepithelial electrical resistance. GBM targeting effect of Lf-PEG-AuNP was quantitatively measured by ICP-MS and IHC. 4W/cm^2 532nm laser were used for photothermal cancer therapy. U87MG was used for stereotaxic orthotopic GBM mouse modeling.

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Conclusion : Therefore, this study presented the possibility of oral formulation PTT agents for GBM treatments.

CONFLICT OF INTEREST

Ownership: National Leading Research Laboratory (NRF-2015R1A2A1A05001832)
Advisory Board: National Research Foundation of Korea
Board of Directors: Korean government.

360 Aptamer-mediated inhibition of EGFR and PDGFRβ blocks vasculogenic mimicry and growth of triple-negative breast cancers

S. Camorani[1], A. Foresta[1], E. Crescenzi[1], M. Fedele[1], A. Zannetti[2], L. Cerchia[1]

[1] Istituto per l'Endocrinologia e l'Oncologia Sperimentale "Gaetano Salvatore", CNR, Naples, Italy
[2] Istituto di Biostrutture e Bioimmagini, CNR, Naples, Italy

BACKGROUND: Triple negative breast cancers (TNBCs) are a heterogeneous group of tumors consisting of different subtypes with unique biology and distinct clinical behavior. They lack of estrogen and progesterone receptors and HER2, excluding an effective targeted therapy. TNBCs of basal- and mesenchymal-like subtypes express EGFR and PDGFRβ, respectively, that are under investigation as therapeutic targets due to their involvement in invasiveness, epithelial-to-mesenchymal transition, and vasculogenic mimicry (VM). However, clinical trials with TKIs against these receptors have not produced satisfactory results. Oligonucleotide aptamers are a valid alternative to antibodies for diagnostic and therapeutic uses. Here, the aptamers that we previously generated against EGFR and PDGFRβ have been reported as efficacious inhibitors of TNBC.

Material and Methods

The capability of aptamers to interfere with cell aggressiveness was evaluated by analysing the growth of TNBC cell lines in both classical 2D and 3D conditions, invasion and vessel-like structures formation on Matrigel. The effects of aptamers on tumor growth were analysed by high-frequency ultrasound and imaging of IntegriSense in MDA-MB-231-derived mouse xenografts upon aptamers intravenous injection. VM was analysed by PAS/CD31 staining of tumors section.

RESULTS: Our result demonstrate that, when TNBC cells are grown on Matrigel, integrin αvβ3 is associated with EGFR and this interaction regulates integrin binding to matrix that is required for VM. The anti-EGFR aptamer, differently from erlotinib and cetuximab, impairs EGFR-integrin complex, thus impeding cell adhesion to vitronectin and tube formation. Consistently, the aptamer inhibits in vivo EGFR-integrin αvβ3 interaction, VM and tumor growth. Moreover, we prove that the anti-PDGFRβ aptamer drastically reduces invasive growth and VM of mesenchymal TNBC cell lines, thus reinforcing the role for this receptor in TNBC malignancy.

Despite having higher rates of response to neoadjuvant chemotherapy, TNBC patients show high risk of recurrence and metastasis. Our result provide the rationale for testing the aptamers in modulating the susceptibility of the cells to conventional chemotherapeutics.

Conclusions

We demonstrated the role of anti-EGFR and anti-PDGFRβ aptamers in the inhibition of VM and tumor growth of TNBCs resistant to TKIs. Our data support the possibility of a new aptamer-based therapeutic approach to manage TNBC in a near future.

NO CONFLICT OF INTEREST

361 UNIPR1331, a pan ephrin receptor antagonist, impairs the glioma stem-like cells-induced vasculogenesis promoting neuronal/glial differentiation

C. Festuccia1 , G. Gravina1 , C. Giorgio2 , S. Martellucci3 , A. Mancini1 , A. Colapietro1 , S. Delle Monache1 , R. Castelli2 , F. Vacondio2 , V. Matteis3 , A. Lodola2 , M. Tognolini2
[1] University of L'Aquila, Experimental Medicine, L'Aquila, Italy
[2] University of Parma, Department of Pharmacy, Parma, Italy
[3] University Consortium "Sabina Universitas- Italy, Department of Experimental Medicine, Rieti, Italy

BACKGROUND: Anti-angiogenic therapy has shown promising but insufficient efficacy on glioblastomas (GBM). This may be attributed to recurrence due to the activation of cancer stem-like cells (SLCs) able to support tumor vasculogenesis through increased vasculogenic mimicry (VM). This phenomenon was previously reported to be promoted by Ephrin receptors (mainly EphA2 and EphB4) activation signaling.

MATERIAL AND METHODS: The aim of the present study was to investigate the effects of the brain penetrating Eph receptor antagonist, UniPR1331, on the VM formation and molecular phenotype of patients derived glioma stem cells. A series of five patient derived glioma stem-like cell lines and U87MG derived stem cells were used in FACS analyses. The luciferase transfected Tumor Promoting cell (TPC8) was used for the in vivo analyses.

RESULTS: We observed that treatment with UniPR1331 was able to reduce stem cell marker expression including CD133, CD44, Thy-1/CD90 and nestin. In addition glial (glial fibrillary acidic protein, GFAP, S100 and galactosylceramidase (Galc) and neuronal (β-tubulin III and neurofilamnts) marker expression was, indeed, increased. We validated the, UniPr1331, as anti-vasculogenic compound by using in vitro and in vivo tubule formation assay. UniPr1331 was also active in the subcutaneous xenografts and in orthotopic intra-brain tumors by reducing vasculogenesis. Disease Free Survival and Overall Survival were synergistically increased in combination with bevacizumab or sunitinib.

CONCLUSIONS: Therefore, our data indicate that UniPR133l may represent a novel therapeutic strategy to tackle GBM tumors increasing or bypassing resistance to VEGF- based anti-angiogenetic treatments reducing vasculomimicry.

NO CONFLICT OF INTEREST

362 miR-126 is downregulated in melanoma cells with acquired resistance to dabrafenib and its restoration impairs proliferation, invasiveness and VEGF secretion

C. Simona[1], E. Alvino[2], A. Amaro[3], P.M. Lacal[1], L. Levati[1], G.C. Antonini Cappellini[4], U. Pfeffer[3], N. Felli[5], A. Carè[6], S. D'Atri[1]

[1] IDI-IRCCS, Laboratory of Molecular Oncology, Rome, Italy
[2] National Council of Research, Institute of Translational Pharmacology, Rome, Italy
[3] IRCCS-AUO San Martino- National Cancer Research Institute, Integrated Molecular Pathology, Genoa, Italy
[4] IDI-IRCCS, Department of Oncology and Dermatological Oncology, Rome, Italy
[5] Istituto Superiore di Sanità, Department of Oncology and Molecular Medicine, Rome, Italy
[6] Istituto Superiore di Sanità, Center of Gender Medicine, Rome, Italy

INTRODUCTION: Previous studies have shown that melanoma cells with acquired resistance to BRAF inhibitors (BRAFi) are endowed with increased invasiveness, as a result of activation/altered expression of effector molecules involved in the metastatic process. Micro-RNAs (miRNAs) may constitute novel targets and therapeutic agents for cancer treatment. However, miRNA alterations occurring in cells resistant to BRAFi remain poorly elucidated. Here we investigated changes of miRNA expression associated with acquired resistance to dabrafenib and their role in the biological behaviour of the resistant cells.

Materials and methods. miRNA expression profile of the BRAF-mutant melanoma cell line A375 and its dabrafenib-resistant subline A375R, displaying increased invasiveness and VEGF secretion, was determined using Affymetrix GeneChip® miRNA 3.1 microarrays. Differential expression of selected miRNAs was confirmed in the two cell lines using qRT-PCR. miR-126, previously shown to negatively regulate proliferation and invasiveness of melanoma cells, was chosen for further studies. These were conducted in A375R cells and in an additional melanoma cell line with acquired resistance to dabrafenib (SK-Mel28R), also showing enhanced invasiveness and VEGF-A secretion as compared with its drug sensitive counterpart. A375R and SK-Mel28R cells were transfected with 50 nM Pre-miR hsa-miR-126 miRNA Precursor (pre-miRNA-126) or Pre-miR miRNA Precursor Negative Control#1 (Ambion®) and proliferation was evaluated 6 days later using the MTT assay. Otherwise, 72 h after transfection, the cells were assayed for invasion of the extracellular matrix and VEGF-A secretion using Boyden Chamber and ELISA assays, respectively.

RESULTS AND DISCUSSION: SAM analysis identified 13 miRNAs up-regulated and 32 miRNAs down regulated (fold change ≥ 2) in A375R cells with respect to A375 cells**.** qRT-PCR confirmed down-regulation of miRNA-126 in A375R and SK-Mel28R cell lines. Restoration of miR-126 in A375R and SK-Mel28R cells significantly inhibited proliferation, invasion and secretion of VEGF-A, a validated target of miR-126.

CONCLUSION: Our result show that the development of secondary resistance to dabrafenib is accompanied by down-regulation of miR-126 and suggest that therapeutic strategies aimed at restoring the expression of this miRNA in dabrafenib-resistant melanomas might have a therapeutic potential.

Supported by the Italian Ministry of Health, grant 5PerMille-2010 and AIRC, IGP 17585.

NO CONFLICT OF INTEREST

363 Identification and characterization of NMS-P830, an ATP-mimetic choline kinase inhibitor

P.Gnocchi[2], N. Avanzi[2], M. Bagnoli[3], E. Casale[4], C. Cristiani[2], U. Cucchi[5], M.L. Giorgini[1], F. Quartieri[4], M. Tato[4], E. Ardini[1]

[1] Nerviano Medical Sciences, Biology, Nerviano, Italy
[2] Nerviano Medical Sciences, Biotechnology, Nerviano, Italy
[3] Fondazione IRCCS Istituto Nazionale dei tumori, Dipartimento di Oncologia Sperimentale e Medicina Molecolare- Unità di terapie Molecolari, Milano, Italy
[4] Nerviano Medical Sciences, Chemical Core Technologies, Nerviano, Italy
[5] Nerviano Medical Sciences, Biothecnology, Nerviano, Italy

INTRODUCTION: Choline kinase alpha (ChoKa), the first enzyme in the Kennedy pathway that catalyzes the phosphorylation of free choline to phosphocholine (PCho), is responsible for the de novo biosynthesis of phosphatidylcholine (PC), the major phospholipid of cellular membranes. Aberrant choline metabolic profiles and concomitant ChoKα upregulation have been described in most human malignancies (i.e. breast, lung, ovary) and has been found to correlate with advanced histological tumour grade. ChoKα depletion, but not of ChoKß isoform, by siRNA or shRNA inhibited growth and migration of different tumor cell lines both in vitro and in vivo. Choline mimetic inhibitors of ChoKα have been described (i.e. MN58b) showing antitumor effects in preclinical models, although their efficacy is hampered by a significant toxicity, possibly due to cross-reactivity with other choline-dependent proteins (transporters, enzymes). In NMS a high throughput screening (HTS) has been performed with the objective to identify ATP-mimetic specific ChoKα inhibitors, which have not been reported yet, in order to avoid the toxicity observed with choline-mimetic compounds.

METHODS: A HTS was performed on 90K compounds belonging to NMS proprietary chemical collection. About 200 compounds with IC_{50}< 10 µM on ChoKα and pertaining to different chemical classes were identified. NMS-P830 was selected for its characterization in term of enzymatic mechanism of inhibition, crystallographic studies, cellular mechanism of action and antiproliferative activity.

RESULTS: NMS-P830 proved to be a competitive inhibitor of ATP as demonstrated by its enzymatic mechanism of inhibition and its crystallographic structure in complex with ChoKα. NMS-P830 showed high selectivity versus ChoKß and a panel of 60 different protein kinases. In several tumor cell lines, the compound was able to inhibit the formation of PCho in agreement with the concentration required to achieve antiproliferative activity. Moreover, the inhibitory effect on PCho and proliferation observed with NMS-P830 mirrors the one observed using siRNA ChoKα specific.

CONCLUSION: NMS-P830, a first-in-class ATP-mimetic ChoKα inhibitor, confirmed the viability of this approach. HTS allowed the identification of several active compounds belonging to different chemical classes and medicinal chemistry activities aimed at improving their biochemical and cellular potency are ongoing.

NO CONFLICT OF INTEREST

364 Nucleotide-modified RNA-aptamer inhibits in vitro cell-proliferation of Burkitt lymphoma, but not B-cell lymphoblastoid or T-cell lymphoma cell lines

I. Holodnuka Kholodnyuk[1], Z. Rudevica[2], M. Cistjakovs[1], S. Kozireva[1], G. Norstedt[3], A. Leonciks[2]

[1] Riga Stradins University, A. Kirhensteins Institute of Microbiology and Virology, Riga, Latvia
[2] Latvian Biomedical Research and Study Centre, Latvian Biomedical Research and Study Centre, Riga, Latvia
[3] Karolinska Institute, Department of Molecular Medicine and Surgery, Stockholm, Sweden

INTRODUCTION: Aptamers are oligonucleotides that can bind the ligands with high affinity and specificity by folding into complex tertiary structures. RNA molecules are unique in their folding topologies, but the applications of RNA-aptamers as therapeutics and diagnostics become possible after the chemical modifications of nucleotides, what protect from nucleases cleavage, have been introduced. The SELEX (Systematic Evolution of Ligands by EXponential enrichment) technology for the generation of aptamers of the interest from random sequences of the combinatorial libraries was described in 1990 by Tuerk&Gold and by Ellington&Szostak. We have elaborated the whole-cell SELEX method modification (Cell-SELEX-FA) for the selection of the nucleotide-modified RNA-aptamers (nmRNA-aptamers) that are targeted to the cell-surface molecules.

MATERIALS AND METHODS: We have applied the Cell-SELEX-FA for the generation of nmRNA-aptamers to the cell-surface molecules of malignant Burkitt lymphoma (BL) cells, using the EBV-positive BL cell line Raji.

To subtract from the cell-surface molecules of non-malignant B cells, we curried out the Negative selections, using the lymphoblastoid cell line (LCL) that was established by EBV infection of normal B cells. The cell proliferation growth-blocking effect was assessed by MTT assay and the Soft-Agar-Growing (SAG) test.

RESULTS: One of the selected nmRNA-aptames, Apt5 and the control AptN (with random sequences) were examined for the cell proliferation growth-blocking effect, using the viability MTT and SAG tests. The viability test has demonstrated that Apt5 (at concentration 4.0 µM) inhibited cell proliferation of highly malignant CD10+ BL cell lines from 65% up to 90%, of lymphoblastoid CD10- cell lines (LCL and BL MutuIII) – up to 28%, but was inactive for the T-cell lymphoma cell lines and normal donor lymphocytes. The SAG test, using Apt5, has demonstrated dramatic inhibition of colonies formation for CD10+ BL cell lines. Apt5-FITC (5'-end labeled) showed the significant cell staining for the CD10+ BL cell lines, but not for the T-cell lymphoma cells, using immunofluorescent microscopy and flow cytometry.

CONCLUSIONS: Applying the elaborated by us the Whole-Cell SELEX method modification (Cell-SELEX-FA), we have selected nucleotide-modified RNA-aptamer with the therapeutic potential. Further broad studies are needed to define the types of B-cell lymphomas that can be suppressed (in vitro, for the first) by this aptamer.

NO CONFLICT OF INTEREST

365 Viscum album Extracts Downregulate the Expression Levels of Hsp27 in Brain Tumors

E. Mertoğlu[1], S.N. Biltekin[1], E. Onay Uçar[1]

[1] Istanbul University- Faculty of Science, Department of Molecular Biology and Genetics, İstanbul, Turkey

BACKGROUND: Cancer is one of the most common disease in the world. Previous studies demonstrate that there is a relationship between cancer cells and Heat Shock Proteins (Hsps), and it is observed that expression levels of Hsps are elevated in different types of cancer. The plant extracts are widely used both in traditional and complementary medicine in the whole world. Viscum album (mistletoe) extracts with their anticancer and antioxidant properties are used in cancer treatment. Biochemical composition of this plant varies depending on the host trees and the harvest time. The aim of this study was to determine whether V.album extracts have effects on Hsps expression level and apoptosis in C6 glioma cell line or not.

MATERIAL AND METHODS: In this study, three different extracts of V.album grown on different hosts (acer, acacia and wild pear) were compared for their potential inhibitory effects on Hsps expression levels. The cytotoxic effects of extracts were determined by MTT test. Different experimental groups were designed and these groups were exposed to heat shock and/or incubated without any heat shock exposure. Overexpression of Hsps was induced by exposure to heat shock at 42°C for 1h in C6 glioma cells. Expression levels of Hsps were determined by Western blot analysis and the apoptosis inducing effect was evaluated by caspase-3 activation.

RESULTS: The expression levels of Hsps are reduced significantly after pretreatment of the cells with non-toxic concentration of V.album extracts prior to heat shock exposure. Also, pretreatment with the extracts prior to heat shock exposure induced apoptosis by caspase-3 activation in C6 glioma cells. These result will be used to determine the effect of different extracts on stress protein expressions.

CONCLUSIONS: These result suggest that different extracts of V.album are able to down regulate expression levels of Hsps, and induce apoptosis. According to these result Viscum album extracts might be regarded as a potential resource of bioactive compounds that can be used in cancer therapy. Targeting Hsps may help to the improvement of the cancer treatment as a complementary strategy.

NO CONFLICT OF INTEREST

366 Acquired resistance to HER2-targeted therapies is associated with EMT

N. Tracey[1], H. Creedon[1], L. Gomez-Cuadrado[1], M. Muir[1], J. Loane[2], T. Klinowska[3], A. Byron[1], V. Brunton[1]

[1] Edinburgh Cancer Research Centre, IGMM, Edinburgh, United Kingdom
[2] Pathology Department, Western General Hospital, Edinburgh, United Kingdom
[3] Oncology iMed, Astra Zeneca, Macclesfield, United Kingdom

INTRODUCTION: Breast cancer is the most common cause of cancer in women. Human epidermal growth factor receptor 2 (HER2) positive breast cancer is responsible for up to 25% of breast cancer cases each year. Targeted therapies against the HER2 receptor are first line treatment, such as trastuzumab, which targets the extracellular domain of HER2, and lapatinib, which inhibits the tyrosine kinase activity of HER2 and other HER family members. Despite the available treatments, patients treated with these therapies often develop resistance and the tumours no longer respond. The mechanism of resistance is poorly understood.

METHODS: Tumour fragments from MMTV-NIC-PTEN were inserted into the fourth mammary fat pad of FVB/N mice. After tumours were established, mice were treated with either vehicle (Tween 80) or pan-HER family tyrosine kinase inhibitor, AZD8931. Mice were culled and tumours were analysed using immunohistochemistry.

RESULTS AND DISCUSSION: In the MMTV-NIC-PTEN genetically engineered mouse model of HER2 driven breast cancer, epithelial to mesenchymal transition (EMT)-associated acquired resistance to AZD8931 was seen in 50% of resistant tumours. Immunohistochemical analysis showed that this was concomitant with a loss of E-cadherin and increased expression of the EMT markers Zeb1 and vimentin. In addition we have been able to show that a loss of membranous HER2 in resistant tumours was associated with the EMT phenotype. Current work is underway to understand the drivers of resistance and in particular the control of HER2 expression.

CONCLUSION: This work identifies resistance mechanisms linking loss of HER2 to EMT. Further work is needed to establish the mechanisms leading to the loss of HER2, and the initiating events leading to a mesenchymal phenotype in response to AZD8931.

CONFLICT OF INTEREST

Other Substantive Relationships: T Klinowska is employed by AstraZeneca

367 Regulation of chemokines CXCR4/CXCL12 axis and HER2 by estrogen and Progesterone in breast cancer cells: Investigation the crosstalk between HER2 and CXCR4 signalling pathways

S. Kakel[1], A. Tyson-Capper[2], Y. Bury[3]

[1] Student, Institute of cellular medicine, Newcastle upon tyne, United Kingdom
[2] Institute of cellular medicine, Medical School, Newcastle upon tyne, United Kingdom
[3] Cellular Pathology, Royal Victoria Infirmary, Newcastle upon tyne, United Kingdom

INTRODUCTION: Overexpression of human epidermal growth factor receptor 2 (HER2) occurs in ~20-30% cases of breast cancer. It is associated with estrogen receptor (ER) positive breast tumours. This relationship between HER2 and ER is likely to be the basic impairment against hormonal therapy. It comes to light that there is other protein which is also overexpressed in breast cancer, termed CXCR4, that is recognized as a prognostic marker in different types of cancer such as breast cancer. CXCR4 and CXCL12 are chemokines associated with tumours and metastasis to organs enriched by its ligand CXCL12 (SDF1-α).

The aim of this study is to define and compare the expression profile of chemokines, CXCR4 and CXCL12 in different breast cancer cell lines, in addition to clinical tumour samples with specific status regarding to HER2 and ER.

MATERIAL AND METHODS: MCF7 and MDA-MB-231 were exposed to β-Estradiol (E2), progesterone and their receptors antagonists; fulvstrant, mifepristone and org31710 respectively at concentration 1μm. Gene expression of HER2 and CXCR4/CXCL12 was detected at mRNA level via qPCR.

Four breast tumour samples were included in the study – subdivided by their grade and receptor status in respect to HER2 and ER. Clinically validated antibodies by IHC were used; anti-CXCR4, anti-CXCL12. Data were analysed by SPSS.

RESULTS: Data observed show that CXCR4/CXCL12 and HER2 are E2 and progesterone targeted genes. Unexpectedly, fulvstrant increases the CXCR4 and HER2 expression at mRNA level, but decreases CXCL12 expression in MCF7, however, mifepristone and org31710 significantly decrease the expression of both CXCR4/ CXCL12. MDA-MB-231 revealed low expression of the three studied genes.

Tumour sections demonstrated high nuclear and cytoplasmic expression for CXCR4 and CXCL12 respectively with high intensity in both positive and negative ER breast tumours.

CONCLUSION: Our result suggest that fulvestrant has the potency to trigger up-regulation of CXCR4/CXCL12 and HER2 in ER-positive breast cancer cells. While mifepristone and org31710 have induced down-regulation effects on these genes expression, therefore, targeting of CXCR4/CXCL12 axis and/or HER2 might be a promising therapy in association with the estrogen effect on the breast cancer growth, since both CXCR4/CXCL12 is considered a prognostic marker in breast tumours.

NO CONFLICT OF INTEREST

368 The repositioning of anti-malarial compounds for anticancer activity in breast cancer

A. Neumann[1], S. Kimani[1], S. Jordaan[2], K. Chibale[2], S. Prince[1]

[1] University of Cape Town, Department of Human Biology, Cape Town, South Africa
[2] University of Cape Town, Department of Chemistry, Cape Town, South Africa

INTRODUCTION: Breast cancer is the most common malignancy in women globally and it is estimated that one in eight women will develop this cancer in their lifetime. Despite enormous efforts to identify breast cancer therapies there has been limited success which is exacerbated by the drug development pipeline being long and costly. An attractive strategy to overcome these challenges has been to reposition non-cancer drugs for anticancer activity. Here we describe the characterisation of an anti-malarial compound (MMV652103) which shows promising anticancer activity in an estrogen-receptor (ER) positive breast cancer cell line.

MATERIALS AND METHODS: The following experiments were performed on breast cancer (MCF7) and normal breast epithelial (MCF12A) cells treated +/- MMV652103: MTT and clonogenic assays to determine effect on short- and long-term cytotoxicity; western blotting to determine the impact on p-p38, cell cycle regulators (p53, p21), apoptosis (PARP cleavage) and autophagy (LC3β); in vivo studies using chicken embryos - briefly, MCF7 cells were grafted onto the chorioallantoic membrane of 9 day old fertilized chicken eggs; tumours were detected 10 days later; varying doses of MMV652103, Paclitaxel (positive control) and DMSO (negative vehicle control) were administered over 10 days; tumour clearance and cytotoxicity monitored.

RESULTS AND DISCUSSION: MMV652103 displayed short- and long-term anticancer activity. It exhibited differential cytotoxicity with an IC_{50} of 6.6μM and 2.2μM in normal and breast cancer cells respectively. Preliminary data show that MMV652103 may exert its anticancer activity via the p38 MAPK pathway and triggering cell cycle arrests in a p21-dependent manner. Its mechanism of cell death was confirmed to be apoptosis and possibly autophagy. MMV652103 displayed no deleterious effects (no abnormal death ratio, no abnormalities) on chicken embryos and no signs of toxicity were observed at the doses tested. Importantly, 4.4μM and 22μM MMV652103 treatment reduced tumour weight by 34.89% and 41.71% respectively, more effective than the current approved chemotherapeutic, Paclitaxel.

CONCLUSION: This study identified MMV652103 as a promising ER positive breast cancer drug which is ready to be advanced to pre-clinical trials. It had reduced activity in normal breast epithelial cells and showed no signs of toxicity in chicken embryos suggesting that it may be specific for breast cancer cells and associated with reduced side-effects.

NO CONFLICT OF INTEREST

369 Increased tumor infiltration by cellular viroimmunotherapy: Mesenchymal stem cells as cell carriers for oncolytic virus

A. Morales-Molina[1], S. Gambera[1], T. Cejalvo[1], D. Kones[1], A.J. Perisé-Barrios[1], J. Garcia-Castro[1]

[1] Instituto de Salud Carlos III, Instituto de Investigacion en Enfermedades Raras IIER - Cellular Biotechnology Unit, Majadahonda, Spain

Oncolytic virotherapy is a novel therapeutic approach for the treatment of cancer that uses oncolytic viruses designed to selectively replicate in cancer cells. The use of cellular vehicles with migration ability to tumors has been considered to increase their delivery to target sites. Following this approach, the antitumoral efficacy of the treatment Celyvir –mesenchymal stem cells (MSCs) infected with the oncolytic adenovirus ICOVIR-5– has been demonstrated in patients with neuroblastoma, as well as in preclinical immunocompetent models. However, the better efficacy of syngeneic or allogenic MSCs as cell vehicles and the specific role of the immune system in this therapy are still unknown.

In this study we use our virotherapy Celyvir with syngeneic (C57BL/6) and allogeneic (C57BL/10) mouse MSCs (mMSCs) to determine their antitumor efficacy in a murine adenocarcinoma model using C57BL/6 mice. Tumor infiltration was studied by flow cytometry and immunohistochemistry. Adoptive transfer of splenocytes from treated mice to new tumor-bearing mice was performed, followed by a secondary adoptive transfer of splenocytes to a third group.

Similar reduction of tumor growth was observed in groups treated with syngeneic or allogeneic mMSCs infected with ICOVIR-5. Flow cytometry analysis of tumor-infiltrating immune cells (CD45+) presented no differences in the three groups. However, a different pattern of infiltration was observed by immunofluorescence in Celyvir-treated groups. Control non-treated tumors present a higher density of CD45+ cells in the periphery of the tumor. By contrast, Celyvir-treated group present a higher infiltration of CD45+ cells in the core of the tumor. It is well-known that higher infiltration in the tumor core induces a better antitumoral response in immunotherapies. Therefore, these result suggest that Celyvir induces a higher infiltration of immune cells in the tumor core that may cause a better antitumoral response. A similar antitumoral response was observed in both adoptive transfers of splenocytes from treated mice, despite no clear differences in localization of infiltrating-cells were observed in these tumors.

Our result show that Celyvir presents an antitumoral response with syngeneic or allogeneic mMSCs that could be induced by a higher tumor infiltration of immune cells in the tumor core.

NO CONFLICT OF INTEREST

370 Dual Inhibition of FLT3 and Src Pathways by ON150030, a Type 1 Inhibitor, as a Novel Strategy for Relapsed and Refractory AML

H. Ghaffari[1], M.V.R. Reddy[2], S. Cosenza[1], R. Vasques del Carpio[1], E.P. Reddy[1]

[1] Mount Sinai Medical Center, Oncological Sciences, New York, USA
[2] Mount Sinai Medical Center, Department of Pharmacological Sciences, New York, USA

Approximately one third of patients suffering from Acute Myeloid Leukemia harbor a FLT3 internal tandem duplication mutation (FLT3-ITD). When mutated, this receptor tyrosine kinase promotes pathways for proliferation and blocks apoptosis. Quizartinib is a 2nd generation FLT3 inhibitor that inhibits FLT3-ITD in AML, but has a median duration response of 12.1 weeks. Studies revealed a secondary substitution mutation in FLT3 at the D835 residue is responsible for relapse because it renders FLT3 constitutively active. Type 2 inhibitors like Quizartinib bind to FLT3 in its inactive state and fail to inhibit FLT3-ITD harboring a D835 mutation. Here, we tested the utility of ON150030, developed by our group, as a novel therapeutic agent to treat AML. Structural studies suggest ON150030 binds to the active form of FLT3 (Type 1 inhibitor) so mutations such as D835X do not affect the inhibitory activity of the compound. In vitro kinase assays demonstrate that ON150030 potently inhibits wildtype and FLT3-D835Y forms, while Quizartinib fails to inhibit FLT3-D835Y. Additionally, ON150030 demonstrated time and temperature dependent inhibition of FLT3, suggesting that the compound could be an irreversible inhibitor of FLT3. Biological studies reveal that ON150030 specifically inhibits the growth of MV4-11 cells harboring the FLT3-ITD mutation (GI50: 10nM). Western blot analysis demonstrates that MAPK and PI3K/AKT pathways in these cells are inhibited by ON150030. JAK independent phosphorylation of STAT5 seen in the context of FLT3-ITD is also reduced in response to ON150030. Mouse xenograft models were used to assess the drug's effect in vivo. Animals treated with ON150030 exhibited dramatic regression of subcutaneous tumors compared to control. Future goals are to use mouse myeloid cells expressing FLT3 and FLT3 mutants to examine the role of FLT3 mutants in inducing IL-3 independence and GCSF sensitivity, and how inhibition of FLT3 affects these processes. ON150030 inhibits SRC, which has been shown to induce resistance to targeted therapies in AML. We will introduce SRC into the cell

lines sensitive to ON150030 and test whether these cells retain their sensitivity to the drug. Next, we will perform cytotoxicity and biochemical assays on patient-derived primary AML cells treated with ON150030. At the conclusion of this project, we hope to demonstrate that ON150030 can be used in all AML patients harboring a FLT3 mutation, and result in sustained remission of disease.

CONFLICT OF INTEREST

Board of Directors: Dr. Premkumar Reddy is on the board of directors at Onconova Therapeutics Inc. Corporate-sponsored Research: At its early stages, this project used funding from Onconova Therapeutics Inc. It's no longer using funds from Onconova Therapeutics Inc.

371 Metronomic topotecan causes mycn inactivation and impedes tumor growth selectively in mycn-amplified neuroblastoma cells in vitro and in vivo by therapy induced senescence

S. Taschner-Mandl[1], M. Schwarz[1], T. Gerber[1], J. Blaha[1], T. Weiss[1], F. Kromp[1], F. Rifatbegovic[1], R. Ladenstein[2], M. Kauer[3], M. Hohenegger[4], I.M. Ambros[1], P.F. Ambros[1]

[1] Children's Cancer Research Institute, Dep. for Tumour Biology, VIENNA, Austria
[2] Children's Cancer Research Institute, Studies & Statistics for Integrated Research and Projects, VIENNA, Austria
[3] Children's Cancer Research Institute, Bioinformatics Core Facility, VIENNA, Austria
[4] Medical University of Vienna, Inst. of Pharmacology, Vienna, Austria

BACKGROUND: Poor prognosis and frequent relapses are major challenges for patients with high-risk neuroblastoma (NB), especially when tumors show MYCN amplification. High-dose chemotherapy triggers apoptosis, necrosis and senescence, a cellular stress response leading to permanent proliferative arrest and a typical senescence-associated secretome (SASP). SASP components reinforce growth-arrest and act immune-stimulatory, while others are tumor-promoting. Studies on low-dose long-term, i.e. metronomic, treatment schedules have demonstrated effects on the tumor microenvironment, but have not comprehensively analysed cellular responses in tumor cells.

Methods and result We evaluated efficacy and mode-of-action of low-dose metronomic, treatment schedules in in vitro and in vivo NB models. And importantly, by using the secretome as a discriminator for beneficial versus adverse effects of senescence, drugs with a tumor-inhibiting SASP were identified. Our preclinical work has demonstrated that low-dose long-term topotecan (TPT) treatment (0.1 mg/kg/d, i.p., daily over 15 weeks) improves survival in mouse NB xenografts and leads to long-term cure in 40% of mice. TPT induces a tumor-inhibiting type of senescence, accompanied by changes in the tumor transcriptome and secretome, specifically in MYCN-amplified NB cells. As senescent NB cells showed a reduced MYCN copy number and strongly down-regulated MYCN expression, we investigated the mechanism of MYCN inactivation. This showed that upon senescence MYCN is epigenetically silenced and gene copies are recruited to the nuclear periphery where changes in the nuclear lamina composition take place.

CONCLUSION: This new mode-of-action of metronomic TPT treatment, i.e. promoting a tumor-inhibiting type of senescence and silencing the oncogenic driver gene, is clinically relevant as metronomic regimens are increasingly implemented in therapy protocols of various cancer entities and are considered as a feasible maintenance treatment option with moderate adverse event profiles.

NO CONFLICT OF INTEREST

372 miRNAs and lncRNAs as molecular biomarkers of response to Sorafenib in human hepatocellular carcinoma cells

T. Faranda[1], G. De Petro[1], A. Salvi[1]

[1] Department of Molecular and Translational Medicine- Division of Biology and Genetics- University of Brescia- Brescia- Italy, Department of Molecular and Translational Medicine, Brescia, Italy

INTRODUCTION: Hepatocellular carcinoma (HCC) is the most frequent malignant tumor of the liver and it is the third most lethal malignancy on the global scale. Often patients with HCC are diagnosed in advanced stages and the poor prognosis is, in part, related to the lack of effective therapeutic agents for inoperable tumors. Sorafenib is the only drug currently approved by the FDA for the treatment of advanced HCC. It is an oral multikinase inhibitor that exerts anti-angiogenic and anti-tumor effects by blocking different growth factors pathways. Moreover recently, ncRNAs have emerged as key regulators of gene expression and appear to play vital roles in human tumorigenesis. The ncRNAs have been grouped into two main classes: small non-coding RNAs (<20nt) including microRNAs (miRNAs), better described, and the long non-coding RNAs (lncRNAs) (> 200nt) that have recently been characterized and include lincRNAs, intronic RNAs, circRNAs, ceRNAs, UCRs, asRNA.

MATERIAL AND METHODS: In this study, we have examined the expression profiles of different miRNAs and lncRNAs in HCC cell line (HA22T/VGH) treated and untreated with Sorafenib using miScript miRNA PCR Array (Qiagen) and RT[2] lncRNA PCR Array (Qiagen). These arrays analyze 84 miRNAs and 84 lncRNAs respectively. The result were elaborated by Web-based Data Analysis Software (Qiagen). The data were validated by qPCR.

RESULTS AND DISCUSSION: Among the miRNAs and lncRNAs examined the data analysis software identified 3 miRNAs and 2 lncRNA that were up-regulated and 1 miRNAs and 17 lncRNAs down-regulated (p value <0.05 in Sorafenib treated cells compared to control cells). We pointed out the attention to those miRNAs and lncRNAs that showed an opposite trend of expression compared to the expression levels of HCC samples, as verified in literature or in bioinformatic databases, and we assume that Sorafenib may mediate such expression changes. 2 miRNAs and 3 lncRNAs (both up- and down-regulated) have been chosen to validate the array data by qPCR. Further in vitro studies are ongoing on different cellular models of HCC treated and untreated with Sorafenib to compare the effects on miRNA/lncRNA expression.

CONCLUSION: Our study provide the bases for the identification of possible molecular markers associated with response and/or resistance to Sorafenib. Increasing knowledge in this context could offer the opportunity to improve the result of the use of Sorafenib in clinic.

NO CONFLICT OF INTEREST

373 Inhibition of bone marrow-derived mesenchymal stem cells homing towards breast cancer microenvironment using an anti-PDGFRβ aptamer

B. Hill[1], S. Camorani[2], A. Greco[3], N. Passaro[1], M. Gramanzini[1], L. Auletta[4], S. Gargiulo[1], S. Albanese[1], L. Cerchia[2], A. Zannetti[1]

[1] CNR, Istituto di Biostrutture e Bioimmagini, Napoli, Italy
[2] CNR, Istituto per l'Endocrinologia e l'Oncologia Sperimentale "G. Salvatore", Napoli, Italy
[3] Università degli Studi di Napoli "Federico II", Dipartimento di Scienze Biomediche Avanzate, Napoli, Italy
[4] IRCCS SDN, IRCCS SDN, Napoli, Italy

INTRODUCTION: Bone marrow-derived mesenchymal stem cells (BM-MSCs) are shown to participate in tumor progression by establishing a favourable tumor microenvironment (TME) that promote metastasis through a cytokine networks. However, the mechanism of homing and recruitment of BM-MSCs into tumors and their potential role in malignant tissue progression is poorly understood and controversial. Therefore, the aim of this study was to inhibit BM-MSC recruitment and their activity in triple-negative breast cancer (TNBC) TME through modulation of PDGFRβ signalling using a specific anti-PDGFRβ aptamer (Gint4.T).

MATERIAL AND METHODS: to investigate whether BM-MSCs have the ability to promote migration and invasion of TNBC cells, MDA-MB-231 and BT-549, a trans-well assay was performed, in which each cancer cell line was exposed to BM-MSCs, added in the lower chamber. Furthermore, we analyzed stem cell markers, such as Sox2 and Nanog in TNBC cells co-cultured with BM-MSCs. PDGFRβ downstream signalling pathways were investigated in BM-MSCs after Gint4.T treatment. Migration and trans-differentiation into cancer associated fibroblasts (CAFs) of BM-MSCs co-cultured with TNBC cells, were assessed in transwell chambers cells, in presence or absence of Gint4.T. To follow the homing of BM-MSCs towards TNBC in vivo, nude mice bearing MDA-MB-231 xenografts, were intravenously injected with VivoTrack 680-labeled BM-MSCs, treated with Gint4.T or a scrambled aptamer, and analyzed by Fluorescence Molecular Tomography.

RESULTS: we found that BM-MSCs increase aggressiveness of TNBC cell lines evaluated as capability to migrate, invade and acquire stemness markers. Importantly, we demonstrate that the treatment of BM-MSCs with a nuclease-resistant RNA aptamer against PDGFRβ causes the inhibition of receptor-dependent signalling pathways thus drastically hampering BM-MSC recruitment towards TNBC cell lines and BM-MSCs trans-differentiation into CAF-like cells. Moreover, in vivo molecular imaging analysis demonstrated the aptamer's ability to prevent BM-MSCs homing to TNBC xenografts.

CONCLUSIONS: our findings suggest a novel therapeutic agent, that targeting PDGFRβ, interferes either with the recruitment of BM-MSCs into TME and with tumor cell-stroma interactions thus potentially hampering TNBC aggressiveness.

NO CONFLICT OF INTEREST

374 Loss of HER2 and gain of tumor aggressiveness in HER2 positive mammary tumors: To go beyond anti-HER2 therapy

A. Palladini[1], V. Giusti[2], L. Landuzzi[2], M.L. Ianzano[1], A. Lamolinara[3], M. Dall' Ora[1], T. Balboni[3], R. Laranga[1], P. Nanni[2], P.L. Lollini[1]

[1] University of Bologna, Department of Experimental- Diagnostic and Specialty Medicine, Bologna, Italy
[2] Rizzoli Orthopedic Institute, Laboratory of Experimental Oncology, Bologna, Italy
[3] "Gabriele d'Annunzio" University, Aging Research Centre, Chieti, Italy

Human HER2 positive tumors can undergo loss of HER2 expression in recurrences or metastases. We found that loss of HER2 expression can occur in vivo and in vitro also in a transgenic mouse model harboring the human HER2 gene.

We studied this process taking advantage of two representative cell lines. MAMBO43 cell line, derived from an HER2 positive tumor, showed an epithelial phenotype and was HER2 positive. These cells were able to lose HER2 in vitro, after long-term trastuzumab treatment, or in vivo transplant. In fact MAMBO38 cell line derived from in vivo growth of MAMBO43 cell line but showed an elongated phenotype and was HER2 negative. All phenotypes discussed below for MAMBO38 cells were also obtained in vitro after trastuzumab treatment of MAMBO43 cells.

Tumor growth of MAMBO38 cells was faster than MAMBO43. MAMBO38 cells induced a high lung metastatic burden whereas no metastases were observed after MAMBO43 i.v. injection. Hence HER2 loss increased the tumorigenic and metastatic phenotypes.

MAMBO38 cells were CD24-CD44+, unlike MAMBO43 cells. The higher stemness of MAMBO38 cells could justify their gain of aggressiveness.

HER2 loss was also associated with the epithelial to mesenchymal transition (EMT). MAMBO38 cells expressed PDGFRB whereas MAMBO43 did not. Sunitinib is a drug that targets PDGFRB and other RTKs. Under conditions promoting HER2 loss, sunitinib was able to inhibit tumor growth. Thus PDGFRB was a potential target to limit the progression from an HER2 positive to an HER2 negative phenotype.

Despite HER2 loss, MAMBO38 cells maintained a strong activation of PI3K signalling. The inhibition of this pathway by GNE-317 reduced in vitro growth of MAMBO38 cells. Moreover, the drug blocked in vivo growth of MAMBO43 cells and inhibited MAMBO38 tumor formation. PI3K inhibitors could be an effective therapeutic strategy in presence of HER2 loss.

In conclusion, this dynamic model of HER2 loss can lead to the identification of relevant molecular mechanisms and to the development of therapeutic strategies to counteract resistance to HER2 target therapeutic agents.

FUNDING: Italian Association for Cancer Research (AIRC) (IG15324 to P-L. Lollini); Department of Experimental, Diagnostic and Specialty Medicine of the University of Bologna (DIMES) ("Pallotti" Fund).

NO CONFLICT OF INTEREST

375 Metronomic combination of 5-Flurouracil and Vinorelbine reveals low-grade toxicity on Human Umbilical Vein Endothelial Cells and Triple Negative Breast Cancer cells. The VICTOR-0 proof-of-concept study

M.G. Cerrito[1], M. De Giorgi[1], D. Pelizzoni[2], N. Digiacomo[2], M. Lavitrano[1], R. Giovannoni[1], M. Cazzaniga[2]

[1] University of Milano-Bicocca, School of Medicine and Surgery, Monza MB, Italy
[2] ASST- Monza, Oncology Unit, Monza MB, Italy

BACKGROUND: Triple Negative Breast Cancer (TNBC) is an aggressive neoplasia with median Overall Survival less than two years. Despite the availability of new drugs, the chance of survival of these patients (pts) did not increase. The combination of low doses of drugs in a metronomic schedule showed efficacy in clinical trials, exhibiting an anti-angiogenic and anti-tumour activity. In VICTOR-2 study we recently evaluated a new metronomic combination (mCHT) of Capecitabine 500 mg 3 times a day and Vinorelbine 40 mg 3 times a week continuously in HER2-ve breast cancer pts showing a disease control rate (CR+PR+SD) of 53.6% with a median PFS of 4.7 months in 28 TNBC pts. In this study (VICTOR-0) we evaluated the effect of mCHT vs standard schedule in TNBC and endothelial cells treated with 5-Flurouracil (5FU) and Vinorelbine (VRL).

MATERIALS AND METHODS: Cell viability and cytotoxicity assays were performed on MDA-MB-231 and human Umbilical Vein Endothelial (HUVEC) cell lines. Cells were exposed to different concentrations of 5FU (10nM-200mM) and of VNR (0,01nM-1mM) for 4 and 96 hours. To simulate the metronomic dosing schedule, we replaced the drug-enriched medium every 24h, while to simulate the standard administration regimen, cells were exposed to VRL/5FU for 4h, and then the medium was replaced with fresh medium without drug every 24h. The concentration of drug that reduced cell proliferation by 50% (IC_{50}) vs. controls was calculated by non-linear regression fit of the mean values of data obtained in triplicate experiments. VNR and 5FU were given simultanously at concentrations corresponding to their IC_{50} or alternatively to 50% or 25% of their IC_{50}.

RESULTS: A significant anti-proliferative activity was observed on HUVEC and MDA cells treated with metronomic VRL/5FU administration. The IC_{50} at 96h was a couple orders of magnitude lower in both HUVEC and MDA treated with VRL/5FU in metronomic schedule compared to exposure at conventional concentrations. Noteworthy, the concentration of combined drugs used to obtain the same effect observed after 96h in metronomic schedule with the conventional treatment induced cytotoxicity and cell death in MDA as well as in HUVEC. This phenomenon could resemble the in vivo settings in which the side effects are often associated to maximum tolerated dose chemotherapies.

CONCLUSIONS: These result are consistent with the hypothesis that the metronomic schedule of VRL/5FU have effect on both vascular and cancer cells.

NO CONFLICT OF INTEREST

376 Inecalcitol induces CD38 expression in multiple myeloma cell lines

E. Menoret[1], C. Kervoelen[1], C. Planquette[2], R. Delansorne[2]

[1] Myelomax, Research Department, Nantes, France
[2] Hybrigenics, Research Department, Paris, France

Targeting CD38 has proven to be an effective strategy for the treatment of multiple myeloma (MM), with the approval of the first therapeutic anti-CD38 monoclonal antibody: daratumumab. Interestingly, CD38 expression levels on MM cells, before start of treatment by daratumumab, were significantly higher in patients who achieved at least partial response (PR) as compared to patients who achieved less than PR (Nijhof IS et al., 2016, Blood). Therefore, increasing the levels of CD38 expression is the next logical pharmacological step to enhance the efficacy of anti-CD38 therapy.

In this context, inecalcitol (INE: 14epi-,19nor-,23yne-,1,25dihydroxy-cholecalciferol) has already been demonstrated to stimulate CD38 expression levels in two MM cell lines (ASH 2016, Abstract#3521). The aim of the present study was to screen a large collection of 38 human MM cell lines representative of chromosomal translocations involving different partners (CCND1, c-MAF or MAFB, MMSET and FGFR3, CCND3). After culture for 72 hours with or without 10 nM INE, cell surface CD38 labeling was measured with a fluorescent anti-human CD38 mouse FITC antiboby (eBiosciences, 11-0389-41). On 3 independent experimental days, cells from 3 different wells of either control (ethanol 0.1% v/v) or treated conditions were analyzed by flow cytometry to measure mean fluorescence intensity (MFI). Paired Student's t test was performed to check the unilateral hypothesis that INE had stimulated CD38 expression.

Basal control CD38 labeling was heterogeneous among the 38 MM cell lines and ranged from minimal (MFI < 150) to very high (MFI > 10,000). Only U266 remained totally insensitive to INE. JIM3 and XG5 showed a heterogenous increase in CD38 labeling. The stimulation of CD38 expression by INE reached statistical significance in all the other 35 MM cell lines, with a mean ± SEM of [5.2 ± 1.38]-fold increase in MFI.

Inecalcitol is a vitamin D receptor agonist characterized by a high anti-proliferative effect and a low calcemic potential (Okamoto R et al., 2012, Int J Cancer; Ma Y et al., 2013, Cell Cycle), allowing its administration at high oral doses to human cancer patients (Medioni J et al, 2014, Clin Cancer Res). The present findings show that INE consistently induces CD38 in 35 out of 38 human MM cell lines (92%), and suggest that INE could optimize the clinical response of a vast majority of MM patients, independently of their molecular subgroups, to anti-CD38 antibodies such as daratumumab.

CONFLICT OF INTEREST

Ownership: RD owns 0.8% of Hybrigenics' shares
Board of Directors: RD is a Director of Hybrigenics' Board
Corporate-sponsored Research: Hybrigenics sponsored Myelomax' research

377 Targeted transduction of cancer cells with cytopathic alphavirus coupled to magnetic nanoparticles

A. Zajakina[1], J. Sorokina[1], O. Trofimova[1]

[1] Latvian Biomedical Research and Study Centre, Cancer Gene Therapy group, Riga, Latvia

INTRODUCTION: Alphaviruses are potential cancer gene therapy vectors capable of providing a high expression of immunomodulating proteins in mammalian cells. However, suboptimal transduction levels in several cancer cell types as well as a broad biodistribution of alphaviruses have been observed after in vivo applications. Magnetic nanoparticles (MNPs) have been shown to increase cell transduction with several viral vectors in vitro under an external magnetic field and enhance magnetically guided viral vector delivery in vivo.

METHODS AND RESULTS: In this work, we examined a panel of MNPs for enhanced cancer cell transduction with Semliki Forest virus and Sindbis virus vectors. Magnetotransduction using positively charged MNPs increased alphavirus transduction in TS/A and 4T1 mouse mammary carcinoma cells in vitro in the presence of fetal bovine serum, known cell transduction inhibition factor. Moreover, positively charged MNPs efficiently captured virus particles independently of capturing medium, and MNPs-virus complexes were successfully separated from suspension by magnetic precipitation.

CONCLUSIONS: The enhanced cancer cell transduction by alphavirus/MNPs complexes under magnetic field holds promise for future applications such as targeted and enhanced virus-based gene therapy with simultaneous tracing of therapeutics by magnetic resonance imaging. Moreover, this study suggests alphavirus capture by positively charged MNPs could represent a rapid and efficient way to isolate, purify and concentrate virus particles by magnetic precipitation independently of capturing medium for preclinical and clinical applications.

NO CONFLICT OF INTEREST

378 p65BTK as a novel therapeutic target in ovarian cancer

D. Conconi[1], R. Fruscio[2], A. Cialdella[1], G. Romano[1], A. Decio[2], G. Damia[2], R. Giavazzi[2], R. Giovannoni[1], M. Lavitrano[1], E. Grassilli[1]

[1] University of Milano-Bicocca, School of Medicine and Surgery, MONZA, Italy
[2] Mario Negri Institute of Pharmacological Researches, Dept. of Oncology, Milano, Italy

INTRODUCTION: Ovarian cancer is one of the most lethal gynecological cancer and the majority of patients die within 5 years, mainly due to diagnosis at advanced stage and chemoresistance. We recently identified a novel oncogenic isoform of the Bruton's kinase (p65BTK) and showed that its targeting restore the response to chemotherapy in drug-resistant colon cancers. Here, we investigated whether p65BTK might be a therapeutic target also in ovarian cancer.

MATERIALS AND METHODS: Anti-p65BTK specific polyclonal antibodies were used to study p65BTK expression by immunohistochemistry (IHC) in Patient-Derived-Xenografts (PDX) tissues and by Western blot in cell lines. RT-PCR analysis was used to confirm p65BTK expression in ovarian cancer cell lines and cells from patients' tissue. Effect of standard of care (SOC) drugs used in therapy (paclitaxel, carboplatin and bevacizumab) and/or BTK inhibitors (Ibrutinib, AVL-292, RN486) on cell viability of ovarian cancer cell lines and primary coltures from patients' or PDX-derived cancer tissues was assessed by MTT or CellTiter-Glo® Luminescent Cell

Viability Assay; long-term cell viability/clonogenicity was assessed by soft agar and colony assay.

RESULTS AND DISCUSSION: We found that p65BTK isoform is expressed in all specimens of ovarian cancer examined. IHC on 36 PDX showed that in the majority of tissues (70%) p65BTK was expressed in more that 50% of cells. Treatement for 72 hs of Caov-3 and OVCAR-3 cell lines or PDX-derived cells with either reversible or irreversible BTK inhibitors or SOC drugs showed that p65BTK inhibition, at variance with SOC, significantly decreases cell proliferation. Moreover, BTK inhibitors also effectively impaired long-term proliferation and clonogenicity. Sensitivity/resistance to BTK inhibition and/or to SOC drugs clinically used as 1st line treatment was assessed on primary cell coltures derived from tissues of 59 patients by testing viability after 72 hs treatment by means of MTT assay. Notably, IBR - the only BTK inhibitor already in the clinic - was effective in inhibiting cell proliferation in 31 patients' samples, 19 of which resistant to SOC drugs treatment.

CONCLUSIONS: Our data indicate that p65BTK can be considered as a novel therapeutic target in ovarian cancer. In particular the finding that IBR has a better anti-proliferative effect than SOC therapy on primary cell coltures from patients' ovarian cancer tissue paves the way for future clinical trials.

NO CONFLICT OF INTEREST

379 The structure-based design, synthesis, and evaluation of potent dual BET-JAK2 inhibitors as a new anticancer therapeutic strategy

N. Lawrence[1], *H. Lawrence*[2], *S. Gunawan*[1], *M. Ayaz*[2], *S. Ember*[1], *J.Y. Zhu*[1], *N. Berndt*[1], *M. Tauro*[3], *C. Lynch*[3], *G. Reuther*[4], *E. Schonbrunn*[1]

[1] *Moffitt Cancer Center, Drug Discovery, Tampa- FL, USA*
[2] *Moffitt Cancer Center, Chemical Biology Core, Tampa- FL, USA*
[3] *Moffitt Cancer Center, Tumor Biology, Tampa- FL, USA*
[4] *Moffitt Cancer Center, Molecular Oncology, Tampa- FL, USA*

BACKGROUND: Bromodomain (BRD)-containing proteins are essential for the recognition of acetylated lysine (KAc) residues of histones during transcriptional activation. Targeting BRD4 [a member of bromodomain and extra-terminal (BET) protein family] with small molecules represents a new way to treat prostate and breast cancer, acute myeloid leukemia (AML), and melanoma. We hypothesize that dual inhibition of BRD4 (which regulates oncogenes e.g. c-Myc) and oncogenic kinases will improve anticancer responses. Several kinase inhibitors that also inhibit BRD4 were identified by co-crystallization screening of kinase inhibitor libraries and serve as starting points for this project.

METHODS: A library of dual BRD4-JAK2 inhibitors, derived from the screening hits, has been designed by structure-based methods and prepared using a range of synthetic chemistry methods. The co-crystal structures of our BRD4-JAK2 inhibitors revealed the inhibitors bound to the KAc site of BRD4-1. The inhibitors were subjected to differential scanning fluorimetry (DSF) and AlphaScreen assay to assess their binding and inhibitory potentials against BRD4.

RESULTS: We report the design, synthesis, structural and preliminary biological analysis of next-generation nanomolar BET-selective and nanomolar dual-activity BET-JAK2 inhibitors, based on the initial co-crystallization screening hits. Structure-activity relationships were developed using DSF and co-crystallization of the inhibitors with BRD4. Several potent BRD4-JAK2 inhibitors have higher BRD4 activity compared to the widely used BRD4 inhibitor JQ1. Additionally, MM.1S cell survival by MTT assay showed good activity with IC_{50} of < 100 nM. Screening against a large panel of cancer cell lines revealed differential growth inhibitory potential, with high activity against bone and blood cancers. Lead compounds exhibit good solubility, high stability in human plasma ($t_{½}$ > 24 h) and other drug-like property assays and significant activity in multiple myeloma mouse models.

CONCLUSIONS: Several highly potent dual BRD4-JAK2 inhibitors have been developed that serve as lead dual targeting anticancer agents. The dual acting agents have the potential for the treatment of cancers with resistance to single activity kinase or BET inhibitors.

CONFLICT OF INTEREST

Ownership: The Moffitt Cancer Center has filed patent applications on compounds described. These have been licensed to Aptose Biosciences Inc.

380 "Blood-brain-barrier spheroids" maintain key barrier characteristics in vitro: A screening platform for brain-penetrating agents

C.F. Cho[1], *J. Wolfe*[2], *C. Fadzen*[2], *D. Calligaris*[1], *K. Hornburg*[2], *E.A. Chiocca*[1], *N. Agar*[1], *B. Pentelute*[2], *S. Lawler*[1]

[1] *Harvard Medical School, Neurosurgery, Boston, USA*
[2] *MIT, Chemistry, Cambridge, USA*

BACKGROUND: The inability of most drugs to cross the blood-brain-barrier (BBB) is a major barrier to effective brain cancer treatment. Therefore, there is an urgent need for the development of next-generation therapeutics with improved brain delivery. In vitro BBB models are crucial tools to enable rapid screening and identification of brain-penetrating drugs. However, reproducibility of in vitro barrier properties, permeability, and ease of culture remain as major challenges. For the first time, we describe here a multicellular BBB spheroid model to enable high-throughput screening.

METHODS: A BBB spheroid was produced through co-culturing brain endothelial cells (ECs), pericytes and astrocytes under low-adhering conditions. These cells self-assemble into a spheroid where the ECs encased the outer surface, acting as a barrier to regulate molecular transport into the spheroid. To test for BBB penetration, the spheroids were incubated with a variety of compounds and the level of influx into the spheroid was assessed by either confocal microscopy or MALDI mass spectrometry imaging. **result** We found that the outer surface of the spheroids formed a tight barrier which can be permeabilized by VEGF. The barrier was also characterized by the presence of intact tight junctions and efflux-pump activity (i.e., P-glycoprotein). Furthermore, we used this model to successfully demonstrate the transport of angiopep-2 (a well-known brain delivery vector) and its conjugates (containing cargoes of various sizes such as peptide, protein and affibody), as well as BKM120 (a BBB-penetrant small molecule that inhibits phosphatidylinositol 3-kinase (PI3K)), thereby displaying the versatility of this model to screen and study a wide range of therapeutic agents. We demonstrated that this model is superior to the conventional transwell model in maintaining essential BBB characteristics (i.e., tight/adherens junctions and P-glycoprotein expression) and in screening for BBB-penetrating compounds. Finally, we utilized the spheroid model to screen a panel of 16 cell-penetrating peptides (CPPs) to identify several candidates with high brain-penetration potential. We then verified the ability of the top 4 candidate CPPs to cross the BBB in mice.

CONCLUSIONS: This facile but robust model can lead to better design and analysis of first-in-class glioma therapeutics, and improve prediction of drug penetration in a living model, paving the way for breakthrough discoveries in brain cancer.

NO CONFLICT OF INTEREST

381 FOXO1 promotes resistance of Non-Hodgkin lymphomas to anti-CD20-based therapy

A. Zerrouqi[1], *B. Pyrzynska*[1], *M. Dwojak*[1], *P. Zapala*[1], *N. Miazek*[1], *M.M. Machnicki*[1], *J. Golab*[1], *M. Winiarska*[1]

[1] *Medical University of Warsaw, Dept. of Immunology, Warsaw, Poland*

Lack of remission or early relapse remains a major clinical concern in diffuse large B cell lymphoma (DLBCL) with a third of patients failing to respond to current regimens or relapsing with resistant disease. One explanation for such incomplete therapeutic success is the considerable heterogeneity of these tumors and the high degree of genomic lesions (point mutations, copy number, translocations) across patients. Genome and transcriptome sequencing studies have identified FOXO1 as one of the recurrent target of somatic mutations in DLBCL (Morin RD et al, Nature, 2011), associated with short survival of patients. These mutations enhance FOXO1 nuclear localization and activity.

Herein, we determine the role of FOXO1 (wild-type and mutated) on the expression of CD20, the target for rituximab, using flow cytometry. CD20 promoter activity was assessed using luciferase reporter, while FOXO1 binding to the CD20 promoter was studied by in Silico analysis of a publicly available ChIP-Seq data and confirmed by both EMSA and ChIP experiments.

Our result show that the activation of FOXO1 using AKT inhibitors (MK-2206 and GDC-0068) led to a marked decrease of CD20 transcript and protein levels, which resulted in a significant resistance to rituximab-mediated complement-dependent cytotoxicity (CDC). Consistently, the expression of FOXO1-AAA mutant (resistant to inhibition by AKT) repressed CD20 to higher extent than the overexpression of wild-type FOXO1. Furthermore, CD20 levels remained high and unchanged by the DNA-binding defective mutant of FOXO1-H215R, suggesting that FOXO1 mediates regulation of CD20 transcription through binding to CD20 promoter, as confirmed by ChIP, EMSA and the analysis of ChIP-Seq data.

In summary, our data highlights the significance of mutations of FOXO1 in non-Hodgkin lymphomas and complement the clinical studies associating FOXO1 mutations to the poor outcome of Rituximab-based therapy. Our study provide a molecular mechanism by which FOXO1 mutations negatively modulate CD20 expression. Testing the effect of each individual point mutation of FOXO1, present in lymphoma specimens, on whole transcriptome would provide further insights on their contribution to DLBCL short survival and resistance to current therapies.

ACKNOWLEDGEMENT: abstract supported by National Science Center, Poland (NCN, grant no: 2013/11/B/NZ5/03240) and STREAM project (Horizon 2020, CSA action, grant no: 692180)

NO CONFLICT OF INTEREST

382 ERRα as new potential target against chemoresistance in human breast cancer

D.C. Belisario[1], *S. Doublier*[2], *I. Roato*[1], *D. Ghigo*[2], *A. Bosia*[2], *R. Ferracini*[1]

[1] *Ospedale S. Giovanni Battista Molinette, Oncologia- laboratori CERMS, Torino, Italy*
[2] *Department of Oncology, University of Torino, Torino, Italy*

INTRODUCTION: multidrug resistant (MDR) phenotype commonly develops in breast cancer cells after neoadjuvant chemotherapy. This phenotype is, at least in part, due to overexpression of P-glycoprotein (P-gp)/MDR1, an efflux pump of anticancer drugs and to hypoxia-inducible factor-1 (HIF-1), a master regulator of the adaptive response to hypoxia. We hypothesized that MDR1 gene transactivation by HIF-1 requires interaction with the estrogen-related receptor-α

(ERRα), a member of the group III nuclear receptor superfamily, that can increase HIF-1 transcriptional activity.

MATERIALS AND METHODS: 3-D spheroids are formed by culturing ER-positive MCF7 cells with human neutrophil elastase. Expression of HIF-1α, ERRα, were verified by Western blot analysis HIF-1 activation was measured by EMSA and ELISA. Modulation of P-gp expression was evaluated by FACS analysis and confocal microscopy assay. Intracellular doxorubicin accumulation was measured by spectrofluorimetric assay. HIF-1/ERRα interaction was evaluated by immunoprecipitation experiments. Binding of HIF-1α to MDR1 gene promoter was evaluated by ChIP assay

RESULTS: We demonstrated that the ER-positive MCF7 3-D spheroids are resistant to doxorubicin and that this resistance is associated with an increased P-gp expression at the plasma membrane via activation of HIF-1. Then, we showed that ERRα bound with HIF-1α in MCF-7 3-D spheroids and CoCl2-treated-SKBR3 (an hypoxia-mimicking condition). Silencing of ERRα, treatment with ERRα inhibitors, and with HIF-1α inhibitor completely abrogated the ERRα/HIF-1α complex formation, and the transcriptional activity of HIF-1, decreasing significantly the expression of P-gp on the cell surface and increases the intracellular doxorubicin accumulation in both MCF-7 3-D spheroids and CoCl2-treated-SKBR3, suggesting that ERRα plays an important role in development of MDR in breast cancer.

CONCLUSIONS: These result identify ERRα as a novel interacting protein with HIF-1α, which is crucial for the regulation of hypoxia-mediated gene transcription involved in the MDR phenotype, and suggest a potential mechanism to induce overexpression of P-gp in MCF-7 3-D spheroids suggesting that the pharmacological modulation of ERRα activity and/or expression may have therapeutic value in the treatment of breast cancer.

NO CONFLICT OF INTEREST

383 Pim kinase: A mechanism of resistance to PI3K-mTOR inhibition in NSCLC

K. Gately[1], *S. Heavey*[1], *S. Cuffe*[1], *S. Finn*[1], *M. O'Neill*[2], *G. Moore*[1]

[1] *Trinity College Dublin/St. James's Hospital, Clinical Medicine, Dublin 8 , Ireland*
[2] *Inflection Biosciences Ltd., Blackrock, Dublin, Ireland*

BACKGROUND: Activated PI3K-mTOR signalling has been implicated in the various hallmarks of cancer and this pathway is known to be dysregulated in several cancer types including non-small cell lung cancer (NSCLC). Efforts to therapeutically target the PI3K-mTOR pathway have shown promising clinical efficacy, however the inevitable emergence of drug resistance inhibits a durable response to treatment. We developed NSCLC cell line models of acquired resistance to PI3K-mTOR inhibitor GDC-0980 to pinpoint specific bypass mechanisms of resistance.

MATERIALS AND METHODS: Resistant cell line models were established using chronic treatments with IC50 drug doses. Alterations to mRNA/ miRNA expression profiles of GDC-0980 resistant cells, H1975GR versus age-matched parent cells H1975P were examined. mRNA expression was screened using an IL-6/STAT3 RT2 gene profiler array containing 84 key genes involved in activation and downstream effects of IL6/STAT3 signalling. Selected genes from the array were validated by qPCR and western blot analysis (n=3). miRNA expression was profiled using an Exiqon array (2100 miRNAs) and a miRNA signature was validated by qPCR. The effect of BEZ235 (PI3K-mTOR inhibitor) and a novel triple targeted therapy IBL301 (PI3K/mTOR/PIM kinase inhibitor) on cell viability and proliferation were measured in H1975GR versus H1975P by CellTitre Blue and BrdU assays respectively. result A number of genes with a 2-fold or more change in expression were identified and 7 of these genes were chosen for validation by qPCR (n=3). PIM1 was upregulated in GDC-0980 resistant cells ($p<0.05$) in addition to genes downstream of PI3K and PIM1: mTOR ($P<0.05$) and BCL-2 ($p<0.001$). TNF-α and its receptor co-stimulatory molecule CD40 were upregulated in GDC-0980 resistant H1975 ($p<0.05$ and $p<0.01$). Furthermore, the cell cycle inhibitor, CDKN1, and JAK-signalling blocker, SOCS1 were downregulated (both $p<0.01$). Elevated PIM1 kinase and other proteins were also validated. H1975GR were less sensitive to BEZ235 and more sensitive to IBL-301 compared to H1975P.

CONCLUSIONS: Our PI3K-mTOR inhibitor resistant NSCLC (H1975GR/P) cell line model demonstrates acquired resistance to both GDC-0980 and BEZ235. Alterations in IL-6/STAT3 signalling including PIM1 kinase provide an escape mechanism to PI3K-mTOR inhibition. A novel PI3K/mTOR/PIM inhibitor IBL301 has shown promising in vitro data that warrants further investigation as a multi-targeted therapeutic strategy for NSCLC.

CONFLICT OF INTEREST

Corporate-sponsored Research: This research was partly funded by a grant from Inflection Biosciences Ltd

384 Targeting the heparin-binding domain of fibroblast growth factor receptor 1 for cancer therapy

L. Ling[1], *V. Nurcombe*[1], *A. Van Wijnen*[2], *S. Cool*[1]

[1] *Agency for Science- Technology and Research, Institute of Medical Biology, Singapore, Singapore*
[2] *Mayo Clinic, Department of Orthopedic Surgery & Biochemistry and Molecular Biology, Rochester, USA*

INTRODUCTION: Aberrant activation of fibroblast growth factor receptors (FGFRs) deregulates cell growth and allows mutant cells escape from apoptosis, predisposing them to tumorigenesis. FGFRs have thus become important targets for intervention. As activation of FGFRs requires the interaction of their heparin-binding domains (HBDs) with heparan sulfate (HS), an abundant glycosaminoglycan on cell surfaces and extracellular matrix, we aimed to develop a neutralizing antibody against FGFR1 designed to prevent its interaction with HS.

MATERIAL AND METHODS: We first screened FGFR1 expression using immunohistochemical staining in 19 common types of human cancer. The levels of FGFR1 were also examined using Western blotting and Taqman QPCR analysis in a range of malignant cell lines. A neutralizing antisera was then manufactured targeting the HBD on FGFR1. Efficacy and specificity were assessed via ELISA and immunoprecipitation. Its anti-cancer efficacy was analysed using GUAVA EasyCyte Flow Cytometry and Annexin V-PI dual staining analysis to assess cancer cell growth rate and survival, respectively. The molecular mechanism of FGFR1 inactivation was explored via microarrays.

RESULT AND DISCUSSION: FGFR1 is significantly up-regulated in various tumors, including breast cancer, lung cancer and lymphoma. Cancer cells often expressed higher amount of FGFR1 than the normal cells from same tissue type. This again validates FGFR1 as an anti-cancer drug target. The FGFR1 antibody exhibited dose-dependent affinity to FGFR1 and minimal cross-reactivity to FGFR2 or FGFR3. It specifically inhibited heparin's interaction with FGFR1 and the kinase activity of the receptor. The basal and FGF2-induced growth of cancer cells were both blocked. Furthermore this antibody induced significant cell death, presumably through the disruption of antioxidative defense networks.

CONCLUSION: Our study supports the idea that FGF/FGFR play key roles in maintaining cancer cell survival and that disrupting HS interaction with extracellular onco-proteins is a promising strategy for cancer intervention.

NO CONFLICT OF INTEREST

386 Suppression of medulloblastoma lesions through forced migration of preneoplastic precursor cells by Cxcl3

M. Ceccarelli[1], *L. Micheli*[1], *F. Tirone*[1]

[1] *National Research Council- Fondazione Santa Lucia, Institute of Cell Biology and Neurobiology, Rome, Italy*

BACKGROUND: Medulloblastoma (MB), tumor of the cerebellum, remains a leading cause of cancer-related mortality in childhood. We previously showed, in a mouse model of spontaneous MB ($Ptch1^{+/-}/Tis21^{-/-}$), that a defect of the migration of cerebellar granule neuron precursor cells (GCPs) correlates with an increased frequency of MB. This occurs because GCPs, rather than migrating internally and differentiating, remain longer in the proliferative area at the cerebellar surface, becoming targets of transforming insults. Furthermore, we identified the chemokine Cxcl3 as responsible for the inward migration of GCPs. As it is known that preneoplastic GCPs (pGCPs) can still migrate and differentiate like normal GCPs, thus exiting the neoplastic program, in this study we tested the hypothesis that pGCPs within a MB lesion could be induced by Cxcl3 to migrate and differentiate.

MATERIAL AND METHOD: We have administered the chemokine Cxcl3 or the vehicle (cerebrospinal fluid solution) by Alzet osmotic minipumps in the cerebellum of the $Ptch1^{+/-}/Tis21^{-/-}$ mice, a mouse model that develops Shh-dependent MB at high frequency. The treatment lasted 2 or 4 weeks and started in 1-month-old $Ptch1^{+/-}/Tis21^{-/-}$ mice, i.e., at a stage when MB lesions are already formed and represent MB at its initial expansion. The proliferating or migrating pGCPs were identified as $BrdU^{+}$ cells, 1 hour or 5 days after an intraperitoneal injection of BrdU, respectively.

RESULTS AND DISCUSSION: We observed that the administration of Cxcl3 for 28 days within the cerebellum of 1-month-old $Ptch1^{+/-}/Tis21^{-/-}$ mice leads to complete disappearance of the lesions. However, a shorter treatment with Cxcl3 (2 weeks) was ineffective, suggesting that the suppression of MB lesions is dependent on the duration of Cxcl3 application. We verified that the treatment with Cxcl3 causes a massive migration of pGCPs from the lesion to the internal granular layer, where they differentiate.

CONCLUSION: Thus, the induction of migration of pGCPs in MB lesions may open new ways to treat MB that exploit the plasticity of the pGCPs, forcing their differentiation. It remains to be tested whether this plasticity continues at advanced stages of MB. If so, these findings would set a potential use of the chemokine Cxcl3 as therapeutic agent against MB development in human preclinical studies.

CONFLICT OF INTEREST

Other Substantive Relationships: A patent was filed by the National Research Council on the possible use of the chemokine Cxcl3 in medulloblastoma therapy. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

387 In vitro Cytotoxicity of Ethanol extracts of Euphorbia hirta on B16F10 human melanoma cell line

S.M. Mishra[1], *A. Pathak*[1]

[1] *barkatullah University, Pharmacy, bhopal, India*

BACKGROUND: Euphorbia hirta has many health benefits for enhancing human health. It exhibits various antioxidant properties. The current research evaluates with the objective to rationalize the cytotoxicity effect of E. hirta ethanolic extract

on B16F10 human melanoma cell line in accordance with the observable changes of cell morphology upon exposure to the extract.

MATERIAL AND METHODS: The cytotoxic activity of the Euphorbia hirta extract on B16F10 human melanoma cell line was investigated in vitro using 3- (4, 5-dimethyl thiazol-2yl) -2, 5-diphenyl tetrazolium bromide (MTT). B16F10 (human Melanoma) cell lines were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 mg/ml) and amphotericin B (5 mg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with Trypsin solution (0.2% trypsin, 0.02% EDTA in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtiter plates. After culturing and proliferation of B16F10 cells, these cells were exposed to the various ethanolic extract of E hirta. The potency of plant extract concentration was calculated in terms of percent decrease in viable B16F10 cells as compared to the control value.

RESULT: The potential use of Euphorbia hirta as therapeutic agent holds great promise as the isolation of one or more cytotoxic chemicals from crude extract and the judicious use of such chemicals can control the progression of cancer. The result of MTT assay test showed that the ethanol extract of Euphorbia hirta was tested for the anticancer activity and it showed most effective inhibition of B16F10 cells proliferation.

CONCLUSION: We assessed the cytotoxic effect of Euphorbia hirta extract in cell culture. In defiance of astonishing advances in modern medicine, such as surgery, radiotherapy, chemotherapy, and immunotherapy, cancer disease remains a worldwide health problem, thus endeavouring the search for the new alternate approach.

NO CONFLICT OF INTEREST

388 Single agent PI3K inhibitors induce robust prostate cancer cell death In vivo via microdevice implantation

A. Patnaik[1], S. Chen[2], M. Whitman[3], O. Jonas[3], R. Langer[3], M. Yaffe[4], G. Bubley[2], L. Cantley[5], S. Balk[2]

[1] *The University of Chicago/Beth Israel Deaconess Medical Center/Harvard Medical School, Medicine-Hematology/Oncology, Chicago- IL, USA*
[2] *Beth Israel Deaconess Medical Center/Harvard Medical School, Medicine-Hematology/Oncology, Boston, USA*
[3] *Massachusetts Institute of Technology, Biological Engineering, Boston, USA*
[4] *Massachusetts Institute of Technology, Biology, Boston, USA*
[5] *Weill Cornell Medical College, Meyer Cancer Center, New York, USA*

BACKGROUND: There has been considerable interest in targeting the PI3K pathway for cancer therapy. However, single-agent inhibitors that perturb the PI3K signaling pathway have demonstrated limited efficacy in clinical trials.

METHODS: We utilized a multi-dimensional approach to unravel the basis for the limited anti-cancer activity of buparlisib (pan-PI3K inhibitor) in prostate cancer mouse models and a human clinical trial in metastatic, castrate-resistant prostate cancer (mCRPC) patients. result We observed limited target inhibition and efficacy of systemic buparlisib administration at maximum tolerated doses in the prostate-specific PTEN/p53–deficient genetically engineered mouse model and human prostate cancer xenograft models. Moreover, p-AKT staining of metastatic bone biopsies from mCRPC patients treated with buparlisib showed minimal PI3K inhibition within the tumor-bone microenvironment. Strikingly, there was a profound induction of apoptosis when buparlisib was administered to prostate cancer xenograft tumors via an implantable microdevice utilized for in vivo drug sensitivity screening.

CONCLUSIONS: These data demonstrate that the limited efficacy of PI3K inhibitors in the clinic is not due to intrinsic drug resistance mechanisms, but rather an intratumoral pharmacodynamic failure to completely suppress the pathway, resulting in inadequate anti-tumor responses.

(Manuscript summarizing this work is currently in preparation)

NO CONFLICT OF INTEREST

389 Lithium chloride alters cell plasticity in primary colon cancer cell cultures

M. De Rosa[1], V. Costabile[1], S. Arpino[1], M. Turano[2], F. Duraturo[1], P. Delrio[3], D. Rega[3], U. Pace[3], C. Dodaro[4], P. Izzo[1]

[1] *University of Naples "Federico II", Department of Molecular Medicine and Medical Biotechnology, Naples, Italy*
[2] *University of Naples "Federico II", Department of Biology, Naples, Italy*
[3] *Istituto Nazionale per lo studio e la cura dei tumori- "Fondazione Giovanni Pascale" IRCCS-Naples, Colorectal Surgical Oncology, Naples, Italy*
[4] *University of Naples "Federico II", Department of Advanced Biomedical Sciences, Naples, Italy*

INTRODUCTION: Colorectal cancer (CRC) is one of the main causes of cancer deaths, with metastases representing the first cause of death. It has been recently demonstrated that epithelial to mesenchymal transition (EMT) could play a critical role in invasion and metastasis of many types of cancer, including CRCs. GSK3β is a multifunctional serine/threonine kinase that may act as anti or proapoptotic in a cellspecific manner (1).

METHODS: Here, we investigate GSK3β as a new druggable target and LiCl as new drug for cancer therapy. To this aim we isolated primary mesenchymal colorectal cancer cells from CRC patients and analysed them by using PCR, real time RT-PCR, immunofluorescence assay and western blot assay.

RESULTS AND DISCUSSION: We demonstrated that these cells expressed epithelial (E-Cadherin and Cytokeratins), mesenchymal (N-Cadherin and Vimentin) and stemness markers, together with high level of epithelial-mesenchymal transition-transcription factors (EMT-TFs) (Snail and Twist), suggesting that these were epithelial cells undergone EMT.

We demonstrated that LiCl was able to induce mesenchymal-epithelial reverting transition (MET) and differentiation in our cell cultures (2), also affecting cell migration.

Our cell cultures were also able to aggregate and form spheroids in suspension and this ability was affected when cells were incubated with LiCl. Furthermore, the inhibition of GSK-3β by LiCl, strongly down-regulated the expression of stem cell markers, such as Oct4, Sox2, Nanog, ALDH1, LGR5, and CD44, in cancer spheroids.

Finally, we demonstrated that LiCl affected cellular plasticity, an important feature in tumor progression and metastasis; indeed, after LiCl incubation cancer cells were unable to switch from one phenotype to another.

CONCLUSIONS: We have performed an experimental system of primary mesenchymal colorectal cancer cell cultures to study the role of EMT in CRCs and molecular basis of cell plasticity during cancer progression and acquisition of resistance to the therapy. Thus, we observed that LiCl induces MET and alters dynamic of cancer spheres formation, indicating a differentiated state that correlate with expression of undifferentiation/differentiation markers, suggesting that GSK-3β and LiCl could represent an eligible target and a potential drug for CRCs therapy.

REFERENCES:

1) De Rosa et al. Oncol Rep. 2015 Sep;34(3):1087-96.

2) Costabile et al. Int J Oncol. 2015 May;46(5):1913-23.

NO CONFLICT OF INTEREST

POSTER SESSION: MOLECULAR AND GENETIC EPIDEMIOLOGY

390 A polymorphism of VEGF -2489C>T is associated with prostate cancer susceptibility in Mexicans

A. Martínez-Rizo[1], M. Padilla-Cristerna[1], C. Peña-George[2], X. Casillas-Rangel[1], M.L. González-Carrillo[3], J. Velázquez-Fernández[4], J. Navarro-Partida[1]

[1] *Autonomous University of Nayarit, Laboratory of Biomedical Research, Tepic, Mexico*
[2] *Autonomous University of Nayarit, Academic Medicine Unit, Tepic, Mexico*
[3] *Mexican Social Security Institute, Delegation Nayarit, Tepic, Mexico*
[4] *Autonomous University of Nayarit, Laboratory of Toxicological Biochemistry, Tepic, Mexico*

INTRODUCTION: The VEGF has been implicated in the development and progression of prostate cancer as the main modulator of the angiogenic and mitogenic properties of vascular endothelial cells.

Aim: Investigate the association of polymorphisms in the VEGF gene on Prostate Cancer patients of western Mexico.

METHODS: Study of cases and controls. 219 patients with histologically confirmed cases of prostate cancer and 292 HBP as controls. Genotyping of VEGF gene polymorphisms at -1154G>A, -2489C>T, and −7 C>T was performed by real time PCR using TaqMan probes. Hardy Weinberg Equilibrium was calculated by chi-square ($\chi 2$) test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) for each SNP were calculated.

RESULTS: All analyzed SNP was in Hardy Weinberg equilibrium. No significant association was found between -1154G>A and −7 C>T VEGF polymorphisms and Prostate Cancer. The genotype frequencies of the -2489C>T were distributed differently in patients with prostate cancer C/C=72, C/T=112, T/T=31 vs. HBP group C/C=116, C/T=121, and T/T=53. The stratification for severity and progression not demonstrate association with any VEGF polymorphisms.

CONCLUSION: This study suggests that VEGF -2489C>T polymorphism is associated with PCa risk however further study is needed.

NO CONFLICT OF INTEREST

391 SOKAL & EUTOS scores are not predictive for clinical outcome in patients with chronic phase chronic myeloid leukemia treated with imatinib

N. Bhutani[1], S.A. Guru[2], P. Yadav[2], N. Gupta[3], A. Saxena[2]

[1] *vardhaman mahavir medical college, department of biochemistry, New Delhi, India*
[2] *Maulana azad medical college, department of biochemistry, new delhi, India*
[3] *Maulana azad medical college, department of medicine, new delhi, India*

BACKGROUND: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by the presence of Philadelphia chromosome (Ph) or BCR-ABL1 chimeric gene; which codes for an abnormal tyrosine kinase leading to malignant proliferation of myeloid cells.Imatinib,a selective inhibitor of this kinase,is the first choice of therapy in patients with CML. Sokal and EUTOS (European Treatment and Outcomes Study), scores are prognostic scores used for predicting response to therapy. This study aimed to determine the predictive value of these scoring systems on the response to imatinib in CML patients.

METHODOLOGY: 30 newly diagnosed BCR-ABL positive CML patients in chronic phase&30 healthy control subjects, all ethnic Indians, were recruited in the study. Patients underwent detailed clinical examination and hematological laboratory tests. Pre-treatment prognostic scores(Sokal score,EUTOS score) were calculated on the basis of spleen size,platelet count& DLC. After initiation of imatinib therapy,hematological response was monitored at regular intervals & molecular response (BCR-ABL1/ABL1 ratio) assessed after 6 or 12 months.

RESULTS AND DISCUSSION: Cases were divided into two groups,high risk(n=27) & low risk (n=3),based on EUTOS score.24(88.88%)patients with low risk achieved CHR by the end of 3 months,whereas 2(66.66%)with high risk had it(p=0.3596). Time to CHR was insignificantly higher in high risk patients as compared to low risk group(p=0.711).An optimal molecular response was seen in 55.55% low risk patients(n=15)&66.66 %(n=2) patients with high risk. In low risk group,18.51% patients had treatment failure(n=5) while 33.33%(n=1) high risk patients had it.(p=0.765).

Cases were divided into three groups, high risk (n=14), intermediate risk (n= 14) & low risk (n=2), based on Sokal score. All patients(100%) with low risk achieved CHR by the end of 3 months, whereas 12(85.71%) with high risk achieved it (p=1.0). Time to CHR was highest in intermediate risk patients (p=0.336). An optimal molecular response was seen in 50% of both low risk (n=1) &high risk (n=7) patients. In low risk group 50% patients had treatment failure(n=1)while7.14%(n=1) high risk patients had it.(p=0.127)

CONCLUSIONS: In this study,both Sokal and EUTOS score were not predictive for haematological and molecular response in CML patients in chronic phase treated with imatinib and seem to be inadequate.Better prognostic models need to be suggested for TKI therapy.

NO CONFLICT OF INTEREST

392 Effect of genetic variation in microRNA binding site in WNT1-inducible signaling pathway protein 1 gene on oral squamous cell carcinoma susceptibility

Y.E. Chou[1,2], S.F. Yang[2], C.W. Lin[3]

[1] Chung Shan Medical University, School of medicine, Taichung, Taiwan
[2] Chung Shan Medical University Hospital, Department of Medical Research, Taichung, Taiwan
[3] Chung Shan Medical University, Institute of Oral Sciences, Taichung, Taiwan

BACKGROUND: Oral squamous cell carcinoma (OSCC), which is the most common head and neck cancer, accounts for 1%–2% of all human malignancies and is characterized by poor prognosis and reduced survival rates. WNT1-inducible signaling pathway protein 1 (WISP1), a cysteine-rich protein belonging to the Cyr61, CTGF, Nov (CCN) family of matricellular proteins, has many developmental functions and may be involved in carcinogenesis. This study investigated WISP1 single-nucleotide polymorphisms (SNPs) to elucidate OSCC susceptibility and clinicopathologic characteristics.

MATERIAL AND METHODS: We genotyped 4 single nucleotide polymorphisms of WISP1 rs16893344, rs2977530, rs2977537, and rs2929970 from 900 OSCC patients and 1200 cancer-free controls. The association between WISP1 expression and WISP1 genetic polymorphism was analysed in the Encyclopedia of DNA Elements (ENCODE) data from the NCBI gene database and confirmed by quantitative real-time PCR.

RESULTS: The WISP1 rs2929970 polymorphism carriers with at least one G allele were susceptible to OSCC. Moreover, compared with wild-type carriers, we observed that among 100 nonsmoker OSCC patients, those carrying WISP1 rs2929970 AG + GG genotypes had later stage OSCC (stages III and IV) and a larger tumor size. In addition, OSCC patients who were betel quid chewers and carried WISP1 rs16893344 (CT + TT) variants had a low risk of lymph node metastasis. Finally, bioinformatics analysis was used to characterize the functional relevance of these variants for the microRNA-99a binding site and transcriptional regulation by the WISP1 3′-UTR and promoter regions.

CONCLUSIONS: The WISP1 SNP of rs2929970 is associated with OSCC susceptibility, and rs2929970 A/G polymorphisms may be correlated with a worse prognosis of OSCC, such as later stage OSCC or larger tumor size. WISP1 rs2929970 may serve as a marker or a therapeutic target in OSCC.

NO CONFLICT OF INTEREST

395 Contribution of BRCA1 large genomic rearrangements to early-onset and familial breast/ovarian cancer in Pakistan

M.U. Rashid[1], N. Muhammad[1], A. Asim Amin[2], A. Loya[3], U. Hamann[4]

[1] Shaukat Khanum Memorial Cancer Hospital and Research Centre SKMCH & RC, Department of Basic Sciences Research, Lahore, Pakistan
[2] Carolinas Healthcare System, Levine Cancer Institute LCI, Charlotte, USA
[3] Shaukat Khanum Memorial Cancer Hospital and Research Centre SKMCH & RC, Department of Pathology, Lahore, Pakistan
[4] German Cancer Research Center DKFZ, Molecular Genetics of Breast Cancer, Heidelberg, Germany

INTRODUCTION: Germline mutations in BRCA1 and BRCA2 (BRCA1/2) account for the majority of hereditary breast and/or ovarian cancers. Pakistan has one of the highest rates of breast cancer incidence in Asia, where BRCA1/2 small-range mutations account for 17% of early-onset and familial breast/ovarian cancer patients. We report the first study from Pakistan evaluating the prevalence of BRCA1/2 large genomic rearrangements (LGRs) in breast and/or ovarian cancer patients that do not harbour small-range BRCA1/2 mutations.

MATERIAL AND METHODS: Both BRCA1/2 genes were comprehensively screened for LGRs using multiplex ligation-dependent probe amplification in 120 BRCA1/2 small-range mutations negative early-onset or familial breast/ovarian cancer patients from Pakistan (Group 1). The breakpoints were characterized by long-range PCR and DNA sequencing analyses. An additional cohort of 445 BRCA1/2 negative high-risk patients (Group 2) was analyzed for the presence of LGRs identified in Group 1.

RESULTS: Three different BRCA1 LGRs were identified in Group 1 (4/120; 3.3%), two of these were novel. Exon 1-2 deletion was observed in two unrelated patients: an early-onset breast cancer patient and another bilateral breast cancer patient from a hereditary breast cancer (HBC) family. Novel exon 20-21 deletion was detected in a 29-year-old breast cancer patient from a HBC family. Another novel exon 21-24 deletion was identified in a breast-ovarian cancer patient from a hereditary breast and ovarian cancer family. The breakpoints of all deletions were characterized. Screening of the 445 patients in Group 2 for the three LGRs revealed ten additional patients harboring exon 1-2 deletion or exon 21-24 deletion (10/445; 2.2%). No BRCA2 LGRs were identified.

CONCLUSIONS: LGRs in BRCA1 are found with a considerable frequency in Pakistani breast/ovarian cancer cases. Our findings suggest that BRCA1 exons 1-2 deletion and exons 21-24 deletion should be included in the recurrent BRCA1/2 mutations panel for genetic testing of high-risk Pakistani breast/ovarian cancer patients.

NO CONFLICT OF INTEREST

396 Genetic susceptibility variants for lung cancer: Replication study and assessment as expression quantitative trait loci

F. Colombo[1], G. Pintarelli[1], C.E. Cotroneo[1], M. Dugo[2], L. Citterio[3], M. Incarbone[4], L. Santambrogio[5], T.A. Dragani[1]

[1] Fondazione IRCCS Istituto Nazionale Dei Tumori - Milano, Medicina Predittiva e per la Prevenzione, Milano, Italy
[2] Fondazione IRCCS Istituto Nazionale Dei Tumori - Milano, Dipartimento di Oncologia Sperimentale e Medicina Molecolare, Milano, Italy
[3] IRCCS San Raffaele Scientific Institute, Genomics of Renal Diseases and Hypertension Unit, Milan, Italy
[4] Ospedale San Giuseppe, Department of Surgery, Milan, Italy
[5] Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, U.O. Chirurgia Toracica e dei Trapianti di Polmone, Milan, Italy

INTRODUCTION: Many single nucleotide polymorphisms (SNPs) have been associated with lung cancer risk and/or prognosis. Sometimes, these studies lack of confirmation, maybe due to the wide genetic heterogeneity of the human population, and of functional characterization. Indeed, the functional characterization of candidate SNPs from genome wide association studies (GWAS) is particularly challenging, since most of them map in non-coding regions. However, these candidate SNPs might have a regulatory function, exerting their effects by modulating the expression of both near and distant genes; when these genes are involved in cancer-related pathways, the SNPs may affect the process of tumorigenesis.

MATERIALS AND METHODS: We retested the association of 56 candidate SNPs with lung adenocarcinoma risk and overall survival in a cohort of 823 Italian patients and 779 healthy controls, by genotyping them using the TaqMan® OpenArray® Genotyping System. We then assessed SNPs function as expression quantitative trait loci (eQTLs), by combining genotype data with normal lung tissue transcriptome data, obtained with HumanHT-12 v4 Expression BeadChip microarrays, from the same patients. Finally, we experimentally tested the eQTL result by searching for differential allelic expression (DAE) of the target genes of the eQTL SNPs.

RESULTS: In the replication study, eight SNPs (rs401681, rs3019885, rs732765, rs2568494, rs16969968, rs6495309, rs11634351, and rs4105144) associated with lung adenocarcinoma risk and three (rs9557635, rs4105144, and rs735482) associated with survival. Five of these SNPs acted as cis-eQTLs, being associated with the transcription of IREB2 (rs2568494, rs16969968, rs6495309, and rs11634351), PSMA4 (rs6495309) and ERCC1 (rs735482), out of 10,821 genes analyzed in lung. For these three genes, we obtained experimental evidence of DAE in lung tissue, pointing to the existence of in-cis genomic variants that regulate their transcription.

CONCLUSION: Our study highlights the relevance of deepening the function of the many loci found associated with human complex traits by GWAS, and points to the role of some of these loci as eQTLs. Our result support the hypothesis that some polymorphisms associated with lung cancer risk or prognosis influence tumor development and progression through the modulation of expression levels of target genes in the normal lung tissue.

NO CONFLICT OF INTEREST

397 Molecular profiling of Non-Small Cell Lung Cancer in a Nova Scotian patient cohort

A. Alwithenani[1,2], M. Forsythe[1], P. Marcato[1], W. Greer[1], M. Castonguay[1], Z. Xu[1]

[1] dalhousie, pathology, halifax, Canada
[2] Umm AlQura University, Laboratory Medicine, Makkah, Saudi Arabia

INTRODUCTION: Most lung cancer patients are diagnosed at an advanced stage, limiting their treatment options to chemotherapies that have low response rate. Therapies that target driver gene mutations (ALK and EGFR), are used to treat patients who have tumors with these mutations. Thus, being able to identify the presence of driver mutations will help select patients benefit from different therapies.

METHODS: A total of 844 Nova Scotian lung cancer samples have been profiled for EGFR, KRAS, BRAF and PIK3CA mutations by SNaPshot genotyping. Immunohistochemistry and fluorescent in situ hybridization identified ALK gene rearrangements. Histological and microscopic examination determined the pathological type and lymphatic\vascular invasion.

RESULTS: Statistical analysis revealed a number of correlations between driver mutations and the patient clinical data. Specifically, women had lung tumors with a significantly greater number of EGFR mutations than men (p value = 0.003). Analysis of the presence of mutations among each type of lung cancer (adenocarcinoma, squamous cell carcinoma, large cell carcinoma) yielded a p-value of <0.0001, which implies strong evidence that different mutations are associated with different cancer types. Invasion of tumor cells to vascular and pleura areas occurred in some cases. Examining the presence of driver mutations against the presence of these invasions yielded p-value of 0.01 for vascular invasion, which implies different mutations are associated with vascular invasion unlike pleural invasion which yielded a p-value of 0.071.

CONCLUSION: A profiling of Nova Scotian lung cancer samples for the presence of these mutations will help the patients to benefit from targeted therapies like EGFR, ALK inhibitors.

NO CONFLICT OF INTEREST

POSTER SESSION: MOUSE MODELS

399 Effects of NO and H2S releasing doxorubicin on a xenograft model of chemoresistant prostate cancer

L. Cinci[1], E. Bigagli[1], M. De Angioletti[2], K. Chegaev[3], C. Riganti[4], M. D'Ambrosio[1], S. Saponara[5], C. Luceri[1]

[1] University of Florence, Neurofarba, Firenze, Italy
[2] Istituto Toscano Tumori, ITT, Firenze, Italy
[3] University of Turin, Drug Science and Technology, Torino, Italy
[4] University of Turin, Oncology, Torino, Italy
[5] University of Siena, Life Sciences, Siena, Italy

BACKGROUND: Prostate cancer is the most prevalent cancer in men worldwide. Advanced diseases are commonly treated with androgen deprivation therapy however patients progress into castration-resistant prostate cancer, which is the most aggressive and lethal form of prostate cancer. In these patients, chemotherapy is not effective due the onset of drug resistance mediated by P-glycoprotein p (Pgp) overexpression, consequently, their prognosis is poor.

MATERIALS AND METHODS: We developed synthetic doxorubicins, containing NO and H_2S-releasing groups (DR6 and CC2790A) that showed an high cytotoxicity on doxorubicin-resistant prostate cancer cells (DU-145). DU-145 cells were injected ($4*10^6$ for animal) in nude mice (Nude-Foxn1nu/Foxn1+). Animals were divided into four groups and treated for three weeks with vehicle, doxorubicin, DR6 and CC2790A (5mg/kg) respectively. Animal weight, tumor volume, drugs accumulation in the tumors, percentage of apoptotic cells, presence of nytrotyrosine residues and SH groups in the tumors, together with the left ventricular wall thickness, were evaluated.

RESULTS AND DISCUSSION: DR6 and CC2790A induced a reduction of 60% of tumor volumes, while doxorubicin didn't exert any effect. The drugs auto-fluorescence revealed a high accumulation of our drugs within the tumor masses. In fact, DR6 and CC2790A, as opposed of doxorubicin, increased the presence of apoptotic cells in both the inner and the outer part of the tumors. Treatments with DR6 and CC2790A increased the presence of nytrotyrosine and SH groups in the tumor masses respectively, suggesting a possible mechanism of Pgp inhibition. Regarding the cardio-toxic side effects, doxorubicin caused two deaths during treatment and significantly increased the left ventricular wall thickness. On the contrary, the treatment with DR6 and CC2790A did not show either macroscopic or microscopic evidences of toxicity.

CONCLUSION: DR6 and CC2790A are potential novel therapeutic strategies against chemoresistant prostate cancer, combining efficacy with reduced cardiovascular side effects. By proposing an innovative strategy to reverse chemotherapy resistance, our study suggests a new tool against androgen independent prostate cancer, aiming at ameliorating the prognosis and improving the quality of life of a significant proportion of patients.

Supported by Ministry of Education, University and Research (MIUR) by the grant FIRB 2012 code RBFR12SOQ1

NO CONFLICT OF INTEREST

400 Establishment of Patient-Derived Xenografts (PDX) to study the biology and therapy of bone sarcomas

C. Cristalli[1], G. Nicoletti[1], M.C. Manara[1], A. Righi[2], A. Mai[3], S. Valente[3], P. Nanni[4], P. Picci[1], P.L. Lollini[4], K. Scotlandi[1]

[1] Rizzoli Orthopaedic Institute, Laboratory of Experimental Oncology, Bologna, Italy
[2] Rizzoli Orthopaedic Institute, Department of Pathology, Bologna, Italy
[3] Sapienza Università di Roma, Dipartimento di Chimica e Tecnologie del Farmaco, Roma, Italy
[4] University of Bologna, Department of Experimental- Diagnostic and Specialty Medicine, Bologna, Italy

BACKGROUND: Osteosarcoma (OS) and Ewing's sarcoma (EWS) are two of the most common pediatric tumors. Both are very aggressive neoplasms with high recurrence rate and tendency to metastasize. Multimodal treatments, including chemotherapy and surgery, have significantly improved prognosis for patients with localized tumors (5-year survival rates up to 70%) but outcome is still grim for patients with metastasis and limited therapy options can be offered after first line therapies. For this reason, there is a urgent need for reliable preclinical models to better understand the biology of these tumors and to test novel therapeutic strategies.

METHODS: A panel of patient-derived xenografts (PDX) was established by subcutaneous implantation of fresh, surgically resected OS and EWS tumor samples in NSG mice. Once tumor growth was observed, pieces of tumor were re-transplanted to next mice generations. At each passage tumor fragments were collected for histopathological and molecular characterization. A model was considered established after observing stable histological and molecular features for at least three passages. To evaluate differences among gene expression between patient and PDX tumors, gene expression profiling on RNA was performed. Representative samples of OS/EWS tumors and their PDXs (1st and 3rd passage) were included in tissue microarrays (TMA). To verify the feasibility of these models to study new compounds, we tested a new DNMTs inhibitor (MC3343) on a OS-PDX.

RESULTS: We implanted 58 OS and 23 EWS tumor samples. 13 out of 37 (35%) primary OS and 6 out of 21 (29%) OS lung metastases successfully engrafted. Among EWS samples, 5 out of 19 (26%) primary samples and 1 out of 4 (25%) metastases were established. Comparison between patient samples and PDX, highlighted that the histology and genetic characteristics of PDX tumor models were stable and maintained over passages, confirmed by immunohistochemistry on TMA. In vivo response to the DNMT inhibitor confirmed in vitro data on OS cells.

CONCLUSIONS: These PDX models have stable histological and molecular features, that recapitulate these diseases and retain the phenotypic and genetic characteristics of the original tumors. These models can contribute to study the biology and to develop preclinical studies for new anticancer treatments for OS and EWS.

Grants from: ER2017 2364984-ERANET;AIRC IG2013_14049 to KS; PRIN 2016 20152TE5PK, AIRC 19162/2016 and IIT Sapienza to AM. Pallotti fund UNIBO to PN.

NO CONFLICT OF INTEREST

401 Patient-derived xenograft (PDX) models of Glioblastoma: From basic research to preclinical studies

A. Golebiewska[1], A.C. Hau[1], D. Stieber[1], A. Oudin[1], F. Azuaje[2], T. Kaoma[2], A. Mock[3], C. Herold-Mende[3], R. Bjerkvig[4], S.P. Niclou[1]

[1] Luxembourg Institute of Health LIH, Department of Oncology- Norlux Neuro-Oncology laboratory, Luxembourg, Luxembourg
[2] Luxembourg Institute of Health LIH, Department of Oncology- Genomics and Proteomics Research Unit, Luxembourg, Luxembourg
[3] University of Heidelberg, Division of Neurosurgical Research- Department of Neurosurgery, Heidelberg, Germany
[4] University of Bergen, Department of Biomedicine- NorLux Neuro-Oncology laboratory, Bergen, Norway

INTRODUCTION: Animal models are essential tools for basic research and preclinical therapeutic interventions. Although numerous clinical cancer trials are being conducted, many fail due to inappropriate selection of compounds at the preclinical stage. Therefore better preclinical models are crucial for predicting successful clinical impact. Orthotropic PDX models are of particular importance for brain cancers, as they allow to better recapitulate the brain tumor environment and the blood brain barrier.

MATERIAL AND METHODS: Glioblastoma PDX models were based on 3D organotypic spheroids, derived from mechanically minced patient material. Spheroids were implanted in the brain of immunodeficient mice and further propagated by serial intracranial transplantations. For detailed molecular characterization each PDX was compared to its original patient tumor at the genetic, epigenetic and transcriptomic levels and intra-tumoral heterogeneity was addressed at the single cell level. We furthered performed proof-of-concept preclinical studies interfering with angiogenesis and autophagy.

RESULTS AND DISCUSSION: Our glioblastoma PDX models starting with viable patient-derived spheroids has a tumor take rate close to 100% and a reproducible phenotype and tumor development time. We observed three distinct histological tumor phenotypes: a highly 'invasive", a highly 'angiogenic" and an 'intermediate" phenotype which combines invasion and vascular abnormalities. Typical glioblastoma characteristics such as pseudopalisading necrosis, invasion or microvascular proliferation we maintained. PDXs retained the genetic and epigenetic profiles of patient tumors through several generations. Transcriptomic profiles of PDXs were similar to patient biopsies and correlated better with TCGA glioblastoma samples than conventional glioma cell lines. In vivo pharmacological inhibition of autophagy significantly increased survival of PDXs and combination treatment with bevacizumab showed a synergistic effect.

CONCLUSION: Here we show that glioblastoma PDXs represent a reliable and clinically-relevant animal model. The model can be applied for analyses at different molecular levels. Importantly, the PDXs can be applied for accurate reproducible pre-clinical trials, including personalized medicine-based treatments. The use of this model should lead to a more realistic evaluation of the efficacy of novel drugs, thereby increasing the success of clinical studies.

NO CONFLICT OF INTEREST

POSTER SESSION: PREVENTION AND EARLY DETECTION

403 Factors associated with the occurrence and prognosis of bladder cancer – environmental and proteomic approaches

K.H. Hsu[1], *P.J. Liao*[2]

[1] *Chang Gung University, Health Care Management, Taoyuan, Taiwan*
[2] *Oriental Institute of Technology, Health Care Administration, New Taipei City, Taiwan*

INTRODUCTION: The study is to explore the relationship of environmental risk factors and proteomic markers with the occurrence and prognosis of bladder cancer. Life style variables, history of chemical exposures, urinary inorganic elements, and proteomic markers were identified and applied to the model constructions.

MATERIAL AND METHOD: This study is a matched case-control design. One hundred bladder cancer patients and 100 controls without bladder cancer in a medical center were collected. In addition to questionnaires, measurements of urinary and blood biomarkers including UBS, NMP22, fibrin/fibrinogen degradation product, surviving, and multi-elements were performed to construct predictive models. Logistic regression and area under curve (AUC) of receiver operator calibration analysis were applied to the analyses.

RESULTS AND DISCUSSION: Statistically significant higher levels were found in As, Cu, and Zn among patients of bladder cancer and BPH as opposed to community healthy controls. However, lower levels of specific inorganic elements were observed in bladder cancer patients including Bi, Tl, and Mn. Whereas As may be a marker for advanced bladder cancer diagnosis. Potential recurrence markers were found among subset of patients with higher levels of As and Ni. Early detection of bladder tumors has identified a variety of potential markers in urine. The NMP22 has a sensitivity of greater than 68% and a specificity >61%. The fibrin/fibrinogen degradation product (FDP) has a sensitivity of 82% and a specificity of >86%. Two innovative proteomic biomarkers were identified in this study for urinary diagnosis of bladder cancer, including UBS and Survivin. The AUC of UBS was found as 0.82 with a potential cut-off point reaching sensitivity of 0.7 and specificity of 0.65. Survivin was also feasible for urinary diagnosis of bladder cancer with sensitivity of 0.9 and specificity of 0.75 (AUC=0.71). The mechanistic study has shown that inorganic elements may be related to uroplakin expression and hence synergistically affect the cancer progression and recurrence.

CONCLUSION: Our findings imply that exploring disease detection with a combination of candidate markers will be the best way to discover the most applicable panels. With the help of proteomic analysis, predisposing environmental exposures, and life-style variables, this study developed models to predict disease progression and assist the treatment of bladder cancer more effective in the future.

NO CONFLICT OF INTEREST

404 Evaluation of novel biomarkers associated to pancreatic carcinogenesis for the early diagnosis of pancreatic ductal adenocarcinoma

M.A. Kamal[1], *I. Siddiqui*[2], *D. Pistillo*[2], *A. Zerbi*[3], *F. Gavazzi*[3], *C. Ridolfi*[3], *G. Carpetti*[3], *S. Schiarea*[4], *C. Chiabrando*[4], *P. Allavena*[1]

[1] *Humanitas Clinical and Research Centre, Department of Immunology and Inflammation, Rozzano Milano, Italy*
[2] *Humanitas Clinical and Research Centre, Humanitas Biobank, Rozzano Milano, Italy*
[3] *IRCCS Humanitas Clinical and Research Centre, Pancreatic Surgery Unit- Department of Surgery, Rozzano Milano, Italy*
[4] *Istituto Mario Negri - Milano, Environmental Health Sciences, Milano, Italy*

INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive forms of cancer and estimated to become a leading cause of cancer death by the year 2030 in United State. Early detection of this cancer is the key determining step to surgery eligibility of PDAC patients, in order to improve survival rate and prognosis. Moreover, less than 20% of PDAC patients are eligible for surgery, because only small tumors (< 3cm) can be surgically resected without major complications. So there is urgent need to identify early stage diagnostic biomarker to detect Pancreatic cancer.

MATERIAL AND METHODS: In this study, we report a differential quantitative proteomic approach for the global identification and quantification of secretome proteins in Human Pancreatic Ductal Epithelial (HPDE) KRasG12V-transformed cells to be compared with Mock-transduced cells. Proteins in cell conditioned media labeled with Stable Isotope Labeling with Amino acids in Cell culture (SILAC) were analyzed by 1DE-LC-mass spectrometry analysis with LTQ Orbitrap XL. Early stage identified candidate diagnostic biomarker were validated by RT-PCR, Immunohistochemistry and ELISA in PDAC patients.

RESULTS AND DISCUSSION: Among the over-expressed proteins by HPDE-KRasG12V-transformed cells, were proteins linked to important biological pathways for tumor cells, which include: matrix-associated proteins, proteins related to KRas-signaling, metabolic enzymes and inducers of the epithelial-to-mesenchymal transition. A panel of 6 candidate proteins: LamininC2 (LAMC2), Tenascin-C (TNC), Stanniocalcin 2 (STC2), RAN-GTPase, Farnesyl Pyrophosphate synthase (FPPS), and Ubiquitin carboxyl terminal hydrolase-L1 (UCHL-1) was further selected to be validated in biological samples. Human PDAC patient's tissue had high mRNA expression for these proteins. The circulating levels of LAMC2 and TNC, measured by ELISA, were significantly higher ($p < 0.001$) in PDAC patients (n=102) compared to healthy individuals (n=80). The ROC curve analysis (0.86 and 0.74, respectively) revealed good specificity and sensitivity.

CONCLUSION: These candidate biomarkers were significantly higher already in early-stage patients, indicating potential for the early diagnosis of PDAC. This work is supported by AIRC 5X1000 project 12182.

NO CONFLICT OF INTEREST

405 CD157 as a potential pleural effusion biomarker for malignant mesothelioma

S. Morone[1], *S. Capano*[1], *A. Giacomino*[1], *S. Augeri*[1], *E. Ortolan*[1], *I. Rapa*[2], *L. Righi*[2], *A. Funaro*[1]

[1] *University of Torino, Department of Medical Sciences, TORINO, Italy*
[2] *University of Torino, Department of Oncology, TORINO, Italy*

INTRODUCTION: Malignant pleural mesothelioma (MPM) is a deadly cancer, which is difficult to diagnose because of nonspecific clinical symptoms. Since most patients with MPM present with pleural effusion (PE), cytological examination is routinely adopted to identify malignant cells for diagnostic purpose. However, cytology has an average sensitivity of only 30–50 % in MPM. Hence, the measurement of biomarkers in PE can play a meaningful role in the diagnosis of MPM.

CD157 glycoprotein was found in the most common types of MPM and its expression levels were associated with exacerbated tumor aggressiveness. In this study we explored the existence of soluble CD157 in PE and evaluated its potential clinical utility as biomarker in MPM patients.

MATERIALS AND METHODS: CD157 expression was analysed by western blot and its levels were measured by an enzyme-linked immunosorbent assay in the culture medium from non-malignant and malignant mesothelial cell lines and in 303 consecutive patients who had developed PE: 56 PE were from MPM, 170 from other tumors and 133 from benign thoracic diseases.

RESULTS AND DISCUSSION: We demonstrated that CD157 is released both in vitro and in vivo either by normal or neoplastic mesothelial cells. CD157 can be shed from the cell membrane by proteolytic cleavage and as exosome-anchored protein. In PE from patients with MPM we found significantly higher levels of sCD157 than in those from patients with lung cancer (P=0.0001) or other tumors (P=0.0095), as well as in those from patients with benign pathologies (P <0.0001), suggesting that PE sCD157 levels could serve as a biomarker for MPM in the differential diagnostic settings. sCD157 evaluation has proven to make a valuable contribution to the diagnosis of MPM in 50% of cytology-negative cases, whereas in the setting of inconclusive effusion cytology, sCD157 had a positive predictive value for MPM of 84.6%. Overall, measurement of sCD157 in combination with cytology evaluation allowed to formulate diagnosis of mesothelioma in 76.8% of patients from a single PE sample.

sCD157 levels resulted below the cut-off value in 5/21 cases of cytology-positive MPM, and sCD157-positive samples were found in PE due to other tumors or benign pathologies, indicating that sCD157 cannot be regarded as a diagnostic tool by itself.

CONCLUSION: This study showed that sCD157 detection in PE may be a way to significantly improve the clinical impact of effusion-based MPM diagnosis limiting diagnostic delay.

NO CONFLICT OF INTEREST

407 Immunoprevention Portfolio of the NCI PREVENT Cancer Program

S. Sei[1], *M. Miller*[1], *E. Glaze*[1], *D. Boring*[1], *B. Dunn*[1], *R. Shoemaker*[1]

[1] *National Cancer Institute- NIH, Division of Cancer Prevention, Rockville- MD, USA*

INTRODUCTION: The NCI PREVENT Cancer Preclinical Drug Development Program (PREVENT) is a peer-reviewed program designed to translate the best ideas in cancer prevention into novel cancer preventive interventions and biomarkers for clinical use (https://prevention.cancer.gov/major-programs/prevent-cancer-preclinical). It focuses on research needs not addressed by the private sector. Applications from US or international researchers in academia, government or industry are accepted. The Program has flexible entry points to the pipeline and supports a continuum of agent development phases from proof of principle and validation studies to IND-enabling studies leading to Phase I clinical trials.

METHODS: The PREVENT utilizes NCI contract resources to support the development of a diverse portfolio of chemical and biological agents selected through peer review and programmatic approval processes. Competitively selected contractor pools with necessary technical expertise are in place to implement approved projects under NCI oversight.

RESULTS AND CONCLUSION: One of the Program's focus areas is cancer immunoprevention. Since its inception, a total of 16 cancer preventive vaccine projects have been selected for funding. Of those, one has progressed to the clinical trial stage with two additional projects currently in the IND-enabling study phase. The PREVENT immunoprevention portfolio consists of a variety of vaccination approaches, targeting oncogenic drivers, early tumor associated antigens, and against infectious agents known to induce cancers. Recent advances in cancer immunology and immunotherapies have shed tremendous light on key determinants of successful immune control of cancer as well as the role of tumor derived immunosuppression in disease progression. While the expanded knowledge base in tumor immunology has uncovered the potential challenges in developing efficacious preventive vaccines, it has also helped delineate key issues surrounding preclinical development of cancer preventive vaccines, including the selection of optimal target antigens, vaccine delivery strategies and schedules, immune biomarkers for vaccine immunogenicity and durability, and of antitumor protection, and the need for suitable animal models. Building on the success of prophylactic HPV vaccines in preventing cervical cancer, the PREVENT partners with researchers to develop novel cancer preventive vaccines for viral as well as non-viral cancers toward the clinical applications.

NO CONFLICT OF INTEREST

408 Bone marrow represents an early sensor of incipient malignancies occurring at distant site

A.M. Tomirotti[1], T.A. Renzi[1], C. Chiodoni[1], M. Dugo[2], V. Cancila[2], C. Tripodo[2], M.P. Colombo[1]

[1] Fondazione IRCCS Istituto Nazionale Tumori, Department of Experimental Oncology and Molecular Medicine, Milan, Italy
[2] University of Palermo School of Medicine, Department of Health Science- Human Pathology Section, Palermo, Italy

INTRODUCTION: Tumour development is not completely cell-autonomous, being dependent on the ability of bystander cells, mostly of bone marrow (BM) origin, to establish a pro-tumourigenic microenvironment. We hypothesize that identification of early BM changes would confirm that BM senses the onset of a neoplastic lesion in peripheral tissues even at very early stages and that such alterations could offer the opportunity to identify key signalling molecules that may represent early biomarkers of cancerogenesis and could allow discovering novel potential therapeutic targets.

MATERIAL AND METHOD: The MMTV-NeuT (NeuT) transgenic mouse model of mammary carcinogenesis was used to study the early phases of tumor development because its progression recapitulates the hyperplasia/dysplasia, carcinoma in situ and invasive carcinoma found in human breast cancer. Mice were analyzed at 6 and 12 weeks of age, time points that reflect early stages of tumor onset, and at 24 weeks of age as representative of late time point when overt tumors are evident. We characterized the BM hematopoietic compartment and the peripheral blood by flowcytometry and BM was also profiled for gene expression.

RESULT AND DISCUSSION: Profound BM modifications in the composition (increase of myeloid/granulocytic cells, contraction of B cells) and spatial arrangement of the hematopoietic populations (displacement of BM niches) characterize NeuT mice with overt carcinomas and some of such modifications are already detectable at earlier stage. These result suggest that the early modifications occurring in the BM at premalignant phases are mainly related to an innate immune cell subset, specifically myeloid cells, being favored in their relocation within BM niches. The BM of NeuT tumor-bearing mice is characterized by an up-regulation of inflammatory and innate immune response processes and a down-regulation of adaptive immune response and B cell receptor signaling pathway. Genes differentially expressed between transgenic and wild type mice were used to design a 'late BM gene signature", validated in two other breast cancer models (PyMT and huHer2D16). Interestingly, this BM signature is able to distinguish young mice (6-12 weeks) with mild dysplasia in their mammary glands from those with sever dysplasia/early carcinomas.

CONCLUSION: BM actually represents an early sensor of pre-malignant transformation occurring at distant site with specific myeloid subsets undergoing expansion and re-localization within BM stromal niches upon sensing of 'danger"-related signals, from incipient malignancies. The commonalities in the processes characterizing the BM during early and late phases of breast tumour development provides the link between the early modification in the transforming tissue and a precise BM transcriptional pattern. NO CONFLICT OF INTEREST

409 Serum miRNAs as biomarkers for early diagnosis of non-small cell lung cancer

P. D'Antona[1], E. Gini[1], S. Grossi[1], M. Nicolis[2], D. Noonan[3], A. Poli[2], L. Dominioni[4], M. Cattoni[5], E. Daffrè[5], P. Campomenosi[1]

[1] University of Insubria, Department of Biotechnology and Life Sciences DBSV, Varese, Italy
[2] University of Verona, Department of Public Health and Community Medicine, Verona, Italy
[3] IRCCS MultiMedica, Scientific and Technological Pole, Milan, Italy
[4] University of Insubria, Department of Medicine and Surgery, Varese, Italy
[5] University of Milan, Department of Medicine and Surgery, Milan, Italy

INTRODUCTION: Lung cancer (LC) is the predominant cause of cancer-related mortality in western countries. Patients with early stage (stage I and II) non-small cell lung cancer (NSCLC), have a much better prognosis than those diagnosed later but unfortunately they represent only 20-25% of all NSCLCs. For these reasons, the development of novel, sensitive and non-invasive methods for screening individuals at high risk for NSCLC (older than 50 years and heavy smokers) is needed. Recently, microRNAs (miRNAs) have been suggested as a novel class of tumor biomarkers, as the levels of some of them are altered in various human cancers, including NSCLC. The presence and stability of miRNAs in biofluids, and alteration in their levels in consequence of diseases, are strong points suggesting their possible application as circulating biomarkers.

We hypothesized that the serum level of specific miRNAs could be used to discriminate patients with early NSCLC (stage I and II) from healthy individuals. The final aim of this study is to develop a diagnostic miRNA signature to be applied as first line, large scale screening of individuals at risk for early stage NSCLC.

MATERIAL AND METHOD: A systematic review of the pertinent literature allowed us to select a panel of miRNAs whose expression in the serum or plasma has been reported to be different, in independent studies, between individuals with and without NSCLC. For measurement of serum miRNAs, droplet digital PCR (ddPCR) was used, as this method provided greater precision and higher throughput of analysis. The level of the following 4 miRNAs was evaluated in serum samples from a cohort of 85 early stage NSCLC patients and 82 healthy donors: let-7a, miR-210, miR-221, miR-320a.

RESULTS AND DISCUSSION: We found significant differences between NSCLC patients and controls in serum levels of three out of the tested four miRNAs ($p<0.01$ for miR-let-7a-and miR-210; $p<0.05$ for miR-320a, Mann-Whitney t-test) and showed fair accuracy in identifying early NSCLC cases. All miRNAs under study showed a decrease in NSCLC patients compared to controls.

CONCLUSION: Three out of four miRNAs that we evaluated as early stage NSCLC biomarkers are expressed at different levels in NSCLC patients compared to controls and may indeed be used for identifying individuals to be further examined for the presence of lung cancer. We are currently performing a critical revision of the literature to identify further miRNAs to be added to our panel.

NO CONFLICT OF INTEREST

410 Identification of circulating microRNAs as early biomarkers of tumor development in a pre-clinical mouse model of mammary carcinogenesis

T.A. Renzi[1], A.M. Tomirotti[1], C. Chiodoni[1], M. Dugo[2], V. Cancila[3], C. Tripodo[3], M.P. Colombo[1]

[1] Fondazione IRCCS Istituto Nazionale dei Tumori, Experimental Oncology and Molecular Medicine, Milano, Italy
[2] Fondazione IRCCS Istituto Nazionale dei Tumori, Functional Genomics and Bioinformatics Core Facility, Milano, Italy
[3] University of Palermo, Department of Human Pathology, Palermo, Italy

BACKGROUND: During tumorigenesis newly transformed cells initiate an active cross-talk with the adjacent stroma through the secretion of a variety of factors, such as microRNAs (miRNA), which can modulate the local microenvironment toward a pro-tumoral phenotype. The identification of circulating miRNAs that are already detectable at early stages during tumorigenesis could be relevant as novel prognostic/predictive biomarkers.

MATERIAL AND METHODS: We used the transgenic MMTV-NeuT (NeuT) mouse model that spontaneously develops mammary carcinomas with a well-defined progression, mimicking the human pathology. Plasma miRNA profile was performed with Agilent microarrays and validated with qRT-PCR and concomitant histopathological analyses have been conducted on mammary primary lesions of each mouse.

RESULTS: To investigate circulating miRNAs deregulation during different phases of mammary cancer progression, we analysed plasma samples of NeuT and control siblings collected at early (6 and 12 weeks) and late (24 weeks) time-points of cancer development and found differentially expressed miRNAs already at early stages of tumor development. Since we observed a considerable degree of individual variability in the onset and extension of nascent lesions within the mammary glands of early time points, we have re-classified the samples on the basis of the severity of the primary lesions, identifying 3 different categories (normal, mild or moderate dysplasia and severe dysplasia/early carcinoma). Class comparison analysis confirmed that specific circulating miRNAs increased along transformation from normal to severe dysplasia/early carcinoma. Moreover, we found a significant correlation among the deregulated circulating miRNAs and the tumor burden, well before the tumor became palpable.

CONCLUSIONS: These result demonstrate that already at early phases of tumor transformation there is a significant modification in circulating miRNAs that correlates with the stage and severity of the disease. Circulating miRNAs could therefore represent not only a potential diagnostic tool of an ongoing neoplastic transformation, but could also be used to discriminate between different stages of breast cancer progression.

NO CONFLICT OF INTEREST

411 Patient navigation: Mitigating the surge of cancer in sub-Saharan Africa

R.C.W. Chidebe[1], C.T. Orjiako[2], I. Okoye[3]

[1] Project PINK BLUE - Health & Psychological Trust Centre, Patient Group & Research, Abuja, Nigeria
[2] University of Nigeria, Psychology, Enugu, Nigeria
[3] University of Nigeria, Radiology, Enugu, Nigeria

BACKGROUND: The sub-Saharan region is noted to have a characteristic cancer profile. The adverse mix of late detection rates and poverty result in a high rate of advanced cancers and high cancer mortality rates. In Nigeria, cancer leads to 72,000 deaths per annum; this number is set to increase given that there are 102,000 new cases of cancer every year. The mortality incidence for breast cancer is 51% with over 70-80% of breast cancer patients present advanced stages III or IV. In an attempt to mitigate the surge of cancer, a metastatic breast cancer (MBC) programme was launched; known as Breast Cancer Navigation and Palliative Programme (BCNPP) with the goal of increasing early diagnosis and reducing late presentation of stage III or IV breast cancer in central Nigeria, we trained breast cancer survivors, and oncology nurses on patient navigation and home-based palliative.

METHOD: Forty-two breast cancer survivors and oncology nurses, from 6 municipal areas of Abuja, central Nigeria were scheduled for training sessions on patient navigation and palliative care. An additional cancer help line was launched in the region to connect urban and rural women to cancer care closest to them.

RESULTS: A mixed methods approach involving qualitative and quantitative analysis revealed that the health care workers improved in their knowledge of patient navigation and an increased commitment on patient navigation. Health centres in the region recorded an increased number of patients reporting for early screening.

CONCLUSION: Cancer awareness, patient navigation trainings and programmes are potential useful tools in the sub-Saharan region in mitigating the surge in cancer deaths in the region.

NO CONFLICT OF INTEREST

POSTER SESSION: RADIOBIOLOGY/RADIATION ONCOLOGY I

413 Adjuvant radiation therapy effects systemic immune response cells in female breast cancer patients

T. Luetragoon[1,2], D. Oliva[3,4], S. Löfgren[1], F. Lewin[3,4], M. Strandeus[4], B. Ake Andersson[1,3], N. Laytragoon-Lewin[1,3]

[1] Division of Medical Diagnostic, Ryhov Hospital, Sweden
[2] Department of Medical Technology, Narasuan University, Thailand
[3] Department of Clinical and Experimental Medicine, Linköping University, Sweden
[4] Department of Oncology, Ryhov Hospital, Sweden

BACKGROUND: Radiation induces DNA damage, leads to cell cycle arrest and cell death. We investigated the effect of adjuvant radiation therapy (RT) on systemic innate and adaptive immune cells in female breast cancer (BC) patients by assessing circulating white blood cells (WBCs) and it's subpopulations.

MATERIAL AND METHOD: Peripheral blood cell numbers from BC patients (n=114, median age 64) who received adjuvant RT (50 Gy) at Dept. Oncology, Ryhov hospital, Sweden, were analyzed. Blood (30 ml) was obtained from BC patients before and after adjuvant RT. The number of total WBCs and its subpopulations were analyzed by Sysmex XE5000 instrument. The phenotype of ex vivo fresh peripheral white blood cell subpopulation were analyzed by BD FACSCanto II flow cytometer and BDFACSDiva software program. Paired T-test was used for comparison between the patients before and after adjuvant RT, Significant p-values <0.05.

RESULTS: After adjuvant RT, the number of WBCs, neutrophils and lymphocytes were significantly decreased.Neutrophil to lymphocyte ratio (NLR) is suggested to be a biomarker for poor prognostic and short survival time for cancer patient. Interestingly, the NLR was significantly increased after treatment, p-values = <0.001.As for total lymphocytes, adjuvant RT decreased the numbers in all T-cell subpopulations studied (CD3+, CD4+, CD8+, and CD3+56+ [NKT cells]) and in CD56+ (NK cells).

CONCLUSION: Our result indicated that adjuvant RT of BC patients induces systemic alterations in number of innate and adaptive immune cells. This might be due to the bystander or distant immunosuppression from adjuvant radiation. Alternative, adaptive and innate immune response cells are redistributed from circulation to the radiation site. The significance of these alteration on clinical outcome need further investigation.

NO CONFLICT OF INTEREST

415 Oesophageal radiotherapy and metallic stenting are effective for the stenosis of advanced oesophageal cancer; Compared to stenting for patients without radiotherapy

T. Toyokawa[1], I. Fujita[1], J. Horii[1]

[1] National Hospital Organization Fukuyama Medical Center, Department of Gastroenterology, Fukuyama city, Japan

BACKGROUND: Stent placement is an attractive modality for improving dysphagia in patients with malignant stenosis due to advanced oesophageal cancer. However, it was reported that the treatment complications, including bleeding, fistula and mediastinitis, increased in patients undergoing radiotherapy. In this study we evaluated the efficacy and safety of the combination of radiotherapy and metallic stenting compared to stenting without radiotherapy in patients with oesophageal cancer.

MATERIAL AND METHODS: We enrolled 29 patients with oesophageal cancer who underwent metallic stenting therapy at our hospital, and all patients were followed-up more over 1 year. We classified patients into two groups; RT group (undergo stenting and radiotherapy) and non-RT group (undergo stenting only) and investigated the patient characteristics (age, gender, clinical stage, the length of stenosis, and the presence of chemotherapy), improvement of symptoms, survival time from stenting and complications (bleeding, fistula and mediastinitis) between these groups. Statistical analysis was performed using the Mann-Whitney U-test and Chi-square test, $p < 0.05$ was considered to be statistically significant.

RESULTS: The RT group included 17 patients, and non-RT group included 12 patients. The mean follow-up period was approximately 96 days after stenting. The patient characteristics were not different between these groups; however, the presence of chemotherapy was more high frequent in the RT group. The improvement of symptoms and survival time from stenting were excellent and similar in both groups. Bleeding occurred in 4 patients and 1 patient, and fistula occurred in 3 patients and 1 patient in the RT group and the non-RT group, respectively. Mediastinitis did not occur in either group. The complication rates were not different between these groups.

CONCLUSIONS: In this study, the efficacy and safety of the combination of radiotherapy and metallic stenting were clarified as equivalent to stenting without radiotherapy in patients of oesophageal cancer. Therefore, it seems that we should not hesitate metallic stenting for patients with oesophageal cancer undergoing radiotherapy.

NO CONFLICT OF INTEREST

416 Alteration of carbohydrate metabolic pathway of hepatoma following radiation therapy

C.S. Chiang[1], C.F. Yu[2], F.H. Chen[3]

[1] National Tsing Hua University, Biomedical Engineering and Environmental Sciences, Hsinchu, Taiwan
[2] Chang Gung Memorial Hospital, Radiation Biology Research Center, Taoyuan, Taiwan
[3] Chang Gung University, Medical Imaging and Radiological Sciences, Taoyuan, Taiwan

Hepatocellular carcinoma (HCC) is the fifth most common liver malignancy and the third leading cause of cancer-related death worldwide. It is also a major cancer in Taiwan. Radiotherapy (RT) is one of modalities for HCC therapy in clinics, but the therapeutic effect is unsatisfied due to multiple reasons. The original aim of this study was to develop a therapeutic platform for double targeting treatment for HCC patients in a proton facility center in Taiwan. A multiple image platform, integrating IVIS, FDG-PET, and MRI imagings was established to measure the response of orthotopic murine BNL hepatoma and human HCC in C57BL/6 and SCID mouse, respectively, following single or fractionated radiation therapy (RT). result show that the tumor volumes measured by IVIS and MRI imaging were consistent for control tumors, but were inconsistent in recurrent tumor following RT. FDG-PET imaging shows the inflammatory response following RT. Further immunohistochemical (IHC) staining shows that the metabolic pathways of glucose metabolism and glycogen synthesis were altered in recurrent hepatoma and also normal liver tissue following RT. The inflammatory response and consequent altered carbohydrate metabolic pathways may compromise the readout of IVIS. Our result not only caution the use of IVIS to monitor the hepatoma response following RT, but also suggest that the strategy for treating primary and recurrent hepatoma may not be the same.

NO CONFLICT OF INTEREST

417 Enhancement of soft tissue sarcoma cell radiosensitivity by poly(ADP-ribose) polymerase-1 inhibitors

M. Sottili[1], G. Salvatore[1], M. Mangoni[1], I. Meattini[1], I. Desideri[1], D. Greto[1], M. Loi[1], G. Beltrami[2], D. Campanacci[2], L. Livi[1]

[1] University of Florence, Department of Experimental and Clinical Biomedical Sciences, Firenze, Italy
[2] Azienda Ospedaliera Universitaria Careggi, Department of Orthopaedic Oncology, Firenze, Italy

INTRODUCTION: Soft-tissue sarcomas (STS) are aggressive tumors with a poor prognosis and there is a major clinical need for new strategies. Poly-ADP ribose polymerase (PARP)-1 promotes base excision repair and DNA strand break repair. PARP inhibitors (PARPi) have shown to enhance the cytotoxic effect of irradiation. We evaluated the effect of PARPi on STS cell lines survival and DNA damage after irradiation.

MATERIAL AND METHODS: Cell proliferation was evaluated on human fibrosarcoma, liposarcoma, leiomyosarcoma and rhabdomyosarcoma cell lines with increasing doses of olaparib (0.25; 0.5; 1; 2; 4 μM). Cells were counted after 5 days and result normalized to control. For clonogenic assays, STS cell lines were irradiated with 2, 4 or 6 Gy, with or without olaparib (1 μM), iniparib (10 μM) or veliparib (5 μM) pretreatment. The impact of PARPi on γ-H2AX and Rad51 foci formation was evaluated by immunofluorescence in rhabdomyosarcoma cells treated with olaparib and irradiated at 4 Gy. Phospho-ERK, cleaved caspases 3 and γ-H2AX protein expression was evaluated by western blot in rhabdomyosarcoma cells treated with olaparib and irradiated at 4 Gy.

RESULTS AND DISCUSSION: The treatment with olaparib for 5 days caused a dose-dependent inhibition of proliferation in all STS cell lines. Significant radiosensitization was observed in all STS cell lines using PARPi. Rhabdomyosarcoma showed the greatest increase in radiosensitivity, with a radiosensitization enhancement ratio at 50% survival (ER50) of 3.41 with veliparib. Fibrosarcoma showed an ER50 of 2.29 with olaparib. Leiomyosarcoma and liposarcoma showed the higher radiosensitization with veliparib (ER50 1.62 and 1.46, respectively). The combination of olaparib and radiation in rhabdomyosarcoma cells caused an increased number of γH2AX and Rad51 foci compared to olaparib or irradiation alone. Rhabdomyosarcoma cells treated with the association of olaparib and irradiation showed an increased cleaved caspases 3 and γH2AX protein expression compared to olaparib or irradiation alone. Olaparib pretreatment decreased radiation-induced phospho-ERK protein expression.

We showed that PARPi are potent radiosensitizers on human STS in vitro models: they reduced cell survival, inhibited DNA damage repair and pro-survival ERK signaling, and induced apoptosis in STS cells when used in combination with irradiation.

CONCLUSION: These data encourage to further study the association of PARPi with irradiation as a promising treatment for STS.

NO CONFLICT OF INTEREST

POSTER SESSION: RADIOBIOLOGY/RADIATION ONCOLOGY II

420 Potential benefit of proton beam therapy in triple negative breast cancer treatment

S. Park[1,2], *C. Choi*[2], *W. Park*[1,2], *D.H. Choi*[1,2]

[1] *Samsung Medical Center, Department of Radiation Oncology, Seoul, South Korea*
[2] *Sungkyunkwan University, School of Medicine, Seoul, South Korea*

BACKGROUND: Treatment of solid tumors with proton therapy is getting more popular with reduced normal tissue toxicity due to its physical properties. Nonetheless, biological consequence of the proton therapy is not fully understood. Triple negative breast cancer (TNBC) accounts for 15-20% of all breast cancers but there are no approved targeted agents or therapies. In this study, we evaluated proton relative biological effectiveness (RBE), a clinically fixed value of 1.1 using human breast cancer cell lines and potential benefit of proton therapy for TNBC treatment. We also identified CHK1 as a genetic factor to modify proton RBE.

MATERIAL AND METHODS: 11 human breast cancer (BC) cell lines including 3 ER+/PR+ BCs, 3 HER2+ BCs and 5 triple negative BCs (TNBC) were tested for this study. Proton beam irradiation was performed at Samsung Medical Center in Seoul, Korea with proton beam (230 MeV) and photon irradiation were performed with a Varian Clinac 6EX accelerator. Clonogenic assay was performed to measure sensitivity to proton or photon irradiation. Radiation-induced cell signaling was determined by western blot analysis. Apoptosis was assessed by flow cytometry with propidium iodide and annexin V staining and western blot analysis. RBE was calculated as the ratio of the physical doses of photon and proton that yield a surviving fraction of 50% and 37%.

RESULTS: Analyses on surviving fraction at 2 Gy of proton or photon irradiation revealed that proton killed more BC cells than photon regardless of genetic background. TNBC had larger variation of SF2 compared to non-TNBC, suggesting heterogeneity of TNBC subtypes may affect radiation sensitivity. Proton RBE_{50} and RBE_{30} of TNBC cells were 1.46 ± 0.34 and 1.40 ± 0.27, respectively, which are higher than those of non-TNBC cells (1.24 ± 0.12 and 1.23 ± 0.09). These data suggest that proton irradiation may be more efficient to kill TNBC cells, ignoring heterogeneous nature of TNBC. In TNBC MDA-MB-231 cells, knockdown of CHK1, a major G2/M cell cycle checkpoint protein, decreased clonogenic survival and increased apoptosis, which was greater in proton-irradiated cells than photon-irradiated cells
CONCLUSIONS: Based on RBE calculations, we concluded that proton therapy may be more effective treatment option for TNBC treatment than conventional photon therapy. Furthermore, CHK1 inhibitors that are now being tested in clinical trials could provide additional benefit to proton therapy for TNBC treatment.

This work is supported by a grant from Samsung Biomedical Research Institute Grant (SMX1170211) and Basic Science Research Program through National Research Foundation of Korea funded by the Ministry of Education (NRF-2016R1D1A1B03935165).

NO CONFLICT OF INTEREST

421 Combination therapy with histone deacetylase inhibitor, panobinostat and proton irradiation is an effective regimen for hepatocellular carcinoma cells

G.H. Lee[1,2], *C. Choi*[2], *H.C. Park*[1,2]

[1] *Samsung Medical Center, Department of Radiation Oncology, Seoul, South Korea*
[2] *Sungkyunkwan University, School of Medicine, Seoul, South Korea*

BACKGROUND: Preclinical studies have demonstrated an anti-tumor activity of histone deacetylase inhibitors (HDACIs) in hepatocellular carcinoma (HCC) models. HDACIs lead to hyperacetylation of histones and various intracellular proteins, resulting in inducing apoptosis in malignant cells and exerting a synergistic effect with radiation therapy. Here, we investigated whether a HDACI, panobinostat radiosensitizes HCC cells and compared cellular responses against two radiation treatment modalities – X-ray and proton irradiation.

MATERIAL AND METHODS: Human hepatocellular carcinoma cell lines, Huh7 and Hep3B were tested in this study. Proton beam irradiation (230 MeV) and X-ray irradiation (6 MV) were performed at Samsung Medical Center in Seoul, Korea. The radiation effect of panobinostat on two HCC cell lines was determined by MTT and clonogenic assay. Western blot analysis was used to investigate the panobinostat-mediated intracellular radiation response. Modes of cell apoptosis were assessed with Annexin V/propidium iodide double staining. Cell cycle progression and levels of intracellular reactive oxygen species (ROS) were analyzed using flow cytometry.

RESULTS: Cell viability assay showed that panobinostat was more cytotoxic in Huh7 cells than Hep3B. The treatment of panobinostat decreased clonogenic survival of Huh7 in combination with proton irradiation. Cell cycle analysis revealed that panobinostat enhanced proton-induced G2/M arrest. Panobinostat induced apoptotic death of Huh7 cells and further enhanced apoptosis when combined with proton irradiation. Panobinostat increased expression of Bax and Bak, pro-apoptotic proteins when combined with proton irradiation whereas it downregulated expression of Mcl-1, Bcl-2 and Bcl-XL, anti-apoptotic proteins. These data suggest panobinostat promotes proton-induced apoptosis by regulating pro-and anti-apoptotic proteins. Panobinostat also increased ROS production and further enhanced it when combined with radiation.

CONCLUSIONS: Panobinostat sensitized HCC cells more effectively to proton irradiation than X-ray irradiation. Panobinostat treatment led to upregulation of Bax and Bak, and downregulation of MCL-1 and Bcl-2, resulting in an increase in apoptosis and a decrease in clonogenic survival after proton irradiation. Panobinostat-increased ROS generation is additionally detrimental to HCC cells. Taken together, our findings suggest panobinostat as a potential radiosensitizer for proton therapy in HCC treatment.

NO CONFLICT OF INTEREST

422 Evaluation of the relative biological effectiveness of proton beam irradiation in hepatocellular carcinoma cell lines

A. Son[1,2], *C. Choi*[2], *G.H. Lee*[1], *H.C. Park*[1,2]

[1] *Samsung Medical Center, Department of Radiation Oncology, Seoul, South Korea*
[2] *Sungkyunkwan University, School of Medicine, Seoul, South Korea*

BACKGROUND: Proton therapy is an emerging therapy in the treatment of hepatocellular carcinoma (HCC). The relative biological effectiveness (RBE) of proton beam irradiation is clinically a fixed value of 1.1 but it is recently suggested that RBE variations depend on biological factors. Here, we evaluated proton RBE for HCC using seven human hepatocarcinoma cell lines and investigated a role of two major double strand break repair pathways - homologous recombination (HR) and non-homologous end joining (NHEJ) in proton RBE for HCC.

MATERIAL AND METHODS: Seven human hepatocellular carcinoma (HCC) cell lines were tested in this study. Clonogenic assay was performed to obtain radiation-dose response curves. Proton beam irradiation was performed at Samsung Medical Center in Seoul, Korea with proton beam (230 MeV) in the middle of spread-out Bragg peak (SOBP) width of 10 cm with a dose rate of 2.14 Gy/min. Photon irradiation were performed with a Varian Clinac 6EX accelerator with a dose rate of 3.96 Gy/min. For RBE calculation, an in-house MATLAB-based software was developed.

RESULTS: Radiation sensitivity that is defined as survival fraction at 2 Gy varied from 0.4 to 0.8. SNU449 is the most radioresistant cell line whereas SK-HEP1 is the most radiosensitive cell line. RBE was determined at three different end points (90%, 50% and 10% survival) and the RBE values were 1.154, 1.105 and 1.100, respectively. Depletion of Chek2, a cell cycle checkpoint protein increased radiation-induced cell killing but diminished RBE, which may be because Chek2 is a downstream of DNA damage pathway. Depletion of BRCA1, a DNA double strand break repair protein implicated in HR repair pathway, did not alter RBE. In contrast, depletion of DNA-PKcs, a key protein in NHEJ pathway greatly increased RBE. These data suggest that NHEJ but not HR repair pathway may be more relevant for modulating effect of proton therapy.

CONCLUSIONS: Proton RBE in various HCC cell lines has been determined for the first time. Clonogenic survival data indicate clinically fixed RBE 1.1 may be a valid approximation for HCC treatment regardless of end points. RNA interference revealed that NHEJ repair pathway may be more important for proton radiosensitization in HCC. Given that DNA-PKcs is usually overexpressed

or activated in advanced HCC, inhibition of DNA-PKcs may be a good therapeutic strategy for enhancing efficacy of proton therapy for HCC patients.

NO CONFLICT OF INTEREST

423 A 25 MeV proton irradiation platform for radiobiological studies

J. Constanzo[1], M. Vanstalle[1], H. Burckel[2], C. Finck[1], D. Brasse[1], G. Noel[2-3], M. Rousseau[1]

[1] Strasbourg University- CNRS- IPHC UMR 7 1 7 8 , DRHIM, Strasbourg Cedex 0 2 , France
[2] Strasbourg University- EA3 4 3 0 - F-6 7 0 0 0 Strasbourg- France, DRHIM, Strasbourg, France
[3] Radiotherapy department- Centre de lutte contre le cancer Paul Strauss- UNICANCER- F-6 7 0 6 5 Strasbourg, DRHIM, Strasbourg, France

Small animal proton therapy (PT) research platforms mimicking human PT conditions are needed. Indeed, pre-clinical studies are important to fully understand the therapeutic limitations and toxicities of PT. We developed a 25 MeV proton beam platform dedicated to small animal experiments, mostly for subcutaneous tumors irradiation. At this energy, dosimetric controls remain very challenging as the proton beam path in water is about 6 mm. In this study we first measured the initial energy that was E_0 = (24.85 ± 0.14) MeV after acceleration and the associated straggling σ_E = (0.127 ± 0.020) MeV. A homogeneous irradiation field with a suitable proton flux was obtained by means of a movable Al-scattering foil (200 µm thick). To allow a passive modulated proton beam, an energy degrader made of variable Al-thicknesses, corresponding to E_{range} = [23.71-4.03] MeV, was set in the air before an adaptable collimator for mouse subcutaneous tumors irradiation. Then, dosimetric measurements were performed using CMOS detector, plastic scintillator and Gafchromic® EBT3 films to qualify the beam line as well as the adapted treatment planning system. The spatial uniformity in air was found better than ± 2% over 9 mm diameter. A preliminary biological experiment was performed to test protocols with mouse glioma 261 cells. Cell survival to proton irradiation was performed and compared to X-rays irradiation. result will be presented and discussed.

NO CONFLICT OF INTEREST

424 Rat brain region-specific sensitivity following localized irradiation

J. Constanzo[1], L. Masson-Côté[2,3], M. Descoteaux[4], M. Lepage[5], L. Tremblay[6], M. Dumont[6], J.M. Longpré[6], K. Kirby[7], S. Geha[8], P. Sarret[7], B. Paquette[2]

[1] Strasbourg University- CNRS- IPHC UMR 7 1 7 8 , DRHIM, Strasbourg Cedex 0 2 , France
[2] Université de Sherbrooke, Center for Research in Radiotherapy- Department of Nuclear Medicine and Radiobiology, Sherbrooke, Canada
[3] Université de Sherbrooke, Service of Radiation Oncology, Sherbrooke, Canada
[4] Université de Sherbrooke, Department of Computer Science, Sherbrooke, Canada
[5] Université de Sherbrooke, Sherbrooke Molecular Imaging Center- Department of Nuclear Medicine and Radiobiology, Sherbrooke, Canada
[6] Université de Sherbrooke, Plateforme d'analyse et visualisation d'images PAVI- Centre de Recherche du Centre Hospitalier Universitaire de Sherbrooke, Sherbrooke, Canada
[7] Université de Sherbrooke, Department of Pharmacology and Physiology, Sherbrooke, Canada
[8] Université de Sherbrooke, Department of Pathology- Centre Hospitalier Universitaire, Sherbrooke, Canada

PURPOSE: Despite its efficiency for treating brain tumors and metastases, stereotactic radiosurgery (SRS) may deliver a significant dose of radiation in the surrounding healthy tissue. This can induce brain swelling, necrosis, and neuronal dysfunction leading to cognitive impairment. In this study, a localized irradiation in the rat brain highlighted region-specific sensitivity that leads to selective behavioral impairments.

RESULTS: 25 male Fisher rats were irradiated targeting the primary somatosensory cortex (S1FL, right hemisphere). Using the Leksell Gamma Knife (GK) Perfexion® with the associated treatment planning software, the prescribed dose was 37 Gy at 30% isodose corresponding to 113 ± 4 Gy in S1FL, 24 ± 10 Gy in the hippocampus, and 41 ± 8 Gy in the primary motor cortex (M1). Rats were scanned with a small-animal MRI scanner (7T) at different time points post-irradiation. In our rat model, necrosis and neovascularization were detected by T_2*-weighted MRI combined to dynamic-contrast-enhancement (DCE) MRI 54 days after GK. Brain-region specific sensitivity to radiation was determined with different behavioral tests assessing: i) motor function (actimetry) and coordination (rotarod), revealed that motor performance (M1-related) was not affected by radiation, ii) anxiety-like behaviors (elevated plus maze) and learning/memory performances (Morris water maze) were significantly decreased in rats exposed to GK, and iii) sensory pain behavior (formalin test) demonstrated a longer lasting pain (S1FL-related) due to inflammation for irradiated rats. Also, diffusion MRI revealed displacements and breakdown in neuronal pathways interconnecting cortical and subcortical structures (confirmed by histopathology).

CONCLUSION: Our result demonstrate that radiation induces region-specific changes in the behavioral response. Radionecrosis and neovascularization were revealed by T_2*-weighted combined with DCE-MRI earlier than behavioral dysfunctions appearance. Also, fiber bundles displacements between functional areas were observed by diffusion MRI, when the volume/severity of the lesion increased. Improved knowledge of specific targets dose response, based on advanced MRI tools, can help in reducing cognitive impairments observed after brain irradiation.

NO CONFLICT OF INTEREST

425 Downregulation of BCL10 enhances radiosensitivity of pancreatic cancer cells through attenuating the activation of NF-κB signaling and double-strand break repair

M.F. Wei[1], Y.H. Chen[1], Y.S. Tzeng[1], H.W. Lee[1], A.L. Cheng[1], S.H. Kuo[1]

[1] National Taiwan University Hospital, Department of Oncology, Taipei, Taiwan

INTRODUCTION: Locally advanced pancreatic cancer (LAPC) is highly lethal, even after combined chemotherapy and radiotherapy. We previously described the molecular linkage that directs both BCL10 overexpression and nuclear translocation in response to inflammation-related AKT/NF-κB signaling pathway. We also reported that nuclear expression of BCL10 in tumor cells was closely associated with the worse overall survival in patients with LAPC. Several studies demonstrated that the activation of PI3K/AKT signaling pathway and DNA repair pathway may be associated with radioresistance. In this study, we assessed whether inhibition of BCL10 can enhance the radiosensitivity of pancreatic cancer cells through the down-regulation of NF-κB signaling pathway and DNA repair pathway.

MATERIAL AND METHOD: Two pancreatic cancer cell lines (BxPC-3, wild-type K-RAS; PANC-1, mutant K-RAS) were used in this study. Both cell lines were transfected with BCL10 short hairpin RNA (shRNA) lentivirus for silencing the expression of BCL10. The expression pattern of BCL10 was assessed by western blot analysis. Clonogenic assay was used to determine whether BCL10 inhibition can enhance radiosensitivity of pancreatic cancer cells. We examined the expression levels of AKT/mTOR signaling molecules and DNA repair signaling molecules by western blotting. The γ-H2AX staining was also evaluated to determine the DNA damage.

RESULTS AND DISCUSSION: In the colony formation study, we found that BCL10 shRNA treatment resulted in a significant decrease of cell survival of both cell lines after irradiation. For DNA damage analysis, BCL10 inhibition resulted in more than 5 fold increase in number of γ-H2AX induced by irradiation when compared with irradiation alone. Furthermore, after irradiation, BCL10 shRNA treatment inhibited the expression levels of AKT/mTOR signaling molecules (p-mTOR, p-eIF4E, and p-rpS6) and DNA double-strand break (DSB) repair-related molecules (p-DNA-PKcs and p-ATM) in both cell lines when compared with irradiation alone. BCL10 shRNA treatment also decreased the irradiation-inducing NF-κB-dependent gene transcription in both cell lines.

CONCLUSION: Our findings revealed that inhibition of BCL10 can increase the radiosensitivity of pancreatic cancer cells through attenuating the activation of AKT/mTOR/NF-κB signaling and DNA-DSB repair. Further investigation of biologic significance of nuclear of BCL10 in mediating radioresistance of pancreatic cancer cells is warranted.

NO CONFLICT OF INTEREST

426 different radioresistant gene expression profiles in two novel established human melanoma cell lines

C. Arienti[1], S. Pignatta[1], M. Zanoni[1], A. Zamagni[1], M. Cortesi[1], L. Medri[2], C. Leonetti[3], S. Serravalle[4], A. Tesei[1]

[1] Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori I.R.S.T., Drug Discovery Unit & Radiobiology, Meldola, Italy
[2] Morgagni-Pierantoni Hospital, Pathology Unit, Forlì, Italy
[3] Regina Elena National Cancer Institute, Department of Research- Diagnosis and Innovative Technologies- Traslational Research Area, Rome, Italy
[4] S.Orsola Malpighi Hospital, Laboratory of Pediatric Hematology Oncology "Lalla Seràgnoli", Bologna, Italy

BACKGROUND: Despite progress made in recent years following the introduction of new drugs and new combinations of chemoimmunotherapy for melanoma, prognosis remains poor. Although considered a radioresistant tumor, radiation treatment is sometimes used in patients with lentigo maligna melanoma, as adjuvant therapy in selected patients with regional metastatic disease, and as palliative therapy, especially in patients with bone and brain metastases. Cell lines represent an important experimental tool in cancer research, providing an infinite supply of relatively homogeneous cell populations that are capable of self-replication. Cell cultures established directly from human tumors serve as unique models for the identification of novel biological and physical therapeutic targets.

MATERIAL AND METHODS: We established two human melanoma cell lines, M66 and M79, and characterized their genome profile with sequence analysis and microarray analysis. Karyotype analyses were performed to clarified structural abnormalities. The tumorigenic potential in vitro and in vivo was investigated on both cell lines. Cells were exposed to radiation to produce radioresistant subclones and clonogenic assay was performed for studying proliferation of treated cells. A library of drugs used in clinical practice was also tested.

RESULTS: M79 and M66 cell lines were isolated from a primary tumor and a lymph node metastasis, respectively, and were propagated in culture. DNA fingerprinting analysis indicated that the cell lines originated from parental tumor tissue. Both cell lines showed anchorage-dependent growth and high tumorigenic capacity, inducing solid tumors in immunodeficient mice. BRAF mutational analysis revealed a V600 mutation in both cell lines. In vitro radiation-induced cytotoxicity was evaluated and the sensitivity against chemotherapeutic agents used alone or in combination with radiation regimens was assessed.

We also established radioresistant cell lines by applying fractionated radiation, then using cDNA microarrary to identify differentially expressed genes in parent

and radioresistant cells. A number of differentially expressed genes were then assessed.

CONCLUSIONS: We established two new melanoma cell lines with different radio-chemo profiles that could be used to investigate the potential of new therapeutic approaches.

NO CONFLICT OF INTEREST

427 Ionizing radiation abrogates the pro-tumorigenic effects exerted by admixed Cancer-Associated Fibroblasts in xenografts

T.Hellevik[1], M. Tunset Grinde[2], J. Vik[3], K.A. Camilio[4], I. Martinez-Zubiaurre[5]

[1] University Hospital of Northern Norway, Oncology, Tromso, Norway
[2] University of Tromso, Clinical Medicine, Trondheim, Norway
[3] University of Tromsø, Clinical Medicine, Tromso, Norway
[4] University of Tromso, Medical Biology, Tromso, Norway
[5] University of Tromso, Clinical medicine, Tromso, Norway

INTRODUCTION: Non-malignant elements of tumors play fundamental roles in both cancer sustainability and therapeutic responses. In the context of clinical radiotherapy (RT), all cell types residing within the tumor mass are exposed to radiation, but the impact of stromal cells - in particular cancer-associated fibroblasts (CAFs) - to treatment outcomes remains largely unexplored. In this study, we have investigated the effects of ionizing radiation on the natural pro-tumorigenic actions of CAFs in vivo.

MATERIAL & METHODS: Human lung CAFs were isolated from freshly resected non-small cell lung carcinomas (NSCLCs). Ionizing radiation produced by clinical linear accelerators was delivered to cultured CAFs, in regimens consisting a single-high dose or fractionated doses. Tumor take and development was compared in murine xenografts, after co-implantation of A549 lung tumor cells along with irradiated or non-irradiated human lung CAFs. Quantitative histology and immunohistochemistry was performed to investigate potential biological mechanisms behind tumor growth regulations.

RESULTS AND DISCUSSION: Viability assays demonstrated that irradiated CAFs are viable and fully functional at the time of implantation. However, the enhanced tumorigenesis observed with admixed control CAFs was nullified in animal tumors established with irradiated CAFs. Quantitative determinations of parameters such as desmoplasia, angiogenesis, inflammation, or tumor cell proliferation revealed enhanced blood vessel density in tumors established with irradiated CAFs. Experiments to ascertain the fate of implanted CAFs showed that both irradiated and non-irradiated CAFs only reside at the implantation site during early stages, suggesting that the regulatory functions of admixed CAFs may take place during initial phases of tumor formation/engraftment.

CONCLUSIONS: In this study, we show that CAFs receiving ionizing radiation lose their pro-tumorigenic potential in vivo, affecting angiogenesis and possibly other mechanisms related to tumor engraftment. This finding represent a previously unknown advantageous effect induced by radiotherapy, which adds to the well-known direct cytotoxic effects on transformed epithelial cells.

NO CONFLICT OF INTEREST

POSTER SESSION: SIGNALLING PATHWAYS I

428 Anti-tumor mechanisms of J2, a novel aliphatic hydroxamate derivative, in FaDu head and neck cancer cells

M.J. Hsu[1], W.J. Huang[2], PY. Lai[3]

[1] Taipei Medical University, Department of Pharmacology, Taipei, Taiwan
[2] Taipei Medical University, Graduate Institute of Pharmacognosy, Taipei, Taiwan
[3] Taipei Medical University, Graduate Institute of Medical Sciences, Taipei, Taiwan

INTRODUCTION: Hydroxamate derivatives have recently attracted much attention in the field of drug discovery due to their broad pharmacological properties including anti-tumor activity.

MATERIAL AND METHOD: In this study, we investigated a novel aliphatic hydroxamate derivative, J2, and characterized its anti-tumor mechanisms in Fadu head and neck cancer cells.

RESULT AND DISCUSSION: J2 induced G2/M cell cycle arrest and apoptosis in FaDu cells. These actions were associated with liver kinase B1 (LKB1), AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase (p38MAPK) activation, p63 phosphorylation, as well as the modulation of p21 and survivin. LKB1-AMPK-p38MAPK signaling blockade reduced J2's effects on p63 phosphorylation, p21 induction and survivin reduction. The binding of p63 to the survivin promoter region increased after J2 exposure, and this was accompanied by a decreased in Sp1 binding to the promoter region. In contrast, J2 increased p63 and Sp1 binding to the p21 promoter region in FaDu cells. J2 also caused STAT3 dephosphorylation, STAT3-luciferase activity reduction and decreased STAT3 binding to the survivin promoter region. Moreover, J2 induced α-tubulin acetylation and interfered with microtubule assembly. Transfection with HDAC6-Flag or HDAC8-Flag abrogated J2's enhancing effect on α-tubulin acetylation. Furthermore, J2 suppressed the growth of subcutaneous xenografts of FaDu cells in vivo.

CONCLUSION: J2-induced FaDu cell death may involve LKB1-AMPK-p38MAPK-p63-survivin signaling pathway. Disruption of microtubule assembly and repression of STAT3 signaling may also contribute to J2's actions in FaDu cells. These result suggest that J2 may be a potential drug candidate and warrant the clinical development in the treatment of head and neck cancer cells.

NO CONFLICT OF INTEREST

429 Signaling mechanisms involved in IL-6-induced epithelial-to-mesenchymal transition in MCF-7 cells

Y.F. Hsu[1], M.J. Hsu[2]

[1] Landseed Hospital, Department of Surgery, Taoyuan, Taiwan
[2] Taipei Medical University, Department of Pharmacology, Taipei, Taiwan

The difficulties in breast cancer treatment are attributable to highly metastatic ability of breast cancer cells. The aberrant activation of epithelial-to-mesenchymal transition (EMT) is involved in the enhanced tumor metastasis. Serum interleukin-6 (IL-6) level increases with tumor grade of breast cancer. Recent study showed that IL-6 induces EMT in breast cancer cells with an epithelial phenotype. However, the underlying mechanisms by which IL-6 induces EMT remain incompletely understood. In this study, we aimed to explore the signaling mechanisms involved in IL-6-induced EMT in MCF-7 breast cancer cells. Treatment of MCF-7 cells with IL-6 result in EMT, as evidenced by induction of the mesenchymal markers twist, snail, slug and repression of the epithelial marker E-cadherin. IL-6 is capable of increasing cell motility in MCF-7 cells. These actions were associated with Src, FAK, ERK and p38 mitogen-activated protein kinase (MAPK) activation, as well as the phosphorylation of STAT3, p65 and C/EBPß. Src-FAK signaling blockade reduced IL-6's enhancing effects in inducing ERK, p38MAPK, STAT3, p65 and C/EBPß phosphorylation and subsequent EMT. In addition, inhibitors of ERK or p38MAPK reduced IL-6-induced p65 and C/EBPß phosphorylation and EMT. STAT3 knockdown by STAT3 siRNA also suppressed IL-6-induced EMT. Furthermore, IL-6 caused increases in STAT3, p65 and C/EBPß binding to the twist promoter region in MCF-7 cells. Taken together, these result indicated that IL-6 may activate Src-FAK-STAT3 signaling cascade, leading to EMT and subsequent breast cancer metastasis. ERK and/or p38MAPK signaling and transcription factors p65 and C/EBPß may also contribute to IL-6-induced EMT in MCF-7 cells.

NO CONFLICT OF INTEREST

430 ITB02, a novel indol derivative, impairs tumor angiogenesis and growth by interfering with VEGF-A/VEGFR2 pathway

S.W. Huang[1], T.F. Huang[1]

[1] National Taiwan University, Graduate Institute of Pharmacology, Taipei, Taiwan

INTRODUCTION: Angiogenesis plays a key role in the pathogenesis of ischemic, inflammatory and immune disorders. It is also required for tumor growth and metastasis. As a critical factor in inducing angiogenesis, vascular endothelial growth factor (VEGF)-VEGF receptor thus represent an attractive therapeutic target for anti-angiogenesis treatment.

MATERIAL AND METHODS: In an effort to develop novel inhibitors to suppress VEGF signaling and angiogenesis, we explored and characterized anti-angiogenic mechanisms of an indol derivative ITB02 using human umbilical endothelial cells (HUVECs).

RESULT AND DISCUSSION: ITB02 concentration-dependently suppressed VEGF-A-induced proliferation, migration and tube formation of HUVECs. ITB02 also inhibited VEGF-A-induced microvessel sprouting from aortic rings ex vivo and suppressed neovascularization of implanted matrigel plugs in vivo. In addition, ITB02 attenuated the phosphorylations of VEGFR2, VEGFR1, mitogen-activated protein kinase (MAPK), focal adhesion kinase (FAK), Src kinase, and phosphoinositide 3-kinase (PI3K)/ protein kinase B (AKT) pathway, in VEGF-A-stimulated HUVECs. Systemic administration of ITB02 decreased tumor angiogenesis and suppressed tumor growth in in vivo mouse xenograft models. Taken together, ITB02 was shown to modulate vascular endothelial cell remodeling through suppressing VEGF-A signaling. These result also support the role of ITB02 as a potential drug candidate for developing anti-angiogenic agent in field of cancer and angiogenesis-related diseases.

CONCLUSIONS: ITB02 regulates vascular endothelial cell remodeling through suppressing VEGF-A signaling. These result also support the role of ITB02 as a potential drug candidate for developing anti-angiogenic agent in field of cancer and angiogenesis-related diseases.

NO CONFLICT OF INTEREST

431 Using retinoic acid to treat triple-negative breast cancer

K.M. Coyle[1], C. Dean[1], D. Vidovic[1], I. Weaver[2], C. Giacomantonio[3], L. Helyer[3], P. Marcato[1]

[1] Dalhousie University, Pathology, Halifax, Canada
[2] Dalhousie University, Psychology and Neuroscience- and Psychiatry, Halifax, Canada
[3] Dalhousie University, Surgery, Halifax, Canada

BACKGROUND: We have identified a novel strategy to identify breast cancer patients who will benefit from an existing anti-cancer agent, retinoic acid (RA).

While RA has not yet achieved success in the treatment of breast cancers, we hypothesized that it can be an effective therapy for a subset of triple-negative breast cancer (TNBC) patients. TNBC is among the most aggressive breast cancers, and lacks targeted therapies. TNBCs can be further subtyped into basal-like and claudin-low, which differ in gene expression and drug sensitivities. Understanding the molecular basis of these subtypes will lead to the development of more effective treatment options for TNBC.

MATERIAL AND METHODS: We performed tumor growth assays on TNBC cell lines and patient-derived xenografts (PDXs) in NOD/SCID mice. DNA methylation was interrogated by Illumina Human Methylation 450 arrays, and we used Affymetrix Human Gene 2.0 arrays to quantify gene expression.

RESULTS: We found that RA treatment decreased the tumor growth of four basal-like TNBC cell lines (MDA-MB-468, HCC70, SUM149, HCC1937). In contrast, RA increased the tumor growth of two claudin-low TNBC cell lines (MDA-MB-231, MDA-MB-436). Gene expression and methylation analysis of these affected cell lines revealed subtype-specific expression of RA-inducible genes due to silencing by DNA methylation, e.g. of the RA-inducible tumor-suppressor gene RARRES1. RARRES1 is silenced by methylation in claudin-low cell lines, but is hypomethylated and expressed in basal-like cells. Use of the subtype-specific expression and methylation profiles allowed us to accurately predict the response of 4 PDXs to RA treatment.

CONCLUSIONS: Continued classification of TNBCs into these two subtypes will enable clinical use of RA, in part due to the subtype-specific hypomethylation of RA-inducible tumor suppressor genes including RARRES1. We have identified additional subtype-specific biomarkers which can predict the response of patient tumors to RA treatment, thus identifying a novel targeted therapy strategy for TNBCs.

NO CONFLICT OF INTEREST

432 The role of Pdk1-dependent signalling pathways in pancreatic cancer

F. Hesse[1], *N. Schönhuber*[1], *D. Saur*[1]

[1] *Klinikum rechts der Isar der Technischen Universität München, Klinik und Poliklinik für Innere Medizin II, Munich, Germany*

BACKGROUND: In pancreatic ductal adenocarcinoma (PDAC) the mutation of the oncogene Kras plays a decisive role. It initiates carcinogenesis via different signalling cascades including the PI3K-Pdk1 pathway. The deletion of the 3-phosphoinositide dependent protein kinase 1 (Pdk1) entirely blocks the formation of precursor lesions and PDAC in a pancreatic cancer mouse model. The aim was to further analyse downstream kinases of Pdk1 to define novel therapeutic targets.

MATERIAL AND METHODS: Pdk1 has two regulatory domains, i.e. the PH-domain activating Akt and the PIF-pocket-domain regulating Sgk, Pkc and Rsk. Using two different Pdk1 mutant alleles, $Pdk1^{K465E}$ and $Pdk1^{L155E}$, we blocked the activation of each domain particularly. Therefor, these alleles were crossed into the genetically engineered endogenous [NR1] $Ptf1a^{Cre/+}$;$LSL\text{-}Kras^{G12D/+}$ pancreatic cancer model. Pancreatic tissue of mice at 3, 6, 9 and 12 month of age were analysed for tumor formation and compared to $Ptf1a^{Cre/+}$;$LSL\text{-}Kras^{G12D/+}$ mice.

RESULTS: Recombination of the mutant Pdk1 alleles in the pancreas was shown by PCR using microdissected pancreatic tissues. Western blot and immunohistochemistry indicated a downregulation of targets of Pdk1 in the respective knock-in mice. Despite expression of oncogenic Kras, inactivation of each regulatory domain resulted in a reduction of preneoplastic pancreatic intraepithelial lesions (PanIN) and blocked cancer development in the animals within one year. Aged $Pdk1^{L155E}$-mutant animals evolved pancreatic atrophy.

CONCLUSIONS: These result support the pivotal role of Pdk1 and imply that both regulatory domains and their downstream kinases are essentially involved in pancreatic tumorigenesis. Further research will help to clarify their potential as targets for therapy in human PDAC.

NO CONFLICT OF INTEREST

433 Receptor-antibody clustering induced endocytosis of HER2 has therapeutic potential in breast cancer

J.M. Wymant[1], *P. Moody*[1], *E.J. Sayers*[1], *A.T. Jones*[1]

[1] *Cardiff University, School of Pharmacy and Pharmaceutical Sciences, Cardiff- Wales, United Kingdom*

BACKGROUND: HER2 is an oncogenic receptor, promoting tumour development and disease progression in 15-25% of breast cancers. HER2 overexpressing tumours are more aggressive and associated with poorer prognosis, thus the receptor is a priority target for anticancer therapy. HER2 is notably 'endocytosis resistant", frustrating targeting approaches where efficacy is contingent on lysosomal delivery e.g. antibody-drug conjugates (ADC) such as trastuzumab-emtansine. We extend our recently published research showing that receptor-antibody clustering induced endocytosis (RACIE) can be strategically used to promote lysosomal delivery and degradation of HER2 in breast cancer cells (Moody et al. 2015; Ogris and Sami 2015).

MATERIALS AND METHODS: HER2-RACIE was examined in two breast cancer cell lines overexpressing this receptor: BT474 and SKBR3 cells. Clustering was achieved by sequential application of biotinylated, fluorescently-labelled trastuzumab and streptavidin. Trastuzumab was clinically sourced and labelled in-house. Following RACIE, downstream analyses were performed: Western blotting to study changes in HER2 levels, activation and downstream signalling and to measure the levels of other HER family members, EGFR and HER3. Live cell confocal microscopy was conducted to monitor endocytosis.

RESULTS AND DISCUSSION: Examination of HER2 levels at time points up to 48 hr demonstrated that after an initial reduction, HER2 levels then recover, potentially allowing for retargeting. Investigation of HER2 activation and downstream signalling revealed HER2-RACIE specific activation of the MEK/ERK pathway. The levels of HER2 dimerization partners were examined and while EGFR levels were unaffected, HER3 was concomitantly downregulated with HER2. Highly biotinylated trastuzumab (6.0 biotins per antibody) was compared with low biotin (1.7 per antibody) for RACIE. The data showed that HER2 and HER3 downregulation was only induced by the highly biotinylated trastuzumab while ERK activation was stimulated by both antibodies.

CONCLUSIONS: Our data suggest that RACIE, induced by antibodies of sufficient valency, represents an effective approach for enhanced delivery of ADCs in $HER2^{+}$ breast cancer. This strategy may also have the potential extend to other receptor targets in a wide range of cancers.

NO CONFLICT OF INTEREST

434 HER3 activating mutations facilitate human mammary epithelial cells resistant to HER2 inhibition

J. Garrett[1], *R. Mishra*[1], *L. Yuan*[1]

[1] *University of Cincinnati, College of Pharmacy, Cincinnati, USA*

BACKGROUND: Recent large-scale genomic studies reported that HER3 (ErbB3) mutations occur in 1 to 3% of primary breast cancers and up to 11% of colon and gastric cancers. We sought to determine if naturally occurring mutations in HER3 enhance HER2-mediated or hormone-driven transformation in human mammary epithelial cells. Furthermore, we examined if HER3 mutations confer resistance to HER2 inhibitors in HER2-dependent MCF10A/HER2 or antiestrogens in ER+ breast cancer cells (MCF-7 and T47D).

MATERIAL AND METHODS: A series of HER3 mutations identified in patient breast cancer tumors were introduced and stable cell lines were generated in MCF10A/HER2 and ER+ T47D and MCF-7 cells using lentiviral transduction. Furthermore, we tested the effect of knocking down HER3 in four cell lines harboring endogenous HER3 mutations. We transfected these cells with either HER3 or control siRNA.

RESULTS: We identified several HER3 mutations that had higher cell proliferation than wild-type (wt) HER3 in MCF10A/HER2 cells including F94L, G284R, D297Y, T355I, E1261A. Furthermore, these mutations counteracted the effect of the HER2 inhibitor lapatinib. These mutations notably maintained high phosphorylated HER2, HER3, AKT and ERK1/2 compared to cells expressing wt HER3 when subjected to lapatinib treatment. Experiments are ongoing to determine if these mutations render resistance to the irreversible HER2 tyrosine kinase inhibitor, neratinib. We hypothesize that these mutations stabilize HER2 via triggering HER3's open confirmation, therefore leading to increased HER2-HER3 heterodimer formation. Experiments are ongoing to test this hypothesis. The T355I mutant has statistically significant increased proliferation compared to wt HER3 in both ER+ MCF-7 and T47D cells. The T355I mutation renders ER+ T47D and MCF-7 cells resistant to 4-hydroxytamoxifen, but not to fulvestrant, indicating the possible role in anti-estrogen therapy. Experiments are ongoing to determine resistance mechanism(s). ER+ cells overexpressing T355I have increased p-ErbB3 and p-ERK1/2 expression compared to wt cells. Phospho-RTK array result indicate that ER+ T47D T355I cells have increased p-ErbB4 suggesting possible activation of downstream MAPK signaling. Additionally, cell lines with endogenous HER3 mutations transfected with HER3 siRNA had reduced proliferation compared to cells transfected with control siRNA.

Conclusions

These data indicate the potential for HER3 mutations to be oncogenic.

NO CONFLICT OF INTEREST

435 Resistance to tyrosine-kinase inhibitors in ALK-positive lymphoma: Key differences with lung cancer suggest new strategies to prolong disease control

J. Schatz[1], *S. Rajan*[2], *A. Amin*[1], *L. Li*[1], *S. Puvvada*[3]

[1] *Miller School of Medicine- University of Miami, Medicine, Miami, USA*
[2] *Miller School of Medicine- University of Miami, Sheila and David Fuente Graduate Program in Cancer Biology, Miami, USA*
[3] *University of Arizona, Medicine, Tucson, USA*

BACKGROUND: Oncogenic fusions of the anaplastic lymphoma kinase (ALK) are found in 70% of anaplastic large-cell lymphoma (ALCL) and ~5% of non-small-cell lung cancer (NSCLC). Three ALK tyrosine-kinase inhibitors (TKIs) are approved for ALK+ NSCLC, and several additional compounds are in development. Resistance mechanisms in NSCLC are well described, while clinical samples of TKI-treated ALCL are not yet available in quantity. We reported previously that unlike NSCLC, ALCL tumor cells have great difficulty finding alternative pathways to replace ALK and instead up-regulate expression of the NPM1-ALK fusion to maintain survival. Upon inhibitor withdrawal, however, high levels of ALK activity drive death through

apoptosis which permitted prolonged disease control in vivo via intermittent dosing.

MATERIALS AND METHODS: We have developed multiple models of ALK+ ALCL with resistance to first-, second-, and third-generation ALK inhibitors. We characterize mechanisms through genomics and extensive functional and biochemical assessments.

RESULTS: All generations of ALK inhibitors drive NPM1-ALK over-expression as the most frequent resistance mechanism in ALCL, but the highly increased ALK activity upon inhibitor withdrawal is universally toxic. ALK-kinase domain mutations also arise but are less frequent and typically weaker drivers of resistance than over-expression. Only one clone has emerged from selections demonstrating evidence of ALK-independent resistance. Genomic analyses show accumulation of a high mutational burden and extensive aneuploidy in this model revealing multiple simultaneous mechanisms conspiring to promote resistance. Analysis of cells that die due to over-stimulation of ALK activity, meanwhile, demonstrates multiple downstream molecular events that result in apoptosis and reveal important roles for ALK's fusion partner NPM1. Among other things, RNAseq analysis reveals hyper-stimulation of protein biogenesis and RNA processing pathways as cells die due to ALK overdose.

CONCLUSIONS: In contrast to ALK+ NSCLC, ALCL cannot easily bypass a need for ALK signaling by activating alternate pathways. NPM1-ALK over-expression driving resistance can be exploited through drug withdrawal. Our data highlight intermittent dosing and reveal novel mechanisms of cell death resulting from ALK over-activation that inform novel therapeutic strategies for ALCL, some of which may also be applicable to other ALK-driven tumor types.

NO CONFLICT OF INTEREST

436 Recombinant anthrax lethal toxin inhibits colon cancer cell proliferation through targeting the MAPK and Rho GTPase pathway

M. El-Sibai[1]

[1] *Lebanese American University, Natural Science, Beirut, Lebanon*

BACKGROUND: Colon cancer is the second most commonly diagnosed cancer in females, the third in males. More than a million cases are yearly detected globally. Previous studies in our laboratory showed that proliferation of AML cells can be inhibited through the inhibition of the mitogen-activated protein kinase (MAPK) pathway using a recombinant anthrax lethal toxin (LeTx).

MATERIALS AND METHODS: In this study, we examined the effect of Rho GTPases, downstream from the MAPK pathway, on cell proliferation and apoptosis in colon cancer cells. We tested the potency of LeTx on a panel of 5 human colon cancer cell lines. We also used siRNA against the Rho GTPases RhoA and Cdc42 to see if the knock down would mimic the effect of LeTx.

RESULTS: The five cell lines showed cytotoxic responses to LeTx and to the specific mitogen-activated protein/extracellular signal–regulated kinase kinase 1/2 (MEK1/2) inhibitor U0126, indicating that LeTx-induced cell death is mediated through the MEK1/2–extracellular signal–regulated kinase (ERK1/2) branch of the MAPK pathway. This cytotoxicity was mimicked in cells transfected with siRNA against the Rho GTPases RhoA and Cdc42. This effect was reversed in cells that were treated with LeTx and transfected with constitutively active Cdc42 or RhoA constructs showing that the effect of LeTx is mediated through the Rho GTPases. We also looked at the activation of Cdc42 and Rho in a pull-down assay and the activation in these cells was significantly reduced after treatment with LeTx proving that the MAPK pathway is activating Cdc42 and RhoA in colon cancer cells.

CONCLUSION: In this study, we have shown that colon cancer cells are sensitive to the LeTx-mediated inhibition of the MAPK pathway and that this was, at least in part, mediated through RhoA and Cdc42 and their downstream effectors.

NO CONFLICT OF INTEREST

437 Kinase recomplementation screen in Pdk1 deleted pancreatic tumor cell lines

L. Liotta[1], *D. Saur*[1], *C. Veltkamp*[1], *G. Schneider*[1]

[1] *Klinikum rechts der Isar, II Medizinische Klinik, Munich, Germany*

BACKGROUND: Pancreatic cancer is a life threatening disease, leading to death despite treatment in 99% of the patients within 5 years. The most common form of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC) which is characterized by activating Kras mutations in >95% of cases. We showed that the PI3K effector Pdk1 is essential for PDAC initiation and its loss leads to complete blocking of PanIN and PDAC formation in the $Kras^{G12D}$ model. Pdk1 is essential for the growth of $Kras^{G12D}$-driven PDAC cell lines and Pdk1-knockout (KO) cells show a significantly decreased growth and a reduced metabolic activity. Up to now, it is not known which Pdk1-dependent signaling pathways are involved in this process as well as how resistance mechanisms against Pdk1 inhibition develop. Therefore, we examined whether expression of specific activated kinases is able to compensate for the loss of Pdk1 and thus rescue the Pdk1-KO phenotype.

MATERIAL AND METHODS: novel inducible dual-recombinase system (DRS) was generated in our lab by combining Flp/frt and Cre/loxP system. Here, we used the DRS to delete Pdk1 in established PDAC cells by tamoxifen administration. Using a library of myristoylated, and thus constitutively activated, kinases, we overexpressed single kinases in Pdx1-Flp;$FSF\text{-}Kras^{G12D/+}$;$FSF\text{-}R26^{CAG\text{-}CreERT2/+}$;$Pdk1^{lox/lox}$ cell lines by retroviral transduction. Cell viability and colony formation were evaluated in Pdk1-KO and control cells for each kinase.

RESULTS: The majority of almost 30 tested kinases was not able to compensate the loss of Pdk1. We showed a complete rescue of Pdk1-KO phenotype both in MTT and Clonogenic Assay by overexpressing AKT1, one of the main direct targets of Pdk1. Akt downstream pathways were activated in AKT1 overexpressing cells both in control and Pdk1-deleted cell lines. A partial rescue of Pdk1 loss was obtained by overexpressing RSK3, RPS6KA5, CKS1B, PKN1, CDK2 and SYK kinases. Previous exome sequencing studies have shown that CKS1B is often overexpressed in human PDAC supporting the importance of our finding.

CONCLUSIONS: We showed that AKT1 as well as other kinases are able to bypass Pdk1 deletion in PDAC cell lines which could help to better understand the mechanisms of resistance against PI3K/Pdk1 inhibition.

NO CONFLICT OF INTEREST

438 P73 induce apoptosis by transcription-independent mechanism

S.U. Choi[1], *B.Y. Kim*[1], *M.K. Yoon*[2], *S.W. Chi*[2]

[1] *Korea Research Institute of Chemical Technology, Drug Discovery Diuision, Daejeon, Korea*
[2] *Korea REsearch Institute of Bioscience and Biotechnology, Disease Target Structure Research Center, Daejeon, Korea*

BACKGROUND: p73 is a structural and functional homologue of the p53 tumor suppressor protein. Like p53, p73 induces apoptosis and cell cycle arrest and transactivates p53-responsive genes, conferring its tumor suppressive activity. In addition, p73 plays unique roles in neuronal development and differentiation. The importance of p73-induced apoptosis lies in its capability to substitute for the pro-apoptotic activity of p53 in various human cancer cells in which p53 is mutated or inactive. Despite the great importance of p73-induced apoptosis in cancer therapy, little is known about the molecular basis of p73-induced apoptosis. Although mechanistic overlap occurs between p73 and p53 in apoptosis, the underlying mechanism for transcription-independent p73-induced apoptosis has not been investigated in the context of the Bcl-2 family of proteins.

MATERIAL AND METHODS: Cell viability, growth inhibition, colony formation, and cytochrome c release assays were performed to analyze p73-induced apoptosis. Immunoprecipitation, GST-pulldown, immunofluorescence, and nuclear magnetic resonance (NMR) experiments were performed to identify the interactions between p73 and Bcl-2 family proteins. Solution structure of the protein complex was determined by using NMR spectroscopy.

RESULTS AND DISCUSSION: In this study, we showed a transcription-independent mechanism of p73-induced apoptosis in which direct interaction occurs between p73 and Bcl-2 family proteins in the mitochondria. Our result showed that, in response to apoptotic stimuli, p73 induces apoptosis in the absence of transcription and translation by triggering cytochrome c release from mitochondria, but the pro-apoptotic activity of p73 is inhibited by interaction with Bcl-2 family proteins in the mitochondria. In addition, the complex structure of p73 and Bcl-2 family protein revealed a molecular basis for regulation of transcription-independent apoptosis of p73.

CONCLUSION: In response to apoptotic stimuli, p73 induces transcription-independent apoptosis as well as transcription-dependent apoptosis. This reveals the molecular basis for the high similarity between p73 and p53 apoptotic function. Although the mechanisms of p73-induced apoptosis remain controversial, with conflicting activities depending on cellular context, our result may contribute to understanding of the multifunctional role of p73 at the molecular level.

NO CONFLICT OF INTEREST

439 Prolonged Cetuximab treatment selects for novel, activating mutations in the ligand-binding domain of EGFR in head and neck squamous cell carcinoma

M. Bredel[1], *S. Nozell*[1], *R. Rajbhandari*[1], *N. Thudi*[1], *H. Trummell*[1], *C. Dates*[1], *C. Willey*[1], *E. Yang*[1], *J. Bonner*[1]

[1] *Univiersity of Alabama at Birmingham, Radiation Oncology, Birmingham, USA*

BACKGROUND: Epidermal growth factor receptor (EGFR) is a protumorigenic receptor tyrosine kinase. Head and neck squamous cell carcinoma are treated with cetuximab, a therapeutic monoclonal antibody, which binds to EGFR and inhibits EGFR signaling. However, some tumors display little/no effect in response to prolonged cetuximab therapy. In this study, we investigated potential mechanisms underlying cetuximab resistance in head and neck squamous cell carcinoma.

MATERIALS AND METHODS: Human head and neck squamous cell carcinoma cells were developed as cetuximab-resistant (CTX-R) cells after several months of continuous exposure to cetuximab. The DNA sequence and status of EGFR gene was established by DNA sequencing. The levels of total and activated EGFR, and downstream signaling events (Akt, mTOR, NF-kB, STAT3) were assessed by western blot analyses, and the levels of pro-tumorigenic target genes (IL-8, IL-6, cyclin D1, NFKBIA) evaluated by qRT-PCR. EGF- and cetuximab-binding were assessed using labeled EGF and cetuximab, respectively, and flow cytometry.

RESULTS: We determined that persistent cetuximab significantly increased the EGFR gene copy numbers, and levels of EGFR mRNA and activated EGFR (phosphorylated EGFR). We identified three mutations in the EGF ligand-binding domain in CTX-R cells, which were non-synonymous and produced amino acid changes (G33S, N56K, and A313V); a single mutation in the tyrosine kinase domain was a synonymous SNP. PyMOL structural modeling analyses predicted that N56K would reduce EGF binding affinity. We confirmed that mutant EGFR has reduced affinity for EGF and, moreover, is unable to bind cetuximab, a therapeutic monocolonal antibody specific for EGFR. Despite having reduced affinity for EGF, the novel EGFR mutant displays constitutively activated EGFR. More interestingly, mutant EGFR activates pro-survival pathways (Akt, mTOR) distinct from those that are activated by wild-type EGFR (NF-kB) in the parental cells; mutant EGFR activation coincided with elevated levels of pro-tumorigenic target genes.

CONCLUSIONS: Our result demonstrate that upon prolonged exposure to cetuximab, cells selected for a novel activating mutation specific to the EGF ligand-binding domain. This mutation reduces the receptor's affinity for EGF, but renders the receptor constitutively activated. More importantly, this novel mutation prevented EGFR from binding cetuximab. These data suggest that prolonged exposure to cetuximab may increase monoclonal antibody therapy resistance in head and neck squamous cell carcinoma.

NO CONFLICT OF INTEREST

441 Differential anti-proliferative activity of isoflavones against Src- and Ras-activated human adenocarcinoma cells

M. Ono[2], *T. Higuchi*[2], *M. Takeshima*[1], *R. Wakimoto*[2], *S. Nakano*[1]

[1] *Nakamura Gakuen University, Graduate School of Health and Nutritional Sciences, Fukuoka, Japan*

Src and Ras oncogenes have been strongly implicated in the development, growth, progression, and metastasis of a variety of human cancers. Although many studies reported that soy isoflavones have potential anticancer activity, the role of isoflavones in their oncogenic activity remains unknown. Using HAG-1 human adenocarcinoma cells transfected with either activated Src or Ras, we investigated here the functional role of those oncogenes in anti-proliferative activity of isoflavones such as genistein, daidzein, glycitin and equol. The growth inhibitory activities of those isoflavones against Src-, Ras-, and vehicle-transfected cells (HAG/src, HAG/ras, and HAG/neo) were investigated using WST-1 cell proliferation assay. Effects of those isoflavones on apoptosis and cell cycle perturbation were evaluated by FACS analyses. The growth of HAG/neo cells was inhibited potently by genistein and equol, but modestly by daidzein and glycitin. Activation of Ras did not alter the cytotoxicity of these isoflavone components, showing a similar IC50 for HAG/neo. In contrast, activation of Src conferred resistance to either daidzein, glycitin or equol, but rendered the cells more sensitive to genistein, compared to HAG/neo; Genistein strongly inhibited the growth of HAG/src cells in a dose–dependent manner with IC50 approximately 25 μM, whereas in other three isoflavone components, the inhibitory effects were minimal without reaching an IC50 even at a dose of 100 μM. Upon treatment with 50 μM genistein for 72 h, HAG/src cells were significantly arrested at the G2/M compared to HAG/neo control cells (37.7% versus 7.0%). By contrast, the same concentration of either daidzein, glycitin or equol could not arrest HAG/src cells at any checkpoint of the cell cycle. The sub-G0/G1 apoptotic cell populations were not increased after 72 h exposure with either isoflavones. Therefore, it appears that growth inhibition by genistein in Src-activated cells would be mediated mainly by the G2/M arrest of cell cycle rather than apoptosis induction. These data suggest that genistein would be the only isoflavone component that may potentially suppress oncogenic activity driven by Src but not by Ras, providing a mechanistic rationale for the potential use of genistein in the prevention and treatment of human cancers with activated Src.

NO CONFLICT OF INTEREST

442 The major histocompatibility complex (MHC) class II mediated signalling increases lipid raft recruitment of adhesion receptors and signal transduction proteins in melanoma cells

G. Barbieri[1], *F. Costantini*[1]

[1] *Istituto di Biomedicina e Immunologia Molecolare IBIM-CNR, Scienze Biomediche, Palermo, Italy*

The major histocompatibility complex (MHC) class II mediated signalling increases lipid raft recruitment of adhesion receptors and signal transduction proteins in melanoma cells

Francesca Costantini and Giovanna Barbieri.

Istituto di Biomedicina e Immunologia Molecolare 'Alberto Monroy", Consiglio Nazionale delle Ricerche (CNR), Via Ugo La Malfa 1 5 3 , 9 0 1 4 6 Palermo, Italy.

BACKGROUND: The Major Histocompatibility Complex (MHC) class II molecules are signalling receptors whose engagement leads to the activation of several signalling proteins often enabled by the lipid raft localisation of these molecules. The lipid rafts are specific microdomains of the plasma membrane enriched in sphingolipids and cholesterol implicated in selective protein–protein interactions as well as in the assembly of transient signalling platforms. Indeed, the aggressive metastatic trend of melanoma is associated to the lipid raft recruitment and to the deregulation of receptor and signalling protein functions that promotes the tumour cells detachment from primary tumour and the tissue borders. Therefore, in the aim to understand the molecular mechanisms used by melanomas to the metastatic progression, we studied in MHC class II constitutive expressing melanoma cell lines, the membrane localization of adhesion receptors and signalling proteins.

MATERIAL AND METHODS: The class II constitutive expressing melanoma cells (A375 and HT144 cell lines) were stimulated with a specific anti-HLA-DR mAb (L243) that mimics the TCR interaction with the class II molecules, for 24h and 48h or left unstimulated. The lipid rafts of stimulated and unstimulated melanoma cells were isolated and analysed by western blot and co-immunoprecipitation as well as through immunofluorescence experiments.

RESULTS: In melanoma cells stimulated with the L243 specific anti-HLA-DR antibody, we showed that the HLA-DR mediated signalling increases the lipid raft localisation of class II molecules, Integrin and CAM adhesion receptors as well as of FAK, AKT and STAT3 signalling proteins. Moreover, we reported the lipid rafts association of some adhesion molecules and signalling proteins in stimulated melanoma cells. Furthermore, we identified the HLA-DR mediated signals depending on lipid rafts integrity through the melanoma cells treatment with methyl-**β**-cyclodextrin (M**β**CD) that disrupt the lipid raft domains through cholesterol depletion.

CONCLUSIONS: Therefore, our result suggest a new model in which the HLA-DR stimulation activates a signalling in melanoma cells that increases the lipid raft localization and association between class II molecules, adhesion receptors and signalling molecules, providing a platform useful to frustrate an effective anti-tumour response.

NO CONFLICT OF INTEREST

443 Screening of novel druggable targets blocking SOD3 function in developing colon adenocarcinoma

M. Laukkanen[1], *G. Mazzoccoli*[2], *A. Parascandolo*[1]

[1] *IRCCS SDN, Research Laboratory, Naples, Italy*
[2] *IRCCS Casa Sollievo della Sofferenza, Scienze Mediche, San Giovanni Rotondo, Italy*

BACKGROUND: According to our data and Oncomine dataset analysis the expression of extracellular superoxide dismutase, SOD3, is frequently upregulated in colon adenomas. SOD3 is a growth regulatory enzyme that mediates RAS oncogene signal transduction by inducing a three-step immortalization process in primary cells: 1) proliferative burst, 2) growth arrest, and 3) escape from premature senescence. The aim of our study is to identify novel drug targets in adenomas to prevent their transformation to adenocarcinomas therefore reducing the need of invasive treatments.

MATERIAL AND METHODS: SOD3 expression was studied in a set of patient derived matching normal, adenoma and adenocarcinoma tissues. Signal transduction pathways were studied in SOD3 immortalized mouse embryonic fibroblast primary cell model (control, SOD3 immortalized, SOD3 transformed) by protoarrays and by Western blotting. Small GTPases analysis was done by pulldown analysis. Luciferase assay was used to study promoter activation.

RESULTS: We analyzed the expression of SOD3 by qRT-PCR in human colon tissues and found over-expression of the enzyme in adenomas. Studies of the signaling pathways activated by SOD3 in primary MEF cells showed variable small GTPase RAS, RAC, RHO, and CDC42 activation in MEF SOD3 cells compared to controls. Consequently, ERK1/2 phosphorylation was decreased in SOD3 transduced cells as compared to controls. Our data demonstrated strongly increased EGFR, PDGFR, Insulin receptor, Tie2, VEGFR3, and AXL tyrosine kinase receptor activation caused by SOD3 immortalization. Intracellular kinase screening indicated signal transduction change in SOD3 expressing cells suggesting increased 1) activation of WNK1 pathway, 2) PKA pathway, 3) JNK-cJUN-cyclin D1 signal activation, 4) Chk1-p53 signaling, which, however, showed lack of p21 expression, and 5) increased STAT3 activation.

CONCLUSIONS: Large adenomas are removed during colonoscopy or by open surgery whereas drugs are meant for advanced and metastasized cancers. Several studies have demonstrated that use of anti-inflammatory drugs reduces adenoma formation and transformation to malignant tumors. Our findings suggesting increased SOD3 expression in the initial phase of colon cell immortalization may indicate that inhibition of survival and proliferative signals activated by SOD3 through single or combined drug treatments could reduce colon tumor formation.

NO CONFLICT OF INTEREST

POSTER SESSION: SIGNALLING PATHWAYS II

444 Anti-proliferative effect of targeting CDK4/6 and mTOR alone or in combination in sarcoma cell lines

X. Wang[1,2], P. Crowe[2], D. Goldstein[3], J.L. Yang[1,2]

[1] University of New South Wales, Lowy Cancer Research Centre- Prince of Wales Clinical School, Sydney- New South Wales, Australia
[2] University of New South Wales, Department of Surgery- Prince of Wales Clinical School, Sydney- New South Wales, Australia
[3] University of New South Wales, Department of Medical Oncology- Prince of Wales Clinical School, Sydney- New South Wales, Australia

INTRODUCTION: There is evidence to suggest that phosphatidylinositide 3kinase (PI3K)/Protein kinase B (PKB or Akt)/ mammalian target of rapamycin (mTOR) signaling pathway activation is central for cancer growth, survival and motility. One of the key restraints on cell growth downstream of many growth factor signaling pathways including PI3K/Akt/mTOR signaling is retinoblastoma (Rb) tumour suppressor. To allow normal cells to grow, the Rb brake is temporarily switched off by the cyclin-dependent protein kinases (CDK) 4/6 protein. However in many sarcomas the Rb brake is permanently inactivated by an amplified CDK4/6 switch. In these cells, blocking the CDK4/6 switch allows the Rb brake to be reapplied to cell growth, which stops growth of the tumour. We hypothesize that combination therapy using mTOR inhibitor and CDK4/6 inhibitor would have a synergistic growth inhibitory effect on sarcoma cell lines.

MATERIAL AND METHODS: The effect of CDK4/6 inhibitor (palbociclib, Pfizer) and mTOR inhibitors (ridaforolimus, Merck) alone or in combination was investigated in a panel of six soft tissue sarcoma (STS) (449b, 778, SW872, SW982, GCT and HT1080) and six osteosarcoma cell lines (143B, HOS, MG63, SJSA, U2OS and Saos-2). Crystal-violet colorimetric and clonogenic assays were used to measure drug effects. Data were analysed using Chou & Talalay's isobologram assay.

RESULTS: The selective CDK4/6 inhibitor palbociclib has shown anti-proliferative activity in a panel of 12 sarcoma cell lines with IC_{50}s ranging from 0.1 – 1.1 μM, similar to the data in the breast cancer where it is now an approved therapy following successful human trials. Ridaforolimus, an mTOR inhibitor, is effective in 10/12 sarcoma cell lines with IC_{50}s ranging from 0.4-26 nM, except on Saos-2 and HOS cell lines, which are resistant with IC_{50}s of 185 and 544 nM respectively. Our current study has also indicated that the combination therapy using CDK4/6 inhibitor palbociclib and mTOR inhibitor ridaforolimus achieved synergistic anti-proliferative effect in the panel of 12 sarcoma cell lines, including applying the drugs in different sequence (together or pre-treatment with one drug for 24-48 hours) and ratio (1:1, 1:2, 1:4, 2:1 or 4:1). The mechanism study is ongoing and the data will be presented in the conference.

CONCLUSIONS: This in vitro study demonstrated that combining CDK4/6 and mTOR inhibitors is an effective treatment to sarcoma.

NO CONFLICT OF INTEREST

445 Effects of the endocrine disrupting chemical bisphenol A (BPA) in human choriocarcinoma placental cells

S.C. Jahn[1], E. Silva[2], E. Karteris[1]

[1] Brunel University London, Life Sciences, London, United Kingdom
[2] Brunel University London, Clinical Sciences, London, United Kingdom

BACKGROUND: Endocrine disrupting chemicals (EDCs) are found in every day products such as food containers and consumer goods. They can disrupt hormone-controlled systems in the body, which in turn increases chances for malignancies to develop. Emerging studies indicated that Bisphenol A (BPA), an EDC, exerts a multitude of adverse effects, especially as a result of developmental exposures, such as mammary tissue abnormalities and pre-cancer lesions and prostate cancer. A crucial transient endocrine organ during pregnancy is the placenta, as it is essential for the exchange of vital nutrients between the mother and the child. To date, our understanding of the carcinogenic potential of EDCs at placental level is still very limited. The purpose of this study is to develop a 3D model using placental cells, as a platform for testing carcinogenic EDCs in humans. We also seek to elucidate the effects of EDCs in vitro by assessing changes in gene transcription and protein expression following exposure to EDCs; using human choriocarcinoma cells (BeWos).

MATERIAL AND METHODS: For in vitro treatments, we are using BPA as an experimental compound. Since BPA exhibits estrogenic properties, we have elucidated the expression and cellular distribution of all estrogen receptors in our models using immunofluorescence, RNA extraction, qRT-PCR, ImageStream and Western blotting. We are using microarray analyses as a non-biased screen to identify changes that BPA can exert at transcriptome level. **result** qPCR confirmed an upregulation of estrogen receptors in BeWo cells treated with BPA. Treatment with BPA (30 and 100 nM) also increased in the phosphorylation status of Akt, p38 and ERK1/2; indicative of cell proliferative response. Microarray analyses revealed that cells treated with BPA (30 nM) upregulated 460 and downregulated 784 genes. Enrichment analysis using GeneSpring software produced 77 main pathways including: signalling by Rho-GTPases, regulation of lipid metabolism, differentiation of adipocytes, and signalling by NGF. Treatment with 100nm BPA, upregulated 210 and downregulated 272 genes, producing 66 pathways including human complement system, signalling by VEGF and TGF-beta as well as mitochondrial gene expression.

CONCLUSIONS: Our data are indicative of involvement of BPA on key pathways involved in metabolism and energy balance, highlighting the importance of EDCs in the aetiopathogenesis of reproductive malignancies.

NO CONFLICT OF INTEREST

446 Involvement of Histone Methyl Transferase DOT1L on estrogen-mediated transcriptional regulation in breast cancer

G. Nassa[1,2], A. Salvati[3], G. Giurato[1,2], R. Tarallo[1], M. Ravo[1], F. Rizzo[1], E. Alexandrova[1], T.A. Nyman[3], A. Weisz[1]

[1] University of Salerno, Department of Medicine Surgery and Dentistry "Schola Medica Salernitana" - Laboratory of Molecular Medicine and Genomics, Baronissi, Italy
[2] Genomix4 Life S.r.l., Laboratory of Molecular Medicine and Genomics - University of Salerno, Baronissi, Italy
[3] University of Oslo, Institute of Clinical Medicine - Proteomics Core Facility, Oslo, Norway

INTRODUCTION: Estrogens play a key role in breast cancer (BC), where the Estrogen Receptor alpha (ERα), a ligand-inducible transcription factor, mediates estrogen signaling in hormone-responsive tumors and is target of specific anticancer therapies. Understanding the molecular mechanisms underlying ERα action in BC cells allows identification of new targets for more effective pharmacological treatment of this cancer. We recently identified by interaction proteomics the DOT1 Like Histone Lysine Methyltransferase (DOT1L), that catalyzes H3K79 methylation, as a nuclear partner of ERα in BC cells.

MATERIAL AND METHODS: To investigate the role of DOT1L in mediating ERα actions in hormone-responsive BC, MCF-7 cells were used to map ERα-DOT1L physical and functional interactions both 'in vitro' and 'in vivo', at the chromatin level, by Chromatin Immunoprecipitation coupled to Mass Spectrometry (ChIP-MS) and Sequencing (ChIP-Seq). Gene silencing and selective pharmacological inhibition of the enzyme followed by RNA- and Nascent RNA-Sequencing were applied, together with cellular and functional assays, to assess the role of this interaction on BC cell progression mediated by estrogen signaling.

RESULTS AND DISCUSSION: Co-immunoprecipitation and proximity ligation assay confirmed the association between these two molecules both 'in vitro' and 'in vivo'. Transcriptome profiling by RNA-Seq before and after DOT1L inhibition showed that DOT1L modulates the ERα-responsive transcriptome. Indeed, a reduced transcription rate of ERα and several estrogen responsive genes upon DOT1L blockage was measured by Nascent RNA-Sequencing. ChIP-MS revealed the co-existence of the two factors in the same chromatin-associated multiprotein complex. Then, global analysis of ERα and DOT1L binding to the genome by ChIP-Seq showed co-recruitment of the two proteins on several chromatin sites, including regulatory sites of the ERα gene promoter itself. This provided an explanation for the inhibition of ERα signaling observed in the absence of DOT1L activity, thus revealing that this association exerts a significant role on cell proliferation and cell cycle progression in BC cells.

CONCLUSION: These evidences indicate a key role of DOT1L in ERα-mediated pathways, suggesting that this enzyme is a potential target for antiestrogen-resistant BC treatment.

RESEARCH SUPPORTED BY: AIRC (IG-17426), CNR Flagship Projects EPIGEN and InterOmics, MIUR (FIRB RBFR12W5V5_003), MOH (GR-2011 02347781 and GR-2011-02350476) and ELIXIRITA HPC@CINECA.

NO CONFLICT OF INTEREST

448 A chemoproteomic approach reveals massive reprogramming of the epithelial cell surface during oncogenic KRAS-mediated transformation

L. Aubert[1], N. Nandagopal[1], S. Nourreddine[1], G. Lavoie[1], T. Houles[1], P. P. Roux[1]

[1] IRIC - University of Montreal, Pathology and Cell Biology, Montréal, Canada

BACKGROUND: KRAS is frequently mutated in human cancers, including ~45% of colorectal adenocarcinoma (CRC). The most promising therapeutic strategy for advanced CRC is the use of monoclonal antibodies (cetuximab, panitumumab) that block activation of the epidermal growth factor receptor (EGFR). However, activating mutations in KRAS were shown to be common drivers of acquired resistance, and recent retrospective studies have shown that they negatively predict responsiveness to anti-EGFR therapy. Despite continuous efforts, oncogenic KRAS is still deemed "undruggable", warranting the need for alternative therapeutic approaches. While oncogenic KRAS is described to regulate many intracellular signaling events that are currently being evaluated as potential therapeutic targets, much less is known about its potential impact on the cell surface. Elucidating how oncogenic KRAS modifies the cell surface proteome (surfaceome) could help understand its complex mechanism of action, and possibly identify new "druggable" targets and/or tumor-specific biomarkers.

MATERIAL AND METHODS: Herein, we have optimized a cutting-edge chemoproteomic approach based on the labeling of cell surface proteins with biotin reagents, their subsequent purification with avidin chromatography, and quantification using label-free quantitative proteomics with liquid chromatography-tandem mass spectrometry (LC-MS/MS).

RESULTS: Using an intestinal crypt epithelial cell model that reflects KRAS-induced malignant transformation, our LC-MS/MS analyses allowed the identification of over 350 cell surface molecules from which 13% and 22% were significantly upregulated and downregulated in KRAS-transformed cells, respectively. Thus, we

found that oncogenic KRAS modulates the surface expression of a large network of proteins, including cell adhesion molecules, receptor tyrosine kinases, G protein-coupled receptors, ion channels, transporters, and peptidases/proteinases. Interestingly, while many of these changes are associated with a KRAS-dependent gene expression signature, we also identified numerous surface proteins that appear to be regulated in a transcription-independent manner.

CONCLUSION: Taken together, these result indicate that oncogenic KRAS leads to a massive reprogramming of the epithelial cell surface, and suggest multiple cell surface proteins as molecular targets or diagnostic markers for KRAS-dependent cancers.

NO CONFLICT OF INTEREST

449 Mitochondrial reactive oxygen species prime T-cell acute lymphoblastic leukemia to cell death by engaging the OMA1-OPA1 axis

M. Silic-Benussi[1], G. Scattolin[2], I. Cavallari[1], L. Urso[3], S. Minuzzo[4], P. Del Bianco[5], G. Basso[2], D. D'Agostino[3], S. Indraccolo[1], V. Ciminale[4]

[1] Istituto Oncologico Veneto, Immunology Section, PADOVA, Italy
[2] Univuersity of Padova, Department of Woman and Child Health, Padova, Italy
[3] Univuersity of Padova, Department of Biomedical Sciences-, Padova, Italy
[4] Univuersity of Padova, Department of Surgery- Oncology- and Gastroenterology, Padova, Italy
[5] Istituto Oncologico Veneto, Sperimentazioni cliniche- biostatistica e nucleo per la ricerca clinica, PADOVA, Italy

Approximately 20% of T-cell acute lymphoblastic leukemia (T-ALL) patients do not respond to current therapy, and their clinical outcome is dismal.

In the present study we show that leukemic cells isolated from T-ALL patients as well as cell lines stabilized in vitro or propagated in vivo as xenografts exhibit high levels of mitochondrial reactive oxygen species (ROS). Interestingly, raising mitochondrial ROS using NS1619, a small molecule that opens the mitochondrial BK K^+ channel, induced death of leukemic cells from T-ALL patients and T-ALL cell lines but not of primary normal thymocytes or PBMC. These effects were enhanced by blunting ROS-scavenging pathways with dehydroepiandrosterone (DHEA), an inhibitor of the pentose phosphate pathway (PPP). The combination of NS1619 and DHEA led to proteolytic processing of OPA1, an inner mitochondrial membrane protein that controls cristae remodeling, cytochrome c release and apoptosis. OPA1 cleavage was dependent upon both ROS and the OMA1 mitochondrial protease. Furthermore, OPA1 cleavage induced by treatment with NS1619 and DHEA primed T-ALL cells to apoptosis induced by TNF-Related Apoptosis Inducing Ligand (TRAIL).

These findings suggest that engaging the OMA1-OPA1 axis by raising mitochondrial ROS may prove to be an effective strategy for apoptotic priming of refractory T-ALL, which poses a major clinical challenge at present.

NO CONFLICT OF INTEREST

450 Decoding cancer heterogeneity: Using an information-theoretic approach to crack patient-specific protein network structures

E. Flashner[1], N. Kravchenko-Balasha[1]

[1] The Hebrew University of Jerusalem, Department for Bio-Medical Sciences, Jerusalem, Israel

BACKGROUND: Turning tumor heterogeneity into information that will enable to translate cancer into a comprehensible language is longed for. We utilize an information-theoretic approach to accurately identify patient-specific aberrations that induce unbalanced processes. We hypothesize that these processes can be viewed as words that constitute the language we are seeking.

MATERIAL AND METHODS: We utilize the surprisal analysis to study a proteomic dataset of 3467 solid TCGA tumors of 11 types. The analysis divides the proteins into groups in which proteins deviate from their balanced levels in a correlated manner. Each group represents a distinct unbalanced biological process that may comprise a few signaling pathways.

RESULTS AND DISCUSSION: 17 unbalanced processes describe the inter-tumor heterogeneity among 3467 tumors. Each tumor is characterized by a specific subset of unbalanced processes. Our findings unmask a surprisingly simple order that underlies the complexity of cancer systems.

Each patient was assigned a unique barcode, denoting the active unbalanced processes in his tumor. 452 distinct barcodes were identified. Only 16 barcodes were relatively abundant (i.e. each characterizes >1% of the population). In line with the vast heterogeneity of tumors, 376 barcodes were extremely rare (i.e. each characterized 5 patients or less), 273 of them each describing only a single patient,

Our findings suggest that the cohort of patients consists of 452 cancer types, rather than only 11. Each cancer type represents a barcode, or a combination of 17 unbalanced processes, and can be unambiguously mapped into this new data space that we defined. Once the information is transformed into a low-dimensional space where we can read it, treating these thousands of tumors becomes at an arm's reach. The barcodes enable the accurate resolution of patient-specific network structures, and the design of patient-tailored drug combinations. Inhibition of the entire set of tumor-specific processes should stop the disease and decrease the chances for the development of drug resistance.

CONCLUSIONS: We present a novel method to break down the high complexity of cancer systems into simple, easy to crack, barcodes. We sort the needles from the haystack, by identifying with high resolution patient-specific unbalanced processes and rewired signaling pathways. This deep understanding of tumor-specific imbalances should greatly advance the fields of cancer research and therapeutics.

NO CONFLICT OF INTEREST

451 ALK-independent repurposing opportunities for ceritinib in lung cancer

U. Rix[1], B. Kuenzi[1], L. Remsing Rix[1], P. Stewart[2], B. Fang[3], F. Kinose[2], A. Bryant[1], T. Boyle[2], J. Koomen[4], E. Haura[2]

[1] Moffitt Cancer Center, Drug Discovery, Tampa- FL, USA
[2] Moffitt Cancer Center, Thoracic Oncology, Tampa- FL, USA
[3] Moffitt Cancer Center, Proteomics, Tampa- FL, USA
[4] Moffitt Cancer Center, Molecular Oncology, Tampa- FL, USA

INTRODUCTION: The second generation ALK inhibitor ceritinib (LDK378) is approved for crizotinib-resistant, ALK-rearranged non-small cell lung cancer (NSCLC). However, little is known about its activity in other NSCLC subtypes, which may entail novel translational opportunities.

MATERIALS AND METHODS: Cell viability and clonogenicity were determined by CellTiterGlo and crystal violet. Cellular targets and signaling pathways modulated by ceritinib were identified by mass spectrometry-based chemical and phosphoproteomics followed by pathway/network analysis. Validation was done by in vitro kinase assays, immunoblotting, RNAi and rescue experiments using ectopic overexpression. Signaling and apoptosis (PARP1 and caspase 3 cleavage) were assayed by immunoblotting and fluorescence microscopy. Drug synergy was determined by Bliss and Chou-Talalay analysis. Target phosphorylation in tissue microarrays was determined by immunohistochemistry.

RESULTS AND DISCUSSION: Cell viability drug identified ceritinib activity against several ALK-negative NSCLC cell lines within clinically relevant concentrations. Chemical proteomics revealed novel ceritinib kinase targets. Combined gene silencing and drug treatment identified a polypharmacology mechanism of ceritinib mediated through simultaneous RSK1/2, IGF1R and FAK1 inhibition. Pathway-based integration with global phosphoproteomics data identified signaling crosstalk between pathways that converge on microtubule regulation. Based on this, we tested the combination of ceritinib with the microtubule inhibitor paclitaxel, which displays synergy and induces apoptosis across multiple cell lines. Analysis of cell lines for expression of key nodes and phosphorylation sites in our network suggested FAK1 phosphorylation to predict a particularly pronounced synergistic response to ceritinib and paclitaxel. Interrogation of lung cancer tissue microarrays suggested FAK1 phosphorylation to be a prevalent signal particularly in lung cancer subtypes with low incidence of actionable oncogene mutations and associated targeted therapy options, which constitute a significant unmet medical need.

CONCLUSION: Ceritinib targets several kinases in addition to ALK and IGF1R, which contribute to its activity in ALK-negative NSCLC. Functional dissection of ceritinib's mechanism of action led to identification of a novel synergistic drug combination with enhanced potential for clinical translation and an associated, mechanism-based biomarker candidate.

NO CONFLICT OF INTEREST

452 Improved response of B-cell malignancies to rituximab upon FOXO1 inhibition

B. Pyrzynska[1], A. Zerrouqi[1], M. Dwojak[1], G. Morlino[2], P. Zapala[1], N. Miazek[1], A. Zagozdzon[1], D. Calado[2], J. Golab[1], M. Winiarska[1]

[1] Medical University of Warsaw, Dept. of Immunology, Warsaw, Poland
[2] The Francis Crick Institute, Immunity and Cancer, London, United Kingdom

Rituximab, a therapeutic anti-CD20 monoclonal antibody, eliminates B-cells by inducing cytotoxicity, executed by complement, NK cells or macrophages. Rituximab is quite effective against B-cell malignancies, when combined with chemotherapy. However, in case of 30-40% patients the resistance to rituximab-based therapy appears to be a serious concern. One of the major mechanisms of resistance relies on the reduced level of CD20 antigen on the surface of tumor B-cells.

While exploring the molecular mechanisms affecting the level of CD20, we found that transcription factor FOXO1 is a negative regulator of MS4A1 expression, the gene encoding CD20 antigen. Using the CRISPR/Cas9 genome-editing technology, we disrupted the loci of FOXO genes in lymphoma cell lines and discovered that ablation of FOXO1 (but not FOXO3) was sufficient for upregulation of the surface level of CD20 by 3-fold. Consistently, the complement-dependent cytotoxicity (CDC), induced by rituximab, was significantly improved in cell clones with abrogated expression of FOXO1, but not FOXO3. Importantly, the treatment with pharmacological inhibitor of FOXO1 activity, AS1842856, resulted in increased levels of CD20 on the surface of both lymphoma cell lines and patients-derived chronic lymphocytic leukemia (CLL) cells cultured ex vivo.

In order to verify our findings in the animal model, we inoculated SCID mice intravenously with Raji cells, what resulted in the development of lymphoma-like tumors. We demonstrated that mice treated systemically with rituximab, administered at a dose of 10 mg/kg, survived longer when inoculated with sgFOXO1-transduced Raji cells as compared with mice inoculated with control Raji cells (median survival 49 days versus 29 days, respectively). These result confirmed

that FOXO1 ablation in lymphoma cells resulted in higher efficacy of rituximab treatment in vivo.

Taken together, these result establish FOXO1 as an important determinant of cell response to complement-dependent rituximab-induced cytotoxicity and indicate that FOXO1 inhibitors could be exploited for the therapeutic purposes, in combination with anti-CD20 monoclonal antibodies. Novel FOXO1 inhibitors with improved potency and selectivity are however urgently needed for further exploration of our discoveries in the near future.

ACKNOWLEDGEMENTS: NCN, Poland (grant no: 2013/11/B/NZ5/03240) and H2020 (STREAM project, CSA action, grant no: 692180).

NO CONFLICT OF INTEREST

453 Epithelial-mesenchymal plasticity drives growth factor discordance and drug resistance in metastatic breast cancer

S.S. Akhand[1], *M. Wendt*[1]

[1] *Purdue University, Medicinal Chemistry and Molecular Pharmacology, West Lafayette, USA*

INTRODUCTION: Metastasis is the major cause of breast cancer (BC) lethality as these lesions often display inherent or acquired resistance to targeted therapies prescribed based upon molecular characterization of the primary tumor. Induction of epithelial-mesenchymal transition (EMT) is a major contributing factor to BC metastasis and several recent studies have demonstrated that induction and recovery from EMT, or mesenchymal-epithelial transition (MET), selects for a population of cells with stem-like characteristics.

MATERIALS AND METHODS: Our lab utilizes transformation models of mammary epithelial cells to induce orthotopic tumor formation and establish defined response patterns to molecular therapies targeting the established driver gene. We have recently established protocols where induction of EMT:MET via stimulation and withdrawal of transforming growth factor b1 (TGF-b1) is sufficient to drive the metastasis of Her2-transformed tumors. Using these systems we can directly compare differential drug response in the primary tumor cells as compared to their metastatic counterparts.

RESULTS AND DISCUSSION: Only after EMT:MET were Her2-transformed HMLE cells capable of metastasis and acquisition of resistance to the anti-Her2 antibody-drug conjugate trastuzumab emtansine (T-DM1), an event that was associated with a diminution in Her2 expression. RNA-sequence analyses of metastases that transitioned through an EMT-MET cycle revealed a unique 86-gene signature that distinguished primary tumor cells from their bone metastatic counterparts. Concomitant with the loss of Her2 expression, and acquisition of resistance to T-DM1 we observe upregulation of FGFR1 and several extracellular matrix proteins. Indeed, we find FGFR1 physically interacts with integrins and FGF signaling is potently increased when metastatic BC cells are cultured within a three-dimensional matrix. To target these metastatic events, we have collaborated on the development of FIIN4, a highly potent covalent inhibitor of FGFR kinase activity. Importantly, in vivo application of FIIN4 effectively delays tumor growth in murine and patient derived xenograft models of BC metastasis. Overall, our work demonstrates that an EMT reaction in the primary tumor can support discordance in growth factor receptor expression patterns within the metastatic lesion.

CONCLUSION: The EMT:MET process contributes to subtype switching in metastatic BC and supports the inherent and acquired drug resistance of these tumors. We are currently exploring combination strategies targeting Her2 and FGFR to prolong response times in metastatic BC.

NO CONFLICT OF INTEREST

455 The Interleukin-1 receptor/Toll-like receptor family member TIR8 is downregulated in chronic lymphocytic leukemia

M.G. Vilia[1], *E. Fonte*[2], *T. Veliz Rodriguez*[2], *M. Tocchetti*[2], *P. Ranghetti*[2], *L. Scarfò*[2], *P. Nikos*[3], *S. Ntoufa*[3], *K. Stamatopoulos*[3], *P. Ghia*[2], *M. Muzio*[2]

[1] *Università degli studi di Cagliari, scienze biomediche, cagliari, Italy*
[2] *IRCCS San Raffaele Scientific Institute, Diuision of Experimental Oncology, milano, Italy*
[3] *Institute of Applied Biosciences, Certh, Thessaloniki, Greece*

BACKGROUND: Chronic Lymphocytic Leukemia is characterized by the accumulation of clonal mature B-lymphocytes in the peripheral blood, bone marrow and secondary lymphoid organs. Among different microenvironmental elements regulating CLL pathobiology, Toll-like receptors (TLR) stimuli play a key role. To better characterize the molecular mechanism regulating TLR signaling in CLL cells we analyzed TIR8 expression in primary patient samples as compared to normal B-cells. TIR8 (also called SIGIRR) is an Interleukin-1 receptor/Toll-like receptor family member that acts as inhibitor of inflammation. We recently showed that lack of TIR8 triggers progression of CLL in a mouse model supporting its role as a novel tumor restrainer.

MATERIALS & METHODS: Leukemic cells were isolated from the peripheral blood of CLL patients and analyzed by Real-time PCR, Flow Cytometry and Immunofluorescence analysis for the expression pattern of TIR8 and distinct TLR as control. TIR8 was also analyzed in normal human B-lymphocytes isolated from buffy coat. We also analyzed the MEC1 CLL cell line before and after treatment with the Azacytidine drug and we measured mRNA levels by Real Time PCR.

RESULTS: We detected low levels of TIR8 mRNA in both MEC1 and different B-cell lymphoma cell lines. Primary CLL cells expressed lower levels of TIR8 mRNA as compared to normal circulating B-lymphocytes ($p<0,01$). Moreover, leukemic cells expressed a lower percentage of TIR8 positive cells and Mean Fluorescence Intensity of TIR8 as compared to normal B-cells ($p<0,0001$). No significant difference was observed among different groups of CLL patients with different clinicobiological profiles. Next, we studied the distribution of distinct polymorphic variants of TIR8 in normal and leukemic samples. The presence of the reference sequence was the least frequent in this small group of normal subjects and in the CLL cohort. Finally, considering the role of DNA hypermethylation in silencing critical regulatory pathways, we analyzed if epigenetics changes concur to TIR8 downregulation. We found that treatment of MEC1 cells with Azacytidine restored higher levels of TIR8 mRNA suggesting a mechanism of repression by methylation which may be targeted in the context of novel therapeutic strategies.

CONCLUSIONS: We herein report that the novel tumor restrainer TIR8 is downregulated in leukemic cells likely as a consequence of aberrant epigenetic regulation.

NO CONFLICT OF INTEREST

456 IQGAP1 is a novel interactor of endothelin-1 receptor/β-arrestin1 network to promote invadopodia in ovarian cancer

L. Chellini[1], *V. Caprara*[1], *F. Spadaro*[2], *A. Bagnato*[1], *L. Rosanò*[1]

[1] *Regina Elena National Cancer Institute, Preclinical Models and New Therapeutic Agents Unit, Roma, Italy*
[2] *Istituto Superiore di Sanità, Section of Experimental Immunotherapy- Department of Haematology-Oncology and Molecular Medicine, Rome, Italy*

BACKGROUND: The "invasive phenotype" of high-grade serous ovarian cancer (HG-SOC) cells is characterized by the formation of actin-based structures, named invadopodia. Receptor activation might cause the assembly of a F-actin rich core and the engagement of integrins and associated proteins, including IQGAP1, to form an "adhesion ring", helping to invadopodia formation and function. Endothelin-1 receptor (ET-1R) axis is recognized as a novel regulator of invadopodia by the transducer function of β-arrestin1 (β-arr1). However, how ET-1R/ β-arr1 network promotes the integration of different key components in the invadopodia formation remains to be addressed.

MATERIAL AND METHOD: The localization and interaction between β-arr1 and IQGAP1 are evaluated in HG-SOC cells expressing endogenous or exogenous β-arr1 as well as IQGAP1, or upon their silencing with shRNA (immunoblotting, immunoprecipitation, gelatin zymography, immunofluorescence, and pull down assays). The invasive capabilities are evaluated by chemoinvasion assay. Formation/activation of invadopodia are analyzed by IF and fluorescent gelatin degradation assay. Analysis of metastasis inhibition is performed in murine orthotopic HG-SOC xenografts. Macitentan, the dual ET-1 R antagonist, is used for in vitro and in vivo experiment.

RESULTS AND DISCUSSION: In HG-SOC cells, expressing high level of IQGAP1, ET-1 promotes the interaction between IQGAP1 and β-arr1, which can be inhibited by macitentan. This molecular complex is involved in the regulation of ET-1-driven invadopodia formation, such as RhoA and RhoC activation, cofilin phosphorylation or Rac1 inhibition. IQGAP1 or β-arr1 silencing, suggests that IQGAP/β-arr1 might orchestrate a crosstalk between multiple Rho GTPase driven by ET-1R. Silencing of IQGAP1 significantly impairs the ET-1-induced localization of actin-rich filaments with invadopodia markers (cortactin, TKS5). At functional level, both macitentan and IQGAP1 silencing inhibited ET-1R-induced protease secretion, ECM degradation, transendothelial migration and cell invasion, demonstrating an effective involvment of IQGAP1 in the ET-1R mediated signaling to promote invadopodia. In vivo, macitentan inhibits metastatic dissemination and invadopodia marker expression.

CONCLUSIONS: Collectively, the data establish a role for the β-arr1/IQGAP1 complex as a novel network of protein driven by ET-1R to direct invadopodia in HG-SOC, that might be impaired by macitentan treatment.

NO CONFLICT OF INTEREST

457 Reduction of SKP2 prevents cell cycle progression and induces differentiation in embryonal rhabdomyosarcoma by increasing p21Cip1 and MYOG levels

S. Pomella[1], *C. Cossetti*[1], *E. Carcarino*[1], *A. Gualtieri*[1], *L. Raimondi*[1], *Z. Walters*[2], *J. Shipley*[2], *L. Miele*[3], *F. Locatelli*[1], *R. Rota*[1]

[1] *Ospedale Pediatrico Bambino Gesù- IRCCS, Dept. Oncohematology, Roma, Italy*
[2] *The Institute of Cancer Research, Sarcoma Molecular Pathology- Diuisions of Molecular Pathology and Cancer Therapeutics, Sutton Surrey, United Kingdom*
[3] *Louisiana State University- Stanley S. Scott Cancer Center, Department of Genetics, New Orleans-Louisiana, USA*

BACKGROUND: Rhabdomyosarcoma (RMS) is a pediatric soft tissue sarcoma whose cells express the myogenic master gene MYOD but are unable to differentiate and proliferate indefinitely. Restoration of differentiation is an anti-cancer strategy in RMS. The levels of the F-box protein SKP2 have been shown up-regulated in the alveolar RMS subtype and induced by the PAX3-FOXO1 expressed oncoprotein. However, the role of SKP2 in the embryonal RMS variant, devoid of any fusion gene, and the mechanism responsible for its deregulated expression remains to be elucidated.

MATERIALS AND METHODS: The expression levels of SKP2 in a large pediatric RMS cohort including fusion negative and fusion positive variants were obtained analyzing gene expression profiling data of 101 RMS patient samples described in the ITCC/CIT data set (Innovative Therapies for Children with Cancer/Carte d'Identite´ des Tumeurs)1 within the R2 online platform. We investigated the role of SKP2 in embryonal RMS cell lines in which the gene has been knocked down with specific siRNAs or with shRNAs expressed by a lentiviral vector pLKO.1. Moreover, NOTCH1 was down-regulated with siRNA or shRNAs to evaluate SKP2 expression. Cells were transfected with plasmids expressing SKP2 promoter-driven luciferase for a luciferase assay .

RESULTS: SKP2 transcripts were up-regulated in RMS patients and cell lines compared to their normal counterparts. SKP2 knockdown in embryonal RMS cell lines resulted in up-regulation of p21Cip1 protein levels and cell cycle arrest. MYOG and MEF2D levels were also up-regulated in SKP2-depleted cells, which showed muscle-like differentiation. SKP2 expression was down-regulated by NOTCH1 silencing while NOTCH1 forced expression activated SKP2 promoter-dependent transcription.

CONCLUSIONS: Our data suggest that SKP2 could be a novel target in embryonal RMS.

NO CONFLICT OF INTEREST

458 Post transcriptional regulation of microRNA processing enzyme Dicer1 in PMA treated K562 cells

P.Muiwo[1], *M.H. Ahmad*[2], *A. Bhattacharya*[1],

[1] *School of Life Sciences, Jawaharlal Nehru University, New Delhi, India*
[2] *Department of molecular cell and cancer biology, Umass medical school, Worcester MA, USA*

INTRODUCTION: Dicer1 is a RNase III family endonucleases that cleaves the precursor miRNA into mature miRNA, which in turn regulates the gene expression (Grishok et al 2001, Kim & Nam 2006). Both Dicer1 and miRNA are deregulated in many cancer cells (Jansson et al 2012). In our previous studies we have shown that the expression of Dicer1 is down regulated in CML model cell line K562 and this could be correlated to overall decrease in miRNAs population when compared with the healthy volunteer PBMCs and HL60 (Vaz et al 2010). The PMA (phorbol-12-myristate-13-acetate) treated K562 cells are differentiated into mature megakaryocytes and these differentiated cells are considered equivalent to normal cells.

MATERIAL AND METHODS: *NGS sequencing:* We outsource our PMA treated and untreated k562 to illumina platform for small RNA sequencing.

Real time RT-PCR: Reverse transcription was done using superscript® III (Invitrogen) reverse transcriptase. The cDNA synthesized was further used for Real time RT-PCR on an Applied Biosystems 7500 Real Time PCR instrument.

Western techniques: Total Cell lysate was prepared in RIPA buffer and run on SDS PAGE and then transferred on a PVDF membrane (Millipore, USA). For loading control we used β-Actin (BD Biosciences). Antibodies DICER1 (#5362), 4EBP1 (#9644s), ERK1/2 (#4695p), phospho ERK1/2(#4370p) were purchased from Cell Signaling® and used according to manufacturer's protocol.

RESULTS AND DISCUSSION: We found that the miRNAs profile are also altered by the treatment. 26.8% of the reference miRNAs goes up, while only 17% goes down when compared with the control (DMSO) sample. And this result can be co-related with increase expression of Dicer under PMA stimulation. It was interesting to find that the stimulation of Dicer1 is at protein level and not mRNA level. We found that eIF4EBP1 (Eukaryotic translation initiation factor 4E binding protein) protein, a translational inhibitory protein was negatively regulating Dicer1 expression. Also 4EBP1 expression was found to be negatively regulated by MAP kinase signalling pathway which is activated by PMA treatment.

CONCLUSION: Dicer are deregulated in many cancer cells, in our system (PMA treated K562 cells) we find that MAP kinase signalling are responsible for modulating the expression of Dicer at post transcription level. This way the expression of tumour suppressor miRNA are inhibited and restore the normalcy of the cells.

NO CONFLICT OF INTEREST

POSTER SESSION: TRANSLATIONAL RESEARCH I

459 Immune stimulation by approved PAMP drugs

U. Hobohm[1]

[1] *THM University of Applied Sciences Giessen, Bioinformatics, Giessen, Germany*

Without doubt, more than 100 years ago, Coley "documented cases of long-term survival of individuals with malignancies that remain a major challenge to treat now" (Mantovani Nature 2008) by injecting bacterial extract in cancer patients in a metronomic (high frequency) fashion. From theoretical considerations we proposed that PAMP (pathogen associated molecular pattern) might be the molecular explanation of Coley's successes (BJC 92 (2005), 421; Crit. Rev. Immun. 2008, 28(2):95-107). At the core of this hypothesis is the proper activation of dendritic cells (DC) to induce a tumour specific T-cell response. DC need both antigen and PAMP to become fully activated. Proper DC activation is required for T-cell activation and clonal expansion. Cancer cells do not provide PAMP, T-cells remain in a state of anergy. The application of bacterial extracts in cancer patients might supply PAMP needed for DC-activation. DC activation involving PAMP leads to expression of co-stimulatory signals required for a full T-cell response against cancer cells, provided, tumour antigens are available to DC, which is the case in many cases of cancer. To test our hypothesis, we applied single and combined PAMP in an autologous mouse cancer model. After tumour cell inoculation and outgrowth of visible tumours, PAMP were applied 10 times over 3 weeks, which corresponds to a treatment over several month in humans. While single PAMP could delay tumour growth, a combination of three PAMP led to complete tumour eradication (Canc. Immun. Immunother. 62 (2013), 1283-1292).

To the best of our knowledge, this is the first study where a cocktail of PAMP has been tested in a „metronomic' fashion similar to Coley's recommendations. Our finding has been discussed in Nature (Nature 504, S4–S5, 19 December 2013, doi:10.1038/504S4a). Here we suggest to replace bacterial extracts by a combination of approved drugs. We focus on drugs which are known to contain bacterial of viral content and where fever is a known adverse event as judged by the respective instruction leaflet (in our context, fever is a wellcome rather than adverse event) (Augmented mistletoe therapy, AMT; Integrative Cancer Therapies April-June 2016, 1-10; PMID 27207233). Meanwhile, three large German clinics have agreed to join in a clinical AMT study. AMT so far has been tested in about 80 patients on a compassionate basis and safety has been determined to be excellent.

NO CONFLICT OF INTEREST

460 Correlation between luminal A molecular subtype and the immunohistochemical characteristics among Jordanian breast cancer patients

L. Hamadneh[1], *A. Al-Omari*[2], *H. Abdel-Razeq*[3], *M. Sughayer*[4], *K. Jaber*[5]

[1] *Al-Zaytoonah University, Pharmacy, Amman, Jordan*
[2] *King Hussein Cancer Center, Office of Scientific Affairs and Research, Amman, Jordan*
[3] *King Hussein Cancer Center, Deputy Director- KHCC, Amman, Jordan*
[4] *King Hussein Cancer Center, Chairman- Department of Pathology and Laboratory Medicine, Amman, Jordan*
[5] *Al-Zaytoonah University, Science, Amman, Jordan*

BACKGROUND: Breast cancer is the most reported cancer among Jordanians females, in which treatment protocols are determined according to patients' immunohistochemistry (IHC), tumor grade and age. In recent years, intrinsic molecular subtyping has been used to subclass breast tumors into five subclasses, luminal A, luminal B, Her 2, basal like and normal like. Luminal A is reported to be the most prevalent subclass among different populations tested.

MATERIALS AND METHODS: After ethical approval from King Hussein Cancer center was obtained. Quantitative real time PCR (RT-qPCR) was used to determine the gene expression profiles of PAM50 genes for 95 retrospective samples from Jordanian breast cancer patients using RNA extracted from paraffin embedded tissues. Hierarchical clustering was used to subclass the gene expression profiles using R package.

RESULTS: Luminal A was seen in 32% of the molecular subtypes produced that is similar to other populations. Comparison between the standard IHC classification (ER, PR and Her2) and luminal A subclass showed concordance between IHC and the molecular subtype in more than 57% of the cases. It is been reported that IHC does not adequately identify the PAM50 gene expression subtypes in Caucasian population and that was matched in this pilot study among Jordanian patients.

CONCLUSION: PAM 50 molecular profiling and luminal A subtype prevalence among Jordanian population was in concordance with Caucasian population and it's correlation with IHC showed similar differences between the molecular and IHC classifications.

NO CONFLICT OF INTEREST

461 A novel rational treatment modality for brain cancer: TMZ and Zn2+

R. Shai[1], *T. Pismenyuk*[2], *M. Yalon*[1], *S. Freedman*[1], *A.J. Simon*[1], *J.K.V. Reichardt*[2], *Y. Mardor*[3], *A. Toren*[1]

[1] *Sheba MC, Pediatric Hemato-oncology, Ramat Gan, Israel*
[2] *YachayTech University, Research, Urcuquí, Ecuador*
[3] *Sheba MC, The Advanced Technology Center, Ramat Gan, Israel*

BACKGROUND: Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant brain tumor. Temozolomide (TMZ), a DNA damaging chemotherapeutic alkylating agent, is the first line treatment for GBM management but has been with limited efficacy. TMZ is effective in presence of active form of p53 (tumor protein 53) transcription factor to directly induce the expression of target genes involved in cellular death. TP53 gene is mutated in a wide range of human cancer cells. Furthermore, it has been shown that zinc is essential for proper p53 function since p53 binds to DNA through a structurally complex domain that is stabilized by zinc cations (Zn^{2+}).

MATERIAL AND METHODS: Using both in vitro and in vivo experimental approaches, we investigated whether addition of zinc to TMZ enhances its cytotoxicity against GBM. **result** In vitro cell viability analysis of U87 and U251, showed that the cytotoxic activity of TMZ was substantially increased with addition of zinc and this response was accompanied by an elevation of p21, PUMA, BAX and Caspase-3 expression and a decrease in growth fraction as manifested by low ki67

and lower colony formation. Analysis of GBM as intracranial xenografts in athymic mice and administration of concurrent TMZ and zinc showed that tumor volume was significantly lower in the combined treatment group, compared to TMZ only treated group. We recently reported that elevated levels of metallothionein (MT), the intracellular heavy metal binding proteins, in GBM patients correlate with poor outcome. The result of this study suggest that this effect may be due to binding Zn2+. In this study we report that Zn2+ significantly enhance the effect of TMZ in in-vitro and in-vivo model systems.

CONCLUSIONS: In conclusion, our investigations have established that TMZ treatment of GBM model systems could be enhanced by the addition of Zn2+. Therefore, we suggest that this novel and rational combination treatment be investigated clinically in GBM patients with the goal of improving the outcomes in the 21st century. Finally, we highlight that our rational translational investigations at Sheba combining in vitro and in vivo studies of GBM rooted in basic laboratory studies may benefit cancer patients through applied science. We note that our research model may well also be fruitful in other cancers too.

NO CONFLICT OF INTEREST

462 Construction a high efficient multi-gene biochip for detection the circulating biomarkers of colorectal cancer patient with early relapse

J.Y. Wang[1]

[1] *Kaohsiung Medical University and Hospital, Department of Surgery, Kaohsiung, Taiwan*

BACKGROUND: Identification of colorectal cancer individuals with high risk for postoperative relapse can be improved. The objectives of the study were to construct a high efficient multi-gene biochip for prediction 2-year risk of postoperative relapse.

MATERIAL AND METHODS: 322 stage I–III colorectal cancer patients underwent curative resection at Kaohsiung Medical University Hospital, Taiwan, between June 2010 to October 2014, were included. During each follow-up, a postoperative surveillance strategy, including ESMO Guidelines Working Group recommendations and a 19 target genes chip, was used. A statistical algorithm was proposed to select biomarkers for construction.

RESULTS: After a 25.0-month median follow-up, 47 patients (14.6%) had postoperative relapse. Of the 19 circulating biomarkers, CHRNB1, ELAVL4, PTTG1, PED6D, BIRC5, PSG2, UBE2C, PSAT1, TK1, MMP13, CEA, and OLFM4 genes were good predictors of postoperative relapse (all $P < 0.05$). However, the accuracy of combination of these biomarkers for predicting postoperative CRC relapse is high than that of any one candidate gene. More advanced, the use of the 11 contemporary biomarkers, included CHRNB1, ELAVL4, PTTG1, PDE6D, BIRC5, PSG2, UBE2C, PSAT1, TK1, MMP13, and CEA genes, resulted in the cutoff value of 6.5 and an area under the curve of 0.968 (95% confidence interval: 0.929-1.000) showing sensitivity, specificity, positive predictive value, negative predictive value and accuracy of respectively 91.5%, 93.1%, 81.1%, 98.5% and 95.7%. Moreover, the median lead time prior to the detection of postoperative relapse was 191.0 days.

CONCLUSIONS: For detecting the 11 contemporary circulating biomarkers from the peripheral blood, the prediction model can be used for earlier identification of 'high-risk" Taiwanese CRC patients for postoperative relapse and might be helpful to prevent complications.

NO CONFLICT OF INTEREST

463 Tailored cell cycle-targeting combinations as an effective first- and second-line therapy in patient-derived models of pancreatic cancer

A. Chou[1], *A. Nagrial*[1], *V. Chin*[1], *A. Morgan*[1], *D. Wohl*[1], *J. Samra*[2], *S. Clarke*[3], *P. Timpson*[1], *A. Gill*[4], *M. Pajic*[1]

[1] *Garvan Institute of Medical Research, Cancer research program, Sydney- NSW, Australia*
[2] *Royal North Shore Hospital, Department of Surgery, St Leonards- Sydney- NSW 2065, Australia*
[3] *Royal North Shore Hospital, Northern Cancer Institute, St Leonards- Sydney- NSW 2065, Australia*
[4] *Royal North Shore Hospital, Department of Anatomical Pathology, St Leonards- Sydney- NSW 2065, Australia*

BACKGROUND AND AIMS: Extensive molecular heterogeneity of pancreatic ductal adenocarcinoma (PDA), plus a lack of effective therapies and almost uniform mortality, makes this disease a prime model for advancing development of tailored therapies. The p16-cyclin D-CDK4/6-retinoblastoma protein (CDK4) pathway, key regulator of cell proliferation, is frequently deregulated in PDA. Here we investigated the potential of targeting CDK4 as a personalized treatment strategy for this disease.

METHODS: Sensitivity to the potent CDK4/6 inhibitor PD-0332991 (palbociclib) was correlated to protein expression and mutation status of CDK4 signaling components in 19 primary patient-derived PDA lines to identify candidate companion biomarkers. We then determined the efficacy of PD-0332991 as mono- and combination therapy in vivo in mice bearing subcutaneous and orthotopic tumors derived from genome-sequenced patient specimens. Retinoblastoma protein expression in 200 pancreatic cancer specimens, sequenced as part of the International Cancer Genome Consortium, was correlated to patient outcome. Finally, the mechanistic effects of mono- and combination therapy were investigated.

RESULT: Here we show that patient-derived models of PDA with activated CDK4 signaling are strongly sensitive to CDK4/6 inhibitor PD-0332991 and clinically-relevant combinations, significantly improving survival over standard-of-care therapy. This was associated with increased quiescence, apoptosis, decreased migration, invasion in vitro and metastases in vivo, with further promising in vivo efficacy when PD-0332991 was utilized as second-line therapy. Immunohistochemical analysis of retinoblastoma protein may present an essential prognostic and predictive biomarker to guide this therapeutic approach.

CONCLUSIONS: This study demonstrates clear advantages in the use of CDK4 inhibition in PDA over the current standard treatment when applied in a molecular subtype-specific context.

NO CONFLICT OF INTEREST

465 Reliable inference of genes associated with resistance to immuno and targeted cancer therapy

P. Jiang[1], *W. Lee*[2], *X. Wang*[3], *X. Li*[4], *C. Johnson*[2], *J. Mi*[5], *J. Liu*[6], *L. Garraway*[3], *M. Brown*[3], *J. Aster*[2], *X. Liu*[1]

[1] *Dana Farber Cancer Institute, Biostatistics and Computational Biology, Boston, USA*
[2] *Brigham and Women's Hospital, Department of Pathology, Boston, USA*
[3] *Dana Farber Cancer Institute, Department of Medical Oncology, Boston, USA*
[4] *Tongji University, School of Life Science and Technology, Shanghai, China*
[5] *Boston University, Department of Biomedical Engineering, Boston, USA*
[6] *Harvard University, Department of Statistics, Cambridge, USA*

BACKGROUND: Drug resistance is a major challenge of immuno and targeted therapy in cancer. To search for genetic drivers of drug resistance, many genomics technologies have been applied to identify alterations in patient samples and cancer cell lines, which has resulted in massive amounts of cancer biology data.

MATERIAL AND METHODS: We present CARE (Comprehensive Analysis of REsistance), a computational method for the identification of genes associated with immuno and targeted therapy resistance from cell line screen and clinical profiling data. The methodology of CARE is based on test of interaction effect in a multivariate regression.

RESULTS: As a first application on immunotherapy, CARE reliably identified genes associated with T cell exhaustion in tumor microenvironment using TCGA tumor profiling data. The gene signature inferred could predict anti-PD1 therapy response at an accuracy of 72%, which is higher than all other signatures collected from previous studies. As a second application on targeted therapy, CARE significantly outperformed other computational methods in predicting drug resistance-associated genes using compound screen data on cancer cell lines. Moreover, the gene signature inferred by CARE can better predict therapy clinical outcome than the signatures from other experimental technologies such as shRNA and CRISPR screens. When finding genes associated with Lapatinib resistance, CARE identified PRKD3 as the top candidate. Experimental validation by both siRNA pool and compounds (KBNB14270, CRT0066101) confirmed that inhibition of PRKD3 significantly sensitizes HER2+ breast cancer cell to Lapatinib.

CONCLUSIONS: CARE is a reliable statistical method to systematically infer genes associated with immuno and targeted therapy resistance. As the volume of data on cell line and clinical studies increase, we foresee CARE as a powerful method to assistant future development of combination therapy to overcome cancer drug resistance.

NO CONFLICT OF INTEREST

466 Community shifts in the oral microbiome across the spectrum from benign breast disease to invasive breast cancer

T. Hieken[1], *J. Chen*[2], *N. Chia*[1], *T. Hoskin*[2], *M. Walther-Antonio*[1], *S. Johnson*[2], *D. Radisky*[3], *A. Degnim*[1]

[1] *Mayo Clinic, Surgery, Rochester- Minnesota, USA*
[2] *Mayo Clinic, Health Sciences Research - Bioinformatics and Statistics, Rochester- Minnesota, USA*
[3] *Mayo Clinic, Cancer Biology, Jacksonville-Florida, USA*

BACKGROUND: Globally breast cancer is a major public health concern for which individualized risk prediction and prevention are needed. Epidemiologic data shows correlations of oral health and disease with breast cancer. While the microbiome is implicated in carcinogenesis, the oral microbiome has not been specifically investigated with genomic deep sequencing methods for associations with breast cancer prompting this prospective study.

METHODS: With IRB approval, we analyzed preoperative buccal swab samples from 46 women operated on for benign (n=21) or malignant (n=25) breast disease. DNA extraction and 16S rDNA hypervariable tag sequencing preceded processing with the IM-TORNADO pipeline. ANOVA and PERMANOVA were used to analyze alpha- and beta-diversity measures. Permutation test based on linear regression on square-root transformed taxa proportions was used to identify differentially abundant taxa; false discovery rate (FDR) control was used for multiple testing correction.

RESULTS: Rarefaction curves to explore sampling depth demonstrated sufficiency of reads (median 80,737 reads/sample, range: 21,365-471,589). A total of 2,018 operational taxonomic units (OTUs) were detected (median 205 OTUs/sample, range: 60-353). The oral microbiome showed a trend toward reduced alpha diversity among women with breast cancer vs benign disease, P=0.15 for observed OTU number, P=0.06 for inverse Simpson index. Permutation testing identified

differential expression in seven taxa from the phyla Actinobacteria, Cyanobacteria, Fusobacteria and Proteobacteria (unadjusted P<0.05). Differences were most striking for increased relative abundance of the phylum Actinobacteria in breast cancer patients, q-value=0.04 (FDR <5%), and driven mainly by the genus A. Rothia. Stratifying patients into three groups as 1) low risk benign disease vs 2) atypical hyperplasia and ductal carcinoma in situ vs 3) invasive breast cancer showed greater similarities between the patients without vs with invasive cancer with respect to alpha diversity and interestingly, a trend toward increased relative abundance of Actinobacteria with progression toward invasive cancer, q=0.06.

CONCLUSIONS: Ours is the first study to report associations of the genomically surveyed oral microbiome with breast cancer. These data suggest that this body niche may provide a microbial biomarker associated with invasive breast cancer with potential value for individualized risk prediction and prevention.

NO CONFLICT OF INTEREST

467 Thyroid hormones and TRβ agonists as potential therapeutic agents in HCC

E. Puliga[1], *A. Perra*[1], *M.A. Kowalik*[1], *V.P. Leoni*[1], *G.M. Ledda-Columbano*[1], *A. Columbano*[1]

[1] *University of Cagliari, Biomedical Sciences, CAGLIARI, Italy*

BACKGROUND: Although targeted therapies have been proposed for the treatment of hepatocellular carcinoma (HCC), the benefits obtained are still disappointing. Previously, we found that rat and human HCCs are characterized by down-regulation of thyroid hormone-β receptor (TRβ), and that exogenous triiodothyronine (T3) induces the regression of preneoplastic hepatic nodules in rats. Here we sought to investigate whether the anti-tumorigenic effect of T3 can be exerted also when it is given at late stages of the process.

MATERIAL AND METHODS: The effect of T3 administration on rat HCC was investigated by immunohistochemistry, qRT-PCR and western blot. The expression of transcription factors associated with hepatocytes differentiation was also investigated in 52 human HCC.

RESULTS: Exogenous administration of T3 to rats bearing HCCs causes their regression as observed both at macroscopic levels, as well as under microscopic examination. While the liver of rats not exposed to T3 displayed well- or poorly-differentiated HCCs, only features of adenomas were observed in T3-treated animals. Moreover, T3 was able to almost completely inhibit lung metastases that were instead observed in 6/7 untreated animals. QRT-PCR analysis showed that reactivation of the T3-TR axis, monitored by an increased expression of the TRβ-target genes Dio1 and G6pc,was associated to loss of markers of putative stem/progenitor cells, such as CK-19, or fetal markers, such as GSTP, and to enhanced mRNA levels of the transcription factors as HNF4, CEBPα and KLF9, known to be involved in hepatocytes differentiation. Moreover, a statistically significant direct correlation between the mRNA levels of TRβ and those of HNF4 and KLF9 was also found in a set of 52 human HCCs.These findings together with the absence of T3-induced cell death suggest that T3 may exert its anti-tumorigenic effect by inducing differentiation of neoplastic hepatocytes towards a more benign phenotype.

CONCLUSIONS: the result suggest that reestablishment of the function of the TR/T3 axis, lost in neoplastic cells, is associated to induction of a differentiation program. It follows that thyroid hormone or its analogues, especially selective agonists of TRβ, devoid of T3-harmful effects, might represent a valuable tool in HCC therapy.

NO CONFLICT OF INTEREST

468 Optical Imaging of αvβ3 integrin expression with a new Near-Infrared Fluorescent RGD probe

A. Cordaro[1], *F. Chianale*[2], *C. Brioschi*[2], *M. Bartolomeo*[2], *G. Valbusa*[3], *F. Blasi*[3], *F. Maisano*[2], *F. Tedoldi*[2], *S. Aime*[1]

[1] *University of Torino, Department of molecular biotechnology and health sciences, Torino, Italy*
[2] *Bracco Imaging SpA, BioImaging group, Colleretto Giacosa, Italy*
[3] *Ephoran Multi Imaging solution, Imaging, Colleretto Giacosa, Italy*

INTRODUCTION: Integrins are a family of transmembrane adhesion proteins that mediate cell adhesion and intracellular signaling. $\alpha_v\beta_3$ Integrin is usually expressed at low or undetectable levels in most adult epithelia, but it is highly up-regulated in tumors and correlates with disease progression. Moreover, unlike in quiescent endothelium, $\alpha_v\beta_3$ is mainly expressed in tumor-associated vessels. Arg-Gly-Asp (RGD) motif-containing peptides are preferentially bound to $\alpha_v\beta_3$ integrin, and are known to interfere with neovasculature formation by competition to extracellular matrix proteins. Thus, RGD-based strategies have been widely adopted to design targeted molecules for cancer therapy and/or diagnosis.

This study employed a new cyclic RGD-based peptidomimetic conjugated with a NIR fluorophore intended for intraoperative use during tumor resection, to enable proper identification of tumor margins by optical imaging. The characterization of this molecule are presented here, to show its affinity and specificity for the target receptor, and its capability to identify the tumor masses.

MATERIAL AND METHODS: ELISA and SPR techniques were adopted to define the affinity of our RGD-based fluorescent probe for both $\alpha_v\beta_3$ integrin and Human Serum Albumin (HSA). Flow cytometry, immunofluorescence and cell assays were used to assess the interaction of the probe with cells, expressing different levels of the target. Nude mice bearing tumors from U87MG human glioblastoma and A431 human epidermoid carcinoma cells were chosen for in vivo optical imaging experiments.

RESULTS AND DISCUSSION: Our RGD-based fluorescent probe exhibited high affinity to isolated $\alpha_v\beta_3$ integrin (IC_{50}<10 nM) and moderate binding to HSA. Different levels of $\alpha_v\beta_3$ expression were found in the cell lines: CHO and WM266 cells were selected as negative and positive controls, respectively, for target expression and for in vitro characterization purposes. Cell adhesion assays and immunofluorescence showed that our probe interfered with $\alpha_v\beta_3$-mediated cell adhesions. In vivo optical imaging displayed higher accumulation of RGD-based fluorescent probe in U87MG tumors than in A431 tumors, in accordance to $\alpha_v\beta_3$ expression and the extent of neo-vascularization.

CONCLUSION: Our NIR fluorescence RGD-based molecule can be used for sensitive detection of tumor lesions in vivo. It is a promising optical imaging probe for fluorescence-guided surgical resection of tumors characterized by variable expression of $\alpha_v\beta_3$ integrin.

NO CONFLICT OF INTEREST

469 Aryl hydrocarbon receptor regulates histone deacetylase 8 expression to repress tumor suppressive activity in hepatocellular carcinoma

S.N. Wang[1], *L.T. Wang*[2], *S.H. Hsu*[2]

[1] *Kaohsiung Medical University Hospital, Division of General and Digestive Surgery- Department of Surgery, Kaohsiung, Taiwan*
[2] *Kaohsiung Medical University, Graduate Institute of Medicine, Kaohsiung, Taiwan*

BACKGROUND & AIMS: Histone deacetylase 8 (HDAC8), a unique member of class I histone deacetylases, shows remarkable correlation with advanced disease stage and multiple malignant tumors However, little is known about the contribution of HDAC8 to the tumorigenesis of hepatocellular carcinoma (HCC). The present study investigated the expression of HDAC8 regulated by the aryl hydrocarbon receptor (AHR) in HCC cell lines and tissues, and the roles of HDAC8 overexpression in cell proliferation, including potentially underlying mechanisms.

METHODS: We assessed the correlation between the clinic-pathological parameters and the expression of AHR and HDAC8. Further, we analyzed the AHR siRNA transfection and HDAC8 inhibitors to explore the functions of HDAC8 in HCC progression in vitro and in vivo. In a panel of 289 HCC patients, HDAC8 was shown to be highly correlated with AHR expression at both mRNA and protein levels.

RESULTS: HCC patients with high AHR expression showed a shorter survival time than that with low AHR expression. We then found that the expression of both AHR and HDAC8 was significantly upregulated in both HCC cell lines and tumor tissues compared to human normal hepatocytes and matched non-tumor tissues. Furthermore, HDAC8 inhibition remarkably inhibited hepatoma cell proliferation and transformation activity via upregulation of RB1 in vitro and in vivo.

CONCLUSIONS: Our data revealed an important role of the AHR-HDAC8 axis in promoting HCC tumorigenesis, thus identifying HDAC8 as a potential therapeutic target for HCC treatment.

NO CONFLICT OF INTEREST

470 Synergistic effect of PI3K and FAK inhibition in squamous lung cancer cells with reduced PTEN level

A. Cavazzoni[1], *S. La Monica*[1], *R. Alfieri*[1], *R. Sciarrillo*[2], *D. Cretella*[1], *N. Van Der Steen*[2], *M. Tiseo*[3], *A. Ardizzoni*[4], *P.G. Petronini*[1], *E. Giovannetti*[5]

[1] *University of Parma, Department of Medicine and Surgery, Parma, Italy*
[2] *VU University Medical Center, Department of Medical Oncology, Amsterdam, Netherlands*
[3] *Azienda Ospedaliero-Universitaria, Medical Oncology Unit, Parma, Italy*
[4] *University of Bologna, Department of Experimental- Diagnostic and Specialty Medicine, Bologna, Italy*
[5] *University of Pisa, Cancer Pharmacology Lab- AIRC/Start-Up Unit, Pisa, Italy*

BACKGROUND: Squamous cell lung carcinoma (SCC) accounts for 30% of patients with NSCLC and the patients have a poorer prognosis than those with adenocarcinoma. Three FDA-approved mAb directed to the immune checkpoint PD-1/PDL-1 (nivolumab, pembrolizumab and atezolizumab) have been recently approved for NSCLC treatment, including SCC, but with reduced clinical benefits. Therefore further research on new oncogene-directed targeted drugs is warranted. Several oncogenic aberrations have been reported in SCC patients, including PI3K point mutations and amplification, and loss or reduced PTEN expression. In order to elucidate the role of PTEN abrogation in SCC cells, we generated cell clones with stable reduction of PTEN levels and we evaluated the efficacy of a combination with the pan PI3K inhibitor buparlisip and the FAK inhibitor defactinib.

METHODS: PTEN and FAK phosphorylation were evaluated by validated immunohistochemical methods on tissue sections from formalin-fixed paraffin-embedded samples obtained from 30 patients affected by SCC (15 with resected and 15 with stage IV disease). Cell clones with reduced PTEN level were generated form SCC SKMES-1 cells by shRNA technology. Phosphorylation levels of the main proteins in PI3K/Akt and FAK pathways were investigated by Western blot and a specific RTK array. Mesenchymal markers and miR-21 levels were determined by RT-PCR.

RESULTS: A significant correlation between PTEN loss and FAK phosphorylation was observed in patients with stage IV SCC. After generation of cancer cell clones with reduced PTEN levels, we observed an increase in Akt and FAK phosphorylation, associated with improved proliferation of cancer cells and tumor spheroids. In these

cell clones we also detected a significantly increased migration and invasiveness, associated to the acquisition of a mesenchymal phenotype and overexpression of the oncomir miR-21. The combined treatment of buparlisib and defactinib induced a synergistic inhibition of cell proliferation and a significant reduction of cell migration and invasion only in cell clones with low PTEN levels. The molecular mechanisms underlying these data showed a reduction of phosphorylation of key kinases such as JNK, GSK-3 α/β, and AMPK-α2, Akt and FAK activation.

CONCLUSIONS: The combination of buparlisib and defactinib was effective against cells with reduced PTEN level and warrants further studies as a novel therapeutic strategy for stage IV SCC patients with loss of PTEN expression.

NO CONFLICT OF INTEREST

471 Mechanisms of acquired resistance to cetuximab: Role of interleukin 1

V. Gelfo[1,2], M. Pucci[2], M. Lindzen[3], M. Mazzeschi[2], M. Bonafè[2], R. Solmi[2], G.M. D'Uva[4], Y. Yarden[3], M. Lauriola[1,2]

[1] University of Bologna- Ospedale S. Orsola Malpighi, DIMES, Bologna, Italy
[2] University of Bologna- Ospedale S. Orsola Malpighi, Center for Applied Biomedical Research CRBA, Bologna, Italy
[3] Weizmann Institute of Science, Department of Biological Regulation, Rehouot, Israel
[4] IRCCS MultiMedica, Scientific and Technology Pole, Milan, Italy

Cetuximab (CX) is a monoclonal antibody targeting the Epidermal Growth Factor Receptor (EGFR), which is commonly utilized to treat patients with metastatic colorectal cancer (mCRC). Unfortunately, clinicians often observe a residual disease, with a population of cells surviving the treatment and eventually enabling CX resistance. Our previous studies, performed with a cohort of 150 CRC xenopatients, associated poor response to CX with increased abundance of a set of inflammatory cytokines, including IL1A, B and IL8. Stemming from these observations, our working hypothesis assumes that, resistance to CX is acquired, in a subset of CRC patients, through cell plasticity and consequent rewiring of signalling networks, which confer to tumors dependency on the IL1 pathway. In order to assess the effect of IL1 activity, we employed a colon cancer model unresponsive to cetuximab, as previously characterized in our laboratory. To inhibit activation of the IL1 pathway we used anakinra, an IL1-receptor antagonist and parthenolide, which modulates the activity of NF-kB, the transcription factor involved in the feed-forward loop of inflammation mediators. Furthermore, we employed a recombinant decoy (IL1R-Fc), namely a soluble protein combining the human immunoglobulin Fc portion linked to the extracellular region of IL1-receptor, with the ability to sequester IL1 directly from the medium. We generated stable clones of CX-resistant cells expressing IL1R-Fc. Our preliminary result show that inhibition of IL1R leads to a proliferation decrease of colorectal cancer cells. These findings support the hypothesis of a compensatory activation of the IL1-receptor pathway in cetuximab-resistant CRC cells. Hence, modulating IL1 signalling might represent a new therapeutic strategy suitable for patients who acquired refractoriness to monoclonal antibody therapy.

NO CONFLICT OF INTEREST

472 Multi-omics profiling of sequential tumor and liquid biopsies allowed identification of biomarker candidates of therapeutic resistance in metastatic colorectal cancer; Q-CROC-01: NCT00984048

K. Gambaro[1], M. Marques[2], R. Morin[3], C. Kleinman[4], M. Witcher[5], S. Turcotte[6], M. Couetoux du Tertre[1], S. McNamara[1], P. Kavan[5], G. Batist[5]

[1] Exactis Innovation and Segal Cancer Centre-Jewish General Hospital, Oncology, Montreal, Canada
[2] Exactis Innovation and Segal Cancer Centre-Jewish General Hospital- McGill University, Oncology, Montreal, Canada
[3] Simon Fraser University, Department of Molecular Biology and Biochemistry and School of Computing Science, Vancouver, Canada
[4] Segal Cancer Centre-Jewish General Hospital, Molecular Oncology, Montreal, Canada
[5] Segal Cancer Centre-Jewish General Hospital, Oncology, Montreal, Canada
[6] Centre Hospitalier de l'Université de Montréal, Surgery, Montreal, Canada

BACKGROUND: Colorectal cancer (CRC) is the 3rd most common cancer worldwide. Clinical responses of metastatic (m)CRC to first-line treatment range from 35 to 60%, but even responders inevitably develop therapeutic resistance. Studies aiming at understanding mechanisms of resistance have largely investigated primary tumors. However, selective pressures during therapy can lead to the expansion of resistant clones and tumor heterogeneity. This highlights the need to characterize the molecular changes of metastasis and plasma over time of treatment and response to decipher tumor evolution and therapeutic resistance mechanisms.

MATERIAL AND METHODS: Fifty-two tissue samples from liver metastasis were collected at baseline (pre-biopsies) and at the time of resistance (post-biopsies) in responder and non-responder mCRC patients undergoing the same standard first-line treatments. Multiple post-biopsies also have been harvested in 4 patients, to allow the assessment of tumor heterogeneity after treatment exposure. Biopsies were profiled using exome and transcriptome sequencing as well as high-density SNP array analysis. Additionally, serial blood samples were collected for proteomic, ctDNA and cytokine analysis.

RESULTS: High-density SNP array analysis of serial tumor biopsies identified significant accumulations of genomic anomalies over time of treatment. In chemo-naïve biopsies, specific CNV regions have been found significantly associated with patient progression free survival by Kaplan-Meier analysis. Transcriptome analysis showed genes consistently dysregulated at resistance, while exome sequencing revealed cumulative somatic mutations during treatment, allowing identification of acquired 'driver" mutation candidates of resistance. Immune gene expression analysis of a test set of 27 metastases revealed strong clustering of 7 metastases due to overexpression of transcripts related to active immune response, allowing to define novel subgroups of patients based on immune response status. Plasma-derived ctDNA analysis was performed to investigate the mutational status during treatment and whether they correlate with their relative levels in biopsies.

CONCLUSIONS: Our study, using a multi-omic approaches to profile serial liver metastasis samples and liquid biopsies in CRC patients, constitutes an innovative and powerful approach to identify clinical biomarkers and molecular signature of resistance, and support the development of personalized therapy.

NO CONFLICT OF INTEREST

473 Investigating the role of circulating tumor cells in biliary tract cancer

C. Reduzzi[1], L. Celio[2], R. Motta[1], K. Dotti[2], P. Miodini[1], A. Martinetti[2], F. Cascone[1], M.G. Daidone[1], F. De Braud[2], V. Cappelletti[1]

[1] Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Experimental Oncology and Molecular Medicine, Milano, Italy
[2] Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Medical Oncology, Milano, Italy

INTRODUCTION: Biliary tract cancer (BTC) is a rare disease with a very poor prognosis and limited therapeutic options. The identification of a high number of actionable mutations in BTC makes this tumor a good candidate for personalized approaches exploiting target treatments, but unfortunately having access to tissue biopsies to perform genotype analysis is often impossible in BTC patients. In this regard, circulating tumor cells (CTCs) can function as surrogate of tumor material. Moreover, the analysis over time of their number, phenotype and molecular profile, using a single cell approach, allows the monitoring of tumor evolution in response to treatment and could give predictive information and indications on new treatment options.

MATERIAL AND METHOD: Blood samples from BTC patients were processed with a novel CTC characterization approach. CTC enrichment was performed using a density based method (OncoQuick) or a method based on cell size and deformability (Parsortix); enriched cells were labeled with epithelial and leukocyte-specific antibodies to enable both positive and negative selection of CTCs using the DEPArray which allows both the visualization and the recovery of single cells. After a whole genome amplification step, a copy number alteration (CNA) analysis was performed by low-pass whole genome sequencing to define the tumoral nature of negatively selected CTCs (cells negative for both epithelial and leukocyte markers).

RESULTS AND DISCUSSION: In 8 samples processed, 7 CTCs expressing epithelial markers and 11 CTCs expressing neither epithelial nor leukocyte markers were identified by positive and negative selection, respectively. CNA profile analysis suggested a genomic intra-patient CTC heterogeneity which will be further investigated by mutational profiling (targeted sequencing of 50 cancer-associated genes).

Preliminary data suggest the presence of only one of the CTC subpopulations (either epithelial or non-epithelial) in single patients. CNA analysis of CTCs identified in 23 more samples will be performed to extend the study.

CONCLUSION: The unbiased enrichment and the addition of negative selection of CTC, allowed the successfully isolation of CTC for molecular studies in 100% of samples from advanced BTC patients. It is possible to perform mutational analysis by next generation sequencing of the collected CTCs which therefore can be used as surrogate of unavailable tissue biopsies.

NO CONFLICT OF INTEREST

474 Targeting cyclin-dependent kinases in osteosarcoma can increase the efficacy of DNA damaging drugs

S. Vella[1], E. Tavanti[2], C.M. Hattinger[1], M. Fanelli[1], P. Picci[1], M. Serra[1]

[1] Orthopaedic Rizzoli Institute, Laboratory of Experimental Oncology, Bologna, Italy

INTRODUCTION: To improve the presently achievable clinical result in osteosarcoma (OS) novel treatment approaches are necessary. Targeting protein kinases emerged as a promising therapeutic strategy in several human tumours. In particular, inhibiting cyclin-dependent kinases (CDKs), which are essential not only for cell proliferation but also for cellular response to DNA damaging agents, has been indicated a promising approach to enhance the efficacy of conventional chemotherapy. For these reasons, several CDKs inhibitors have been developed and are now available for clinical use. In this study, we have tested whether targeting CDKs may increase the efficacy of DNA damaging drugs on a panel of drug-sensitive and -resistant human OS cell lines.

MATERIAL AND METHOD: Clinical relevance of CDKs 1-9 was assessed in OS clinical samples, which were stratified according to each kinase expression level. In vitro efficacy of the CDKs inhibitor roscovitine (commercial name "seliciclib or CYC202®") was evaluated by MTT assay, cell cycle perturbations and apoptosis by flow cytometry. The effects on drug-induced DNA damage repair were assessed with the alkaline COMET assay. The type of interaction (synergism, antagonism or additivity) of roscovitine with conventional drugs was calculated with the Chou-Talalay equation.

RESULTS AND DISCUSSION: In OS clinical samples, event-free survival analyses showed that high expression of CDK1, CDK2 and CDK7 at diagnosis was associated with a trend toward a worse outcome. We therefore assessed the in vitro efficacy of roscovitine, which inhibits CDK1, CDK2 and CDK7 in addition to CDK5 and CDK9. Roscovitine proved to be active in all OS cell lines, being cytostatic and moderately apoptotic, without evidence of cross-resistance with doxorubicin, cisplatin or methotrexate. The best interactions with DNA damaging drugs were obtained when roscovitine was sequentially administered after doxorubicin or cisplatin. This positive interaction was mainly due to the fact that roscovitine negatively interacted with the CDKs-mediated DNA damage repair control, delaying the activation of DNA repair systems and consequently increasing cell sensitivity to DNA damaging agents.

CONCLUSION: Our result indicated that roscovitine combined with conventional OS chemotherapeutic drugs may represent a new intervention approach, which may be considered to enhance OS cell sensitivity to DNA damaging drugs.

NO CONFLICT OF INTEREST

475 Development and characterization of microfluidic models of the tumour microenvironment for translational research

M. Virumbrales-Munoz[1], L. Alodia[1], J.M. Ayuso[2], T. Randelovic[1], S. Olivan[1], M. Doblare[1], L. Fernandez[1], I. Ochoa[1]

[1] Aragon Institute for Engineering Research, Mechanical Engineering, Zaragoza, Spain
[2] Morgridge Institute for Research, Wisconsin Institutes for Medical Research, Madison, USA

BACKGROUND: There is compelling evidence demonstrating that the tumor microenvironment (TME) plays a key role in tumor initiation, development and response to therapy. This contributes to a high heterogeneity within the same cancer type, and hinders the process of finding effective treatments.

In this context, microfluidics has proven capable of creating comprehensive and personalized cancer in vitro 3D models reproducing the TME more accurately than traditionalsetups. Microfluidics also permits a high degree of control over the setup, combining different cell types in an orderly manner, as well as different physical and biochemical cues. Furthermore, it facilitates optical inspection and diminishes sample sizes. All in all, microfludics is an interesting toolbox for drug testing, research and development.

MATERIALS AND METHODS: We used COP injection to fabricate microdevices. Cells were seeded at a very high concentration within the microdevices using collagen gels to mimic TME in a microfluidic platform, while two lateral channels served as auxiliary blood vessels. Confocal microscopy was used for the characterization of the TME model. The characterization was performed in terms of hypoxia, proliferation rates, reactive oxygen species concentration, apoptosis rate and glucose uptake using fluorescent probes. Furthermore, we carried out pharmacodynamic and drug efficiency studies in these newly-established models. Finally, we used an enzymatic procedure to extract cells from the microfluidic device and subject them to downstream tests.

RESULTS AND DISCUSSION: We developed a microfluidic tumor-slice model, in which we reproduced and characterized two separate TMEs: colon cancer and glioblastoma. Thereafter, we extracted the cells and verified that quality RNA can be extracted from confined cells for downstream gene expression assays to broaden the characterization of the TME. Cells could be sorted by flow cytometry and examined by this very technique. We validated our models and observed a different TME for each cell type. On the other hand, we observed a higher glucose uptake in case of the colon cancer cells and a higher response to TRAIL formulations.

CONCLUSIONS: All in all, we developed a comprehensive microfluidic model which could be used for translational studies. This model is capable of comprehensive reproduction of the TME, both to unravel molecular mechanisms of cancer, and to characterize of tumor signatures by means of traditional benchtop methods.

CONFLICT OF INTEREST

Advisory Board: I. Ochoa and L.J. Fernandez are both promotors and consultants for BeOnChip S.L. (Zaragoza, Spain).

476 The association of Palbociclib with PI3K inhibitors as a new therapeutic strategy in the treatment of Mesothelioma

G. Digiacomo[1], C. Fumarola[1], D. Cretella[1], R. Alfieri[1], F. Quaini[1], P.G. Petronini[1], M. Bonelli[1]

[1] University of Parma, Medicina e Chirurgia, Parma, Italy

INTRODUCTION: Malignant pleural mesothelioma **(**MPM) is a progressive, incurable malignancy of the pleura associated to the exposure of asbestos fibers. Based on the increasing incidence and the poor prognosis of MPM novel therapeutic strategies are urgently needed. The most frequently inactivated tumor suppressor gene in MPM is cdkn2a/arf, encoding for the cell cycle inhibitors $p16^{INK4a}$ and $p14^{ARF}$, deleted in about 70% of MPM cases. Considering the high frequency of alterations in cdkn2a/arf gene, we tested the efficacy of Palbociclib (PD-0332991), an highly selective inhibitor of CDK4/6, that prevents the progression of the cell cycle by inhibiting Rb protein hyper-phosphorylation. Currently, Palbociclib has been approved by the US FDA, for the treatment of estrogen-positive metastatic breast cancer in association with Letrozole.

MATERIALS AND METHODS: The experiments were performed on MPM cell lines and on cells isolated from pleural effusion of patients with MPM. All the cell lines analyzed presented the predictive factors of response to Palbociclib: Rb and Cyclin D1 expression and low levels of $p16^{INK4a}$.

RESULTS AND DISCUSSION: MPM cells were sensitive to Palbociclib with a significant blockade of the cells in G0/G1 phase of cell cycle. The inhibition of cell proliferation was associated with the acquisition of the senescent phenotype. Moreover, the treatment with Palbociclib reduced the phosphorylation levels of CDK6 and Rb and the expression of myc. After 24 h of treatment with Palbociclib, we also observed an increased phosphorylation of AKT. On the basis of this result we tested the efficacy of a sequential combined treatment of Palbociclib with PI3K/inhibitors (24 h of Palbociclib followed by 48 h of Palbociclib/PI3Ki associated treatment). The sequential association of Palbociclib with PI3K inhibitors determined a synergistic effect on the inhibition of cell proliferation and strongly increased the percentage of senescent cells. Under this condition AKT activation was repressed and cells showed increased levels of p53 and p21. Interestingly, we demonstrated that the inhibition of cell proliferation and the senescent phenotype obtained after two cycles of sequential drug treatment were maintained after drugs withdrawal.

CONCLUSIONS: Our result indicate that the sequential association of Palbociclib with PI3K/inhibitors may represent a possible new therapeutic approach for the treatment of MPM.

NO CONFLICT OF INTEREST

477 Potential biomarkers for personalized oncology radiation in uterine cervical cancer

P. Moreno-Acosta[1], S. Carrillo[1], O. Gamboa[2], A. Romero-Rojas[3], J. Acosta[4], D. Mayorga[1], M. Molano[5], M. Cotes[6], N. Magne[7]

[1] National Cancer Institute, Research Group in Radiobiology Clinical- Molecular and Cellular, Bogota, Colombia
[2] National Cancer Institute, Analysis Unit- Research Group in Radiobiology Clinical- Molecular and Cellular, Bogota, Colombia
[3] National Cancer Institute, Pathological oncology Group, Bogota, Colombia
[4] National University of Colombia, Pathology Group, Bogota, Colombia
[5] The Royal Women's Hospital, Microbiology and Infection Diseases, Melbourne, Australia
[6] National Cancer Institute, Radiotherapy Group- Research Group in Radiobiology Clinical- Molecular and Cellular, Bogota, Colombia
[7] Institut de Cancérologie Lucien Neuwirth, Département de Radiothérapie, Saint-Priest en Jarez cedex, France

BACKGROUND: Uterine cervical cancer (UCC) is one of the most prevalent malignant neoplasms in the world. UCC develops beyond the stage in situ and is frequently treated by a combination of intracavitary radiation therapy and external beam radiation therapy; 30 to 40% of patients with similar prognosis factors not respond equally to a comparable standard treatment. Therefore, the study and identification of prognostic biomarkers, which indicate the probable course of the disease in an untreated individual, and predictive biomarkers, which allow identification of subpopulations of patients most likely to respond to a given therapy, would be extremely useful in the selection of patients for the development of innovative and effective therapies for locally advanced, metastatic and refractory uterine cervical cancer. Within work that we have been developing, reported that gene expression of IGF1R is a strong predictive marker for lack of response to radiotherapy (p=0.018, 95% CI (1.7-41.2)), patients HPV16 (+) have 28.6 times higher risk of failure treatment; consequently, with the present study, we proposed to determine, whether expression of IGF-IR, GAPDH, HIF-1 alpha, Survivin, GLUT1, CAIX, HKII, and clinic-pathological parameters can be used as prognostic and predictive biomarkers and as possible molecular targets for individualized treatment.

PATIENTS & METHODS: This prospective cohort study included 149 patients with squamous cell carcinomas of the uterine cervix in FIGO stages IIB (n= 53) and IIIB (n=96). Of the 149 patients, 61 were treated with radiotherapy and 88 with concurrent radio-chemotherapy. Expression of the proteins CAIX, GLUT-1, HIF1α, HKII, IGF-IRα, IGF-IRβ and Survivin, was determined by immunohistochemistry in biopsies taken before treatment. result An overexpression was found for GAPDH (100%), followed of Survivin (87%), IGF-IRα (76.5%), IGF-IRβ (74.5%), HIF1α (74.1%), IGF-IRα and IGF-IRβ (73%); strong expression was observed with low frequency for GLUT-1 (31.1%), CAIX (16.2%), HKII (10.6%). Hgb level was significantly correlated with treatment response (p=0.01). With a median follow-up of 2.1years, OS was decreased for patients over-expressing IGF-1R β (p=0.04). The OS of the sub-group of patients with anemia (Hgb < 11g/dL) and concomitantly over-expressing IGF-1R and GLUT-1 was significantly decreased compared to the opposite control group (p=0.015).

CONCLUSIONS: The expression of GLUT-1, IGF-1R and Hgb (≤ 11g/dl) are associated with poor prognosis, and thus appear to be interesting biomarkers of radiation resistance.

NO CONFLICT OF INTEREST

478 Immunohistochemical profiling of c-Met, VEGF, TGF-ßRII and PTEN, and EGFR mutation in surgically resected Non-Small Cell Lung carcinoma: Their correlation and prognostic significance

J. Yoo[1], S.Y. Park[2]

[1] The Catholic University of Korea, Pathology, Suwon, South Korea
[2] Stanford University Hospital, Radiology, Stanford, USA

BACKGROUND: Recurrence after curative surgery is common in patients with non-small cell lung carcinoma (NSCLC), and only 25~70% of stage I~III patients survive 5 years. In the present study, we retrospectively investigated EGFR, c-Met, VEGF, TGF-ßRII, and PTEN to determine their correlation and prognostic significance in NSCLC patients who underwent surgical resection.

MATERIALS AND METHODS: A total of 214 NSCLC patients who underwent curative surgery were enrolled in this study. Clinical information was obtained from a computerized retrospective database from tumor registry. Immunohistochemistry was performed for the expression of c-Met, VEGF, TGF-ßRII, and PTEN proteins. PNA-mediated real-time PCR products were used for EGFR mutation status.

RESULTS: Fifty patients (23.4%) suffered from tumor recurrence during the follow-up period (18.9% of early stage NSCLC vs. 29.9% of advanced disease, p=0.063). The median relapse-free survival (RFS) was 22.9 months (42.9 months in early stage NSCLC vs. 12.5 months in advanced tumor, p=0.001). c-Met, VEGF, TGF-ßRII, and PTEN proteins were expressed in 53.7%, 57%, 65%, and 51.4%, retrospectively. EGFR mutation was detected in 53%. VEGF expression and EGFR mutation were not associated with each other, but both correlated with c-Met, TGF-ßRII, and PTEN, respectively. RFS was found to be significantly associated with c-Met (p=0.007), TGF-ßRII (p=0.031), PTEN (p=0.045), EGFR (p=0.001), SUVmax on ^{18}F-FDG PET/CT (p=0.049), tumor differentiation (p=0.001), lymphovascular invasion (p=0.001), resection margin (p=0.001), and tumor stage (p=0.001). In multivariate analysis, patients with high c-Met expression were more likely to experience recurrence than those with low c-Met expression (HR=2.23, p=0.021), whereas patients with mutant EGFR were less likely to experience recurrence than those with wild-type EGFR (HR=0.886, p=0.002). In addition, tumor differentiation and tumor stage were risk factors for poor RFS (well/moderate vs. poor, p=0.028 and I~IIA vs. IIB~III, p=0.002, respectively).

CONCLUSIONS: Our data demonstrated that c-Met expression was an independent unfavorable prognostic marker for RFS, and that EGFR mutation was an independent favorable prognostic indicator for RFS in curatively resected NSCLC patients. Further investigation of their roles for treatment planning is warranted, especially in the adjuvant setting.

NO CONFLICT OF INTEREST

479 Prognostic significance of PD-L1, PD-L2, Caspase 3, and Ki-67 expressions in resected Non-Small Cell Lung carcinoma

J. Yoo[1], S.Y. Park[2]

[1] St. Vincent's Hospital- The Catholic University of Korea, Pathology, Suwon, South Korea
[2] Stanford University Hospital, Radiology, Stanford, USA

BACKGROUND: Non-small cell lung carcinoma (NSCLC) comprises 75~85% of all lung cancers. Despite advances in cancer treatment, 5-year overall survival after diagnosis of NSCLC remains at only 15%, and approximately 25~50% of curatively resected NSCLC patients develop recurrence. In the present study, we evaluated the prognostic significance of PD-L1, PD-L2, Caspase 3, and Ki-67 expressions in NSCLC patients who underwent surgical resection.

MATERIALS AND METHODS: Tissue microarrays of resected tumors from 214 NSCLC patients with (n=50) or without (n=164) tumor recurrence after curative surgery were examined for PD-L1, PD-L2, Caspase 3, and Ki-67 by immunohistochemistry. Clinical information was obtained through a retrospective database from our tumor registry.

RESULTS: The mean overall survival (OS) was 35.9 months (50.6 months in early stage NSCLC vs. 19.4 months in advanced tumor, p=0.001). Fifty of the 214 patients (32%) experienced recurrence (18.9% of early stage NSCLC vs. 29.9% of advanced disease, p=0.063). PD-L1, PD-L2, Caspase 3, and Ki-67 expressions were detected in 19.6%, 25.2%, 33.6%, and 29.4%, respectively. Both PD-L1 and PD-L2 expressions correlated with Caspase 3 (p=0.001, respectively) but not with Ki-67 (p=0.121 and p=0.620, respectively). In univariate analysis, OS was significantly associated with PD-L1 (p=0.041), Caspase 3 (p=0.008), and Ki-67 (p=0.001). Other poor prognostic factors included tumor differentiation (p=0.001), tumor stage (p=0.001), lymphatic invasion (p=0.003), resection margin (p=0.006), and performance status (p=0.001). Cox regression multivariate analysis demonstrated that Caspase 3 expression (<25% vs. p=0.007), Ki-67 expression (<20% vs. p=0.001), tumor differentiation (well/moderate vs. poor, p=0.001), tumor stage (I~IIA vs. IIB~III, p=0.001), and resection margin (R0/R1 vs. R2, p=0.001) were independent unfavorable factors. Of these, Ki-67 was a risk factor for poor disease-free survival (p=0.056).

CONCLUSIONS: High expressions of Caspase 3 and Ki-67 were independent poor prognostic factors for OS in curatively resected NSCLC patients.

NO CONFLICT OF INTEREST

480 The effect of accumulation of mutated gene, K-RAS, in gastrointestinal cancer cells and IGF-1R blockades

Y. Adachi[1], Y. Sasaki[2], H. Koide[1], S. Shigeru[3], Y. Hiro-O[2], T. Tokino[2], D. Carbone[4], K. Imai[5], H. Nakase[1]

[1] Sapporo Medical University, Dept of Gastroenterology, Sapporo, Japan
[2] Sapporo Medical University, Medical Genome Sciences- Research Institute of Frontier Med, Sapporo, Japan
[3] Sapporo Medical Univuersity, Dept of Gastroenterology, Sapporo, Japan
[4] The Ohio State Univ Med Ctr, James Cancer Ctr, Columbus, USA
[5] The Univ of Tokyo, The Institute of Medical Science Hosp, Tokyo, Japan

BACKGROUND & AIMS: Mutation in K-RAS (K-RAS-MT) plays important roles in both tumor progression and resistance to anti-epidermal growth factor receptor (EGFR) therapy in gastrointestinal carcinomas. Insulin-like growth factor-1 receptor (IGF-1R) is required for tumorigenicity and progression of many malignancies as well. We have previously shown successful therapy for gastrointestinal tumor cell lines bearing a K-RAS mutation using an anti-IGF-1R monoclonal antibody. In this study, we sought to evaluate effects of additional K-RAS-MT expression on gastrointestinal cancer cells representing a possible second resistance mechanism for anti-EGFR strategies and IGF-1R targeting for these transfectants.

METHODS: We made stable transfectants of K-RAS-MT in 2 gastrointestinal cancer cells, pancreatic BxPC-3 and colorectal RKO. We assessed the effect of over-expression of K-RAS-MT on proliferation, apoptosis, migration, and invasion in gastrointestinal cancers. Then we assessed anti-tumor effects of dominant negative IGF-1R (IGF-1R/dn) and an IGF-1R inhibitor, picropodophyllin (PPP), on the K-RAS-MT transfectants. result Accumulation of gentic changes, addition of K-RAS-MT, in gastrointestinal carcinoma cell lines led to more aggressive phenotypes, with increased proliferation, decreased apoptosis, and increased motility and invasion. IGF-1R blockades suppressed cell growth, colony formation, migration, and invasion, and enhanced chemotherapy-induced apoptosis of gastrointestinal tumor cells, even when K-RAS-MT was over-expressed. IGF-1R inhibitions blocked the Akt pathway more than MAPK pathway in the K-RAS-MT transfectants. IGF-1R/dn, moreover, inhibited the growth of murine xenografts expressing K-RAS-MT.

CONCLUSIONS: K-RAS-MT might be important for progressive phonotype observed in gastrointestinal carcinomas. IGF-1R targeting strategy is a candidate molecular therapeutic approach for gastrointestinal tumors even if K-RAS is mutated.

NO CONFLICT OF INTEREST

481 The role of pancreatic cancer metabolic rewiring in driving the expression of CES2, a predictor of response to FOLFIRINOX therapy

M. Capello[1], J.F. Fahrmann[1], M. Rios Perez[2], Y. Kang[2], H. Xu[1], S. Ferri-Borgogno[3], W. Huamin[3], P.J. Chiao[4], J.B. Fleming[2], S.M. Hanash[1]

[1] University of Texas MD Anderson Cancer Center, Clinical Cancer Prevention, Houston, USA
[2] University of Texas MD Anderson Cancer Center, Surgical Oncology, Houston, USA
[3] University of Texas MD Anderson Cancer Center, Pathology, Houston, USA
[4] University of Texas MD Anderson Cancer Center, Molecular & Cellular Oncology, Houston, USA

BACKGROUND: We previously uncovered a role for the serine hydrolase CES2, expressed in pancreatic ductal adenocarcinoma (PDAC), in mediating intratumoral activation of the inactive prodrug irinotecan, a constituent of FOLFIRINOX, into its active form. In this study we investigated molecular mechanisms associated with CES2 expression in PDAC, which have the potential to improve PDAC patient stratification for FOLFIRINOX therapy.

MATERIAL AND METHOD: CES2 expression in PDAC tissues was evaluated by immunohistochemical analysis. PDAC cell line untargeted metabolomics profiling was performed in triplicate using high-resolution mass spectrometry. PDAC cell in vitro growth was evaluated by live cell imaging confluency analysis, spheroid and colony formation assays. In the in vivo study PDAC cell lines were orthotopically injected into the pancreas of nude mice (n=5/group), and tumor growth monitored by bioluminescence imaging.

RESULTS: CES2 expression in PDAC tissues (N=114) showed a significant positive association with a diagnosis of type 2 diabetes (OR [95% CI] = 4.78 [1.33-17.24]; P=0.01). Ingenuity Pathway Analysis of genes positively correlated with CES2 expression in three independent PDAC gene expression datasets (TCGA, ICGC and Oncomine) identified a gene network involved in lipid and xenobiotic metabolism regulated by the nuclear receptor HNF4A (z-score=4.85; P=4.97×10^{-11}). HNF4A knockdown affected CES2 expression in PDAC cell lines and a significant positive correlation (Pearson R=0.7) between CES2 and HNF4A protein expression was observed in PDAC patient-derived xenograft tissues (PDXs, N=15). To identify metabolic adaptations underlying CES2 expression in PDAC cells, an untargeted metabolomics profiling of PDAC cell lines (AsPC-1 and Su.86.86.) with different levels of CES2 expression was performed after stable overexpression or knockdown of the protein. This metabolomics analysis suggested a role for CES2 in promoting lipid hydrolysis and eicosanoid biosynthesis. CES2 knockdown affected PDAC cell line growth both in vitro and in vivo.

CONCLUSIONS: Our result suggest that CES2 expression in PDAC is positively associated with a diagnosis of type 2 diabetes and triggered by the nuclear receptor HNF4A to regulate PDAC cell lipid metabolism.

NO CONFLICT OF INTEREST

482 Effect of combining osimertinib with trastuzumab emtansine (T-DM1) or pemetrexed in NSCLC cell lines carrying EGFR activating mutations

R. Alfieri[1], S. La Monica[1], D. Cretella[1], M. Bonelli[2], C. Fumarola[1], A. Cavazzoni[2], G. Digiacomo[1], M. Tiseo[2], P.G. Petronini[1]

[1] University of Parma, Department of Medicine and Surgery, Parma, Italy
[2] University Hospital of Parma, Medical Oncology Unit, Parma, Italy

BACKGROUND: Osimertinib is a selective and irreversible third generation inhibitor of mutated EGFR. Osimertinib has been approved for patients with EGFRm T790M NSCLC following progression on TKI therapy. We recently demonstrated that combination of gefitinib and pemetrexed prevents the acquisition of gefitinib resistance in NSCLC cell lines carrying EGFR mutation. The aim of this study is to explore the therapeutic potential of combining, with different schedule of administration, osimertinib with T-DM1 or with pemetrexed in NSCLC cell lines with EGFR activating mutation and with T790M or HER-2 amplification as mechanisms of resistance to first generation TKIs.

MATERIAL AND METHOD: PC9 cells, PC9 T790M (generated in our lab) and PC9HER2c1 cells (provided by Dr. W. Pao) were used. Cell viability was evaluated by MTT assay, colony formation was evaluated by crystal violet assay, cell cycle, cell death and HER2 expression were determined by flow cytometry. Protein expression was evaluated by Western blotting. ADCC was evaluated by Cytotox assay.

RESULTS: A concomitant treatment with osimertinib and T-DM1 or pemetrexed exerted an additive effect in both PC9 and PC9T790M cell lines as evaluated by the Bliss interaction model. With the aim to optimize the sequence of treatment of osimertinib combined with pemetrexed or T-DM1, different schedules of drug association were studied in PC9 and in the PC9T790M cell lines. The best schedules in terms of inhibition of cell proliferation, inhibition of colony formation and induction of apoptosis were when the combined treatment was given as first treatment. Differently, when osimertinib was the first treatment, resistance to subsequent pemetrexed or T-DM1 exposure was observed. Interestingly, PC9HER2c1 were significantly less responsive to osimertinib than parental cells and the combination with T-DM1 was able to overcome osimertinib resistance.

CONCLUSIONS: The combination of osimertinib with T-DM1 or pemetrexed exerted an additive effect in cell assays when given as concomitant administration. However, when cells exposed to osimertinib were subsequently treated with T-DM1 or with chemotherapeutic agents an antagonist interaction was observed. The combination of T-DM1 with osimertinib overcame osimertinib resistance in cells with HER-2 amplification. To confirm these results, in vivo study testing different protocols of administration is ongoing.

CONFLICT OF INTEREST

Ownership: This study has been partially supported by AstraZeneca

483 Tumour inherent IFN regulators as biomarkers of metastasis and immunotherapeutic response in breast cancer

N. Brockwell[1], K. Owen[1], D. Zanker[1], A. Spurling[1], J. Rautela[2], S. O'Toole[3], B. Parker[1]

[1] La Trobe Institute for Molecular Science, Biochemistry and Genetics, Melbourne, Australia
[2] Walter and Eliza Hall Institute of Medical Research, Immunology, Parkville, Australia
[3] Garvan Institute, Translational Breast Cancer, Sydney, Australia

INTRODUCTION: There is an urgent need for new therapeutic strategies aimed at decreasing rapid metastasis and mortality in patients with triple negative breast cancer (TNBC). A promising approach for this subype is the use of immunotherapeutics aimed at activating an anti-tumour immune response. However, predicting patients that are likely to benefit from specific immunotherapies is critical. We previously identified tumour inherent IFN regulators (IRFs) as key breast cancer metastasis regulators and here studied their prognostic and therapeutic potential in this subtype.

MATERIALS AND METHODS: Using 4T1 and E0771 immunocompetent mouse models of metastasis, we tested the efficacy of the IFN inducer and viral mimetic poly I:C in neoadjuvant and advanced treatment settings. Using these models, we investigated the interplay between type I IFN signalling and the PD1/PD-L1 axis in the anti-tumour immune response and anti-metastatic response. Using two independent TNBC cohorts representing over 500 breast cancer patients, we used immunohistochemistry (IHC) to test if primary tumour expression of IRF7 and IRF9 correlated with risk of metastasis in TNBC patients.

RESULTS AND DISCUSSION: We reveal that IFN-based therapies are potent anti-metastatic agents when administered in a neo-adjuvant treatment setting, whereby overt metastases have not been established. Additionally, we reveal that stimulating an IFN response leads to enhanced expression of PD-L1 and that combining poly I:C with anti-PD1 leads to a sustained anti-tumour T cell activation and anti-metastatic response. Importantly, we reveal that anti-PD1 as a stand-alone therapy is ineffective as an anti-metastatic in the immunocompetent models utilised in this study. Our prognostic studies reveal that IRF9 is an independent prognostic marker, whereby loss of this marker predicts shortened survival and an increased risk of metastasis in patients with TNBC. Restored IFN signalling using IFN-based therapies may be beneficial in such patients.

CONCLUSION: Our study suggests that future combination immunotherapy trials may hold most promise in a neoadjuvant treatment setting with the aim of targeting minimal residual disease and that such strategies may not work in TNBC patients with advanced metastases. Future work will determine if IRF serves as a prognostic marker to predict patients that could benefit from such immunotherapeutic approaches.

NO CONFLICT OF INTEREST

484 In vivo and in vitro activity of a new Anti-Cancer Phenolato Titanium(IV) Complex

O. Braitbard[1], N. Ganot[2], S. Meker[2], J. Hochman[1], E. Tshuva[2]

[1] Hebrew University of Jerusalem, Department of Cell and Developmental Biology -Institute of Life Sciences, Jerusalem, Israel
[2] Hebrew University of Jerusalem, Institute of Chemistry, Jerusalem, Israel

Cisplatin is a widely used platinum-based metallo-chemotherapeutic drug that is considered an efficient treatment mainly for testicular and ovarian cancers. However, its narrow activity ranges and substantial side effects triggered studies of other potent transition metal complexes. Two titanium(IV) anti-cancer agents that have been previously studied extensively as cisplatin alternatives were titanocene dichloride and budotitane. Despite their high activity, these complexes failed clinical trials mainly due to their poor stability and formation of unidentified aggregates in aqueous solutions. Our research group focuses on development of new, hydrolytically stable, better-suited families of anti-tumor Ti(IV) complexes. Previously we reported the synthesis, characterization, and in vitro cytotoxicity of 'salan" type Ti(IV) complexes. The well defined hydrolytic behaviour, high stability, and enhanced cytotoxicity of these compounds are strongly correlated to their particular structure.

Lately, we have been working on a new family of more inert Ti complexes based on readily available phenolato ligands. We developed a group of compounds with incredibly high hydrolytic stability for weeks in water. Furthermore, our lead compound, SM23, demonstrated cytotoxic activity towards the NCI-60 cell lines with an average GI50 of 4.7±2μM. Based on these findings, the purpose of this study was to evaluate in vivo: (a) the safety and (b) the growth inhibitory and anti-metastatic activity of SM23. We observed in vivo efficacy towards lymphoma cells in a murine model, and towards a Human colon cancer (HT-29) model in immune deficient (nude) mice. It is noteworthy that no clinical signs of toxicity were observed in the treated mice. Also, preliminary studies using SM23 in combination with Cisplatin indicate synergism between the two compounds.

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 681243)

NO CONFLICT OF INTEREST

485 Dissecting primary resistance to anti-EGFR monoclonal antibodies in RAS and BRAF wild-type metastatic colorectal cancer (mCRC): A case-control study

C. Cremolini[1], F. Morano[2], R. Moretto[1], R. Berenato[2], E. Tamborini[2], F. Perrone[2], D. Rossini[1], A. Gloghini[2], A. Busico[2], G. Zucchelli[1], C. Baratelli[3], E. Tamburini[4], I. Capone[2], C.C. Volpi[2], M. Milione[2], M. Di Maio[5], G. Fontanini[6], F. De Braud[2], A. Falcone[1], F. Pietrantonio[2]

[1] Unit of Medical Oncology 2 - Azienda Ospedaliero-Universitaria Pisana- University of Pisa, Department of Translational Research and New Technologies in Medicine and Surgery, Pisa, Italy
[2] Fondazione IRCCS- Istituto Nazionale Tumori, Medical Oncology, Milan, Italy
[3] San Luigi Hospital, Oncologia Medica, Orbassano- Turin, Italy
[4] Ospedale Infermi, Oncologia Medica, Rimini, Italy
[5] Ospedale Mauriziano- University of Turin, Oncologia Medica, Turin, Italy
[6] Università di Pisa, Dipartimento di Patologia Chirurgica- Medica e Molecolare e dell'Area Critica, Pisa, Italy

BACKGROUND: Almost half of RAS and BRAF wt mCRC patients do not achieve response to anti-EGFRs. Different molecular alterations have been suggested as predictors of resistance but their impact has not been validated.

MATERIAL AND METHOD: We conducted a case-control study to prospectively demonstrate the negative predictive impact of HER-2 amplification or mutations, MET amplification, NTRK/ROS1/ALK/RET rearrangements, and mutations activating MAPKs or PI3K/Akt axis. Patients with RAS and BRAF wt mCRC resistant (cases) or sensitive (controls) to anti-EGFRs were included. HER-2 status was evaluated by immunohistochemistry (IHC) and silver in-situ hybridization (SISH); MET amplification by SISH; gene rearrangements were screened by IHC followed by RNA-based NGS confirmation. Other candidate mutations were investigated by NGS (Hotspot Cancer Panel v2, Life Technologies).Hypothesizing a prevalence of candidate alterations of 0% and 15% among controls and cases, 47 cases and 47 controls were needed to be able to reject the null hypothesis of equally prevalent alterations, with a-error 0.05 and b-error 0.20. Since hypermutated tumors may hardly rely on a single pathway for their growth, the impact of microsatellite instability was also evaluated.

RESULTS: 47 cases and 47 controls were included. Primary endpoint was met: mentioned alterations were reported in 19 (40.4%) cases and 1 (2.1%) control ($p<0.001$). MSI-high was significantly more frequent among resistant than sensitive patients (15% vs 0%, $p<0.001$).

Molecular alteration	Cases (Resistant patients) N=47	Controls (Sensitive patients) N=47
HER-2 amplif	7*	0
HER-2 mut	1 (G776V, exon 20)	0
MET amplif	5*	0

Molecular alteration	Cases (Resistant patients) N=47	Controls (Sensitive patients) N=47
NTRK rearrang	2 (SCYL3-NTRK1 and TPM3-NTRK1)	0
ALK rearrang	0	0
ROS1 rearrang	0	0
RET rearrang	1 (CCDC6-RET)	0
PIK3CA mut (exon 20)	1 (A1035V, exon 20)	1 (H1047R, exon 20)
PTEN mut	3 (L247S, R233stop and del P248, exon 7)	0
Total n. of patients with candidate alterations	19	1
Microsatellite instability (MSI-high)	6	0
RAS mut at low allele fraction **	3 (KRAS G12V, exon 2, 6%; NRAS Q61R, exon 3, 10%; KRAS Q61H, exon 3, 8%)	0
New RAS mut	3 (2 KRAS L19F, exon 2; KRAS T50I, exon 3)	0
* in 1 case HER-2 and MET amplification co-occurred ** previously defined as wt by pyrosequencing		

CONCLUSIONS: This is the first prospective demonstration that the combined assessment of these rare alterations allows to better select patients for anti-EGFRs, while opening the way to new tailored therapies.

NO CONFLICT OF INTEREST

486 Developing an NIR heatable EDGS films to simultaneously detect, quantity and remove hydrogen peroxide in solution with index by fluorescent intensity

T.W. Chung[1], C.Y. Chang[1], L.T. Chen[1]

[1] National Yang-Ming University, Biomedical Engineering, Taipei, Taiwan

INTRODUCTION: Developing a biocompatible and heatable H_2O_2 theranostics system, which can simultaneously detect, quantify, and remove hydrogen peroxide (H_2O_2) in solutions indexed by an indicator, is an important for various diseases treatments including for cancer treatments. Therefore, a multicomponent biocompatible EDGS film consisted of graphene oxide (G), horseradish peroxidase (E), poly-dopamine (D) and silk fibroin (S) was designed to achieve the objective.

MATERIALS AND METHODS: Multicomponent micro-particles or clusters (EDG MPs) which contain G adhered by E and D were first fabricated. The MPs were then blended with S to prepare EDGS films. The films were treated by ethanol and then dried at room temperature for examining/removing various concentrations of H_2O_2 in solution. The optimally working temperature of the measuring system could be adjusted by NIR irradiation the films at 808 nm.

RESULTS: Graphene oxide was successfully fabricated, and characterized by Raman, FTIR and UV-VIS absorption spectra as other report [1]. The images and sizes of EDG MP were characterized by TEM, AFM and light scattering size analyzer. Accordingly, the size of EDG MPs was about 1.6 μm. The EDG MPs were consisted of 0.24 and 0.38 mg of E and D per mg of G, respectively. The EDGS films were further characterized by FTIR spectra to analyze their compositions. Since HRP (E) of a EDGS film can catalyze the reactions of H_2O_2 and tyrosine of S to produce di-tyrosine compounds in the film that emits 400 nm fluorescence, the intensity of fluorescence can be used to quantify the concentration of H_2O_2 in buffer solution. Moreover, EDGS films could be irradiated by NIR to increase 10 ^{0}C of solution within 5 minutes, providing an optimal temperature to support high biochemical reactivity of E in EDGS films. The result showed that the linear detection ranges of H_2O_2 concentrations by EDGS films in the buffer solution were from 5 to 100 μM with associated fluorescent intensity from 0 to 2.8 x10^4 (an arbitrary unit), respectively, at 37 ^{0}C. The removing capacity of H_2O_2 was 10 μmole H_2O_2/gram of an EDGS film.

CONCLUSION: A NIR heatable H_2O_2 theranostics EDGS film was successfully fabricated, which can display the removal of H_2O_2 concentrations in buffer solutions by emitting various intensity of 400 nm fluorescence. The EDGS films can be potentially employed for cancer diseases treatment.

REFERENCE: Carbon 2009; 47(1):68-72.

NO CONFLICT OF INTEREST

487 Universal consent for bio-specimens: A novel electronic/video consent

A. Naeim[1], N. Wenger[1], A. Petruse[1], L. Sanchez[1], A. Sharif[2], S. Dry[1]

[1] UCLA, David Geffen UCLA School of Medicine, Los Angeles, USA
[2] DSI- Precision Medicine Biobanking, Precision Medicine Biobanking, Cambridge, USA

BACKGROUND: Developing innovative, efficient and institutionally scalable bio-specimen consent for remnant tissue that meets the NIH consent guidelines for genomic and molecular analysis is essential for precision medicine efforts in cancer. Solutions in this arena need to satisfy the needs of patients, researchers, ethicists, IRB and compliance leadership, while fitting as seamlessly as possible into existing clinical workflows.

METHODS: UCLA developed a video-application kiosk-based approach for providing universal consent to repurpose clinical remnant bio-specimen for research. The process was designed to be self-service, comprehensive yet fast (mean shorter than 5 minute for completion). The consent additionally asked the patient if they were willing to be contacted directly for future research projects. This approach was piloted with 474 patients who were coming in for routine services in laboratory medicine, radiology, oncology, and hospital admissions. Of the pilot population, 175 individuals had targeted surveys to evaluate drivers for opting-in or opting-out of the consent for allowing the collection and use of their remnant tissue for research. The cognitive survey was online and presented immediately after the consent process was completed.

RESULTS: The opt-in rate for the pilot was 90.7%, and 56% agreed to direct contact for future research. Only 7% needed help navigating the online consent process. Of the subgroup of pilot population who completed the targeted survey, there was no difference between individuals who opted in and out regarding ease of use, of the consent application with about 75% stating it provided mostly or very useful information, 90% stating it was mostly or very easy to understand, and 85% stating they trusted the information. However, there were significant differences between those that opted-in and opted-out in their beliefs concerning usefulness of tissue, trusting researchers, importance of contributing to science and privacy risk with those opting in strongly supporting these beliefs (>90%) compared to those that opted out (<40%), p<0.001.

CONCLUSIONS: Video-application approach for allowing individuals to consent for remnant specimens to be collected and used for research, including cancer research, can be efficient, patient-centric and meet the NIH requirements. This method could increase the availability of blood and tissue for cancer research and should be tested for scalability as an enterprise solution.

NO CONFLICT OF INTEREST

488 miRNA targets of CXCR4 in synovial sarcoma

L. Pazzaglia[1], S. Pollino[1], M. Vitale[1], A. Conti[1], P. Picci[1], M.S. Benassi[1]

[1] Istituto Ortopedico Rizzoli, Laboratory of Experimental Oncology, Bologna, Italy

INTRODUCTION: Synovial sarcoma (SS) is a rare soft tissue tumor, containing a characteristic translocation (X;18)(p11;q11). The 5-year survival rate of SS is about 60%, while the 10-year survival rate is about 20%. The primary treatment for SS is surgery with adjuvant or neoadjuvant radiotherapy. In advanced or metastatic disease chemotherapy (Doxorubicin and/or Ifosfamide) might be recommended. However, considering the side effects and the lack of sensitivity to chemotherapy, new cytotoxic agents and novel therapeutic strategies are needed. Chemokine receptor 4 (CXCR4) is a seven-transmembrane G protein-coupled chemokine receptor. This molecule is most commonly expressed in tumour cells and it is involved in cell migration, invasion, as well as in angiogenesis. Dysregulation of miRNAs is strongly associated with a malignant phenotype and several data showed a strong association between microRNA expression and Soft Tissue Sarcoma prognosis.

MATERIAL AND METHOD: We identified, by in silico analysis, two miRNA regulators of CXCR4, miR-133b and miR-494. The miRNA expression was analyzed by Real Time PCR in 42 SS primary samples and 20 tissues from non oncologic patients. Then, in vitro studies on SW982 cell line with miRNA precursors were performed.

RESULTS: A significant lower expression of miR-133b and miR-494 ($p < 0.0005$) when compared to non tumor tissue was found. In vitro studies with miR-133b precursor have been shown a CXCR4 downregulation and a decrease of cell proliferation.

CONCLUSION: Our preliminary data confirmed a miR-133b and miR-494 downregulation suggesting a potential role in SS. Correlation with clinical data, with CXCR4 expression and in vitro studies in several SS cell lines are on-going.

NO CONFLICT OF INTEREST

489 Development of an Immuno-PCR assay combining broad assay range and excellent sensitivity to support development of a immuno modulator antibody drug

M. Spengler[1], C. Pieper[1], M. Adler[2], D.H. Lee[3], S. Shi[3]

[1] Chimera Biotec, Project Management, Dortmund, Germany
[2] Chimera Biotec, Assay Development, Dortmund, Germany
[3] Merck Research Labs, Bioanalytics, Kenilworth, USA

BACKGROUND: Biologics such as therapeutic antibody drugs are often administered in wide concentration ranges for dose-response evaluation. Safety and potency considerations frequently demand low dosing in clinical trials and therefore very sensitive ligand-binding assays (LBA) for PK analyses. In contrast, significantly higher dosing has to be applied in pre-clinical safety assessment for fast cleared drugs needing high upper limits of quantification. Here we describe an ultra sensitive Imperacer® assay combining a broad assay range of greater than 4 logs with excellent sensitivity in the low pg/ml range, to support development of a TNF-receptor agonist antibody drug.

MATERIAL AND METHODS: Immuno-PCR (IPCR) based Imperacer® LBA technology in sandwich-format, using surface immobilized capture reagent in combination with marker-DNA tagged detection conjugate for qPCR signal generation.

RESULTS: As part of a technology evaluation study, an Imperacer assay to quantify a therapeutic antibody for cancer treatment was developed with a preliminary assay range of 2 – 32,768 pg/ml and an LLOQ at 6 pg/ml with good accuracy and precision. Selectivity testing at 6 - 10 pg/ml was tested in 10 human serum samples from healthy volunteers to define LLOQ level. Prozone (hook) effect was found starting around 1 µg/ml, however dilution linearity confirmed that concentrations up to 10 µg/ml can be diluted into the assay range with acceptable recovery. This assay as developed for a feasibility approach has the potential to significantly reduce time and effort in LBA PK sample testing support for this Biologic, as the sponsor uses two different platforms to measure the range of concentrations in serum. The assay is not yet fully optimized and can be adapted towards further study requirements prior to method validation.

CONCLUSIONS: Exponential signal amplification driven combination of excellent sensitivity and broad assay range on the Imperacer platform can significantly reduce bioanalytical sample testing time and effort during all phases in the development of Biologics.

NO CONFLICT OF INTEREST

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490 Phase I clinical trial of high purity and activity NK cells therapy in combination with IgG1 antibody in patients with gastric or colorectal cancer

T. Ishikawa[1], *T. Okayama*[1], *N. Sakamoto*[1], *M. Ideno*[2], *K. Oka*[1], *F. Sakai*[2], *T. Enoki*[2], *J. Mineno*[2], *Y. Naito*[1], *Y. Itoh*[1], *T. Yoshikawa*[3]

[1] *Kyoto Prefectural University of Medicine, Gatroenterology and Hepatology, Kyoto, Japan*
[2] *Takara Bio Inc., CDM center, Kusatsu, Japan*
[3] *Kyoto Prefectural University of Medicine, Cancer ImmunoCell Regulation, Kyoto, Japan*

BACKGROUND: Natural killer (NK) cells exhibit strong cytotoxic activity against tumor cells without prior sensitization, and produce numerous cytokines resulting in the subsequent activation of the adoptive immune systems. Although NK cell is considered ideal cell for adoptive cancer immunotherapy, it is difficult to obtain large numbers of fully functional NK cells. We successfully generated large numbers of activated NK cells from small quantities of blood and also determined that the expanded cells were safe to administer in a monotherapy (J Transl Med (2015) 13:277). In this study, we are conducting clinical trials combining them with IgG1 antibodies.

PATIENTS AND METHODS: Patients with unresectable gastric cencer (GC) or colorectal cancer (CRC) who have administered IgG1 antibody (i.e. trastuzumab for HER2 positive GC or cetuximab for RAS-wild CRC). NK cells were expanded from PBMCs with the same method in previous clinical trial, briefly ex vivo by stimulating PBMCs with OK432, IL-2, and modified FN-CH296-induced T cells. After two days of administering IgG1 antibody, patients were administered autologous these NK cells three times at triweekly intervals in a dose-escalating manner (dose 0.5 × 10(9), 1.0 × 10(9), 2.0 × 10(9) cells/injection, three patients/one cohort). We evaluated the safety and efficacy of the combination therapy. To assess the immunological response, immunomonitoring including whole blood cytokine assay (Int. J. Cancer (2013):133) was performed in all patients.

RESULTS: At the present time (Jan.,2017), 8 patients have completed the study treatment. Of those patients, total NK cell population had a median expansion of 409-fold (range 247-656), with a significantly purity, median 90.3% (range 66.9 - 99.7%). This combination therapy was very well tolerated with no severe adverse events. Among six evaluable patients, 1 presented PR and 2 presented SD. In immunomonitoring analysis, whole blood IFN-gamma production level was increased and the proportion of Treg (Treg/CD4) in peripheral blood was decreased after the combination therapy.

CONCLUSIONS: Adoptive NK cell therapy is expected to enhance the effect of IgG1 antibodies in cancer treatment. In this study, the NK therapy-related severe adverse events did not occur so far. According to the result of immunomonitoring, tumor immunity is expected to be improved by the combination therapy. We are now considering a Phase2 trial to assess the effect of the combination therapy.

CONFLICT OF INTEREST

Other Substantive Relationships: Takeshi Ishikawa, Tetsuya Okayama, and Toshikazu Yoshikawa are affiliated with a department funded by donations from TAKARA BIO Inc.

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491 Bioanalytical PK support for immunotherapeutics: The need for sensitivity combined with broad assay range – case studies

M. Spengler[1]

[1] *Chimera Biotec, Project Management, Dortmund, Germany*

BACKGROUND: Immunotherapeutic concepts play an increasingly important role in modern drug development. The most common class of immune-modulatory drug candidates are antibodies or recombinant constructs thereof, which can pose mayor difficulties in dosing. The therapeutic dosing regime of immunotherapeutics may vary significantly from fairly low to rather high dosing, depending on safety considerations, binding targets, potency, clearance rate, physiological effects and other considerations. Hence, for optimal trial support, not merely sensitivity but likewise expansive assay range of the supporting method is key. However, most ligand-binding assay (LBA) technologies either lack sufficient sensitivity or have considerable restrains in continuous dynamic range.Here we present state-of-the-art bioanalytical approaches to overcome these limitations in GxP regulated study support, discussing several case studies from the development of immunotherapeutic compounds. Related aspects, e.g. LBA support of studies with limited sample volume availability, as e.g. typical in Ophthalmology are discussed as well.

MATERIAL AND METHODS: Imperacer LBA technology in sandwich-format, using surface immobilized capture reagent in combination with marker-DNA tagged detection conjugate for exponential qPCR signal amplification and read out.

RESULTS: 1) Immunomodulator Agonist Antibody Drug: An LBA assay, using two anti-ideotypic antibodies was developed for quantification of an immunomodulatory antibody drug candidate in human serum samples for phase I oncology trial support.

2) Bi-Specific Antibody Drug: Using the recombinant binding target of one arm as capture and an anti-idiotypic antibody as detector, this specifically developed immunoassay supports the development of a bi-specific antibody for oncology therapy with 1 pg/ml – 6 ng/ml assay range.

3) Diabody Drug: For clinical PK sample testing support of a bi-specific diabody under development as oncology therapy, an ultra sensitive assay ranging from 100 fg/ml – 800 pg/ml was developed.

CONCLUSIONS: Due to exponential signal amplification driven combination of sensitivity, broad assay rage and tolerance for sample dilution, the Imperacer IPCR platform is well suited for LBA sample testing support of biologics.

NO CONFLICT OF INTEREST

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492 Bioanalytical method for ultra sensitive quantification of interleukin-6 to support clinical phase III trial

M. Spengler[1], *C. Pieper*[1], *M. Adler*[2]

[1] *Chimera Biotec GmbH, Project Management, Dortmund, Germany*
[2] *Chimera Biotec GmbH, Assay Deuelopment, Dortmund, Germany*

BACKGROUND: Pharmacodynamic (PD) markers have great impact on the development of Biotherapeutics for treatment of various diseases. While cytokines are highly important PD markers, patient levels are often below sensitivity limits of standard ligand-binding assay (LBA) technologies. More so, pre-clinical PD biomarker assessment, in addition to sensitivity, often demands for minimal sample volume requirement (>5-10 µl). Exemplarily, Interleukin-6 (IL-6) is a multifunctional cytokine that plays a major role in the regulation of immune response. IL-6 therefore is an important PD marker in a variety of therapeutic areas, including cancer and autoimmune indications.

MATERIAL AND METHODS: Imperacer assay development and bioanalytical method validation (BMV) in support of clinical phase-II and phase-III trials. Adaptation for use of the assay in microsampling study support is demonstrated and discussed in comparison to an ultrasensitive chemiluminescence reference assay.

RESULTS: The full-validated Imperacer method for clinical phase-II/III bioanalysis fulfills all BMV guidelines for biomarker LBA methods, with an assay range of 0.3 – 5000 pg/ml (LLOQ: 0.3 pg/ml). The method allows duplicate testing with sub-pg/ml sensitivity from 35 µl sample. In contrast, an exploratory IL-6 chemiluminescence assay (Chimera Biotec), with an assay range of 0.5 – 1500 pg/ml, has approx. 80% higher sample requirement of 200 µl for duplicate runs. Extreme sensitivity of the Imperacer IL-6 method in combination with +4 log assay range leads to excellent performance in clinical study support of 9000+ samples. Imperacer exponential signal amplification allows further reduction of sample requirement by appropriate sample dilution. An exploratory IL-6 Imperacer assay with 1µl sample requirement per duplicate run was developed. A separate IL-2 Imperacer microsampling assay allows parallel bioanalysis of IL-2 and IL-6 from less than 4 µl sample for two separate duplicate runs at approx. 3 pg/ml sensitivity.

CONCLUSIONS: The clinical IL-6 Imperacer method combines sub-pg/ml bioanalysis and low sample requirement. Exponential signal amplification on the Imperacer platform allows further reduction of sample volume to a few microliter, still maintaining single digit pg/ml sensitivity for study support wherever sample volume is critical.

NO CONFLICT OF INTEREST

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493 Xenospheres: A comprehensive patient-derived in vitro model to study response and resistance to targeted therapies in metastatic colorectal cancer

P. Luraghi[1], *V. Bigatto*[1], *G. Reato*[1], *E. Cipriano*[1], *F. Orzan*[1], *F. Sassi*[1], *A. Bertotti*[1], *L. Trusolino*[1], *P.M. Comoglio*[1], *C. Boccaccio*[1]

[1] *Candiolo Cancer Institute - FPO IRCCS- Universityof Turin, Experimental Clinical Molecular Oncology, Candiolo TO, Italy*

INTRODUCTION: From patient-derived xenografts ("xenopatients") of colorectal cancer liver metastases, we generated primary sphere cultures ("xenospheres"), which were previously shown to display properties of cancer stem-like cells in vitro,

and to form biological and pharmacological phenocopies of original patient tumors in vivo ("spheropatients"). A panel of more than 40 different xenospheres were genetically annotated for mutations affecting the RAS pathway, and characterized for response to anti-EGFR targeted therapy to identify mechanisms of response and resistance, and to set-up alternative therapeutic strategies.

MATERIAL & METHODS: An extensive exome-sequencing analysis was performed on 10 xenospheres for which data were already available on the corresponding xenopatients, thus allowing a complete and robust comparison. In xenospheres, the ability of major mitogenic growth factors to induce in vitro proliferation and resistance to anti-EGFR therapy (cetuximab) was assessed. The ability of the EGF-family ligands in sustaining cetuximab resistance was analyzed in vitro and in vivo.

RESULTS: Exome sequencing showed that xenospheres faithfully retain the genetic make-up of their matched xenopatients, and maintain it through multiple passages. Xenospheres display also gene expression profiles very similar to their matched xenopatients.

While xenospheres harboring mutations in genes of the RAS pathway displayed self-sustained proliferative ability and insensitivity to cetuximab, RAS^{wt} xenospheres were strongly growth factor-dependent and sensitive to cetuximab. By stimulating RAS^{wt} xenospheres with EGF family ligands, we found that neuregulin (NRG1) displayed the most potent mitogenic activity and the ability to fully protect against cetuximab treatment. Coherently RAS^{wt} xenospheres overexpressing NRG1 displayed sensitivity to lapatinib (a dual EGFR/ERBB2 small molecule inhibitor) but not cetuximab. By injecting parental and genetically-modified RAS^{wt} xenospheres in mice, we found that NRG1 strongly increased the tumorigenic potential and that tumors were resistant to cetuximab but sensitive to lapatinib.

CONCLUSIONS: Xenospheres are a robust patient-derived in vitro model, amenable to study molecular mechanisms of response and resistance to targeted therapies. We showed that NRG1 bypasses the requirement for EGF in colorectal cancer stem-like cells and that concomitant inhibition of multiple EGFR family members can be advantageous in the clinical setting.

NO CONFLICT OF INTEREST

494 Chimeric Antigen Receptor (CAR) T-cell immunotherapy for MUC1-positive breast cancer

A. Gavriil[1], D. Achkova[1], L. Whilding[1], R. Zhou[2], M. Yazdanifar[3], S. Papa[1], J. Taylor-Papadimitriou[3], J.M. Burchell[3], P. Mukherjee[2], J. Maher[1]

[1] King's College London, Division of Cancer Studies at Bermondsey, London, United Kingdom
[2] University of North Carolina at Charlotte, Department of Biological Sciences, Charlotte- North Carolina, USA
[3] King's College London, Division of Cancer Studies at Guy's Cancer Centre, London, United Kingdom

BACKGROUND: Cancer immunotherapy using chimeric antigen receptor (CAR) T-cells has shown exceptional promise in the treatment of patients with refractory B-cell malignancy. In this approach, patient-derived peripheral blood T-cells are engineered to express a cell surface receptor, which confers specificity for a tumour-associated (TA) antigen. An important drawback of this approach is potential for on-target off-tumour toxicity, since most targets are expressed by some normal tissues. Mucin-1 (MUC1) is a large transmembrane glycoprotein that is overexpressed and aberrantly glycosylated in 90% of breast cancers. Antibodies such as TAB004 and HMFG2 are considered to bind preferentially to TA-MUC1.

Aim: The aim of this project is the development of a CAR T-cell approach for MUC1-positive breast cancer. Herein a new 2nd generation MUC1 CAR is tested, named TAB28z, derived from the TAB004 scFv. TAB28z has been compared with two other previously developed MUC1-specific CARs, H28z and HDF28z, both of which are derived from the HMFG2 antibody. In addition, the expression of TA-MUC1 on human breast cancer cell lines and PBMCs has been investigated.

METHODS: The anti-tumour potency of the three signalling-intact MUC1 CARs was investigated in vitro by performing co-cultivation experiments of CAR T-cells with human breast cancer cell lines expressing various levels of MUC1. Additionally, a cytokine release assay was performed to quantify the production of IFN-γ. In parallel, the surface expression of TA-MUC1 was investigated on activated T-cells by flow-cytometry.

RESULTS: TAB28z CAR T-cells caused significant elimination of MUC1+ve tumour cells, accompanied by release of IFN-γ. There was no significant difference in anti-tumour activity between T-cells that expressed TAB28z, H28z or HDF28z. TAB28z CAR T-cells showed minimal killing of normal breast and other epithelial cells. However, expansion of these CAR T-cells was compromised by the fact that TA-MUC1 was also expressed on activated human T-cells, leading to fratricide and T-cell enrichment.

CONCLUSIONS: There is clear evidence in vitro that the TAB28z CAR T-cells can successfully target MUC1+ve breast cancer cells in an antigen specific manner. Future experiments are planned to explore this activity in breast cancer animal models and investigate if TAB28z will outperform H28z and HDF28z CAR T-cells. We are currently exploring strategies to mask MUC1 expression on activated T-cells during CAR T-cell expansion.

CONFLICT OF INTEREST

Ownership: OncoTAb Inc. (this owns the license to TAB004 antibody and its uses thereof). Advisory Board: CSO of OncoTAb Inc. Board of Directors: OncoTAb Inc.

Other Substantive Relationships: Leudic Bio. (Dr John Maher is the chief scientific officer of Leucid Bio)

495 Targeting Chronic Myeloid Leukemia Stem Cells with the ERK5 pathway inhibitors

I. Tusa[1], G. Chelon[3], N.S. Gray[2], A. Gozzini[3], P. Dello Sbarba[1], E. Rovida[1]

[1] University of Firenze, Department of Experimental and Clinical Biomedical Sciences, Firenze, Italy
[2] Dana Farber Cancer Institute, Harvard Medical School, Boston, USA
[3] AOU Careggi, Hematology Unit, Florence, Italy

BACKGROUND: Tyrosine kinase inhibitors (TKi) targeting BCR/ABL are very effective for the treatment of Chronic Myeloid Leukaemia (CML). However, resistance mechanisms or their inefficacy on CML leukaemia stem cells (LSC) may lead to relapse. Therefore, there is urgent need to identify new molecular targets. The Extracellular signal-Regulated Kinase 5 (ERK5) is a Mitogen-Activated Protein Kinase involved in cancer. Our aim was to study a possible role of the ERK5 pathway in CML LSC.

MATERIAL AND METHODS: Cells used were: human CML cell lines K562 and KCL22 that express constitutively active ERK5; CML patient-derived cells and CD34+ cells from heathy donors (after informed consent had been obtained). Cells were incubated in normoxic (routine) or hypoxic (0.2% O_2) primary cultures (LC1) in the presence or the absence of drugs. Day-7 LC1 cells were transferred to drug-free, non-selective normoxic secondary cultures (LC2), to measure LC2 repopulation as a read-out of progenitor/stem cell potential (CRA assay). ERK5 pathway inhibition was achieved using ERK5 and MEK5 inhibitors. BCR/ABL tyrosine kinase inhibition was obtained using Imatinib (IM) or Dasatinib (DASA).

RESULTS: We previously showed that stem cell potential of CML LSC is maintained in severe hypoxia. In K562 and KCL22 cells and in primary cells derived from 10 CML patients, the treatment in hypoxic LC1 with ERK5 or MEK5 inhibitors, but not with IM or DASA, impaired progenitor/stem cell potential. The same result were obtained by combined treatment of ERK5 inhibitor with IM. Importantly, pharmacological inhibition of ERK5 did not affect progenitor/stem cell potential of CD34+ cells from healthy donors. In colony formation ability assays ERK5 inhibition decreased colony formation by human primary CML cells to a higher extent than that by normal human CD34+ hematopoietic cells. Interestingly, in hypoxia, combined treatment ERK5 inhibitor/IM decreased the expression of genes relevant for stem cell maintenance such as p21, nanog and c-myc. Moreover, either ERK5 or MEK5 inhibitor in combination with IM decreased the expression of CD26, a CML LSC marker.

CONCLUSIONS: MEK5-ERK5 pathway inhibitors impaired LSC maintenance of CML cell lines and primary CML cells while combined treatment ERK5 pathway inhibitors and IM markedly reduced CML cell growth in hypoxia. The existence of several ERK5 inhibitors, open the possibility for a rapid translation to the clinic.

NO CONFLICT OF INTEREST

496 Phase I/II CANON study: Oncolytic immunotherapy for Non-Muscle Invasive Bladder Cancer (NMIBC) using Intravesical Coxsackievirus A21

N. Annels[1], D. Mansfield[2], G. Simpson[1], S. Sandhu[3], A. Melcher[2], K. Harrington[2], H. Mostafid[1], D. Shafren[4], H. Pandha[1]

[1] University of Surrey, Clinical and Experimental Medicine, GUildford, United Kingdom
[2] Institute of Cancer Research, Targeted Therapy Team, London, United Kingdom
[3] Kingston Hopsital, Urology, Kingston, United Kingdom
[4] Viralytics Ltd, Viralytics Ltd, Sydney, Australia

INTRODUCTION: Local live biological (bacille calmette Guerin, BCG) therapy is already well established in the treatment of non-muscle invasive bladder cancer (NMIBC). Intravesical instillation of oncolytic virotherapy may provide similar therapeutic opportunities. Coxsackievirus A21 (CVA21, CAVATAK™) is a novel intercellular adhesion molecule-1 (ICAM-1)-targeted immunotherapeutic virus which exerts potent oncolytic activity against NMIBC cell lines and ex-vivo human bladder tumour. CVA21 in combination with low doses of Mitomycin C enhances CVA21 viral replication and oncolysis by increasing surface expression levels of ICAM-1.

MATERIALS AND METHODS: A Phase I/II trial (CANON) studying the tolerance and biological effects of escalating intravesicular (IV) doses of CVA21 administered alone or in combination with MitomycinC (10mg) in 16 first-line NMIBC cancer patients prior to TURBT surgery was completed. Cystoscopy photography was performed before and after treatment. Tissues were analysed for CVA21 replication, apoptosis, changes in immune cell infiltrates (multi-spectral imaging) and immune-checkpoint molecules.

RESULTS: Intravesical CAVATAK was well tolerated with no adverse events. Anti-tumour activity was demonstrated by direct visualisation including a complete response observed in one of 3 patients receiving monotherapy. In every patient, tumour targeting by CVA21 was shown by detection of secondary viral load peaks in the urine and by immunohistochemical analysis of TURBT tissue displaying tumour-specific viral replication. Nanostring analysis revealed an upregulation of interferon-response and immune checkpoint inhibitory genes in CVA21-treated tissues compared to untreated historical controls. Increase in immune cell infiltrates and expression of PD-L1 within the CVA21-treated NMIBC tissue were also observed. Increased urinary levels of the chemokine, HMGB1, was observed in six of eleven patients following exposure to CVA21.

CONCLUSIONS: The potential utility of CVA21 as a potent immunotherapeutic agent was demonstrated by the observed tumour targeting and viral replication in

all patients. Upregulation of checkpoint molecules following CVA21 exposure may also allow potential sequential combination therapies with checkpoint targeting.

NO CONFLICT OF INTEREST

POSTER SESSION: TRANSLATIONAL RESEARCH II

497 Gastro-esophageal Patient-Derived Xenografts (PDXs) as a tool to improve treatment of HER2+ tumors and identify predictors of resistance

M. Apicella[1], C. Migliore[1], S. Menegon[1], M. Cargnelutti[1], T. Capeloa[1], A. Sapino[2], E. Pectasides[3], A.J. Bass[4], S. Corso[1], S. Giordano[1]

[1] Candiolo Cancer Institute-FPO-IRCCs- University of Turin, Department of Oncology, Candiolo, Italy
[2] Candiolo Cancer Institute-FPO-IRCCs- University of Turin, Department of Medical Science, Candiolo, Italy
[3] Division of Molecular and Cellular Oncology- Dana-Farber Cancer Institute- Harvard Medical School-, Division of Molecular and Cellular Oncology, Boston, USA
[4] Division of Molecular and Cellular Oncology- Dana-Farber Cancer Institute- Harvard Medical School-Associate Member- Broad Institute, Division of Molecular and Cellular Oncology-, Boston, USA

BACKGROUND: Gastric cancer is the third leading cause of cancer mortality worldwide. Surgery is the only curative treatment strategy and conventional chemotherapy has shown limited efficacy. Trastuzumab (a HER2 targeting mAb), is the only therapy targeting molecular alterations approved so far in gastric cancer, for HER2+ patients with advanced disease. However only a fraction (<20%) of HER2 amplified patients benefits from treatment.

MATERIAL AND METHODS: We have recently generated a molecularly annotated colony of gastro-esophageal PDXs (>90 PDXs). The platform also comprises PDX-derived primary cell lines and 3D-coltured organoids. At present, we have identified 6 HER2+ PDXs (> 8 gene copies) and generated the corresponding in vitro derivatives.

RESULTS: Five HER2+ PDXs, showing 3+ HercepTest score and > 8 HER2 gene copies (thus theoretically sensitive to trastuzumab), were selected to undergo 'xenotrials' with different anti-HER2 drugs/combos: Trastuzumab; Pertuzumab (anti-HER2 mAb disrupting HER2 heterodimers with other HER family members); Lapatinib (a dual HER2-EGFR tyrosine kinase inhibitor TKI). According to RECIST-like criteria, Trastuzumab induced tumor regression only in 1 out of 5 HER2+ PDXs, whereas in the other PDXs we observed either a disease stabilization or no response. Interestingly, in 3 out of five tumors the combos Trastuzumab+Pertuzumab and Trastuzumab+Lapatinib were able to bypass resistance to Trastuzumab monotherapy and to induce tumor regression. Notably, the combo treatment resulted not only in increased but also in a durable response as in one PDX we did not observe tumor relapse in the absence of combined treatment for at least 40 days.

CONCLUSION: We identified six HER2 amplified PDXs. The response to anti-HER2 compounds in mice mirrors what happens in humans: only a minor fraction of them benefits from the anti HER2 monotherapy, despite the presence of high level HER2 gene amplification. We show that association of either Lapatinib or Pertuzumab to Trastuzumab not only strongly improves treatment efficacy but it also impairs tumor relapse. Through sequencing a panel of 250 gastric-specific genes we have recently identified genes possibly involved in resistance to anti-HER2 drugs that are now under investigation.

NO CONFLICT OF INTEREST

498 Novel targeted therapies for castrate-resistant prostate cancer; the potential of Serum Response Factor

M. Prencipe[1], A. O'Neill[1], G. O'Hurley[2], A. Fabre[3], W. Gallagher[4], C. Morrissey[5], E. Kay[6], W. Watson[1]

[1] Conway Institute of Biomolecular and Biomedical Research- University College Dublin- Belfield- D4 - Dublin- Ireland, UCD School of Medicine, Dublin, Ireland
[2] NovaUCD- Belfield Innovation Park- Belfield- D4 - Dublin- Ireland, OncoMark Lmt, Dublin, Ireland
[3] St Vincent's University Hospital- Dublin- Ireland, Department of Pathology, Dublin, Ireland
[4] Conway Institute of Biomolecular and Biomedical Research- University College Dublin- Belfield- D4 - Dublin- Ireland, UCD School of Biomolecular and Biomedical Science, Dublin, Ireland
[5] University of Washington- Seattle- Washington- USA, Department of Urology, Seattle, USA
[6] RCSI Education and Research Centre- Beaumont Hospital- Dublin- Ireland, Department of Pathology, Dublin, Ireland

BACKGROUND: Despite the emergence of next-generation anti-androgens, castrate-resistant prostate cancer (CRPC) remains challenging to treat with the androgen receptor (AR) still the main target and key player of resistance. Understanding the mechanisms of AR interaction with co-factors and co-regulators will identify new therapeutic approaches which are not susceptible to AR resistance mechanisms.

METHODS AND RESULTS: Using a combination of transcriptomics and bioinformatics, we identified Serum Response Factor (SRF) as a lead target in an in vitro model of CRPC. The relevance of SRF to patients was validated by immunohistochemistry (IHC). We showed that SRF is associated with CRPC using a TMA of castrate-resistant prostate resections and also with survival from time of diagnosis with CRPC. We also showed a negative feedback loop between AR and SRF in vitro which was confirmed by IHC showing a negative correlation between AR and SRF expression in CRPC metastases (n=42, Pearson correlation= -0.208, p=0.01) and a positive correlation in hormone-naïve prostatectomies (n=340, Pearson correlation=0.362, p<0.001).

Cell proliferation analysis following SRF inhibition via the small molecule inhibitor CCG-1423 showed that CCG-1423 was as effective as AR inhibition (Enzalutamide) in decreasing cell viability in the castrate-resistant cells. Combination of CCG-1423 and Enzalutamide was significantly more effective than monotherapies (viability decrease from 20% to 40%, p<0.01). We are currently validating these result in vivo using prostate patient derived xenografts.

To study the molecular mechanisms behind SRF/AR cross-talk, we performed a String search looking for functional partners in common to SRF and AR and identified Transgelin (TGLN) as a possible link. An actin-binding protein and tumour suppressor, TGLN is a transcriptional target of SRF and an inhibitor of AR. Preliminary data showed a significant increase in TGLN protein expression following SRF down-regulation with siRNA and a significant decrease in TGLN post SRF up-regulation. To identify additional SRF/AR interactors, we are currently performing mass spectrometry experiments following immune-precipitation of SRF and AR.

CONCLUSIONS: We have identified SRF as a transcription factor relevant to CRPC both in vitro and in vivo. Due to SRF cross-talk with AR, its inhibition in combination with current anti-androgens represents a promising therapeutic approach.

NO CONFLICT OF INTEREST

499 CXCL12 driven-circulating tumor cells diversion by a "metastases trap"

C. Ierano[1], L. Portella[2], C. D'Alterio[1], M. Napolitano[1], L. Mayol[2], S. Giarra[2], A. Luciano[3], A. Barbieri[3], C. Arra[3], R. Pacelli[4], G. DeRosa[2], S. Scala[1]

[1] National Cancer Institute Pascale, Functional Genomic, Naples, Italy
[2] University of Naples- Federico II, Department of Pharmacy, Naples, Italy
[3] National Cancer Institute Pascale, Animal Facility, Naples, Italy
[4] Federico II University School of Medicine- and Istituto di Biostrutture e Bioimmagini - C.N.R, Department of Advanced Biomedical Sciences, Naples, Italy

BACKGROUND: An intense cross talk between tumor and microenvironment favors the inefficient process of metastasis through the establishment of the so called 'pre-metastatic niche". The chemokine CXCL12 regulates the pre-metastatic niche recruiting CXCR4 positive cells from bone marrow and circulating tumor cells expressing CXCR4. Herein we evaluated the capability of a 'fake" pre-metastatic niche to distract and capture circulating tumor cells, inhibiting the formation of metastasis in a metastatic murine melanoma model.

MATERIALS AND METHODS: CXCL12 embedded hyaluronic acid (HA) hydrogel has been tested in vitro and in vivo for the capability to recruit and capture circulating tumor cells (CTCs). In vitro, the capability of human hematologic neoplastic cell (CEM) and murine B16 melanoma tumor cells transfected with human CXCR4-GFP (B16-hCXCR4-GFP) to migrate toward an hydrogel-CXCL12 embedded was evaluated by chemotaxis assay. In vivo, murine B16 melanoma tumor cells transfected with hCXCR4-GFP were intravenously injected in C57BL/6 mice five days after the subcutaneous injection of hydrogels. CXCL12-loaded versus empty hydrogels were subcutaneously injected in the flank of mice. CTCs (defined as cells GFP+/CXCR4+/CD45-) were assessed in the blood at day 10 and 21 by flow cytometry. The mice were sacrificed after 21 days and the presence of B16-hCXCR4-GFP in the lung and in the hydrogels was evaluated by flow cytometry, histological analysis and gene expression.

RESULTS: In vitro, CEM and B16-hCXCR4-GFP cells migrated toward CXCL12 loaded hydrogel as demonstrated by cell detection within the hydrogels and in the bottom of the transwell filter. B16-hCXCR4-GFP tumor cells were retrieved through flow cytometry in CXCL12 loaded hydrogels recovered from in vivo experiments. Moreover, flow cytometry and histological analysis of lungs derived from mice B16-CXCR4 injected indicated that the mice loaded with CXCL12-loaded hydrogel, but not empty hydrogel, reduced significantly the percentage of B16-hCXCR4-GFP positive tumor cells in the lung. The metastases number was reduced by 70% in mice receiving a CXCL12-loaded hydrogel versus mice undergoing an empty hydrogel ($N=5$, $P < 0.05$).

CONCLUSION: Our study suggests that a device made by a CXCL12 embedded hydrogel can divert B16-hCXCR4-GFP circulating cells reducing lung metastasis of CXCR4 positive cells in a metastatic murine melanoma model. It suggests that hydrogel-CXCL12 trap may be used as therapeutic /diagnostic tool to examine the biology of circulating tumor cells in conjunction with 'niche" cells enabling the development of patient-specific treatments.

NO CONFLICT OF INTEREST

500 Mutational analysis of BRCA1 and BRCA2 genes in circulating-free DNA in advanced stage epithelial ovarian cancer

L. Paracchini[1], I. Craparotta[2], R. Fruscio[3], V. Fotia[4], L. Beltrame[2], L. Mannarino[2], T. Grassi[3], G. Caratti[2], M. D'Incalci[2], S. Marchini[2]

[1] IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Oncology, Milano, Italy
[2] IRCCS "Mario Negri" Institute for Pharmacological Research, Oncology, Milan, Italy
[3] San Gerardo Hospital, Osbtetric and Gynecologyc Division, Monza, Italy
[4] "Papa Giovanni XXIII" Hospital, Oncology, Bergamo, Italy

INTRODUCTION: About 80% of epithelial ovarian cancer (EOC) cases are diagnosed in an advanced stage when the disease has spread to the abdominal cavity. Although the majority of the patients initially respond to first-line therapy, most of them relapse. The high tumor heterogeneity and the fact that the tumor

changes over time and the molecular characteristics of the synchronous lesions are different from those of the primary tumor hamper the development of reliable prognostic biomarkers and effective therapies. The analysis of circulating-free DNA (cfDNA) may offer the opportunity to easily detect in patients' plasma, somatic mutations related to therapy response and to follow the evolution of the disease.

MATERIALS AND METHODS: cfDNA was isolated from the plasma of three patients of whom blood samples and ovary tissue biopsies were available at time of diagnosis. To evaluate the integrity of cfDNA, the number of genome equivalent (GE) was calculated by PCR using LINE-1 primers. Matched gDNAs were purified from tumor biopsies and blood samples. BRCA1 and BRCA2 genes were sequenced using MiSeq (Illumina). Somatic variants were identified using two variant calling algorithms (supported by bcbio pipeline) on the basis of: i) minimum coverage of 2000x; ii) allelic fraction (AF) >1%; and iii) variants for which Poly-Phen-2 predicts a probable impact on the protein function.

RESULTS: The BRCA1 and BRCA2 genes were entirely sequenced in both solid and liquid biopsies. A GE >1000 per ml was set as the threshold for analysis of liquid biopsies. Unlike other tumors characterized by hematic dissemination, the number of GE/ml in EOC was low. In the first patient, we identified one germline variant in BRCA2 (AF 33.37, depth 22778); one somatic mutation in BRCA2 (AF 1.73, depth 5332) was detected in cfDNA, but not in the matched ovary or synchronous lesion. The second patient had two somatic mutations in BRCA2 in cfDNA (ch13_32929253-32929254,AF 1.12, depth 4908 and ch13_32944548-32944549, AF 1.83, depth 7224). No germline or somatic variants were detected in the third patient.

CONCLUSION: Our preliminary study indicates that liquid biopsy is feasible in EOC patients. It may recapitulate the systemic and heterogeneous nature of EOC as well as its evolution after treatment providing useful information, e.g. BRCA status, to select the best therapy.

NO CONFLICT OF INTEREST

501 Omics data integration approaches to investigate high grade serous ovarian cancer spatial heterogeneity

L. Mannarino[1], G. Caratti[2], I. Craparotta[3], L. Paracchini[2], M. D'Incalci[2], S. Marchini[2], M.G. Pezzotta[2], F. Villa[3], C. Romualdi[4], L. Beltrame[1]

[1] IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Oncology, Milano, Italy
[2] Alessandro Manzoni Hospital, Pathology, Lecco, Italy
[3] Alessandro Manzoni Hospital, Oncology, Lecco, Italy
[4] University of Padova, Biology, Padova, Italy

INTRODUCTION: High Grade Serous Epithelial Ovarian Cancer (HGS-EOC) is characterized by a massive spread of multiple synchronous implantation sites, which exhibit a high level of both inter- and intra-patient mutational heterogeneity. Through a cohort of 8 patients from which multiple biopsies were taken at both different sites of the ovary and neighboring tissues, with a mean of 4 biopsies for patient, we investigated spatial heterogeneity through the integration of mutational, gene expression and miRNA expression data.

MATERIALS AND METHODS: Exome sequencing data were processed through a readily available and tested pipeline, bcbio-nextgen (http://github.com/chapmanb/bcbio-nextgen) on a high performance cluster computing platform (Cloud4CARE project) as previously described (Beltrame et al., 2015). Array experiments for gene and miRNA expression were performed using standardized procedures (Marchini S et al., 2013). Raw data were processed and normalized as previously described (Calura E. et al., 2013). Inter- and intra-patient spatial heterogeneity was exploited through the construction of either a Jaccard distance matrix and a Pearson correlation matrix for genomic variants and expression data, respectively. Data comparability was assessed with the Mantel test. With the aim of identifying potential relationships between mutations and expression, mutated genes with an allelic fraction of at least 30% were combined with the sample distribution of gene expression data (unsupervised clustering). Considering the characteristics of the cohort, gene and mirna expression data were integrated through the evaluation of anticorrelated relationships and their identification on public databases. Finally, analyses were replicated in an independent dataset (TCGA).

RESULTS: Through our approach, we showed that HGS-EOC is characterized by a high level of spatial heterogeneity, present in both biopsies of the patient and within patients. This heterogeneity does not emerge at gene and miRNA expression levels, thus pushing for a further investigation of common features of synchronous lesions, which could become important for the evaluation of new therapeutic strategies.

CONCLUSIONS: Our result suggest that our multi-omics bioinformatics approach is suitable for the investigation of spatial heterogeneity on EOC samples.

NO CONFLICT OF INTEREST

502 Overexpression of MAP3K1 is closely associated with the prognosis of patients with hormone receptor-positive early breast cancer

S.H. Kuo[1,2,3], M.F. Wei[1,2], Y.S. Lee[4], Y.S. Tzeng[1], A.L. Cheng[1,2,3], C.S. Huang[5]

[1] National Taiwan University Hospital, Department of Oncology, Taipei, Taiwan
[2] National Taiwan University College of Medicine, Graduate Institute of Oncology, Taipei, Taiwan
[3] National Taiwan University College of Medicine, National Taiwan University Cancer Center, Taipei, Taiwan
[4] National Taiwan University Hospital, Department of Pathology, Taipei, Taiwan
[5] National Taiwan University Hospital, Department of Surgery, Taipei, Taiwan

BACKGROUND: We recently reported that single nucleotide polymorphism of MAP3K1 rs889312 (C/C) was significantly associated with poor disease-free survival (DFS) in hormone receptor (HR)-positive early breast cancers. MAP3K1 participates in the MAPK signal transduction pathway, responding to a number of mitogenic and metabolic stimuli, including estrogen, which may influence breast cancer susceptibility. In this study, we assessed whether overexpression of MAP3K1 may promote proliferation and migration of breast cancer cells, and thus affect the prognosis of HR-positive early breast cancer.

MATERIALS AND METHODS: A total of 154 patients who had HR-positive and HER2-negative breast cancer with T1 to T2 and negative or 1 to 3 nodal metastases were included. The expression of MAP3K1 protein was assessed by the immunohistochemistry. Furthermore, two HR-positive and HER2-negative breast cancer cell lines (MCF-7 and T47D) with overexpressing MAP3K1 were transfected with MAP3K1 short hairpin RNA (shRNA) plasmids to assess the proliferation effect and the migration of MAP3K1 and its mechanism.

RESULTS: At a median follow-up period of 9.3 years (95% CI, 9.05 to 9.55), 140 patients (90.9%) were alive, and 14 (9.1%) had died. Expression of MAP3K1 protein was found in 65 (42.2%) of 154 cases. There were no significant differences in age, sex, grade, status of estrogen receptor or progesterone receptor, positive lymph nodes, and chemotherapy between MAP3K1-positive and MAP3K1-negative groups, except MAP3K1-positive patients had greater tumor size. Patients with MAP3K1 overexpression had significantly poorer 10-year distant disease-free survival (68.2% vs. 90.2%, P = 0.002), DFS (63.3% vs. 87.7%, P = 0.004), and overall survival (79.9% vs. 94.9%, P = 0.032) than those without. Furthermore, we found that inhibition of MAP3K1 with MAP3K1 shRNA significantly reduced the cell growth rate and the migration of both breast cancer cell lines (MCF-7 and T47D). In addition, MAP3K1 shRNA treatment enhanced the cytotoxicities of doxorubicin, docetaxel, and tamoxifen in both breast cancer cell lines.

CONCLUSIONS: Our result indicate that overexpression of MAP3K1 is a strong predictor of poor prognosis in patients with HR-positive early-stage breast cancer. Additional investigation of the biologic significance of MAP3K1 in drugs resistance of HR-positive breast cancer is warranted.

NO CONFLICT OF INTEREST

503 Genomic and transcriptomic signature integration to investigate spatial heterogeneity in High Grade Serous Epithelial Ovarian Cancer

G. Caratti[1], L. Mannarino[1], I. Craparotta[2], L. Paracchini[1], M. D'Incalci[1], C. Romualdi[2], E. Bonoldi[3], S. Corso[4], L. Beltrame[1], S. Marchini[1]

[1] IRCCS Istituto di Ricerche Farmacologiche "Mario Negri" Milano Italy, Dept. of Oncology, Milan, Italy
[2] University of Padova, Dept.of Biology, Padova, Italy
[3] A. Manzoni Hospital, Division of Pathology, Lecco, Italy
[4] A. Manzoni Hospital, Dept. of Obstetrics and Gynecology, Lecco, Italy

BACKGROUND: At diagnosis, stage III/IV epithelial ovarian cancer (EOC) is characterized by multiple synchronous lesions spread in different anatomical sites. These are usually the main targets of the adjuvant chemotherapy and the cause of the re-growth of a resistant disease, since the primary lesion in the ovary is usually removed during debulking surgery. To study the clonal relationship among these different tumor lesions (i.e., spatial heterogeneity), in order to develop novel therapeutic approaches based on genomic information, whole exome sequencing data as well as miRNA and gene expression signatures were generated and integrated in an unique multidimensional level.

MATERIALS AND METHODS: 37 snap frozen tumor biopsies, collected during first surgery from ovary and synchronous lesion of eight patients affected by high grade serous (HGS) epithelial ovarian cancer (EOC), were collected at Manzoni Hospital (Lecco, Italy) and stored in Pandora tumor tissue collection. Exome sequencing run were performed at 200x coverage on NextSeq500 (Illumina) benchtop sequencer. Genes and miRNA expression signatures were generated with commercially available arrays (Agilent Technologies).

RESULTS: Exome sequencing data revealed high level of private mutations (close to 90%) among ovarian cancer biopsies and their own synchronous lesions. Most of selected mutations were with a allelic frequency ranging from 5 to 20%, with the exception of TP53 and BRCA1/2 genes. Unsupervised cluster analysis revealed that patients alive with no evidence of disease (NED) clustered differently from those alive with tumor (AWT). When we analyzed the total number of somatic mutations per megabase, biopsies from NED patients were characterised by lower numbers of mutations than those from AWT patients. Differently, genes and miRNA expression profile were homogeneous across tumor lesions of the same patient.

CONCLUSIONS: Omics data obtained in our cohort HGS-EOC patients suggest that:

1. Mutational profile of synchronous lesions is characterised by a marked spatial heterogeneity. It appear that a great genomic instability is associated to a worse prognosis.
2. Genes and miRNA expression profiles overlap among synchronous lesions of each patient. The role of epigenetic mechanism require further studies.

NO CONFLICT OF INTEREST

504 Whole exome sequencing of tumors samples from a Skin Cancer Biorepository: Fresh/frozen versus formalin-fixed paraffin embedded

C. Amato[1], *A. Applegate*[1], *J. Hintzche*[1], *W. Robinson*[1]

[1] *University of Colorado Denver AMC, Medical Oncology, Aurora, USA*

INTRODUCTION: Biorepositories and biobanking are an integral aspect of translational cancer research. Proper collection, storage, and maintenance of biological material is necessary in the era of next generation sequencing and big data analysis.

MATERIAL AND METHOD: Patients at the University of Colorado Cancer Center with metastatic melanoma were consented under institutional review board approved protocols. Nineteen patients had sections of their tumor collected and processed using two methods: fresh/frozen at -80 degrees and formalin fixed paraffin embedded (FFPE) samples. Genomic DNA was isolated from both methods of archived tumor. WES was performed with exome capture on an Illumina HiSeq sequencer and mutation analysis was performed using the IMPACT pipeline. Quality control analysis was performed at several steps between the fresh/frozen and FFPE specimens: sample histology, DNA quantity, WES library preparation, sequence coverage, and concordance/discordance of variant calls.

RESULTS AND DISCUSSION: Nucleic acid isolated from FFPE material was often fragmented, and required more concentrated starting material and a DNA repair step prior to WES library preparation. Fresh/frozen and FFPE source material produced similar sequence coverage, but DNA isolated from fresh/frozen often produced sequences with better read quality. On average, more variants were detected in DNA from FFPE samples versus their fresh/frozen counterpart. Most of these variant usually occurred at the ends of sequencing reads as determined by visualizing the data sets in IGV (Integrative Genomics Viewer).

CONCLUSION: Fresh/frozen material is the ideal method for storing biological material, however the majority of cancer samples are fixed in formalin for histopathological analysis then archived for long-term storage as paraffin blocks. Here we demonstrate that FFPE samples are adequate for whole exome sequence analysis as long as proper quality assessment measures and data analysis oversight are incorporated into the workflow.

NO CONFLICT OF INTEREST

505 A genome-wide screen identifies VAMP2 as a novel mediator of paclitaxel resistance in breast cancer

T. Huynh[1], *M. Sultan*[1], *M. Thomas*[1], *K. Coyle*[1], *D. Vidovic*[1], *C. Giacomantonio*[1], *P. Marcato*[1]

[1] *Dalhousie University, Pathology, Halifax, Canada*

INTRODUCTION: Breast cancer is the leading cause of cancer-associated mortality affecting women worldwide. Paclitaxel is a principle chemotherapeutic used to treat breast cancer; however, some patients fail to respond to paclitaxel treatment. Biomarkers which predicts patient response to paclitaxel prior to drug administration will improve treatment efficacy. An in vivo genome-wide RNAi screen was performed to identify genes that mediate resistance or sensitivity to paclitaxel treatment. Vesicle-associated membrane protein 2 (VAMP2) was identified as a potential novel paclitaxel resistance gene. We tested the effect of VAMP2 knockdown in paclitaxel-mediated apoptosis and inhibition of cell proliferation of breast cancer cells to validate its proposed role in paclitaxel resistance.

MATERIALS AND METHODS: Knockdown of VAMP2 was generated with shRNA lentiviral infection in MDA-MB-231 breast cancer cells and knockdown efficiency was confirmed by quantitative PCR (QPCR). The cellular growth rate of knockdown and shRNA scramble control cells with or without 2.5nM paclitaxel treatment was determined by a trypan blue dye live cell exclusion assay. Apoptosis induced by paclitaxel in the knockdown and control cells was quantified by staining of cells with annexin V-647nm and 7-AAD and flow cytometry analysis. The paclitaxel inhibitory concentration 50% (IC50) of the knockdown and control was determined by the CellTiter-Blue cell viability assay.

RESULTS: Knockdown of putative resistance gene VAMP2 decreased the growth of MDA-MB-231 cells post paclitaxel treatment to a greater extent than the shRNA scramble control cells. Knockdown of VAMP2 significantly reduced the paclitaxel IC50 and compared to the control. VAMP2 knockdown increased the percentage of apoptotic cells upon paclitaxel treatment compared to the control.

CONCLUSION: VAMP2 was identified as a novel paclitaxel resistance gene in breast cancer in an in vivo RNAi screen and confirmed using a panel of functional assays. Ongoing paclitaxel tumor regression studies will further substantiate it role in paclitaxel resistance. VAMP2 is a potential novel biomarker for paclitaxel resistance that may have prognostic value for determining treatment efficacy.

NO CONFLICT OF INTEREST

506 LKB1 expression correlates with increased survival in advanced non-small cell lung cancer patients treated with chemotherapy and bevacizumab

G. Nardo[2], *L. Bonanno*[2], *A. De Paoli*[3], *E. Zulato*[1], *G. Esposito*[1], *G. Sozzi*[4], *M. Moro*[4], *A. Amadori*[3], *P. Conte*[2], *S. Indraccolo*[1]

[1] *IOV-Istituto Oncologico Veneto, IDMO, Padova, Italy*
[2] *IOV-Istituto Oncologico Veneto, Medical Oncology 2 , Padova, Italy*
[3] *IOV-Istituto Oncologico Veneto, Clinical trials and biostatistics Unit, Padova, Italy*
[4] *Istituto Tumori Milano, Tumor genomics Unit, Milano, Italy*

BACKGROUND: Inhibition of angiogenesis is one of the strategies to improve the outcome of advanced non-small cell lung cancer (aNSCLC) patients, but no predictive markers are currently available to select patients who will benefit most from the treatment. LKB1 has a fundamental role in the cell response to stress conditions and may be involved in tumor cell adaptation to metabolic perturbations induced by angiogenesis inhibition. We investigated the role of LKB1 as potential predictive marker of sensitivity to bevacizumab in aNSCLC.

MATERIAL AND METHODS: We retrospectively analyzed LKB1 expression by immunohistochemistry in 98 samples from 125 aNSCLC patients, including 59 patients treated with chemotherapy (CT) and 39 treated with CT plus bevacizumab. IHC intensity was re-coded in two classes (negative/weak versus moderate/intense) and correlated with outcome according to treatment arm. Patient-derived tumor xenografts (PDXs) were used to investigate mechanisms involved in preclinical models.

RESULTS: In the whole study population (125), median OS and PFS were 11.7 (95%IC: 9.1-15.3) and 6.7 (95%IC: 5.7-7.2) months, respectively. Differential impact of the marker on outcome of the 98 patients was highlighted according to treatment. Patients with negative/weak LKB1 status had not a statistically significant benefit from bevacizumab added to CT (HR for patients treated with bevacizumab: 0.89, 95% CI: 0.51-1.56, p=0.6803), whereas patients expressing moderate/intense LKB1 and receiving bevacizumab had significant lower risk of death compared to those not receiving bevacizumab (HR: 0.26, 95% CI: 0.10-0.64); p=0.0035). Loss of LKB1 was associated with reduced AMPK activation in PDXs and increased tumor necrosis following bevacizumab administration, highlighting impaired control of the metabolic stress caused by this antiangiogenic drug.

CONCLUSIONS: This retrospective analysis indicates LKB1 as potential predictive marker of increased benefit from antiangiogenic treatment in aNSCLC. Differential impact on overall survival of the marker according to the administration of bevacizumab was highlighted.

NO CONFLICT OF INTEREST

507 Molecular characterization of spatial and temporal heterogeneity in epithelial ovarian cancer: Implications for treatment

S. Ballabio[1], *I. Craparotta*[2], *L. Beltrame*[2], *L. Mannarino*[2], *G. Caratti*[2], *L. Paracchini*[2], *L. Ceppi*[2], *R. Fruscio*[2], *M. D'Incalci*[2], *S. Marchini*[2]

[1] *IRCCS - Istituto di Ricerche Farmacologiche Mario Negri, Oncology, Milano, Italy*
[2] *San Gerardo Hospital, Clinic of Obstetrics and Gynaecology, Monza, Italy*

BACKGROUND: Epithelial ovarian cancer (EOC) is a heterogeneous disease with different histological subtypes, pathogenesis, clinical outcome and molecular features. In this complexity, for the optimization of treatment protocols, it is important to understand the molecular characteristics responsible for the disease and the relapse. To fill this knowledge gap towards the identification of molecular signatures associated to drug resistance, the aim of this study is to identify molecular signatures associated to drug resistance, by examining both spatial and temporal heterogeneity.

METHODS: A cohort of 38 tumor biopsies taken from 11 stage III/IV HGS-EOC patients were selected from Pandora tumor tissue collection. For each patient, multiple biopsies were available at time of primary surgery, when tumor was naïve to chemotherapy, and at relapse, after one or more lines of chemotherapy. For each biopsy, chromosomal alterations were analyzed by array-CGH technology (CytoSure™Cancer + SNP Array (4x180k)).

RESULTS: A high level of concordance was observed in all biopsies taken at the first surgery in all patients and between the primary sample and relapse. With GISTIC algorithm, it was possible to identify mutated chromosomal regions that were mainly represented in all cohort. 8q24.3 resulted in gain in the vast majority of cases. In this region, the two genes that resulted more altered were GLI4 and TOPO1MT. These were selected for next validation experiments with ddPCR. Also C-MYC was validated because of its proximity to region 8q24.3 and its relevance in HGS-EOC. The percentage of validation was very high: >90% for all three genes in spatial and longitudinal analysis.

CONCLUSIONS: These data have identified that structural genomic changes shared among different patients and that are independent from spatial and temporal heterogeneity, that arise at the beginning of the clinical history of the tumor. Further studies will be focused on coding and non-coding genes mapped on these genomic regions.

NO CONFLICT OF INTEREST

508 Predictive power of hERG1 potassium channel expression for response to Bevacizumab in metastatic in colorectal cancer patients

A. Arcangeli[1], F. Di Costanzo[2], L. Antonuzzo[2], L. Messerini[3], E. Lastraioli[1], J. Iorio[1], G. Petroni[1], L. Boni[3], L. Tofani[4], R. Coppola[5], G. Perrone[6], D. Caputo[5], M. Francesconi[6]

[1] University of Firenze, Department of Experimental and Clinical Medicine, Firenze, Italy
[2] AOU Careggi, SOD Oncologia, Firenze, Italy
[3] AOU Careggi, Clinical Trial Center, Firenze, Italy
[4] Universty of Firenze, Department of NEUROFARBA, Firenze, Italy
[5] Campus Biomedico, General Surgery, Roma, Italy
[6] Campus Biomedico, Pathology, Roma, Italy

Colorectal cancer (CRC) represents the most prevalent cancer type in both sexes and the second cause of cancer related deaths worldwide. Although changed in the last years for the advent of targeted therapeutic regimens, the outcome for CRC patients affected by metastatic disease is still unsatisfactory. While RAS mutations are consistently associated with increased treatment failure rates among patients treated with anti-EGFR antibodies, no genetic and molecular markers for anti-angiogenesis therapy (Bevacizumab, BV) have been found. Thus, the identification of novel biomarkers to predict or monitor the efficacy of BV represents a challenging field with immediate spin-off for the clinical practice. We have contributed in this field through our studies on the role of hERG1 potassium channels in human cancers. hERG1 is aberrantly expressed in several types of human cancers and precancerous lesions, where it regulates different aspects of tumor cell behavior. Furthermore hERG1 expression can be exploited for diagnostic and prognostic purposes in different human cancers1-10. Recently, we have identified a novel signaling pathway, centered on hERG1 channels, that sustains angiogenesis and progression in CRC cell lines in vitro and in preclinical mouse models. This pathway is triggered by integrin-mediated adhesion and leads to VEGF-A secretion. Based on the above premises we have conducted a biomolecular study with the aim to evaluate the predictive values of such signaling pathway in the clinical response to Bevacizumab in mCRC patients. 42 mCRC patients with synchronous metastases enrolled at the SOD Oncology of Careggi Hospital of Florence and at the Campus Biomedico, Rome, and treated in first line with Bevacizumab, were analyzed by immunohistochemistry for the expression, in the primary tumor tissue, of the following markers: hERG1, β1 integrin, pAKT, p53, NFkB, HIFα, VEGF-A, Glut-1 and CAIX. The response to BV was evaluated as either the "best response" and the 'progression free survival" (PFS). It emerged that hERG1 identifies a subset of patients with longer PFS (544.72 days vs 288.6; p= 0.00103, log rank test). Overall, we conclude that hERG1 might represent a novel predictive biomarker for better response to BV-based therapies in mCRC patients.

NO CONFLICT OF INTEREST

509 Molecular architecture of tolerance to neoadjuvant treatment in breast cancer

A. Zapater-Moros[1], L. Trilla-Fuertes[2], A. Gámez-Pozo[1,2], G. Prado-Vázquez[1], S. Llorente-Armijo[1], R. López-Vacas[1], P. Main[3], P. Zamora[4], E. Espinosa[4], J.A. Fresno Vara[1]

[1] Hospital Universitario La Paz, INGEMM, Madrid, Spain
[2] Biomedica Molecular Medicine SL, R&D Department, Madrid, Spain
[3] Complutense University of Madrid, Department of Statistics and Operations Research- Faculty of Mathematics, Madrid, Spain
[4] Hospital Universitario La Paz, Medical Oncology, Madrid, Spain

BACKGROUND: Breast cancer is a diverse and heterogeneous disease. Clinical data has uncovered the existence of different tumor responses to neoadjudvant chemotherapy, allowing the classification of patients in different groups. Gene expression profile description of the different patient groups provide essential information in the clinical decision making as well as to allow a deeper knowledge of this disease. The use of neoadjuvant treatments has improved the prognosis of localized breast cancer. However, the molecular basis of neoadjuvant treatment response and resistance remaini unknown.

MATERIALS AND METHODS: Differentially expressed genes between different patient groups were selected by a Significance Analysis of Microarrays (SAM) and gene ontology analyses were done. In addition, functional structure was performed using probabilistic graphical models with local minimum Bayesian Information Criterion. Data analyses were carried out using MeV, BRB Array Tools, R, Cytoscape software suites and DAVID web tools (https://david.ncifcrf.gov/).

RESULTS: SAM between 'stable disease patient group" against others patient groups uncovered 66 differentially expressed genes with a functional enrichment in immune response. Using a probabilistic graphical model approach, we expect to characterize differential processes in different tumor groups that may help to explain its differential response to neoadjuvant chemotherapy.

CONCLUSION: This type of approach allows seeing differences at process levels rather than at the individual level. Moreover, description of gene expression profile among to each clinical patient group can provide a classifying and predicting method that can avoid exposure to chemotherapy to certain patients in which it isn't effective. Tumoral immune status seems to be related with the stable disease phenotype, and could be related with neoadjuvant treatment resistance.

CONFLICT OF INTEREST

Ownership: AGP, EE and JAFV are stakeholders of Biomedica Molecular Medicine
Board of Directors: AGP, EE and JAFV are part of the board of directors of Biomedica Molecular Medicine
Other Substantive Relationships: LTF is an employee of Biomedica Molecular Medicine

510 Functional characterization of colorectal cancer molecular subtypes

S. Llorente-Armijo[1], L. Trilla-Fuertes[2], A. Gámez-Pozo[1,2], A. Zapater-Moros[1], G. Prado-Vázquez[1], R. López-Vacas[1], H. Navarro[3], J.M. Arevalillo[3], J. Feliú[4]

[1] Hospital Universitario La Paz, INGEMM, Madrid, Spain
[2] Biomedica Molecular Medicine SL, R&D Department, Madrid, Spain
[3] University Nacional Educacion a Distancia UNED, Operational Research and Numerical Analysis, Madrid, Spain
[4] Hospital Universitario La Paz, Medical Oncology, Madrid, Spain

BACKGROUND: Colorectal cancer (CRC) not only is the second most common cancer in Europe but the second leading cause of cancer death in Europe as well. CRC is a heterogeneous disease which involves several outcomes and drug responses. To resolve inconsistencies among the reported gene expression-based CRC classifications and facilitate clinical translation in November 2015 an international consortium was formed and four consensus molecular subtypes (CMSs) were identified.

MATERIAL AND METHODS: A total of 7 CRC data sets (n=1439) from public sources (GSE13067, GSE13294, GSE14333, GSE17536, GSE33113, GSE35896, GSE39582) were selected. These datasets were gathered in a new dataset after removing batch effects. 2,500 most variable genes were selected for subsequent analyses. Then, a probabilistic graphical model approach will be applied. Previous experience with this tool allowed the identification of multiple gene clusters, grouped on the basis of their molecular function, in a functional network. Differences between groups and relations of these functional nodes with prognosis will be assessed. On the other hand, a Significance Analysis of Micorarrays (SAM) was performed in order to characterize differentially expressed genes between subgroups.

RESULTS: Gene expression-based subtyping is a widely accepted source of disease stratification. In the present study, we will construct a functional network in order to explore the molecular differences between these subtypes in depth. Differences in the functional network will be assessed between previously described CMSs and new suggested subgroups. In addition, SAM result showed genes with different expressions patterns between groups, most of them related with immune response and extracellular matrix.

CONCLUSION: Functional networks can provide a relevant molecular knowledge which complements the CMSs classification. Relationships between genes will be identified and associated to the different CRC subtypes. Probabilistic graphical modes could allow a better understanding of tumor behavior and CMSs molecular uniqueness. Besides, this deep knowledge will allow a more accurate prediction of outcome and can also be used for diagnostic purposes. The fact that genes related with immune response were showed in SAM could be due to a multilevel information layer related with patient immune response capability.

CONFLICT OF INTEREST

Ownership: AGP and JAFV are stakeholders of Biomedica Molecular Medicine
Board of Directors: AGP and JAFV are part of the board of directors of Biomedica Molecular Medicine
Other Substantive Relationships: LTF is an employee of Biomedica Molecular Medicine

511 New translational method for tumor's rheological and microenvironment evaluations: Optical Flow tracing and Particle Image Velocimetry methods applied to contrast ultrasound imaging

P. Giustetto[1], D. Fuchs[2]

[1] Freedom Waves Srl, R&D, Torino, Italy
[2] Visualsonics Fujfilm, Market Deuelopment & Pre Clinical Applications Manage, Amsterdam, Netherlands

BACKGROUND: Tumor vessels are usually leaky and tortuous, which further decreases tissue perfusion, promoting malignant progression, metastasis diffusion, and reducing the efficacy of cancer therapies.In parallel, vessel leakiness together with vessel compression cause a uniformly elevated interstitial fluid pressure that hinders the delivery of blood-borne therapeutic agents, lowering the efficacy of chemo and nanotherapies. Moreover, shear stresses exerted by flowing blood, and interstitial fluid modulates the behavior of cancer and a variety of host cells. Knowing these physical forces can improve therapeutic outcomes in many types of cancer.

The aim of our work is to integrate the US imaging methods with molecular modeling, drug design and post processing with Optical Flow (OF) method to understand the effects and the action mechanisms of diagnostic, therapeutic and theragnostic agents on cells, tissues and microenvironment of cancer.

MATERIAL & METHOD: Particle Image Velocimetry (PIV) and Particle Tracking Velocimetry (PTV) are innovative and non-intrusive way technology that we have implemented on the MATLAB platform.PIV gives valuable information on how velocity field changes in 2D plane, at regular time intervals. All exams were performed with VEVOLAZR system with 1–24 MHz transducer, evaluating subcutaneous syngeneic breast cancer. We analyzed several aspects of cancer rheology, with the impact of MM1 micro-bubble bolus.

RESULTS: PIV involves two processes, capturing the 2D images for the visualization and the images analysis in order obtain 2D and 3D parameters values, as vector flow, velocity, vorticity, shear and strain rate data. Sub-volumes with the distinct property's inside tumors can now be identified with OF analysis showing velocities magnitude with its components, shear rate, viscosity, vorticity and strain rate

numerical quantification. Strain rate values and trend in various points of tumor vector map are showed; vessel bifurcation and bending as well as diameter reduction also produce local vortices with increased mass transfer rates and are therefore considered as possible docking points for circulating cancer cells.

CONCLUSION: The goal of the project is to develop a volumetric strategy for real-time monitoring and characterization of tumor blood flow using microbubble contrast agents and ultrasound imaging for clinical use, improving sensitivity and resolution of imaging equipment in multimodal multiscale imaging. Early evidence of vascular phase is critical to the study of tumor growth because microcirculation plays a significant role in the growth, metastasis, detection, and treatment of tumors.This method gets a complete information on rheological phenomena which are involved in the throughout the tumor, during diagnostic and drug administration processing or follow-up controls.

NO CONFLICT OF INTEREST

512 PI3K-C2A regulates mitotic spindle assembly and chemotherapy response in breast cancer

M. Martini[1], M.C. De Santis[1], F. Gulluni[1], L. Annaratone[2], D. Di Salvatore[3], G. Bertalot[4], M. Compagno[2], F. Montemurro[5], P. Meraldi[6], C. Marchiò[2], S. Pece[7], A. Sapino[2], R. Chiarle[1], P.P. Di Fiore[3], E. Hirsch[1]

[1] *Università degli Studi di Torino, Department of Molecular Biotechnology and Health Sciences, Torino, Italy*
[2] *Università degli Studi di Torino, Department of Medical Sciences, Torino, Italy*
[3] *IFOM- the FIRC Institute for Molecular Oncology Foundation, IFOM- the FIRC Institute for Molecular Oncology Foundation, Milano, Italy*
[4] *IEO- European Institute of Oncology, Program Of Molecular Medicine, Milano, Italy*
[5] *IRCC Candiolo., Unit of Investigatiue Oncology-, Candiolo TO, Italy*
[6] *University of Geneva, Department of Cell Physiology and Metabolism, Geneva, Switzerland*
[7] *University of Milan, Department of Oncology and Hemato-oncology DIPO, Milano, Italy*

Introduction

Proper organization of the mitotic spindle is key to genetic stability but the molecular components of inter-microtubule (MT) bridges that crosslink kinetochore fibers (K-fibers) are still largely unknown. Here, we identify class II phosphoinositide 3-OH kinase a (PI3KC2a) as a limiting scaffold protein organizing the clathrin and TACC3 complex crosslinking K-fibers.

MATERIAL AND METHODS: Pik3c2a$^{+/-}$ mice were intercrossed with a transgenic strain expressing the activated HER-2/Neu oncogene in the mammary gland. Mice were weekly followed for survival, tumor appearance and growth. Primary Murine Mammary Epithelial Tumor (MMET) cells were derived from early and late stage tumors. Truncating PI3KC2α mutants were generated and interaction with TACC3 was tested. Levels of PI3K-C2α expression were assessed by IHC in breast cancer tissue microarrays (TMA) and correlated with response to chemotherapy.

RESULTS AND DISCUSSION: Loss of PI3K-C2α expression is a frequent occurrence in breast cancer patients (48%) and correlates with local recurrence and metastatic disease. The heterozygous loss of PI3K-C2α initially delays tumor onset but, on the long run, leads to the convergent evolution of aggressive clones with mitotic checkpoint defects. In line with this, downregulation of PI3K-C2α promotes spindle alterations and aneuploidy, indicating that PI3K-C2α expression is a key determinant of genomic stability. As a consequence of the altered spindle, reduction of PI3K-C2α expression increases the sensitivity to anti-MT drugs, such as paclitaxel, in pre-clinical models and in breast cancer patients.

CONCLUSION: Given the role of PI3K-C2α in controlling genome stability and spindle organization, PI3K-C2α expression could be exploited to stratify patients for novel clinical protocols with reduced side effects.

NO CONFLICT OF INTEREST

513 EGFR pathway dysregulation in EphA2 cells and correlation with colorectal cancer progression, prognosis and prediction of therapy response

M. De Robertis[1], L. Loiacono[2], C. Fusilli[3], M.L. Poeta[1], T. Mazza[3], M. Sanchez[4], G. Lamorte[5], A.L. Vescovi[5], J. Garcia-Foncillas[6], V.M. Fazio[7]

[1] *University of Bari, Department of Bioscience- Biotechnology and Biopharmaceutics, Bari, Italy*
[2] *IRCCS "Casa Sollievo della Sofferenza", Laboratory of Oncology, San Giovanni Rotondo FG, Italy*
[3] *IRCCS "Casa Sollievo della Sofferenza", Unit of Bioinformatics, San Giovanni Rotondo FG, Italy*
[4] *Istituto Superiore di Sanità, Department of Cell Biology and Neurosciences, Rome, Italy*
[5] *IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo FG, Italy*
[6] *Oncohealth Institute- FIIS-Fundacion Jimenez Diaz, Translational Oncology Division, Madrid, Spain*
[7] *University Campus Bio-Medico of Rome, Laboratory of Genetic and Clinical Pathology, Rome, Italy*

BACKGROUND: EphA2 receptor is involved in multiple cross-talks with other cellular networks including EGFR, FAK and VEGF pathways, with which it collaborates to stimulate cell migration, invasion and metastasis. Colorectal cancer (CRC) EphA2 overexpression has also been correlated to tumor stem-like properties of cells. Here, we investigated the molecular crosstalk and microRNAs modulation of the EphA2 and EGFR pathway studying a panel of genes and miRNAs EGFR/EphA2-related in EphA2+ murine CRC cells. We also explored the potential role of such EphA2/EGFR pathway mediators as prognostic factors or predictors of cetuximab benefit in CRC patients.

MATERIAL AND METHODS: Stem-like/differentiation property of EphA2high cells isolated from CRC of AOM/DSS murine model was evaluated by gene expression and immunohistochemistry (IHC) analysis of stemness (Lgr5, Ascl2) and differentiation (Krt20) markers. Gene and microRNA expression analysis was performed using low-density TaqMan Assays (LifeTechnologies®) on FACS-isolated EphA2high cells. Expression data were evaluated by statistical and bioinformatics tools. Six independent cohorts of CRC patients (GEO public data repository and The Cancer Genome Atlas (TCGA)) were analysed and stratified by EphA2 expression to assess the prognostic/predictive role of the EphA2/EGFR signature and its effect on cetuximab treatment response.

RESULTS: The expression analysis of genes and miRNAs identified in the sorted EphA2high tumor cells revealed a specific involvement in EGFR related pathway. The EphA2/EGFR-related signature (EphA2/Efna1/EGFR genes, linked to a possible control by mir-200a and mir-26b) was further validated in human samples (public dataset) revealing a prognostic and predictive significance in relation to anti-EGFR treated patients.

CONCLUSIONS: These data offer an EphA2/EGFR-related gene/miRNA signature which could be proposed as novel CRC prognostic biomarkers. Additionally, EphA2 could be linked to a mechanism of resistance to cetuximab.

NO CONFLICT OF INTEREST

514 CAMLs and CTM in women treated for breast cancer: Understanding the clinical value of circulating tumor associated cells

S. Di Cosimo[1], S. Bottelli[2], C. Reduzzi[3], G. Galli[3], G. Bregni[3], A. Martinetti[3], B. Paolini[4], P. Verderio[2], V. Cappelletti[1]

[1] *Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Experimental Oncology and Molecular Medicine, Milano, Italy*
[2] *Fondazione IRCCS Istituto Nazionale dei Tumori, Unit of Medical Statistics- Biometry and Bioinformatics, Milano, Italy*
[3] *Fondazione IRCCS Istituto Nazionale dei Tumori, Medical Oncology Unit, Milano, Italy*
[4] *Fondazione IRCCS Istituto Nazionale dei Tumori, Department of Pathology, Milano, Italy*

INTRODUCTION: Much is currently known about the role of circulating tumor cells (CTCs) in diverse types of cancer. However, the molecular characterization of CTCs remains a challenge because of their rarity, heterogeneity and technological difficulties in enrichment and isolation. In this explorative study, we evaluated prospectively circulating cancer-associated macrophage-like cells (CAMLs) and circulating tumor microemboli (CTM) to ascertain their prevalence and response to treatment in relation to breast disease status at clinical presentation.

MATERIAL AND METHODS: Blood collected from non-metastatic (M0) patients undergoing neoadjuvant therapy and from metastatic (M1) patients starting a new line of treatment was processed at baseline and prior to surgery or at the time of first tumor assessment. All samples were processed within 1 hour after collection and using the ScreenCell® Cyto kit according to manufacturer instructions. For each patient, three separate blood filtrations corresponding to 9 mL of total blood were stained with hematoxylin/eosin or with May-Grunwald Giemsa and evaluated using cytomorphological criteria.

RESULTS: A total of 37 M0 and 24 M1 BC patients were analyzed. For both CTM and CAMLs, patients were classified as positive if at least one cell were detected and negative otherwise. CAMLs were found at baseline in both M0 and M1 cases regardless of BC subtype with a similar distribution (13.5% versus 25%, p= 0.315). Apparently treatment induced no change of CAMLs in M1 breast cancer patients, though one third of M0 breast cancer patients turned CAMLs positive after neoadjuvant chemotherapy. This data supports the hypothesis that CAMLs are associated with the phagocytosis of cancer cell debris resulting from cytotoxicity occurring at the tumor site and may increase in cases who are responsive to systemic therapy. CTM were differentially found in M0 and M1 BC patients at baseline (68% versus 25%, of p= 0.002). No association was found with BC subtype. No change over time in CTM counts in relation to systemic therapy was found implicating relative resistance to cytotoxic drugs for cells. Of note, CAMLs and CTM were not associated with each other, nor with tumor shrinkage.

CONCLUSION: These preliminary findings suggest that both CAMLs and CTM are detectable in patients with BC at different stages. CAML and CTM may have differential utility in monitoring BC patients. In particular, CAMLs appear to be modulated by chemotherapy and may serve as surrogate biomarker of patient outcome, whereas CTM are optimal candidates for specific targeted treatment. Due to the low number of events, data on association with long term clinical outcome will be presented at the meeting.

NO CONFLICT OF INTEREST

515 New treatment modality for non-regressive oral papillomatosis in canine patient

E. Signori[1], F. Maglietti[2,3], M. Tellado[4], S. Michinski[2,3], N. Olaiz[2,3], G. Marshall[2,3]

[1] *CNR-Institute of Translational Pharmacology, Biomedical Sciences, Roma, Italy*
[2] *Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales., Departamento de Física., Buenos Aires, Argentina*
[3] *CONICET- Universidad de Buenos Aires, Instituto de Física del Plasma INFIP, Buenos Aires, Argentina*
[4] *Universidad de Buenos Aires, Facultad de Ciencias Veterinarias-, Buenos Aires, Argentina*

BACKGROUND: Papillomaviruses are species and tissue specific set of viruses responsible for the formation of benign lesions like warts in the skin, oral cavity, larynx and anogenital region. Some papillomavirus types are associated with high risk of progression to malignancy, ie: HPV-6, HPV-11, HPV-16 and HPV-18. The impact of this disease can be much more severe in immunocompromised patients. Canine oral papillomavirus resembles benign forms of the HPV, such as recurrent laryngeal papillomatosis and its ability to cause persistent infections in certain individuals, and in some cases, due to accumulations somatic mutations, some lesions can progress to a squamous cell carcinoma. Intense research has been

performed to understand the biology and immunity related to the virus responsible for this disease. Only recently it has been propagated in vitro. Animal models represent the gold standard by which the in vitro models should be assessed, and canine patients are considered a good translational model due to the development of spontaneous diseases we find in humans.

MATERIAL AND METHOD: The patient is a female cross-breed dog, 8 months old, with a severe oral papillomatosis of 4 months of evolution confirmed by biopsy. Surgery was not the first option, due to the presence of multiple big sized lesions. Interferon administration failed. Azithromycin treatment showed no response. Auto-vaccine did not achieve positive results. The pain of the lesions was hard to control with analgesia. Administration of bleomycin in three sessions of electrochemotherapy (ECT) have been performed in the majority of the lesions, to reduce the extension of the disease and make the surgery possible. The response was evaluated by clinical examination.

RESULTS AND DISCUSSION: After three sessions of ECT, the treated lesions regressed significantly, as well as the non-treated lesions. The ECT was effective by triggering the immune response, so inducing a general regression of the disease. After the third session of ECT, while the lesions were still in reduction, a new lesion of 2.5x1.8x1.8 cm appeared in the left side of the mandible. The biopsy revealed a squamous cell carcinoma which was treated with a mandibulectomy with excellent results. The patient is disease-free for 16 months up to writing of this abstract.

CONCLUSION: ECT can be an effective approach for patients with non-regressive disease, that does not preclude other treatments.

NO CONFLICT OF INTEREST

516 MDM2-inhibition sensitizes dedifferentiated liposarcomas to radiotherapy through enhanced senescence

S. Das[1], *G. Iliakis*[2], *S. Bauer*[3]

[1] *Sarcoma Center- Department of Medical Oncology- West German Cancer Center- University Hospital Essen- Duisburg-Essen University, Sarcoma Center- Department of Medical Oncology, Essen, Germany*
[2] *Institute of Medical Radiation Biology-Medical School-University of Duisburg-Essen, Institute of Medical Radiation Biology, Essen, Germany*
[3] *Sarcoma Center- Department of Medical Oncology- West German Cancer Center- University Hospital Essen- University Duisburg-Essen, Sarcoma Center- Department of Medical Oncology, Essen, Germany*

BACKGROUND: Well- and dedifferentiated liposarcomas (WDLPS/DDLPS) represent a common sarcoma subtype characterized by amplification of the 12q13–15 region that includes the MDM2 and CDK4 genes. Small molecules that reactivate p53 by disrupting the MDM2/p53 interaction have recently been associated with clinical activity in LPS. Radiotherapy is an integral component of treatment in localized liposarcoma yet it is not known if p53 reactivation may sensitize LPS for radiotherapy.

MATERIALS AND METHOD: Three WDLPS/DDLPS cell lines (MDM2 amp/ tp53 wt) and one undifferentiated liposarcoma (tp53 mutant) were evaluated for radiosensitization by nutlin-3, an inhibitor of MDM2/p53 interaction, using long term clonogenic assays. Biological consequences of a combined radiotherapy (4 Gy) / MDM2 inhibition were measured using immunoblotting of cell cycle and apoptosis related intracellular pathways, proliferation and apoptosis assays, cell cycle analyses by flow cytometry, and senescence assays.

RESULTS AND DISCUSSION: Combined treatment of the two DDLPS cell lines lead to a sensitization enhancement ratio (SER) of >1 as measured via clonogenic survival assay supporting a radiosensitization by nutlin-3 (5μM). No effect was seen in the WDLPS and p53 mutant cells. While combination treatment did not enhance apoptosis it stabilized p53, resulted in a G1 arrest and an increased senescence. An increased ≥4N G1 population was found in cells that received both radiation and nutlin via the process of endo reduplication which lead to a senescence-like phenotype (SLP). Sorting of populations with different ploidy (2N, 4N and >4N) and analysis of their eventual fate by long-term clonogenic assay demonstrated attenuated clonogenic ability of the tetraploid or polyploid cells.

CONCLUSION: Here we report for the first time a potential radiosensitizing effect by MDM2 inhibition in dedifferentiated liposarcomas. This effect is exerted via increased senescence and a reduced clone forming ability.

NO CONFLICT OF INTEREST

517 Evaluation of single cell co-expression profiles of immune checkpoint therapeutic targets in the tumor microenvironment of Non-Small Cell Lung Cancer

E. Park[1], *K. Wilkens*[1], *J. Kim*[1], *N. Li*[1], *M.X. He*[1], *X.J. Ma*[1]

[1] *Advanced Cell Diagnostics, R&D, Newark, USA*

INTRODUCTION: Immune-checkpoint inhibitors have demonstrated therapeutic efficacy and durable response for several tumor types including non-small cell lung cancer (NSCLC). In addition to efforts on biomarkers to identify responsive tumors, adaptive resistance to individual checkpoint blockade suggests the need to target multiple immune checkpoints.

MATERIAL AND METHOD: We evaluated single cell expression profiles of therapeutic checkpoint targets in the tumor microenvironment (TME) of 56 archived NSCLC FFPE tissues. Applying RNAscope® in-situ hybridization assays, specific checkpoint target molecules are visualized at single molecule detection sensitivity.

RESULTS AND DISCUSSION: Here we report visual representation of immune checkpoint targets expressed in the TME. The evaluation of PD-L1 expression in each tumor tissue showed a dynamic expression of PD-L1 mRNAs in the tumor and stromal regions. Duplex RNAscope analysis of PD1 (or PD-L1) with other therapeutic targets such as TIM3 or LAG3 revealed co- expression of multiple checkpoint markers in the same tumor environment, primarily in the highly inflamed tumors. Most importantly, single cell co-localization of therapeutic targets are represented and quantified in inflamed and non-inflamed tumors.

CONCLUSION: The expression and co-localization of multiple therapeutic immune checkpoint targets are visualized and quantified in single cells within intact tissues, suggesting potential resistance mechanisms which may help guide in stratifying patients for different combinatorial approaches.

CONFLICT OF INTEREST

Ownership: We are employed at Advanced Cell Diagnostics and own stocks.

518 Valproic Acid sensitizes colorectal cancer to chemotherapy by targeting cancer stem cell compartment

M.S. Roca[1], *A. Leone*[1], *C. Vitagliano*[1], *F. Tatangelo*[2], *A. Avallone*[3], *A. Budillon*[1]

[1] *Istituto Nazionale Tumori Fondazione G. Pascale - IRCCS, Experimental Pharmacology Unit, Napoli, Italy*
[2] *Istituto Nazionale Tumori Fondazione G. Pascale - IRCCS, Pathology Unit, Napoli, Italy*
[3] *Istituto Nazionale Tumori Fondazione G. Pascale - IRCCS, Abdomen Medical Oncology Unit, Napoli, Italy*

INTRODUCTION: Colon Rectal Cancer (CRC) is an heterogeneous disease in terms of clinical presentation and response to therapy. Cancer Stem Cells (CSCs) showing the ability to drive tumor initiation and growth, have been identified in many human cancers, including CRC, and are associated with resistance to chemotherapy and tumor relapse. Epigenetic alterations have been shown to play an important role in CSC phenotype. Since epigenetic modifications are generally reversible, epigenetic manipulation by drugs such as histone deacetylase inhibitors (HDACi), represent an attractive approach to sensitize colon CSCs to conventional chemotherapy. Recently, valproic acid (VPA), a weak HDACi, has been studied by our group in combination with capecitabine and radiotherapy (RT) in breast cancer and CRC models, demonstrating potent synergistic antitumor effect. These studies have been the "proof-of-concept" of the ongoing phase I/II V-SHORT clinical trial, evaluating preoperative VPA, capecitabine and short-course-RT in locally advanced rectal cancer patients (NCT01898104).

MATERIAL AND METHODS: Using primary spheroid CRC cultures, transduced with a TOP-GFP Wnt reporter, we tested the combination of VPA with standard chemotherapy on apoptosis, cell proliferation and clonogenistic capability in both differentiated and CSCs cells within the same population. We also tested VPA impact on cells morphology change and on the modulation of differentiation and CSC markers, by RT-PCR, western blotting and immunohistochemistry (IHC). CSC markers on tumor tissues from patients enrolled in V-SHORT study were evaluated by IHC.

RESULTS AND DISCUSSION: We found that low doses of VPA, corresponding to plasma levels easily reached in treated patients, sensitized CSCs to oxaliplatin treatment in several primary cell lines with a different genetic backgrounds. Moreover, VPA treatment induced changes in cell morphology consistent with the observed increase of differentiation markers and decrease of CSCs markers. Interestingly, healthy primary cell lines resulted not sensitive to VPA treatment. Finally, we evaluated selected CSCs markers on tumor tissue from rectal cancer patients enrolled within neoadjuvant V-SHORT study, demonstrating that a reduction of selected markers correlated with pathologic complete response.

CONCLUSION: We propose a new combination therapeutic strategy to target CSCs compartment and potentiate conventional chemotherapy and/or RT approach in CRC patients by using VPA.

NO CONFLICT OF INTEREST

519 V-ATPase proton pump profiling reveals two different classes in IDH wild type lower-grade glioma

A. Terrasi[1], *I. Bertolini*[2], *M. Formica*[1], *G. Gaudioso*[1], *S. Bosari*[3], *V. Vaira*[2]

[1] *Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico- University of Milan, Pathophysiology and Transplantation, Milan, Italy*
[2] *Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico- Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Inuernizzi"- University of Milan, Pathophysiology and Transplantation, Milan, Italy*
[3] *Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Pathophysiology and Transplantation, Milan, Italy*

INTRODUCTION: Recent work of our group shown that ATP6V1G1, subunit of vacuolar H^+ ATPase (V-ATPase) proton pump, is upregulated in glioblastoma multiforme (GBM) patients. Moreover, we identified that V-ATPase plays major roles in glioma stem cells (GSC), governing clonogenicity, cell motility and survival. IDH1/2-wild-type (IDHwt) lower-grade gliomas (LGG) are a subset of LGG characterized by poor prognosis and GBM-like features. To better clarify the role in supporting tumorigenesis of V-ATPase pump in diffuse glioma we investigated the public dataset generated by The Cancer Genome Atlas (TCGA).

METHODS: Analysis of differentially expressed V-ATPase genes was performed using the 'samr" package of Significance Analysis of Microarray (SAM) in IDH mutated (IDHmut) primary LGG and GBM dataset (n=902). The differentially

expressed genes were used for Principal Component Analysis (PCA) in IDHwt LGG cohort (n=77). These findings were integrated with clinical and molecular data. Expression profiles of 12717 genes were analysed and SAM selected genes were subjected to bioinformatics analysis using Gene Set Enrichment Analysis (GSEA) tools. To investigate the link between V-ATPase subunits and identified genes we used neurospheres (NS) (enriched in GSC) and differentiated cells (diff) isolated from GBM patients.

RESULTS: SAM analysis (q value <0.01) of V-ATPase genes between GBM and IDHmut LGG showed a switch expression of proton pump subunits. PCA analysis uncovered two IDHwt LGG classes; IDHwt class A (n=26) and IDHwt class B (n=51). Shorter overall survival correlation independent of clinical variables was found in class B (p.val <0.05; HR= 0.4). SAM analysis and GSEA between LGG IDHwt classes identified differentially expressed genes related to cell cycle, cancer system morphogenesis and stemness pathways; in particular, HOX gene family was found strongly enriched in IDHwt class B subtype and GBM patients. Moreover, HOX are upregulated in NS compared to diff and are enriched in NS with higher level of ATP6V1G1 expression.

CONCLUSION: Our result suggest a role of V-ATPase genes in diffuse gliomas progression as possible regulator of critical signalling pathways. These findings point to this gene family as a novel clinically independent marker in IDHwt LGG. For the first time we identify two molecular distinct classes with a different survival prediction. The IDHwt LGG class B is molecularly and clinically similar to GBM suggesting higher tumor aggressiveness.

NO CONFLICT OF INTEREST

520 Humanized JAA-F11, a highly specific anti-Thomsen-Friedenreich pancarcinoma antibody: Preclinical specificity and efficacy analysis

K. Rittenhouse- Olson[1,2], J. Abdullah[3], S. Tati[2], J. Fisk[2], T. Chrisikos[2], P. Philbin[2], L. Karacosta[2], S. Morey[4], M. Sesay[4], J. Olson[2,5]

[1] *University at Buffalo, Biotechnical and Clinical Laboratory Sciences, Buffalo, USA*
[2] *For-Robin- Inc, Research and Development, Buffalo- NY, USA*
[3] *University at Buffalo, Microbiology, Buffalo, USA*
[4] *Goodwin Biotechnology, research and development, Plantation- Florida, USA*
[5] *University at Buffalo, Pharmacology and Toxicology, Buffalo, USA*

BACKGROUND: Thomsen-Friedenreich Antigen (TF-Ag) is on tumor cell surfaces of adenocarcinomas and is involved in metastasis. Mouse JAA-F11(mJAA-F11) is highly specific for the TF-Ag alpha-linked tumor marker and decreases metastasis and increases survival in mouse breast cancer model. The objective herein is to humanize mJAA-F11 and to test the specificity and biology efficacy of the humanized JAA-F11 (hJAA-F11) constructs.

METHODS: mJAA-F11 was humanized using complementarity determining regions (CDR) grafting supplemented with computational carbohydrate threading data. Immunogenicity was predicted using the T20 scoring method. The chemical specificity was shown using a glycan array by the Consortium for Functional Glycomics. Immunohistochemistry (IHC) analysis of tumor and normal tissue and in vivo microPET imaging were utilized to show biologic specificity. Antibody-dependent cellular cytotoxicity (ADCC) was shown using the lactate dehydrogenase released from cancer cells. Internalization into cancer cells was determined using both enzyme linked immunoassay (ELISA) and immunofluorescence microscopy. In vivo efficacy of both naked hJAA-F11 and hJAA-F11-maytansine conjugate (DM1) was investigated in SCID mice with a human triple negative breast tumor.

RESULTS: The hJAA-F11 constructs have low predicted immunogenicity and high specificity for the TF-Ag alpha-linked tumor marker with no binding to TF-Ag beta-linked structures which can be on normal tissues. mJAA-F11 reacted with 79-94% of the human cancer specimens including those of breast, lung, colon, prostate and bladder. JAA-F11 staining of normal tissues was limited to those areas also stained with the isotype control antibody. ^{124}I-hJAA-F11 microPET imaging confirmed in vivo tumor reactivity and specificity. Killing by ADCC was achieved by the humanized constructs. One of the hJAA-F11 constructs internalized rapidly into tumor cells and when linked to DM1 was able to significantly decrease breast tumor progression in the in vivo mouse model.

CONCLUSIONS: These result support the conclusion that hJAA-F11 binding to TF-Ag has potential in adjunct therapy either as a direct immunotherapeutic or as part of an antibody drug conjugate to treat cancer, including triple negative breast cancer which currently has no targeted therapy.

CONFLICT OF INTEREST

Ownership: Kate Rittenhouse-Olson primary owner and founder of For-Robin, Inc. which has licensed the patent for the JAA-F11 antibody.

Board of Directors: James Olson on Board of Directors of For-Robin, Inc. which has licensed the patent for the JAA-F11 antibody.

521 P21 activated kinase 4 as a novel therapeutic target for mTOR inhibitor Resistant Pancreatic Neuroendocrine Tumors

I. Mugbil[1], W. Senapedis[2], B. Erkan[2], M. Kauffman[2], S. Shacham[2], R. Mohammad[1], P. Philip[1], A. Azmi[1]

[1] *Wayne State University- Karmanos Cancer Institute, Oncology, Detroit, USA*
[2] *Karyopharm Therapeutics, Researcher and Development, Newton, USA*

BACKGROUND: Patients with advanced unresectable pancreatic neuroendocrine tumors (PNETs) have limited therapeutic options. Despite our knowledge of the frequent MEN1, DAXX/ATRX mutations and understanding of the de-regulations in the serine-threonine kinase mammalian target of rapamycin (mTOR) pathway in PNET subsistence, the FDA approved mTOR inhibitor (mTORi) Everolimus (RAD001), shows only marginal increase in progression-free survival, and not overall survival. Failure of the majority of PNET pathway targeted therapies indicates that there is a void in our understanding of the mechanisms of resistance. Our preliminary studies in pNET cell lines demonstrate hyper-activation of the Rho GTPase effector p21 activated kinase 4 (PAK4). PAK4 protein is recognized to regulate myriad signaling pathways including mTORC1, mTORC2, PI3K, IGF-1 and FAK making it a viable therapeutic target for mTOR inhibitor resistant pNETs.

MATERIALS AND METHODS: pNET cell lines QGP-1 and Bon-1 were exposed to either PAK4 siRNA or PAK4 allosteric modulators (PAMs KPT-9274 a Phase I drug; KPT-7532 and PF-3738309 +ve control) in the presence or absence of mTOR inhibitors RAD001 or INK128. Gene expression profiling and phospho-proteomic analyses were performed to capture molecular changes post PAM or combination treatment. The anti-tumor activity of lead PAM-RAD001 was evaluated in subcutaneous xenograft derived from QGP-1 and Bon-1 cells.

RESULTS: PAK4 RNA interference suppressed proliferation and restored mTOR inhibitor sensitivity in pNET cell lines. PAM analogs were effective in reducing proliferation and could synergistically enhance the anti-tumor activity of RAD001 or INK128 [combination index <1]. Molecular analysis of combination treatment showed down-regulation of known evorulimus resistance drivers mTORC1, mTORC2, PI3K, ERK, FAK, RICTOR, β-catenin and IGF-1. Forced PAK4 expression resulted in the activation of FAK and phospho-FAK that was reversed by PAM treatment. PAM KPT-9274 given i.v. or orally at maximum tolerated dose (140 mg/kg 5 days a week for 4 weeks) dramatically inhibited the growth of QGP-1 and Bon-1 sub-cutaneous tumors.

CONCLUSIONS: This is the first report demonstrating the role of PAK4 in pNET therapy resistance. PAM KPT-9274 is currently in Phase I evaluation (NCT02702492). Our result build solid pre-clinical rationale for Phase II clinical study involving PAM and mTOR inhibitor combination for the treatment of difficult to treat pNETs.

CONFLICT OF INTEREST

Ownership: Michael Kauffman, Sharon Shacham, Yosef Landesman, William Senapedis, Erkan Baloglu have ownership in Karyopharm Therapeutics
Advisory Board: Michael Kauffman, Sharon Shacham, Yosef Landesman, William Senapedis, Erkan Baloglu have ownership in Karyopharm Therapeutics
Board of Directors: Michael Kauffman, Sharon Shacham, Yosef Landesman, William Senapedis, Erkan Baloglu have ownership in Karyopharm Therapeutics
Corporate-sponsored Research: Michael Kauffman, Sharon Shacham, Yosef Landesman, William Senapedis, Erkan Baloglu have ownership in Karyopharm Therapeutics
Other Substantive Relationships: Michael Kauffman, Sharon Shacham, Yosef Landesman, William Senapedis, Erkan Baloglu have ownership in Karyopharm Therapeutics

522 The Androgen Receptor is a negative regulator of eIF4E phosphorylation at S209: Implications in the use of mTOR inhibitors in advanced prostate cancer

P. Ghosh[1], L. D'Abronzo[1], M. Crapuchettes[2], R. Beggs[2], S. Siddiqui[2], Y. Wang[1], B. Durbin-Johnson[1], C.X. Pan[1]

[1] *University of California- Davis, MED: UROLOGY, Sacramento- CA, USA*
[2] *Veterans Affairs Northen California Health Care System, Research, Mather, USA*

INTRODUCTION: Recurrent prostate cancer (PCa) is usually treated with androgen receptor (AR) antagonists, but the treatment ultimately fails, resulting in the development of castration resistant PCa (CRPC). CRPC patients frequently continue to express an active AR despite castration resistance. We previously showed upregulation of mammalian target of rapamycin (mTOR) activity upon AR inhibition contributing to acquired resistance to therapy, and that a combination of an mTOR inhibitor and an AR antagonist overcoming resistance to AR antagonists alone (Wang et al, Oncogene, 2008;27(56):7106-17). Based on our data, a Phase II clinical trial was conducted to determine the efficacy of the combination of the mTOR inhibitor RAD001 and the AR antagonist bicalutamide in bicalutamide-naïve CRPC patients (ClinicalTrials.gov: NCT00814788), showing a response rate of 75% against the historical control of 25%. The goal of this project was to define pathways that result in resistance to combinations of mTOR and AR inhibitors in CRPC patients.

MATERIAL AND METHOD: Comparison of various mTOR inhibitors either alone or in combination with AR antagonists in prostate derived cell lines including C4-2, PC-346C, 22Rv1 and CWR-R1, identified resistant cell lines (CWR-R1, PC-346C) vs sensitive (22Rv1, C4-2) ones. We then investigated the molecular profile of these cell lines before and after treatment to determine indicators of resistance.

RESULTS AND DISCUSSION: Base-line molecular profile demonstrated that cell lines with high levels of phosphorylated eIF4E (S209) were resistant to mTOR inhibitors. Inhibition of eIF4E phosphorylation resulted in sensitivity of CRPC cells to mTOR inhibitors with AR antagonists. Investigation of the mechanism by which eIF4E phosphorylation levels increased in certain CRPC cells but not in others revealed that expression and transcriptional activity of the AR negatively correlated with the levels of eIF4E phosphorylation. In cells with high basal levels of phospho-eIF4E, bicalutamide further increased eIF4E phosphorylation, whereas those with low eIF4E levels were not further affected. The ability of AR inhibition to suppress eIF4E phosphorylation was mediated by MAP kinase interacting kinase (Mnk).

CONCLUSION: based on these studies, we predict that patients with high basal PSA who express low levels of Mnk phosphorylation are the ones who are likely to respond to the combination of an mTOR inhibitor and an AR antagonist

NO CONFLICT OF INTEREST

523 Actionable targets identified through a molecular profiling of advanced paediatric tumors within the monocenter feasibility study (TRICEPS)

F. Khater[1]

[1] *CHU Sainte-Justine, Hemato-Oncology, Montreal, Canada*

INTRODUCTION: Childhood cancer is a group of heterogeneous complex diseases. Although 80% of these children are cured with conventional therapies, it remains the first cause of death among children in Western countries. A significant number of refractory/relapse patients will eventually succumb to their disease and the lack of therapeutic advances for these patients is even more worrisome. Indeed, no significant progress has been noted over the last decade for these patients, urging the need for new and more effective therapeutic approaches. Precision medicine and more effective personalized targeted therapies (PTT) are a major breakthrough leading to increased cure rates and decreased treatment-related morbidity and mortality for the patients with refractory or relapsed tumours. To address this challenge, the TRICEPS study was initiated on April 2014 at the Sainte-Justine UHC (Montreal, Canada) with an overreaching goal to explore the feasibility of performing genomic-driven targeted therapy in pediatric and adolescent (aged 0-21 years) patients with relapsed or refractory childhood cancer.

MATERIAL AND METHOD: This study offers in-depth genomic and transcriptomic investigation of patient's tumoral material to identify patient-specific alterations and actionable driver mutation(s) that can be targeted with approved targeted drug and within a reasonable clinically-relevant timeframe to assess the feasibility of going from biopsy to a detailed tumour analysis report.

RESULTS AND DISCUSSION: Over a period of 30 months, 44 relapsed/refractory cancer patients were recruited. Twenty-two of them underwent extensive genomic investigation (exomic and transcriptomic sequencing) within a median timeframe of 9.7 weeks from patient enrolment to return of results. Patient screen failures occurred due to benign/necrotic tumor biopsies or low tumour purity resulting in suboptimal DNA/RNA quantity or quality for genomic analysis. In all 22 patients, we have identified clinically relevant genomic alterations (SNVs, indels, fusions, CNAs) and relapse-specific mutations influencing patient management and providing options for personalized interventions. We assessed the functional impact of some of these cancer-specific alterations. This was the case of a novel relapse-specific rearrangement, identified on relapsed childhood ETP-ALL, and leading to asparagine synthetase (ASNS) up-regulation through a promoter exchange. The expression of this fusion was associated with reduced apoptosis following l-asparaginase treatment.

CONCLUSION: This study shows that PPT based on next generation sequencing technology is a powerful approach that could be implemented in the clinic within a foreseeable future to guide treatment of hard-to-treat childhood cancers and to further improve patient care and outcomes.

NO CONFLICT OF INTEREST

524 Clinicopathological correlations of CD44, EGFR and p16 expressions in patients with oropharyngeal squamous cell carcinoma treated by radiation therapy

M. Slavik[1], *M. Hermanova*[2], *J. Sana*[3], *T. Shatokhina*[2], *A. Parwez*[3], *P. Slampa*[1], *O. Slaby*[3]

[1] *Masaryk Memorial Cancer Institute and Medical Faculty of Masaryk University, Department of Radiation Oncology, Brno, Czech Republic*
[2] *St. Anne´s University Hospital and Medical Faculty of Masaryk University, 1 st Department of Pathological Anatomy, Brno, Czech Republic*
[3] *CEITEC- Masaryk University, Department of Molecular Oncology, Brno, Czech Republic*

Backround: The objective of this study was to analyse the expression of epidermal growth factor receptor (EGFR), CD44 and p16 in patients with oropharyngeal squamous cell carcinoma (OSCC) and to assess the significance of expression profiles on the radiation treatment outcomes.

MATERIALS AND METHODS: This retrospecitve study included 80 OSCC patients, of those 73 patients had stage 4 of disease according to AJCC classification. There were only 5 and 2 patients with stage 3 and 2 respectively . All patients underwent definitive radiation therapy in curative intent as a main treatment modality. Concomitant platinum based chemotherapy or targeted therapy with cetuximab was administered in 29 (36%) and 26 (33%) patients respectively. Expressions of CD44, EGFR, and p16 were immunohistochemically examined in primary tumor biopsy specimens of all patients, analyzed, and correlated with clinicopathological parameters including treatment outcomes.

RESULTS: The positive tumor expression rates of p16, EGFR, and CD44 were 24,8 %, 28,8 %, and 8 %, respectively. With median follow-up of 39 months, significant correlation was found between p16 expression and both disease free survival (DFS) (median 20 months for P16- vs not reached for p16+; p=0.0259) and overall survival (OS) (median 31 months for P16- vs not reached for p16+; p=0,004). CD44+/p16- displayed the worst (median 5 months), CD44-/p16- the middle (median 25 months) and CD44-/p16+ the best (median not reached) parameters in DFS (difference in subgroups with p=0,0393). Concordantly, same differences were observed in OS: CD44+/p16- displayed the worst (median 18 months), CD44-/p16- the middle (median 34 months) and CD44-/p16+ the best (median not reached) parameters in OS (difference in subgroups with p=0,0059). With combining p16 and EGFR, the worst OS was identified in subgroup of EGFR+/p16- patients (p=0,033).

CONCLUSION: Immunohistochemically detected molecular profiles combining CD44, p16, and EGFR expressions seem to be efficient in prediction of prognosis and treatment outcomes in patients with OSCC.

This work was financially supported by the Czech Ministry of Health, grant no. 15-31627A, project MZ CR – RVO (MOU, 00209805), by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

NO CONFLICT OF INTEREST

525 The RNA binding protein LARP1 is a post-transcriptional regulator of cisplatin resistance in ovarian cancer

C. Stavraka[1], *T. Hopkins*[1], *S. Ghaem-Maghami*[1], *L. Buluwela*[2], *M. Mura*[1]

[1] *Imperial College London, Surgery and Cancer, London, United Kingdom*
[2] *Oxford University, Oncology, Oxford, United Kingdom*

INTRODUCTION: Ovarian cancer (OC) is the most lethal gynaecological malignancy causing 152,000 deaths annually worldwide. The majority of patients present with advanced disease and eventually develop resistance to cisplatin (CDDP) chemotherapy. Resensitising OC to cisplatin is still an unmet clinical need. The RNA-binding protein LARP1 is highly expressed in OC and is a post-transcriptional regulator of cell survival and tumorigenesis. LARP1 depletion using RNA interference (RNAi) restores platinum sensitivity in cisplatin-resistant OC cell lines showing a synergistic anti-tumour effect with cisplatin.

MATERIALS AND METHODS: The LARP1 protein complex was explored using immunoprecipitation (IP) followed by ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) in the CDDP sensitive OVCAR3 and the resistant OVCAR8 cell lines before and after CDDP treatment. LARP1 interaction with target proteins was validated using western blotting, Duolink Proximity Ligation Assay and Immunofluorescence. Key interactors were selected for further study based on the mass spectrometry score and their biological function in platinum resistance. As LARP1 is a master regulator of mRNA homeostasis, the RNA-dependence of protein-protein interactions was further investigated with comparative immunoprecipitations in the presence and absence of RNAses. To identify mRNA targets synergistically regulated by LARP1 and its key interactors, we comparatively analysed transcriptomic deep sequencing data, followed by further validation with RNA IP and RT PCR.

RESULTS: PABPC1, PABPC4 and YB-1 were identified as strong, RNA dependent LARP1 interactors in both cell lines. These interactions were maintained upon CDDP treatment in the resistant (OVCAR8) but not in the sensitive (OVCAR3) cell line. As YB1 has been reported to play a key role in platinum resistance, its interaction with LARP1 was further investigated. LARP1 and YB-1 are both in complex with, and regulate the mRNA transcripts of the efflux ATPases ATP7A/B, the DNA damage binding protein 2 (DDB2) and the pro-survival factor BCL2, which play a pivotal role in cisplatin resistance.

CONCLUSION: Here, we have identified a key interaction between LARP1 and YB1, which may play an important role in mediating cisplatin resistance via the regulation of key mRNA transcripts. Such interactions could be targeted and lead to a novel therapeutic avenue for the treatment of cisplatin resistant ovarian cancer.

NO CONFLICT OF INTEREST

526 VHLdb: A database of von Hippel-Lindau protein interactors and mutations

G. Minervini[1], *F. Tabaro*[1,2], *F. Sundus*[1], *E. Leonardi*[3], *D. Piovesan*[1], *F. Quaglia*[1], *S.C.E. Tosatto*[1,4,5]

[1] *University of Padova, Biomedical Sciences, Padova, Italy*
[2] *University of Tampere, Institute of Biosciences and Medical Technology, Tampere, Finland*
[3] *University of Padova, Woman and Child Health, Padova, Italy*
[4] *CNR, Institute of Neuroscience, Padova, Italy*
[5] *University of Padova, CRIBI Biotechnology Center, Padova, Italy*

INTRODUCTION: von Hippel-Lindau disease (VHL) is a dominantly inherited familial cancer syndrome displaying a broad spectrum of different phenotypes resulting from mutation in VHL gene. However, only some portion of the interfamilial tumor susceptibility can be correlated with mutations in the disease-causing gene. VHL is a hub protein known to interact with hundreds of different interactors and to be involved in different cellular pathways, which may explain the difficult in attributing a proficient mutations-phenotypes correlation. To shed

light on VHL disease, we presented VHLdb, a database focusing on VHL mutations and interactors.

MATERIAL AND METHOD: VHLdb stores interaction data retrieved from the most important biomedical databases. Two levels of annotation, i.e. manual and automatic, are presented to the final user according to their quality. Germline and somatic mutations have been collected from different sources and annotated with predictions on protein stability. The final mutation dataset is made up of 1074 entries and to the best of our knowledge, it represents the largest publicly available repository of pathogenic pVHL variants. VHLdb is based on MongoDB database engine and uses a mobile-ready interface, allowing VHLdb to be natively accessed from any kind of device.

RESULTS AND DISCUSSION: VHLdb offers simple yet powerful ways to access its data. The home page features a map, redirecting the user to interface-specific pVHL interaction lists. The interaction page features a graphical representation of the manually curated pVHL interaction network organized by interacting surface and a sortable, searchable and filterable table. The mutation page lists all coding variants in a user-friendly modifiable table. Statistical analysis shows that the interactors distribution differs throughout the pVHL interfaces. We also found interactors competing for the same interface suggesting pVHL functions beyond the well-known HIF-1α degradation. We also found that 190 mutations affect this area address for three main VHL phenotypes.

CONCLUSION: VHLdb can be particularly helpful for mutation-correlation studies and to optimize VHL syndrome therapies. To this regard, information in VHLdb may serve the scientific community to decipher data derived from tumor genome sequencing projects as well as to provide high quality data to be included in novel tailored patients-specific therapeutic approaches.

NO CONFLICT OF INTEREST

527 Whole exome sequencing identifies novel drug target in mucosal melanoma

J. Hintzsche[1], N. Gorden[1], C. Amato[1], J. Kim[1], K. Wuensch[2], C. Kasey[1], T. Medina[1], K. Wells[1], A.C. Tan[1], W. Robinson[1]

[1] University of Colorado Anschutz Medical Campus, Medical Oncology, Denver, USA

INTRODUCTION: A rare subtype of melanoma is mucosal melanoma which lacks a UV signature and have a poor prognosis due to the absence of common driver mutations found in cutaneous melanoma. By understanding the molecular landscape of these rare melanomas, we unlock the potential to find novel therapeutic targets.

MATERIAL AND METHODS: The largest known cohort of mucosal melanoma patient samples (39 tumor samples from 19 patients) underwent whole exome sequencing (WES). For comparison, 135 sun-exposed cutaneous melanomas also underwent WES. Matched peripheral blood samples were used when available. Variants were called using the IMPACT WES pipeline. Chi-square tests compared the genes mutated between these cohorts, where a p-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION: The mucosal melanoma cohort had 161 genes that were enriched compared to cutaneous melanoma ($p < 0.05$). Within these genes, KIT and NF1 were found more frequently in mucosal melanoma and were often commutated (32%). The most significantly mutated gene was SF3B1(7/19, 37%), with recurrent mutations at R625H/S/C that were validated by PCR. SF3B1 has previously been found to be a driver in uveal melanoma but has never been identified in vulvovaginal mucosal melanoma. Mutations in other spliceosome pathway genes were also found to be enriched in mucosal melanoma. Four genes were found to be alternatively spliced in SF3B1 mutant samples compared to SF3B1 wild-type samples.

CONCLUSION: Recurrent R625 mutations in SF3B1 as well as the co-mutation of NF1 and KIT have expanded the knowledge of this rare disease. This study is the largest WES project of mucosal melanomas to date, and the first to validate SF3B1 mutations in vulvovaginal mucosal melanoma. This study also validated that alternative splicing is occurring in SF3B1 mutant samples in several genes and proposes SF3B1 as a novel drug target in mucosal melanoma.

NO CONFLICT OF INTEREST

528 The deubiquitinase USP13 as a novel therapeutic co-target in EGFR mutant non-small cell lung cancer

P. Giron[1], C. Eggermont[1], E. Teugels[1], G.J. Gutierrez[2], J. De Grève[1]

[1] Vrije Universiteit Brussel- UZ Brussel, Laboratory of Molecular Oncology and Department of Medical Oncology- Oncologisch Centrum, Brussel, Belgium
[2] Vrije Universiteit Brussel, Laboratory of Pathophysiological Cell Signaling, Brussel, Belgium

BACKGROUND: In 15-30% of non-small cell lung cancers (NSCLC), mutations in the epidermal growth factor receptor (EGFR) mutations lead to a constitutive activation of the receptor involved in cancer survival, proliferation and metastasis. Targeted therapies developed against EGFR have only resulted into transient better clinical outcomes for patients who ultimately relapse due to the limited efficacy of the treatments.

We postulate the existence of intrinsic mechanisms in NSCLC responsible for the early insensitivity and limited efficacy of EGFR inhibitors. Further, co-targeting of those mechanisms should considerably improve treatments efficacy. In this study, we aimed to identify and characterize proteins involved in early insensitivity against EGFR inhibitors in EGFR mutant NSCLC.

MATERIALS AND METHODS: We have performed an siRNA high-throughput screen targeting kinase and ubiquitin-related libraries in the presence of sub-lethal doses of afatinib in EGFR mutant NSCLC cells. Targets identified on the screen were validated in vitro using cells viability and apoptosis assays. EGFR signaling pathways and the molecular crosstalk between EGFR and USP13 were analyzed using classical biochemical assays such as immunoblotting and immunoprecipitation.

RESULTS: We have identified the ubiquitin specific peptidase 13 (USP13) as a target that when downregulated strongly synergizes with afatinib in drastically reducing the survival of NSCLC cells in vitro. The combination of USP13 siRNA or USP13 inhibitor (spautin-1) with 5 nM afatinib led respectively to only 7±1.2% and 11±0.7% viability compared to vehicle control in PC9 cells. We also found that co-targeting of EGFR and USP13 strongly induced apoptosis. Moreover, co-targeted cells displayed decreased EGFR phosphorylation and downstream signaling but remarkably also showed reduced total levels of EGFR.

Interestingly, a time-course stability analysis of EGFR using cycloheximide showed that both USP13 siRNA and spautin-1 led to a reduced half-life of EGFR. Since USP13 acts as a deubiquitinase, we further evaluated the effect of USP13 inhibition on EGFR ubiquitination and found increased levels of EGFR ubiquitination upon USP13 inhibition.

CONCLUSIONS: We have identified USP13 as a co-target in NSCLC cells treated with afatinib. Remarkably, USP13 seems to regulated EGFR signaling by directly controlling EGFR stability in cells.

NO CONFLICT OF INTEREST

529 A new monoclonal antibody detects down-regulation of Protein Tyrosine Phosphatase Receptor Type γ in chronic myeloid leukemia patients

M. Vezzalini[1], A. Mafficini[2], L. Tomasello[1], M. Krampera[2], M. Yassin[3], N. Al-Dewik[4], M.A. Ismail[5], A. Al Sayab[3], M. Monne[6], C. Sorio[1]

[1] University of Verona, Department of Medicine, Verona, Italy
[2] University and Hospital Trust of Verona, ARC-Net Research Centre, Verona, Italy
[3] Hamad Medical Corporation, National Center for Cancer Care and Research NCCCR, Doha, Qatar
[4] Hamad Medical Corporation, Qatar Medical Genetics Center, Doha, Qatar
[5] Hamad Medical Corporation, Interim Translational Research Institute, Doha, Qatar
[6] "San Francesco" Hospital-, Centro di Diagnostica Biomolecolare e Citogenetica Emato-Oncologica, Nuoro, Italy

INTRODUCTION: Protein Tyrosine Phosphatase Receptor Gamma (PTPRG) is a ubiquitously expressed member of the protein tyrosine phosphatase family known to act as a tumor suppressor gene in many different neoplasms with mechanisms of inactivation including mutations and methylation of CpG islands in the promoter region. Although a critical role in human hematopoiesis and an oncosuppressor role in chronic myeloid leukemia (CML) have been reported, only one polyclonal antibody (named chPTPRG) has been described as capable of recognizing the native antigen of this phosphatase by flow cytometry. Protein biomarkers of CML have not yet found applications in the clinic and in this study we have analyzed a group of newly diagnosed CML patients before and after treatment.

MATERIAL AND METHOD: TPγ B9-2 monoclonal antibody was produced by ARETA International srl (Gerenzano VA, Italy) from BALB-C mice that were challenged with the purified extracellular domain of human PTPRG. Fresh leukapheresis or peripheral blood samples were obtained with written informed consent from patients with CP CML at diagnosis prior to treatment or non-CML donors. PTPRG was detected by Immunoflurescence, immunohistochemistry, western blotting and flow cytometry

RESULTS AND DISCUSSION: We characterized a newly developed murine monoclonal antibody specific for the PTPRG extracellular domain (named TPγ B9-2) to better define PTPRG protein down-regulation in CML patients. TPγ B9-2 specifically recognizes PTPRG (both human and murine) by flow cytometry, western blotting, immunoprecipitation and immunohistochemistry. Co-localization experiments performed with both anti-PTPRG antibodies identified the presence of isoforms and confirmed protein down-regulation at diagnosis in the Philadelphia positive myeloid lineage (including $CD34^+$/ $CD38^{bright/dim}$ cells). After effective tyrosine kinase inhibitors (TKI) treatment its expression recovered in tandem with return of Philadelphia negative hematopoiesis. Of note PTPRG mRNA levels remain unchanged in TKI non-responder patients, confirming that down-regulation selectively occurs in primary CML cells

CONCLUSION: These result confirm a role of PTPRG in the pathogenesis of CML and suggest potential application in a clinical setting.

NO CONFLICT OF INTEREST

530 Correlation of circulating tumor DNA (ctDNA) level and Non-Small Cell Lung Cancer (NSCLC) tumor burden as approximated by RECIST criteria

V. Lam[1], J. Zhang[2], L. Li[2], H. Tran[1], W. Rinsurongkawong[3], K. Banks[4], R. Lanman[4], J. Wang[2], V. Papadimitrakopoulou[1], J.J. Lee[3], J. Heymach[1]

[1] MD Anderson Cancer Center, Thoracic/Head and Neck Medical Oncology, Houston, USA
[2] MD Anderson Cancer Center, Dept. of Bioinformatics and Comp Biology, Houston, USA
[3] MD Anderson Cancer Center, Dept. of Biostatistics, Houston, USA
[4] Guardant Health, Medical Affairs, Redwood City, USA

BACKGROUND: Studies to date have not detailed how absolute ctDNA levels may correlate with tumor burden in NSCLC.

MATERIALS AND METHODS: We reviewed ctDNA result of NSCLC patients that were tested at our institution between November 2015 and December 2016 with Guardant 360™, a ctDNA assay that detects single nucleotide variants and other important alterations in 70-73 cancer genes. 138 cases with evaluable imaging were selected for this analysis, enriching for EGFR and KRAS mutated cases to facilitate comparisons of major driver mutations (Table 1). Tumor burden was approximated using the sum of the longest diameters (SLD) per RECIST v1.1 criteria. Interactions between ctDNA level (defined as the maximum variant allele frequency detected) and SLD were analyzed.

RESULTS: Overall, there was a statistically significant correlation of moderate strength between ctDNA level and SLD (Spearman's rho = 0.35, $p < 0.001$). In a subgroup analysis of major mutation types, KRAS positive cases significantly strengthened this correlation (Spearman's rho = 0.52, p = 0.001), while EGFR positive cases had a trend towards weaker correlation (rho = 0.21, $p < 0.24$) compared to wild-type (rho = 0.45, $p < 0.001$). Adenocarcinoma histology also contributed to this correlation (rho = 0.35, p = 0.003), but not significantly different to the squamous cell carcinoma subgroup. Multi-variate regression that included stage, histology, and mutation status confirmed the predictive value of ctDNA level for SLD (p = 0.03).

CONCLUSIONS: In this primarily metastatic cohort, circulating tumor DNA level correlated with the radiographic assessment of tumor burden per RECIST criteria. This correlation was mediated in part by histology and the presence of key driver mutations. These findings have potential implications for the application of ctDNA in NSCLC early detection and minimal residual disease monitoring.

Table 1: Baseline characteristics

Characteristic	Number (%)
Stage	
II	3 (2.2)
III	7 (5.1)
IV	128 (92.8)
Histology	
Adenocarcinoma	101 (73.2)
Squamous cell	37 (26.8)
Alteration	
TP53 mutation	63 (45.7)
EGFR mutation	34 (24.6)
KRAS mutation	34 (24.6)
ALK fusion	4 (2.9)

CONFLICT OF INTEREST

Other Substantive Relationships: Kim Banks and Richard Lanman are employees of Guardant Health

531 The EMT blocking via increasing E-cadherin expression

M. Davoodzadeh Gholami[1], R. Falak[1], E. Safari[1], G.A. Kardar[2]

[1] Iran Uniuersity of Medical Sciences, School of Medicine- Department of Immunology, Tehran, Iran
[2] Tehran Uniuersity of Medical Sciences, Immunology Asthma & Allergy Research Institute, Tehran, Iran

INTRODUCTION: In spite of many advances in cancer treatment, unfortunately the majority of deaths caused by metastasis in these patients. Ephitelial mesenchymal transition (EMT) process have an important role in the metastasis of cancer. In this process one of critical change is down regulation of E-cadherin in epithelial cells.

METHODS: In this study, we designed a DNA vaccine that prevents down regulation of E-cadherin by interferencing Snail effect on E-box of E-cadherin promoter. After transfecting of A549 cell lines with pIRES2-ZF-eGFP construct, the EMT in these cells induced by TGFβ and expression of EMT process genes like E-cadherin, Vimentin, N-cadherin, and β-cathenin were evaluated by real-time PCR. The transfection efficiency was monitored by Inverted Fluorescent Microscope.

RESULTS: data were shown transfection efficiency was more than 70% in transfected cells. The result of relative expression of studies gene were shown in transfected group, in compare of EMT induced only, N-cadherin and Vimentin were decreased and E-cadherin and β-cathenin were increased.

CONCLUSION: the result of this study shown by advantage of a DNA vaccine strategy can block EMT and metastasis of cancer cells. This is a new therapeutic strategies to cancer gene therapy.

NO CONFLICT OF INTEREST

532 CCR5 blocking by Maraviroc inhibited microenvironmental interactions and exerted antitumor activity in tumor xenograft model of classical Hodgkin Lymphoma

N. Casagrande[1], C. Borghese[1], M. Mongiat[1], A. Colombatti[1], D. Aldinucci[1]

[1] CRO Aviano National Cancer Institute, Department of Experimental Oncology 2 , Aviano- Pordenone, Italy

The complex interactions between cancer and normal cells accumulating in classical Hodgkin Lymphoma (cHL) tissues promote tumor growth, immunosuppression and drug resistance. cHL lymph nodes are characterized by high levels of CCL5, a chemokine associated with cancer progression.

Using neutralizing anti-CCL5 antibodies and the CCR5 antagonist Maraviroc (MVC), we demonstrated that CCL5 secreted by cHL cells recruited Mesenchymal Stromal cells (MSCs), as well as monocytes. Treatment or 'education" of MSCs (E-MSCs) and Monocytes (E-mon) with cHL cells conditioned medium increased the proliferation of MSCs and monocytes. In turn, CCR5 ligands secreted by E-MSCs and E-mon, increased the clonogenic growth of cHL cells. Only CM from E-mon inhibited the proliferation of PHA activated lymphocytes, demonstrating an 'immunosuppressive" potential.

MVC decreased the proliferation of cHL cells, affected the cell cycle phases, synergized with doxorubicin and exerted additive effects with gemcitabine, cisplatin and vinorelbine.

Moreover, we set up a new tridimensional (3D) approach to study the tumor microenvironment (TME) interactions. For this purpose, we cultured cHL cells, together with MSCs and monocytes, in non-adherent conditions. Tumor and normal cells self-assembled and formed compact 3D hetero-spheroids, mimicking the TME. MVC counteracted the formation and reduced cell viability of hetero-spheroids. Finally, MVC alone led to a 50% tumor growth reduction of L-540 derived tumor xenografts without toxicity.

This study demonstrated that CCR5 ligands secreted by cHL cells, as well as by recruited and then educated MSCs and monocytes, may contribute to build a protumorigenic and immunosuppressive TME. Therefore, the repurposed drug MVC, inhibiting the proliferation of cHL cells and affecting TME interactions, may represent a new therapeutic option for relapsed-refractory cHL.

NO CONFLICT OF INTEREST

533 Molecular profiling of circulating tumour DNA to stratify patients to early phase clinical trials within the MCRC TARGET trial

D.G. Rothwell[1], M. Ayub[1], B. Kilerci[1], S. Gulati[1], A. Wallace[2], C. Dive[1], N. Cook[3], E. Dean[3], F. Thistlethwaite[3], M.G. Krebs[3], G. Brady[1]

[1] Clinical & Experimental Pharmacology Group, CR-UK Manchester Institute University of Manchester, UK
[2] Manchester Centre for Genomic Medicine, St. Mary's Hospital, University of Manchester
[3] The Christie NHS Foundation Trust and Division of Molecular and Clinical Cancer Sciences, Faculty of Biology, Medicine and Health

INTRODUCTION: The Tumour chARacterisation to Guide Experimental Targeted Therapy Trial (TARGET), a large multi-team trial being run at the Manchester Cancer Research Centre (MCRC) tests the hypothesis that molecular profiling of both archival/fresh tumour and circulating tumour DNA (ctDNA) can be used to stratify patients to early phase trials of targeted therapies to maximise patient benefit. Here we present data generated in the validation of NGS analysis of ctDNA from the initial 100 patients.

MATERIALS AND METHODS: Patients were consented for molecular analysis of tumour and blood. Tumour was analysed by Sequenom OncoCarta and/or a targeted 24 gene Next Generation Sequencing (NGS) panel. ctDNA was subjected to NGS and bioinformatic analysis of a panel of >600 genes known to be frequently mutated in cancer. Concordance of variant calls between the two was evaluated. Clinical reports from tumour and blood were generated for discussion in a monthly Molecular Tumour Board (MTB) to identify possible driver aberrations and to aid clinicians in selection of relevant experimental medicine trials.

RESULTS AND DISCUSSION: Initial work focused on the development and optimisation of ctDNA sequencing, bioinformatics analysis and making the ctDNA workflow clinically compliant. The current ctDNA pipeline identified at least one mutation within ctDNA from 84% (84/100) of samples. For the first 40 samples concordance between tumour and ctDNA was 81% (17/21 mutations identified in tumour also picked up in ctDNA). Validation of clinically relevant mutations detected only within ctDNA was confirmed by droplet digital PCR and/or repeated NGS, with mutation calls confirmed in all cases (4/4). The reporting time from collection of blood sample to generation of ctDNA NGS report has been minimised to be clinically relevant, with the intention of achieving a 12-day turnaround.

CONCLUSIONS: Our result support the use of ctDNA for routine molecular characterisation of phase I patients. The success of the overall approach for the initial 100 patients will be presented, the data for which has led to the scale up of patient recruitment to ~450 patients over the next 3 years. The focus of ongoing

work will be to achieve GCLP compliance for the workflow and facilitate the allocation of patients to clinical trials based on ctDNA and/or tumour molecular profiling. Monitoring of treatment response and emerging resistance mechanisms using serial blood samples will also be established.

NO CONFLICT OF INTEREST

534 Using a mouse glioma model to study the participation of tumor vasculature in the response to therapy

E. Hernandez-SanMiguel[1], R. García-Ferreras[1], B. Herranz[1], B. Segura[1], J.M. Sepulveda[2], A. Hernandez[2], V. Perez-Garcia[3], P Sanchez-Gomez[1]

[1] Instituto de Salud Carlos III, Madrid, Spain
[2] Hospital 1 2 de Octubre, Madrid, Spain
[3] Universidad de Castilla la Mancha, Ciudad Real, Spain

Glioblastoma (GBM) is the most common primary brain tumor. Although it is a rare disease, it has a very bad prognosis. Standard treatment for GBM consist of an aggressive surgery, followed by radiotherapy and concomitant Temozolomide (TMZ) treatment. However, GBM is a very resilient tumor and the overall survival of patients with this type of cancer is 15 months. Forty percent of GBMs show amplification of *EGFR* and, in half of those, the extracellular domain of the receptor is deleted, giving rise to a truncated isoform (EGFRvIII) that has a stronger tumorigenic potential. We have recently participated in a Phase II trial with dacomitinib, a second-generation tyrosine kinase inhibitor that binds irreversibly to the receptor and inhibits the wild type (wt) and the truncated isoform. However, despite the good result obtained in patient-derived xenograft (PDX), only a very small percentage of patients responded in a preclinical study.

One of the aspects that complicates GBM treatment is the brain location and the presence of a specialized blood brain barrier (BBB) that prevents drug delivery. However, profuse endothelial proliferation and disruption of the BBB are some of the histological features of GBM. Interestingly, these characteristics are rarely seen in the commonly used PDX models. Moreover, tumor-vasculature has been involved in the proliferation, survival and invasion of tumor cells. Still, the crosstalk between human glioma cells and the mouse microenvironment is far from being optimal. In order to evaluate the role of vascular cells in the innate chemoresistance of GBM, we have generated a new glioma model by overexpressing EGFRwt or EGFRvIII in p16/p19 KO mouse neural progenitors. Co-culture studies in the presence of a mouse endothelial cell line suggest a protective role of these cells in response to chemotherapy. Moreover, analysis of the response of subcutaneous and intracranial allografts to TMZ and dacomitinib, show striking differences between EGFRwt and EGFRvIII expressing tumors. Preliminary data suggest that the vasculature of these two models is behind these differential results. We are confident that our model could help us to understand the poor response of GBM to chemotherapy, and probably to find predictive markers associated with the tumor-vessel architecture and/or function.

NO CONFLICT OF INTEREST

535 Characterizing metformin treatment response and sensitivity biomarkers in breast cancer

L.Anthes[1], J. Salsman[1], G. Dellaire[1,2]

[1] Department of Pathology, Dalhousie University, Halifax, Canada
[2] Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax, Canada

INTRODUCTION: The anti-diabetic drug metformin has been shown to exhibit broad in vitro and in vivo anti-cancer activity against multiple tumour types, including breast cancer. Multiple clinical trials are underway to evaluate metformin as an adjuvant to existing chemotherapies. However, there are currently no targeted biomarkers to predict tumour sensitivity to metformin, or to monitor cancer response during and after metformin treatment.

MATERIALS AND METHODS: We have recently conducted gene expression profiling and quantitative proteomics of metformin-conditioned MDA-MB-231 breast cancer cells to identify genetic markers of metformin response. We are evaluating candidate genes in a panel of 13 breast cancer cell lines through gene and protein quantification, cDNA expression, and short-hairpin RNA knockdown/CRISPR-Cas9 knock-out studies. Work is currently underway to evaluate these genes as predictive clinical biomarkers for metformin response in a cohort of metformin-treated breast cancer patients.

RESULTS AND DISCUSSION: We have discovered a novel gene in connection to metformin, aldo-keto reductase 1C3 (AKR1C3), an enzyme involved in steroid metabolism and modulation of oxidative stress. We have found that AKR1C3 is upregulated in several breast cancer types in vitro with both acute and long-term metformin treatment. In a panel of 13 breast cancer cell lines, we have found that endogenous AKR1C3 expression correlates with metformin sensitivity.

CONCLUSION: Our result indicate that AKR1C3 is potentially a predictive biomarker of metformin sensitivity in breast cancer, and that it may also serve as a therapy response marker that is upregulated with metformin treatment.

NO CONFLICT OF INTEREST

POSTER SESSION: TUMOUR IMMUNOLOGY I

536 Selective expression of a potent anti-survival protein, Clusterin, in monocytes allows exquisite chemotherapeutic targeting of Myeloid-Derived Suppressor Cells in breast cancer

J. Djeu[1], T.L. Trinh[1], N. Tu[1], J.M. Zhou[1], D. Gilvary[2], D. Coppola[2], S. Wei[1]

[1] Moffitt Cancer Center, Immunology, Tampa- Florida, USA
[2] Moffitt Cancer Center, Pathology, Tampa- Florida, USA

INTRODUCTION: Chemotherapy can ideally cause immunogenic cell death of tumor cells, providing antigens to activate monocytes/dendritic cells. However, such antigen presentation fails in a tumor microenvironment because of predominance of myeloid-derived suppressor cells (MDSCs). This study focused on eliminating immunosuppressive MDSCs and uncovered a novel pathway mediated by Clusterin (CLU).

MATERIAL AND METHOD: Mice bearing subcutaneous 4T1 breast tumors were treated with docetaxel (DTX) or curcumin and splenocytes were evaluated for presence of CD11b+Gr1+ MDSCs and their subsets, Ly6G+ PMN-MDSCs and Ly6C+ M-MDSCs by flow cytometry. Tumor-associated macrophages, CCR7+F4/80+ MI and Dectin1+M2 cells, were also evaluated. CLU and active Bax were evaluated by western blot analysis of cell lysates and by immunohistochemistry of tissue sections. Anti-sense CLU was transfected into Ly6C+MDSCs or RAW264.7 macrophages prior to analysis of chemosensitivity to DTX or curcumin.

RESULTS AND DISCUSSION: Both curcumin and DTX prevented tumor growth in vivo and tumor regression was accompanied by a loss of PMN-MDSCs and M2 cells but not M-MDSCs with a gain in M1 cells. IFNg+ CD4 and CD8 T cells were also induced, with effective tumoricidal function. Mechanistically, both drugs directly caused apoptosis in PMN-MDSCs. M-MDSCs were spared because of selective expression of a potent anti-apoptotic protein, CLU, allowing their maturation into M1 cells. Chemoresistance was reversed by anti-sense CLU transfection into M-MDSCs or RAW264.7 macrophages, verifying the key role of CLU in protection of monocytic cells. Of note, both drugs activated Bax but it was tightly bound by CLU in the cytoplasm, sequestering Bax from translocation to mitochondria to cause apoptotic cell death. Histochemical analysis of 20 human breast cancer samples detected major infiltration of immature myeloid cells, not seen in normal breast tissues. More importantly, mature CD68+ M1 macrophages but not CD33+ immature myeloid cells expressed high levels of CLU in the human tumor tissues.

CONCLUSION: Our studies demonstrate that chemotherapeutic agents can reverse immunosuppression by directly targeting the dominant PMN-MDSCs which lack CLU. Conversely, M-MDSCs and M1 cells are spared because CLU prevents active Bax from translocating to mitochondria to trigger the caspase cascade. Thus, CLU plays a defining role in positive immune modulation by chemotherapeutic agents against cancer.

NO CONFLICT OF INTEREST

537 Microenvironment in tumor-draining lymph nodes from patients with HPV-related vulvar cancer

M. Heeren[1], S. Samuels[2], H. Zijlmans[2], J. Rotman[1], J. Van der Velden[3], K. Van de Vijver[4], M. Bleeker[5], G. Kenter[6], E. Jordanova[6], T. De Gruijl[7]

[1] VU University Medical Center-Cancer Center Amsterdam, Centre for Gynecologic Oncology Amsterdam/ Medical Oncology, Amsterdam, Netherlands
[2] AuL-NKI, Centre for Gynecologic Oncology Amsterdam, Amsterdam, Netherlands
[3] Academic Medical Center AMC, Centre for Gynecologic Oncology Amsterdam, Amsterdam, Netherlands
[4] AuL-NKI, Pathology, Amsterdam, Netherlands
[5] Academic Medical Center AMC/VU University Medical Center, Pathology, Amsterdam, Netherlands
[6] VU University Medical Center-Cancer Center Amsterdam, Centre for Gynecologic Oncology Amsterdam, Amsterdam, Netherlands
[7] VU University Medical Center-Cancer Center Amsterdam, Medical Oncology, Amsterdam, Netherlands

INTRODUCTION: Persistent infection with human papillomavirus (HPV) is responsible for circa 40% of all vulvar tumors. Detailed studies are lacking on the effects of primary tumor development and metastatic spread on the microenvironment of vulvar tumor-draining lymph nodes (TDLN). Nevertheless, such studies may yield vital information for the development of effective immunotherapies to halt metastatic spread.

MATERIAL AND METHOD: We investigated the microenvironment of TDLNs of patients with HPV-related vulvar cancer by comprehensive four-color flowcytometry-based phenotyping and enumeration of different T cell and myeloid subsets. Tumor-negative (LN- n=11) and tumor-positive TDLN (LN+ n=4) were obtained after surgery as primary treatment.

RESULTS AND DISCUSSION: We found significantly higher $CD4^-CD8^-$ T cell and FoxP3+ regulatory T cell (Treg) rates in LN+ as compared to LN-. Assessment of the expression of the immune checkpoints CTLA-4 and PD-1 on the T cell subsets showed selective up-regulation of $CTLA4^+CD4^+$ T cell rates in tumor involved versus tumor negative LN ($p<0.03$). Frequencies of all studied conventional and plasmacytoid dendritic cell (DC) subsets were significantly increased in LN+. Of note, CD1a+ migratory DC subsets were suppressed in LN+ with significantly lower levels of CD40, CD83 and PD-L1, whereas lymph node (LN-)resident DC (both CD14-conventional and CD303+ plasmacytoid DC subsets) showed signs of activation with higher levels of CD80/CD86 and of CD40, respectively.

CONCLUSION: Higher frequencies of potentially suppressive T cell subsets ($CD4^-$ $CD8^-$ T cells and Tregs) and of $CTLA4^+CD4^+$ T cells are present in LN+ as compared to LN- from patients with HPV-related vulvar cancer. Interestingly, while all DC subsets were more abundantly present in LN+ than in LN-, migratory and LN-resident subsets were differentially affected, with the former showing signs of suppression and the latter of activation in LN+. Clearly, the numbers of included samples in this study will have to be increased to obtain higher statistical power, but our preliminary data clearly point to the potential of (local) CTLA-4 blockade in the treatment of early-stage or locally advanced HPV-related vulvar carcinoma, in order to counter apparent immune suppression.

NO CONFLICT OF INTEREST

538 Distinct immune status in patients with adenocarcinoma and squamous cell carcinoma: Implication for immunotherapy of non-small cell lung cancer

N. Hradilova[1,2], O. Palata[1,2], L. Sadilkova[2], D. Mysikova[3], H. Mrazkova[3], R. Lischke[3], R. Spisek[1,2], I. Adkins[1,2]

[1] Charles University and University Hospital Motol, Department of Immunology- 2 nd Faculty of Medicine, Prague, Czech Republic
[2] Sotio, Research department, Prague, Czech Republic
[3] Charles University and University Hospital Motol, Thoracic and Lung Transplantation Division- 3 rd Department of Surgery- 1 st Faculty of Medicine, Prague, Czech Republic

Lung cancer is the leading cause of cancer mortality worldwide therefore understanding the biological role of immune cell infiltrates in the tumor microenvironment is of an eminent interest.

In this study we compared immune cell populations, T cell responses and secreted cytokines in primary tumors and non-tumoral lung tissue from more than 40 adenocarcinomas (AC) and 40 squamous cell carcinomas (SCC) of non-small cell lung cancer (NSCLC) patients undergoing neoadjuvant surgery. Moreover, we compared immune suppressive populations such as $CD4^+CD25^+Foxp3^+$ T regulatory cells and myeloid-derived suppressor cells (MDSC) and the expression of immune checkpoint molecules PD-1, TIM3, LAG-3 and CTLA-4 in the blood of these patients.

In both tumor subtypes we observed similarly higher infiltration of B cells, memory T cells, dendritic cells, NK cells, monocytes/macrophages, mast cells and $CD4^+CD25^+Foxp3^+$ T regulatory cells compared to non-tumoral tissue. However, immune cells seemed to be suppressed functionally more in SCC than AC as we detected significantly lower IFN-γ-positive T cells and production of proinflammatory cytokines after stimulation. PD-1, TIM3 and LAG-3 expression on T cells in blood and PD-1 on intratumoral $CD8^+$ T cells of SSC patients were significantly elevated compared to AC patients. Similarly, the high number of MDSC, which correlated with Arginase 1 mRNA levels and downregulation of CD3z in T cells, was detected only in SCC.

These result suggest that immune system of SSC patients might be subjected to a higher systemic and tumor–associated immune suppression than in AC patients. This should be taken into consideration in designing lung cancer immunotherapeutic approaches.

NO CONFLICT OF INTEREST

539 Formyl peptide receptor 1 inhibits gastric cancer angiogenesis and growth by controlling omega-3 and omega-6 polyunsaturated fatty acids metabolism

N. Prevete[1], F. Liotti[2], E. Pone[2], A. Illiano[3], A. Angela[3], P. Pucci[3], A. De Paulis[1], R.M. Melillo[2]

[1] University of Naples Federico II, Translational Medical Sciences, Naples, Italy
[2] University of Naples Federico II, Molecular Medicine and Medical Biotechnology, Naples, Italy
[3] University of Naples Federico II, Chemistry, Naples, Italy

INTRODUCTION: Formyl peptide receptors (FPR1, 2 and 3) are innate immune receptors expressed by leukocytes and non-immune cells recognizing exogenous/endogenous danger signals. We recently demonstrated that human FPR1 controls xenograft growth of gastric cancer (GC) cells by inhibiting angiogenesis. FPR1 blockade in GC cells caused a drop in the production of Resolvin D1 (RvD1) and Lipoxin B4 (LXB4), lipidic specialized proresolving mediators (SPMs) involved in the resolution of the inflammatory process. RvD1 efficiently suppressed spontaneous and induced angiogenesis in parental and FPR1-silenced GC cells through the interaction with the GPR32 receptor. Accordingly, FPR1 activation in GC cells maintained the SPMs level required for the control of angiogenesis and tumor growth. As SPMs derive from the activity of lipoxygenases (ALOX5 and 15) on ω-6/3 essential polyunsaturated fatty acids (PUFA), we asked whether PUFA could affect GC cell neoplastic features, including angiogenesis.

MATERIAL AND METHODS: ω-6/3 modulation of proresolving pathways and angiogenesis was studied in in vitro cultures of GC cells genetically engineered to overexpress or silence FPR1, ALOXs, GPR32; ω-6/3 effects on GC cell tumorigenesis were evaluated by xenotransplants in immunodeficient mice fed balanced, ω-3- or ω-6-rich diets; ω-6/3 modulation of lipidic metabolites was evaluated by lipidomic analysis.

RESULTS AND DISCUSSION: Arachidonic Acid (AA - ω-6), Docosahexaenoic Acid (DHA - ω-3) and Eicosapentaenoic Acid (EPA - ω-3) treatment restored SPMs production and inhibited angiogenic activity of FPR1-silenced GC cells. The antiangiogenic effect of PUFA could be ascribed to their metabolization to SPMs, as it was abolished by ALOX5/15 or GPR32 blockade. AA, DHA and EPA significantly reduced GC cell viability and growth when used at supraphysiological concentrations (50 μM), being this effect independent from their metabolization to SPMs. Mice administration of ω-3 or ω-6 PUFA-enriched diets enforced endogenous production of SPMs and inhibited xenograft growth of FPR1-silenced GC cells by reducing their angiogenic activity and proliferation index.

CONCLUSION: We describe a novel mechanism controlled by FPR1 in GC linking ω-6/3-derived SPMs with angiogenesis. As SPMs production can be largely determined by diet, increasing ω-3 or ω-6 consumption might be useful in the management of GC.

NO CONFLICT OF INTEREST

540 Immune microenvironment of experimental rat C6 gliomas resembles human glioblastomas and is modulated by tumor-derived Osteopontin

A. Gieryng[1], D. Pszczolkowska[1], M. Kloss[1], M. Dabrowski[2], J. Mieczkowski[1], B. Kaminska[1]

[1] Neurobiology Center- The Nencki Institute of Experimental Biology- PAS, Laboratory of Molecular Neurobiology, Warsaw, Poland
[2] Neurobiology Center- The Nencki Institute of Experimental Biology- PAS, Laboratory of Bioinformatics, Warsaw, Poland

BACKGROUND: Cancer immunotherapies exploit various approaches, ranging from stimulating effector mechanisms to counteracting inhibitory and suppressive mechanisms. Reliable animal models are critical for evaluating immunotherapies and for defining tumor immunology mechanisms. Histopathological and flow cytometry analyses of human GBM and rodent experimental gliomas showed cell heterogeneity of the tumor microenvironment, and its infiltration with microglia and peripheral macrophages, granulocytes, myeloid-derived suppressor cells (MDSCs) and T lymphocytes. In the present study, we analyzed the immune composition of rat C6 gliomas, global transcriptomes of glioma-bearing hemispheres and $CD11b^+$ cells immunosorted from C6 gliomas. We evaluated effects of knockdown of Osteopontin (Spp1, secreted phosphoprotein 1), a potent immune cell attractant and activator, secreted by glioma cells.

MATERIAL AND METHOD: Glioma C6 cells, shSpp1 and shNeg cells were intracranially implanted to Wistar rats. Lentivirally delivered shRNA were used to permanently knockdown Spp1 in glioma cells. After 14 or 21 days both tumor-bearing hemispheres and CD11b+ cells infiltrating gliomas were sorted from gliomas and processed. Transcriptome profiling with Affymetrix Rat Gene 2.1 ST microarray and quantitative RQ-PCR were performed.

RESULT AND DISCUSSION: Composition of immune infiltrates in C6 gliomas indicates early and predominant accumulation of microglia followed by macrophages and MDSCs. Transcription profiling of tumor infiltrating $CD11b^+$ cells shows non-inflammatory, immunosuppressive activation. Knockdown Spp1 in glioma cells does not affect microglia accumulation, but blocks protumorigenic activation and significantly reduces tumor growth. $CD11b^+$ cells sorted from control and Spp1 depleted gliomas show different gene profiles and moreover, the expression of genes characteristic for the protumorigenic activation is reduced in Spp1 depleted gliomas. **Conclusions**. Overexpression of invasion and immunosuppression-related genes in glioma-bearing hemispheres and immunosorted microglia suggests that rat C6 gliomas employ similar immune evasion strategies as human GBMs and represent a good model of an immunocompetent host for preclinical studies. Moreover, our result confirm a critical role of tumor-derived Spp1 in shaping glioma microenvironment.

Supported by 2012/04/A/NZ3/00630 grant from the National Science Center.

NO CONFLICT OF INTEREST

541 Liposomal phosphodiester (PO)-CpG enhanced recombinant lipidated HPV E7-induced antitumor immunity

S.J. Liu[1,2], K.Y. Shen[1], H.Y. Liu[1], M.H. Lee[1], C.H. Leng[1]

[1] National Health Research Institutes, National Institute of Infectious Diseases and Vaccinology, Miaoli County, Taiwan
[2] China Medical University, Graduate Institute of Biomedical Sciences, Taichung, Taiwan

BACKGROUND: Recombinant lipoimmunogens with toll-like receptor 2 (TLR2) agonist activity can trigger Th1 immune responses and induce antigen-specific cytotoxic T lymphocytes against tumor growth. Our previous result demonstrated that a lipidated human papillomavirus E7 inactive mutant (rlipoE7m) combine with TLR9 agonist, phosphorothioate (PS)-CpG could synergistically enhanced anti-tumor immunity. To avoid the safety concern of PS-CpG, we develop a liposome-encapsulated native phosphate (PO)-CpG formulation to combine with rlipoE7m for enhancing anti-tumor immunity.

MATERIAL AND METHODS:

The recombinant lipoimmunogens, rlipoE7m, was expressed using E. coli expressing system and purified by Ni-NTA resin column. The 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP, chloride salt) was used to prepare DOTAP liposome using lipid film method. After encapsulation process of PO-CpG in DOTAP liposome, the encapsulation rate was calculated. The bone marrow-derive dendritic cells were used to evaluate the immune-stimulating activity of liposomal formulations. To assess the therapeutic effects of liposomal formulations, TC-1 cells (2×10^5 per mouse) were injected subcutaneously into the left flanks of naïve C57BL/6 mice at 14 days before treatment. All animal experimental protocols were approved by the IACUC of National Health Research Institutes.

RESULTS: The encapsulation rate of PO-CpG/liposome reached to ~98% and ~70% of rlipoE7m could be adsorbed with PO-CpG/liposome, respectively. The rlipoE7m combined liposome-encapsulated PO-CpG formulation could stimulate bone marrow-derived dendritic cells (BMDCs) to secrete IL-1b and IL-12p70 with dose dependent manner. The rlipoE7m combined liposome-encapsulated PO-CpG formulation also could up-regulate co-stimulatory molecules expression on BMDCs, especially CD83. Furthermore, the rlipoE7m combined liposome-encapsulated PO-CpG formulation could elicit high level of IFN-g secreting cells after immunization. Furthermore, the rlipoE7m combined liposome-encapsulated PO-CpG formulation could significantly inhibit tumor growth and prolong the survival rate after treatment.

CONCLUSION: These result suggested that the rlipoE7m combined liposome-encapsulated PO-CpG formulation could be translated into clinical studies to reduce the possible side effects of PS-CpG.

NO CONFLICT OF INTEREST

542 PD-L1 is a therapeutic target of the BET protein inhibitor JQ1 and a promising prognostic biomarker in neuroblastoma when combined with HLA class I

O. Melaiu[1], M. Mina[2], M. Chierici[3], R. Boldrini[4], G. Jurman[3], P. Romania[1], V. D'Alicandro[1], C. Furlanello[3], F. Locatelli[1], D. Fruci[1]

[1] Ospedale Pediatrico Bambino Gesù, Paediatric Haematology/Oncology, Rome, Italy
[2] University of Lausanne, Computational Biology- University of Lausanne- Lausanne- Switzerland., Lausanne, Switzerland
[3] Fondazione Bruno Kessler, Fondazione Bruno Kessler, Trento, Italy
[4] Ospedale Pediatrico Bambino Gesù, Pathology, Rome, Italy

BACKGROUND: The prognostic value of tumor-infiltrating immune cells has been extensively demonstrated in several human cancers. This anticancer immunity can be impaired by a variety of immune-suppressive pathways, including the expression of inhibitory checkpoint receptors and their ligands. Despite the promising result on certain tumors in adults, little is known about the therapeutic potential of immune checkpoint inhibitors in neuroblastoma, the most common pediatric extra-cranial solid tumor, accounting for 15% of childhood cancer-related death. This study sought to evaluate expression of PD-L1 and HLA class I on neuroblastoma cells and PD-1 and LAG3 on tumor-infiltrating lymphocytes to better define patient risk-stratification and understand whether this tumor may benefit from therapies targeting these immune-checkpoint molecules.

MATERIAL AND METHODS: In situ immunohistochemical staining for PD-L1, HLA class I, PD-1 and LAG3 was assessed in 77 neuroblastoma specimens, previously characterized for tumor-infiltrating T cell-density (Mina et al. 2015), and correlated with clinical outcome. Surface expression of PD-L1 was evaluated by flow-cytometry and immunohistochemistry in neuroblastoma cell lines and tumors genetically and/or pharmacologically inhibited for MYC and MYCN. A dataset of 477 human primary neuroblastomas from GEO and ArrayExpress databases was explored for PD-L1, MYC and MYCN correlation.

RESULTS: Multivariate Cox regression analysis demonstrated that the combination of PD-L1 and HLA class I tumor-cell density is a prognostic biomarker for predicting overall survival in neuroblastoma patients. MYC and MYCN control the expression of PD-L1 in neuroblastoma cells both in vitro and in vivo. Consistently, abundance of PD-L1 transcript correlates with MYC expression in primary neuroblastoma.

CONCLUSIONS: The combination of PD-L1 and HLA class I represents a novel prognostic biomarker for neuroblastoma. Pharmacological inhibition of MYCN and MYC may be exploited to target PD-L1 and restore an efficient anti-tumor immunity in high-risk neuroblastoma.

NO CONFLICT OF INTEREST

543 Deciphering HLA motifs across HLA peptidomes correctly predicts neo-antigens

M. Bassani-Sternberg[1], C. Chong[1], P. Guillaume[1], M. Solleder[2], H. Pak[2], P. Gannon[1], L. Kandalaft[1], G. Coukos[1], D. Gfeller[2]

[1] CHUV and the Ludwig Cancer Research Center, Department of Oncology, Lausanne, Switzerland
[2] UNIL, Department of Fundamental Oncology, Lausanne, Switzerland

INTRODUCTION: Cancer immunotherapy reprograms the inherent capacity of cytotoxic T cells to eliminate tumor cells by recognizing molecular entities expressed specifically on tumors but not on normal cells. Recent data show that recognition of mutated neo-antigens, plays a key role. So far, even though the precision of available prediction tools is modest, the discovery of neo-antigens relies mainly on prediction-based interrogation of the 'mutanome'. Currently, mass spectrometry (MS) is the only unbiased methodology to comprehensively uncover the repertoire of HLA binding peptides presented in vivo. Recently, we have developed an in-depth MS-based immunopeptidomics (HLAp) approach combined with exome sequencing to directly identify eleven neo-antigens from human melanoma tumors (Bassani-Sternberg et.al., NatCommun 2016). Four of mutated ligands proved to be immunogenic and neo-antigens specific tumor-reactive T-cells were detected in the patient`s blood and among the tumor infiltrating T cells.

The limiting factor of MS-based HLAp is the availability of sufficient amount patients' tissue samples. Therefore, direct neo-antigens identification by MS is not feasible for substantial number of patients. In a proof-of-concept study we showed that incorporation of deconvoluted HLAp data in ligand prediction algorithms can improve their accuracy (Bassani-Sternberg and Gfeller, J Immunol 2016). We hypothesized that training HLA binding predictors on very large HLAp datasets will significantly improve prediction of neo-antigens.

METHODS AND RESULT: We assembled an in-depth HLAp dataset comprising ten newly generated and forty publicly available datasets. This collection contains 252,165 unique HLA-ligand interactions, which makes it the largest currently available both in term of number of peptides and diversity of HLA-I molecules. By taking advantage of co-occurring HLA-I alleles we can rapidly and accurately identify HLA-I binding motifs and map them to their corresponding alleles without any a priori knowledge of HLA-I binding specificity. Our novel approach uncovers new motifs for several alleles that up to now had no known ligands. HLA-ligand predictors trained on such data substantially improve neo-antigen predictions in four melanoma and two lung cancer patients, indicating that unbiased HLAp data are ideal for in silico identification of neo-antigens (Bassani-Sternberg et. al., bioRxiv 2017).

CONCLUSIONS: This novel approach may facilitate identification of clinically relevant targets for development of cancer immunotherapy, especially when direct identification of neo-antigens with MS cannot be done experimentally.

NO CONFLICT OF INTEREST

544 Indoleamine 2,3-dioxygenase regulates anti-tumor immunity in lung cancer by metabolic reprogramming of immune cells

J. Deshane[1], C. Schafer[3], Y. Wang[2], K. Hough[2], S. Grant[1], V. Thannickal[1], J. Zmijewski[1], S. Ponnazhagan[3]

[1] University of Alabama at Birmingham, Department of Medicine/ Diuison of Pulmonary Allergy and Critical Care Medicine, Birmingham, USA
[2] University of Alabama at Birmingham, Medicine, Birmingham, USA
[3] University of Alabama at Birmingham, Pathology, Birmingham, USA

INTRODUCTION: Myeloid Derived Suppressor Cells (MDSCs) are major contributors of immunosuppression by inducing oxidative stress and by modulating amino acid metabolism. Indoleamine 2,3-dioxygenase (IDO) is a key regulator of tryptophan (Trp) metabolism. Poor prognosis in lung cancer patients is associated with elevated IDO expression and activity. We have previously shown that a combination strategy of gemcitabine and a superoxide dismutase mimetic promoted anti-tumor immunity by enhancing metabolism of $CD8^+$ memory T cells and prolonging survival in mice with lung cancer. In this study, we aim to elucidate how modulation of IDO pathway alters metabolic signaling in the TME and influence mitochondrial dynamics to promote long term immunity against lung cancer.

METHODS: Lewis Lung Carcinoma cells were injected by intracardiac route into wild type (WT) and $IDO^{-/-}$ mice. We assessed tumor burden and infiltration of MDSCs and $CD8^+$ T cells by flow cytometry. Protein analyses of metabolic signaling pathways were performed on whole tissue and sorted cell lysates. Peripheral blood samples from Stage III–IV lung cancer patients and healthy normal relatives were used to measure serum IDO activity and percent circulating MDSCs.

RESULTS: irculating human MDSCs and serum IDO activity correlated with lung cancer. In aIn a preclinical lung cancer mouse model, MDSCs were the significant contributors of IDO in the TME. Combination therapy targeted IDO signaling, specifically in MDSCs, tumor cells, and $CD8^+$ T cells infiltrating the TME. Deficiency of IDO caused significant reduction in tumor burden, tumor-infiltrating MDSCs, GM-CSF, MDSC survival and infiltration of programmed death receptor-1 (PD-1)-expressing $CD8^+$ T cells compared to controls. $IDO^{-/-}$ MDSCs downregulated nutrient-sensing AMP-activated protein kinase (AMPK) activity, but $IDO^{-/-}$ $CD8^+$ T cells showed AMPK activation associated with enhanced effector function. Additionally our studies show that IDO pathway directly modulated mitochondrial dynamics to enhance memory T cell response.

CONCLUSIONS: Our study reveals a novel role for IDO in regulation of AMPK activation, distinct from Trp sufficiency and deficiency signaling. These data also provide mechanistic evidence that a combination treatment of gemcitabine and a SOD mimetic can metabolically reprogram the cellular components of the TME including suppressor cells, effector T cells, and tumor cells.

NO CONFLICT OF INTEREST

545 Resistance mechanisms to PD-1 therapy in melanoma: Three cases

K. Wells[1], C. Amato[1], J. Hintzsche[1], W. Robinson[1]

[1] University of Colorado Anschutz Medical Campus, Medical Oncology, Aurora, USA

INTRODUCTION: Immunotherapy has rapidly altered the treatment paradigm of multiple cancers, especially melanoma. Treatment with nivolumab or pembrolizumab, antibodies to PD-1 (anti-PD1), are currently a standard treatment for patients with metastatic melanoma. Unfortunately, less than a half of patients respond to current immunotherapy and others will develop progressive disease while on treatment. Current knowledge about resistance to anti-PD1 therapy in the literature comes from small case series. Here we present additional molecular evidence of secondary resistance to anti-PD1 therapy.

MATERIAL AND METHOD: We identified three metastatic melanoma patients with progressive disease while on anti-PD1 therapy. DNA was extracted from

the tumor samples from these patients before anti-PD1 therapy and after oligo-progression during treatment on anti-PD-1 immunotherapy. WES was performed with exome capture on an Illumina HiSeq sequencer and mutation analysis was performed using the IMPACT pipeline.

RESULTS AND DISCUSSION: We analyzed each patients paired tumor samples with WES. Unique mutations were identified in those samples taken after progression on anti-PD1 therapy. Similar to published reports, we found a truncating mutation in beta-2 microglobulin (B2M) in one of the samples. In addition, we identified mutations in the gamma interferon receptor (IFNGR1). This is a novel finding, but prior work has shown alterations in the interferon-JAK pathway in resistant samples. Lastly, we identified a KRAS mutation in Exon 4, similar to other less common driver mutations found in colon cancer. One sample had significant variability between samples with 229 new mutations acquired in the sample from an oligo-progressive adrenal nodule.

CONCLUSION: Understanding the mechanisms of resistance to immunotherapy, especially anti-PD1 therapy, will be critical to treating patients with metastatic melanoma. Here we show additional evidence of tumor immune evasion through modulation of antigen presentation, interferon signaling, and a new driver mutation.

NO CONFLICT OF INTEREST

546 Immune microenvironment changes in EGFR tyrosine kinase inhibitor resistant mutant hEGFR bitransgenic mice model

S.H. Hong[1], *J. Deng*[2], *H. Asahina*[2], *S. Li*[2], *T. Chen*[2], *W. Xiaoen*[2], *P. Gao*[2], *K.K. Wong*[2]

[1] *Seoul St. Mary's hospital- Catholic University of Korea, Internal medicine, Seoul, Korea*
[2] *Dana-Farber Cancer Institute, Internal medicine, Boston, USA*

BACKGROUNDS: EGFR mutant NSCLC was reported that immunosuppressive immune checkpoint protein such as PD-L1 was increased in tumor cells. However, Immune microenvironment in EGFR-TKI resistant tumor could be different before EGFR –TKI therapy. We evaluated tumor immune microenvironment changes after developing EGFR-TKI resistance.

METHODS: After 4 weeks Erlotinib (50mg/kg) on and 4 weeks off treatment for 6 months to CCSP-rtTA/Tet-op-hEGFR Del-Luc bitransgenic mice model, we generated EGFR-TKI resistance mutant hEGFR tumor bearing mice by confirmed progressed disease based on MRI imaging on treatment period and acquired T790M mutation. We investigated immune cell analysis by multicolor flow cytometry.

RESULTS: We found increased alveolar macrophage infiltration in tumor area in H&E stain and FACS analysis. These alveolar macrophages increased expression of CD80, CD86 and PD-L1 expression (*p<0.005) compared to EGFR-TKI naïve mutant hEGFR tumor bearing mice. $CD3^+$T lymphocytes increased PD1 and CTLA4 expression. Double positive PD1 and CD3 was 17.0 ± 2.1% in EGFR TKI naïve mice, 33.5 ± 7.1% in Erlotinib resistant tumor bearing mice. Double positive for CTLA4 and CD3 was 13.9 ± 5.6% in Treatment naïve, 20.7 ± 6.41% in Erlotinib resistant tumor bearing mice. PDL1 expression of $epicam^+$ tumor cells tended to be increased expression however statistically not significant.

CONCLUSIONS: Immune microenvironment of EGFR-TKI resistant tumors are different from EGFR-TKI naive tumors especially in immune function of alveolar macrophage and T cell. Alveolar macrophage could be one of targets for immunotherapy in EGFR- TKI resistant EGFR mutant NSCLC.

NO CONFLICT OF INTEREST

548 Targeted Interferon gene delivery reprograms the tumor microenvironment and induces protective immunity against multiple neo-antigens

G. Escobar[1], *L. Barbarossa*[1], *A. Ranghetti*[1], *T. Plati*[1], *C. Brombin*[2], *D. Cittaro*[3], *A. Annoni*[1], *B. Gentner*[1], *L. Naldini*[1]

[1] *San Raffaele Telethon Institute for Gene Therapy, Regenerative Medicine Stem Cell and Gene Therapy, Milan, Italy*
[2] *Vita-Salute San Raffaele University, CUSSB-University Center for Statistics in the Biomedical Sciences, Milan, Italy*
[3] *San Raffaele Scientific Institute, Centre for Translational Genomics and Bioinformatics, Milan, Italy*

Immunotherapy is emerging as a new pillar of cancer treatment with the potential to cure. However, many patients fail to respond to these new therapies, either due to the failure to generate tumor-specific T cells or the existence of an immunosuppressive tumor microenvironment, which imparts resistance to blockade of CTLA4 or PD1/PDL1 checkpoints. To extend the benefits of current therapies, we express an IFNα transgene in the tumor infiltrating monocyte progeny of transplanted, genetically engineered hematopoietic stem cells and test whether our IFN cell/gene therapy could boost the induction and maintenance of anti-tumor responses and synergize with other immunotherapies in a novel mouse B-cell lymphoblastic leukemia (ALL) model mimicking human ALL. When we challenged the mice with our ALL model we found tumor growth inhibition in IFN mice. Administration of αCTLA4 blocking antibody (Ab) had no effects in control mice but significantly improved ALL inhibition in IFN mice, suggesting an immune contribution to the observed response of IFN mice. To investigate the induction of anti-tumor immunity we generated an ALL variant expressing the ovalbumin (OVA) model antigen and showed effective OVA-ALL growth inhibition in IFN mice accompanied by induction of OVA-specific T-cells, with increased cytotoxic activity. Of note, a fraction of IFN mice generated durable responses and spreading of the immune repertoire to multiple tumor-associated antigens, which conferred protection against both OVA+ and parental ALL. Depletion of CD8 T cells abrogated efficacy, confirming their major contribution to the anti-tumor response. In contrast, control mice generated fewer endogenous OVA-specific T cells which were hypo-functional and experienced high rates of immune-escape due to loss of OVA expression. Analysis of the tumor microenvironment revealed an ISG/Th1 gene signature and M1-skewed immune cell infiltrate in IFN mice. Remarkably, adoptive transfer of OVA-specific T cells (OT-I) had limited efficacy in control mice, and showed lack of expansion and phenotypic evidence of exhaustion, similarly to the endogenous OVA-specific T cells. Conversely, when we combined our IFN cell/gene therapy with the adoptive transfer of OT-I cells or with αCTLA4 blocking Abs, we significantly improved survival rates. These findings warrant the clinical testing of our strategy in patients undergoing disease remission after standard therapy, with the aim to provide long-term disease control.

CONFLICT OF INTEREST

Ownership: L.N. and B.G. are inventors of patents filed by the San Raffaele Scientific Institute and Telethon Foundation related to technologies described in the present abstract and own equity on Genenta Science, a biotechnology startup aimed at developing IFN gene therapy by tumor-infiltrating monocytes (www.genenta.com).

549 Visualization of intravital melanoma-T cell interaction in human cell derived xenografts

Y. Wolf[1], *S. Kalaora*[1], *T. Feferman*[1], *G. Shakhar*[1], *Y. Samuels*[1]

[1] *The Weizmann Institute of Science, Molecular Cell Biology, Rehovot, Israel*

CD8+ tumor infiltrating lymphocytes (TILs) have emerged in recent decades as powerful effector cells capable of killing tumor cells. Exploiting TIL reactivity against tumor cells stands at the basis of immunotherapies such as checkpoint blockade and adoptive cell therapy. However, little is known concerning the human TIL biology due to lack of in vivo settings. Here, we use the NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ immunocompromised mice, which are used routinely for human patient and cell-derived xenografts, as recipients of the human melanoma cell line 12T-gfp, which was previously characterized in our lab for its neoantigen landscape using HLA peptidomics. Following the transplant, we inject TILs derived from the original source of the 12T cell line that have been fluorescently labeled, and follow their interaction with the tumor cells in vivo using 2-photon microscopy. The TILs are assessed for their velocity, cluster formation, tumor cell contacts and tumor cell death, in parallel with in vivo tumor rejection assays. These approaches will also allow us to compare in vivo the interactions of melanoma and the bulk TIL population to tetramer-enriched TILs which are neoantigen specific. Our observations will shed light on TIL biology in a more physiological setting which mimics the human condition further.

NO CONFLICT OF INTEREST

550 UCP2-regulated immunostimulatory shift in the tumor microenvironment of melanomas

W.C. Cheng[1,2], *Y.C. Tsui*[2], *P. Romero*[2], *P.C. Ho*[1,2]

[1] *Université de Lausanne, Fundamental Oncology, Lausanne, Switzerland*
[2] *Ludwig cancer institute, Cancer Research, Epalinges, Switzerland*

BACKGROUND: The combined checkpoint blockade with anti-CTLA-4 antibody and anti- PD-1 antibody display promising therapeutic outcomes in advanced melanoma patients.. However, only a restricted fraction of patients has clinical benefit to these treatments. Recent studies uncovered that lack of T cell infiltration into tumors represents a major barriers for effective therapeutic responses of checkpoint blockade treatment. We found that mRNA expression level of mitochondrial uncoupling protein 2 (UCP2) positively correlates with signatures of superior anti-tumor immune responses, including elevated CD3ε, IFNγ and TNFα mRNA expression levels in the TCGA database. Therefore, we postulate that elevated UCP2 expression could result in T cell infiltration into the tumor microenvironment and aim to test the therapeutic effect of UCP2 inducer in combination with checkpoint blockade treatment in melanoma mouse models.

MATERIALS AND METHODS: We established B16-OVA melanoma cells express either control vector (3Flag) or UCP2-expressin vector (3Flag-UCP2) under the control of doxycycline-inducible promoter and use co-engraftment mouse model to check tumor growth rates, tumor weight, and immune infiltrates.

RESULTS: UCP2 overexpression in B16-OVA melanoma cells suppressed tumor growth and elevated the $CD8^+$ T cell infiltration into the tumor microenvironment. Moreover, we confirmed that UCP2 overexpression failed to suppress B16-OVA tumor progression in WT mice depleted with $CD8^+$ T cells by antibody injection and Rag1-KO mice (T and B cell-deficient mouse strain). We further found that overexpressing UCP2 in melanoma cells promoted the production of IFN-γ and IFN-γ-responsive cytokines, but suppressed the expression of angiopoietin-2, M-CSF, IL-10 and VEGF in vivo. Our data uncovered that UCP2 overexpression in melanoma cells can induce a $CD8^+$ T cell-dependent anti-tumor immunity to restrain tumor growth. Further, our result suggested that UCP2 overexpression in melanoma cells might hinder angiogenesis and other immunosuppressive features in tumors.

CONCLUSION: Inducing UCP2 expression in melanoma cells can promote $CD8^+$ T cell-dependent anti-tumor immunity, and lead an immunostimulatory shift to the tumor microenvironment.

NO CONFLICT OF INTEREST

551 Immunomodulatory action of hypomethylating agent Guadecitabine in ovarian cancer

M. Natoli[1], K. Flower[1], A. Karadimitris[2], S. Ghaem-Maghami[1]

[1] Imperial College London, Department of Surgery and Cancer, London, United Kingdom
[2] Imperial College London, Department of Medicine, London, United Kingdom

BACKGROUND: Ovarian cancer (OC) is regarded as being an immunogenic cancer, however, the bulk of the immune responses associated with OC are suppressive in nature. This suggests that strategies to overcome these suppressive mechanisms and enhance immune function in patients will confer benefit and improve prognosis. Immunotherapy has been shown to be most promising when combined with other therapeutic approaches. The purpose of this study is to test the hypothesis that treatment with epigenetic modifying drugs, such as DNA methyltransferase inhibitors (DNMTi), can lead to improved immune activation in OC. We hypothesise that the demethylating and DNA damaging effects of treatment with next generation hypomethylating agent guadecitabine can induce the re-expression of immunomodulatory genes and endogenous retroviruses (ERVs), which shifts ovarian cancer cells towards a more immunogenic expression profile.

MATERIALS AND METHODS: Ovarian cancer cell lines SKOV3, OVCAR4, Kuramochi and OVSAHO were treated with low dose guadecitabine and assessed for increased immunogenicity. Expression of immune biomarkers and ERVs was assessed at different time points at mRNA levels by qPCR and protein levels by Flow Cytometry. Treated cell lines were functionally tested for increased immunogenicity by co-culture with γκ T cells, which were isolated from healthy donors and expanded by zoledronic acid selection. MTT and cytotoxicity assays were performed to assess cell viability and T cell killing following co-culture. IFNγ and Granzyme B release from T cells and chemokine release from tumour cells were assessed by ELISA.

RESULTS: Our result show up-regulation of key immunogenicity biomarkers and ERVs in our panel of OC cell lines, following treatment with guadecitabine. Co-culturing guadecitabine-treated cell lines with γκ T cells resulted in increased tumour cell killing with a synergistic mechanism.

CONCLUSIONS: There is clear potential for guadecitabine to increase immunogenicity in OC. We now plan to employ a genomic approach to study the immunomodulatory potential of this promising new epigenetic agent in OC by performing RNA-sequencing of guadecitabine-treated OC cell lines and to further identify and validate immunomodulatory genes and dsRNAs which contribute to the observed increased immunogenicity. Moreover, we plan to test the in vivo feasibility of a guadecitabine/γκ T cell combination therapy, which if successful might be further optimised for a clinical trial.

NO CONFLICT OF INTEREST

552 Myeloid and lymphoid dendritic cells in type I and type II of ovarian cancer

I. Wertel[1], J. Surowka[1], K. Okła[1], M. Bilska[1], G. Polak[1], R. Tarkowski[1], W. Bednarek[1], J. Kotarski[1]

[1] Medical University, I Chair and Department of Oncological Gynaecology and Gynaecology, Lublin, Poland

BACKGROUND: Ovarian cancer (OC) is often diagnosed at an advanced stage and requires effective chemotherapy treatment. According to the classification proposed in 2004 by Kurman and Shih, OC can be divided into two subtypes with different genetic and clinicopathologic features: type I and type II. Type II tumors are diagnosed at high stage of the disease; they develop rapidly and are highly aggressive. While type I tumors are less aggressive, with low-grade and better prognosis. Still, the interactions between the different immune cell subsets in type I and type II OC remain unclarified. The aim of this study was to estimate myeloid (M) and lymphoid (L) dendritic cells (DC) in the peripheral blood (PB), peritoneal fluid (PF) and ovarian cancer tissue of women with type I and type II OC.

MATERIAL AND METHODS: The study group consisted of 176 women with histologically confirmed ovarian cancers that were classified according to Kurman and Sigh classification into the type I (n=78) or type II (n=98). The patients ranging in age from 22 to 89 years, 55 of women was before and 121 after menopause. The reference group consisted of 50 women with serous cyst of the ovary. MDC and LDC were identified by flow cytometry as BDCA-1 $(CD1c)^+CD19^-$ cells or BDCA-2^+CD123^+ cells, respectively. result are expressed as percentages of MDC and LDC in mononuclear cells.

RESULTS: The percentage of MDC and LDC and the ratio of MDC to LDC in the PB and tissue did not differ significantly ($p>0.05$) between patients with type I and type II OC. Also the percentage of MDC in the PF did not differ significantly ($p>0.05$) before patients with type I and type II OC. However, the percentage of LDC in the PF of ovarian cancer type II was significantly higher ($p=0.005$) than in women with type I OC. Moreover, the ratio of MDC to LDC in the PF of ovarian cancer type II was significantly lower ($p=0.002$) than in patients with type I OC.

CONCLUSIONS: The accumulation of lymphoid DC in the PF of type II OC patients may play an important role in the pathogenesis of this disease, especially in induction of immune tolerance and neoangiogenesis. Interference with immune system function by influence on LDC subsets may become attractive potential target for future treatment of type II ovarian cancer patients.

NO CONFLICT OF INTEREST

554 Elucidating microglia/macrophages heterogeneity in glioblastoma by single-cell transcriptomics

A. Michelucci[1], A. Golebiewska[1], S.K. Poovathingal[2], A. Oudin[1], R. Balling[2], A. Skupin[2], S.P. Niclou[1]

[1] Luxembourg Institute of Health LIH, Department of Oncology, Luxembourg, Luxembourg
[2] University of Luxembourg, Luxembourg Centre for Systems Biomedicine, Esch-sur-Alzette, Luxembourg

BACKGROUND: Glioblastoma (GBM) is an aggressive brain tumour characterized by prominent genetic and histopathological heterogeneity. A major contributing factor to malignant gliomas development and progression is their ability to evade the immune system. Neoplastic cells release factors that recruit resident microglia, macrophages and other peripheral immune cells to the tumour site and transform them into tumour-supportive cells. Here, we aim to elucidate the variation in molecular profiles of microglia/macrophages (MMs) during tumour progression and with respect to different histopathological GBM features, such as invasion and angiogenesis.

MATERIAL AND METHODS: To address this question, we took advantage of our established orthotopic patient-derived xenograft (PDX) models generated in nude mice representing different stages of tumour progression as well as recapitulating strong inter- and intra-tumour heterogeneity present in patients. The 'invasive' tumours present an apparent normal brain vasculature while the 'angiogenic' ones display necrotic areas and microvascular proliferation. The 'intermediate' tumours share characteristics of invasive behaviour and vessel abnormalities. MM phenotypes were analysed by combining immunohistochemistry with multicolour flow cytometry and single-cell mRNA sequencing. The latter allows for an unbiased, surface marker-free approach to decipher MM transcriptional signatures at the single-cell level.

RESULTS: Depending on the GBM phenotype observed in the PDX models, we found that MMs displaying different characteristics accumulate within and around the tumour core. The ratio of $CD11b^{pos}CD45^{low}$ microglial cells and $CD11b^{pos}CD45^{high}$ macrophages in the different tumour phenotypes reflects histological tumour hallmarks. Taking advantage of high-throughput single-cell sequencing, we present here specific MM transcriptional profiles associated with invasive and angiogenic features, thus revealing high stromal heterogeneity related to specific tumour compartments.

CONCLUSIONS: The application of single-cell mRNA sequencing enabled the elucidation of MM subtypes correlating to temporal and spatial histopathological heterogeneity in GBM. These analyses will pave the way to novel targeted immune-therapeutic approaches to be tuned according to tumour stage and histopathology.

NO CONFLICT OF INTEREST

555 Neuronal autoantibodies are prognostic in small cell lung cancer patients and predictive for response to immunochemotherapy

M. Hardy-Werbin[1], O. Arpí[2], A. Taus[2], P. Simoes[2], C. Guardia[1], F.J. Sánchez-Martín[1], D. Joseph-Pietras[3], A. Rovira[1], C.H. Ottensmeier[4], E. Arriola[2]

[1] Institut Mar d´Investigacions Mèdiques, Cancer Research Program, Barcelona, Spain
[2] Hospital del Mar, Department of Medical Oncology, Barcelona, Spain
[3] University of Southampton, Southampton NIHR Experimental Cancer Medicine Center, Southampton, United Kingdom
[4] University of Southampton, Faculty of Medicine, Southampton, United Kingdom

BACKGROUND: Small cell lung cancer (SCLC) can trigger autoimmune neurological paraneoplastic syndromes, and is then usually associated with circulating neuronal autoantibodies (NAAs). Ipilimumab, an anti-CTLA-4 antibody associated with autoimmune adverse events is currently being tested in SCLC. Our aim was to assess the prevalence and patterns of change of NAAs in SCLC patients treated with standard chemotherapy vs. those treated with chemotherapy plus ipilimumab.

MATERIAL AND METHODS: We evaluated 2 cohorts (C) of patients: in C1, 47 patients were treated with standard platinum/etoposide (PE), in C2, 42 patients with ipilimumab, carboplatin and etoposide (ICE). No neurological symptoms were present at diagnosis.

Serum samples at baseline and subsequent time points were analyzed for the presence of NAAs with an immunoblotting commercial kit (RAVO Diagnostika®, Freiburg, Germany) to recombinant Gad65, Sox1, Amphiphysin, CRMP5, Ri, Yo and HuD with samples diluted 1:200. Data was processed for quantification with ImageJ® 1.51h (NIH, USA) and statistical analysis was performed with SPSS (SPSS Inc®, Chicago, IL, USA).

RESULTS: NAAs were detected at baseline in 25 patients (53 %) in C1 and 21 (50 %) in C2. The most prevalent autoantibody was anti-Sox1 (C1:38.3% vs C2:33.3%), followed by anti-Yo (C1:17% vs C2:7.1%) and anti-HuD (C1:6.4 vs C2:11.9%). Eleven patients showed 2 reactivities, and three, 3 or more. In 37 of 46 patients with pre-existing NAAs, titers decreased after initiation of treatment.

The presence of NAAs at baseline was associated with higher response to immuno-chemotherapy (76%) compared to chemotherapy alone (42%; p=0,026). Overall

survival in patients with NAAs at baseline was 17.7 months (95% CI: 12.2-23.2), compared to 12.1 (95% CI: 9.7-14.5) in negative cases (p=0,066). No significant differences were observed when analyzing each cohort separately.

CONCLUSIONS: NAA positivity, most commonly against SOX1, is found in around 50% of patients with SCLC without neurological symptoms. Successful tumor treatment is associated with reducing levels of antibody. NAAs predict for better response to chemo-immunotherapy. NAAs at baseline identify patients with better prognosis irrespective of whether immune-chemotherapy or chemotherapy is used.

NO CONFLICT OF INTEREST

556 A differentiated CD47 therapeutic antibody recognizing a novel epitope and sparing erythrocytes and platelets

T. Guo[1], Z. Wang[1], L. Fang[1], J. Zang[1]

[1] Third Venture Biopharma, Discouery, Shanghai, China

Tumor cells overexpress CD47 which engages signal-regulatory protein (SIRPα) on macrophages (MΦ) to deliver a 'do not eat" signal to avoid being phagocytosed. Blocking CD47 using SIRPα-Fc or anti-CD47 antibodies (Ab) has emerged as a promising strategy to neutralize CD47 and promote tumor eradication. However, CD47 is also expressed on red blood cells (RBC) and platelets (PLT) which can act as a large Ab sink. Previous CD47-targeting agents were found to cause anemia and thrombocytopenia in animal studies and phase I trials, which is of serious concern. Here we report the discovery of a CD47 Ab with a novel epitope that endows it with enhanced phagocytic and RBC-sparing properties, thus differentiating itself from current CD47-targeting therapies.

A naïve human single chain variable fragment (ScFv) phage library was subjected to panning for binders to a human CD47-extracellular domain (ECD) fragment. All unique ScFv binders were converted to full IgG1 and screened against human RBC and PLT binding followed by hemagglutination confirmation assays. The resulting Abs were further screened for their ability to enhance phagocytosis of Raji cells and human primary acute myeloid leukemia (AML) cells by CD14+ MΦ. A lead Ab (1F8) was used to treat immunodeficient NSG mice engrafted with luciferase-positive Raji tumor cells. In addition, hematological and pharmacokinetic parameters were assessed in cynomolgus monkeys receiving a single intravenous infusion of 1F8 at 15 mg/kg. To determine the binding pose and epitope, the CD47-ECD/1F8-Fab complex was co-crystallized and subjected to X-ray diffraction. The crystallographic data collected at 3.1 Å were used to solve the structure.

1F8 is a fully human anti-CD47 Ab that blocks the interaction of CD47 and SIRPα (IC_{50} 0.26 μg/ml) leading to enhanced MΦ phagocytosis of various $CD47^+$ tumor cell lines and primary AML cells. Treatment of 1F8 eradicated tumors xenograft and induced MΦ M1 shift in vivo. Of note, 1F8 treatment spared RBC and PLT, as evidenced by a lack of binding or hemagglutination as well as an unchanged hematological profile in monkeys treated with a high dose regimen. The unique functional properties of 1F8 can be explained in part by its structure when complexed with CD47, revealing almost straight head-to-head binding and a novel conformational epitope that encompasses SIRPα interaction domain.

We have successfully discovered a CD47-blocking therapeutic Ab devoid of the hematological liabilities seen in competitor molecules while maintaining anti-tumor efficacy and promoting M1 polarization. 1F8 represents potentially a differentiated, best-in-class molecule and is undergoing preclinical development with an aim to enter clinical studies in 2018.

NO CONFLICT OF INTEREST

557 Theranostic evaluation of the combination of hypofractionated radiotherapy and IL-2/anti-IL-2 complexes in tumor-bearing mice

H. Jing[1], M. Hettich[1], M. Bartholomä[2], G. Niedermann[1]

[1] University Clinics Freiburg, Dept. of Radiation Oncology, Freiburg, Germany
[2] University Clinics Freiburg, Dept. of Nuclear Medicine, Freiburg, Germany

BACKGROUND: Combinations of radiotherapy (RT) and immune checkpoint inhibition (ICI) are effective in a fraction of patients with advanced malignancies. However, the majority of patients still do not respond. For these patients, and for patients with preexisting autoimmune diseases or ICI-induced autoimmune-like symptoms, alternative treatments are required. Combinations of RT with IL-2 can be very potent, but IL-2 has only a short half-life. It also stimulates immunosuppressive CD4+ regulatory T cells and can lead to vascular leak syndrome, both depending on IL-2 binding to its high-affinity receptor CD25. To circumvent these disadvantages, we tested combination treatments of hypofractionated RT (hRT) and stable IL-2/anti-IL-2 complexes (IL-2c); the complexes prevent IL-2 binding to CD25 but preserve its binding to the low-affinity IL-2 receptor CD122.

MATERIAL & METHODS: Mice with established melanomas were treated with local hRT (2 × 12 Gy) and IL-2 or IL-2c. Besides tumor sizes and survival, the frequencies and quality of the tumor-specific T cells were assessed by flow cytometry and ELISA. In addition, a novel PET tracer was developed for the detection of IL-2c and the ligand CD122. For this purpose, the IL-2c were conjugated with the chelator NOTA and further labeled with the radioisotope ^{64}Cu.

RESULTS: Treatment of mice harboring relatively large, established melanomas with hRT + IL-2c was superior to treatment with hRT + IL-2 or hRT alone. Treatment with IL-2c alone was not effective. The better activity of the hRT/IL-2c combination treatment was reflected by an enhanced number of tumor-specific CD8+ cytotoxic T cells. The novel PET tracer allowed visualization of the whole-body distribution of the IL-2c and of the bound CD122 receptor. We also non-invasively visualized the side effects of IL-2c treatment by using different PET tracers and contrast-enhanced CT.

CONCLUSIONS: We developed a novel combination treatment consisting of hRT and high-molecular-weight IL-2c, which resulted in long-term tumor control and sometimes cures in mice with large, established melanomas. Since similar high-molecular-weight complexes have recently been developed using humanized anti-human IL-2 antibodies, combination treatments with hRT could soon be evaluated in clinical trials. In addition, a novel PET tracer for the theranostic evaluation of the IL-2 complexes and their receptor has been developed by us.

NO CONFLICT OF INTEREST

558 Nivolumab treatment of metastatic renal cancer patients impairs Tregs and potentiates NK function: The role of CXCR4 inhibition (The "ReVoluTion" Trial)

S. Santagata[1], A.M. Trotta[1], M. Napolitano[1], L. Portella[1], S. Rossetti[2], S. Perdonà[2], S. Pignata[2], S. Scala[1]

[1] Istituto Nazionale Tumori Fondazione "G.Pascale", Genomica Funzionale, Napoli, Italy
[2] Istituto Nazionale Tumori Fondazione "G.Pascale", Uro-Gynecological, Napoli, Italy

BACKGROUND: Despite encouraging results, in metastatic renal cancer (mRCC) nivolumab response is not as wide as expected. Among mechanisms of resistance to immune-mediated therapies, T-regulatory cells (Tregs) and Natural Killer (NKs) function plays a central role. Indeed, tumoral Tregs in RCC patients have been shown to be more suppressive than healthy donors Tregs. The ReVoluTion clinical trial is an osservational trial aiming to identify biomarkers predictive of nivolumab efficacy through evaluation of Tregs and NKs function. In addition, since tumoral Tregs express high level of CXCR4, antagonism to this receptor will be evaluated in vitro as possible inhibitor of Tregs suppressive activity. We report the preliminary result on 9 mRCC patients enrolled and evaluated up to six months of treatment.

PATIENTS AND METHODS: Tregs and NKs function was evaluated at day 0, 14, 28, 90, and 180 in patients affected by mRCC undergoing to nivolumab treatment as second line treatment. Functional Tregs and NKs were phenotypically identified as $CD4^+CD25^+127^{low}FOXp3^{high}CD45RA^-$ and $CD3^-CD56^+CD107a^+$, respectively. Tregs activity was evaluated through the soppression of T effector proliferation and NKs activity through K562 dependent cell cytotoxicity. The effect of CXCR4 antagonism was evaluated ex vivo on Tregs and NKs activity.

RESULTS: At this time 11 patients were analyzed and 9 were evaluable: 2 died of disease (DOD), 2 progressed at six months (PD6), 3 presented partial response at 3 months (PR3) and 2 patients showed stable disease at three months (SD3). In 2 patients initial T effector anergy was revealed but recovered during nivolumab treatment. In 4/9 patients the PD/DOD corresponded to increase in Tregs activity while in 3/9 patients with a PR3 increase in Teff proliferation, compatible with a decreased Tregs activity, and ex vivo Tregs inhibition through CXCR4 antagonists was detected. Though the total peripheral number of Tregs was unaffected, a significant decrease in the suppressive Tregs ($CD4^+25^+Foxp3^{hi}127^{low}45RA^-$) was revealed. Suppressive Tregs (high PD1 and CXCR4) at time 6 months displayed a reduction in the expression of PD-1 and CXCR4 during nivolumab treatment ($p<0.001$ and $p<0.05$, respectively). NK function increased during nivolumab treatment with a concomitant reduction in the expression of the inhibitory receptors CD158a (p=0.02), PD-1 (p=0.04) and CXCR4 (p=0.24). In the 4/9 patients that experienced PD/DOD the higher Tregs function accompanied a reduction in NK cytotoxicity as revealed through in vitro K562 cytotoxicity.

CONCLUSION: Nivolumab treatment in mRCC patients determined detectable variations on Tregs and NKs function. Tregs suppressive function was impaired by inhibition of CXCR4 receptor suggesting that CXCR4 antagonism reverted Tregs suppressive activity.

NO CONFLICT OF INTEREST

559 The interplay between anti-cd20 therapeutic antibodies and human natural killer cells: Impact of antibody fc engineering

C. Capuano[1], C. Pighi[2], R. Molfetta[2], R. Paolini[2], S. Battella[2], G. Palmieri[1], G. Giannini[2], F. Belardinilli[2], A. Santoni[2], R. Galandrini[1]

[1] Sapienza Uniuersity of Rome, Experimental Medicine, Rome, Italy
[2] Sapienza Uniuersity of Rome, Molecular Medicine, Rome, Italy

BACKGROUND: Obinutuzumab is a next-generation glycoengineered anti-CD20 monoclonal antibody (mAb) with a modified crystallizable fragment (Fc) domain designed to increase the affinity for FcγRIIIA/CD16 and consequently the killing of mAb-opsonized targets. Natural Killer (NK) cell-mediated Antibody Dependent Cellular Cytotoxicity (ADCC), based on the recognition of IgG-opsonized targets by the low affinity receptor for IgG, CD16, represents one of the main mechanisms by which anti-CD20 therapeutic mAbs mediate their anti-tumor effects. Beside ADCC, CD16 ligation also result in cytokine production, in particular, NK-derived IFN-γ is endowed with a well recognized role in the shaping of adaptive immune

responses. However, the impact of CD16 ligation in optimized affinity conditions on NK functional program is not completely understood.

Here we aim to compare the ability of anti-CD20 Abs with different CD16 binding affinity to affect CD16 dynamics and NK cell functions.

MATERIALS AND METHODS: CD16 aggregation was obtained by rituximab or obinutuzumab-opsonized CD20$^+$ B lymphoma Raji cell line. Anti-CD20 'experienced" NK cells were obtained by negative selection on streptavidin-beads upon short- or long-term co-culture of primary NK cells with biotin-loaded anti-CD20-opsonized targets and then used for further analysis.

RESULTS AND DISCUSSION: Our data show that the interaction of NK cells with obinutuzumab-opsonized cells result in enhanced IFN-γ production as compared with parental non-glycoengineered mAb or the reference molecule rituximab. We observed that affinity ligation conditions strictly correlate with the ability to induce CD16 down-modulation and lysosomal targeting of receptor-associated signaling elements. Indeed, a preferential degradation of FcεRIγ chain and Syk kinase was observed upon obinutuzumab stimulation independently from CD16-V158F polymorphism. Although the down-regulation of FcεRIγ/Syk module leads to the impairment of cytotoxic function induced by NKp46 and NKp30 receptors, obinutuzumab-experienced cells exhibit an increased ability to produce IFN-γ in response to different stimula.

CONCLUSION: These data indicate a relationship between CD16 aggregation conditions and the ability to promote a degradative pathway of CD16-coupled signaling elements associated to the shift of NK functional program.

NO CONFLICT OF INTEREST

560 A three-dimensional ex vivo platform for assessing targeted immunotherapeutic agents

F. Chiovaro[1], T. Tanos[1], I. Agarkova[1], J. Kelm[1], P. Guye[1], S. Dhar[1]

[1] InSphero AG, Oncology, Schlieren, Switzerland

BACKGROUND: Multicellular tumor spheroid (MCTS)-based 3D ex vivo platforms are extensively used for the evaluation of cytotoxic drugs, targeted molecules, and antibodies activity. To increase the translational value of this model, it is of great importance to maintain tumor microenvironment-like conditions (immune and other stromal cells).

Material &Methods
We have established a 96 well based 3D ex vivo system using well known tumor cell lines derived from non-small cell lung cancer and breast cancer expressing wide range of PDL1 levels.

RESULTS: The cells were cultured with stromal and immune cells to replicate tumor-like microenvironment. Upon treatment with CD3/CD28 stimulated PBMCs, efficient infiltration of immune cells was observed using an immunohistochemical approach. Subsequently, these MCTS were exposed to known PD1 inhibitors, resulting in an increased elimination of target cells within the MCTS.

CONCLUSIONS: Currently, we are evaluating FDA approved targeted small molecules to study their ability in enhancing the T-cell infiltration in tumor cell lines and thereby sensitizing these cells to immunomodulatory agents including PD1 and PDL1 inhibitors. This study is being carried out using targeted molecules alone and in sequential combination with immunotherapeutic agents.

In conclusion, this study demonstrates the power and versatility of our 3D MCTS platform for the assessment of antibody-based targeted tumor cell elimination.

NO CONFLICT OF INTEREST

POSTER SESSION: TUMOUR IMMUNOLOGY II

561 A chimeric DNA vaccine against CSPG4 for the treatment of malignant melanoma: An effective way to overcome immune tolerance in dogs and humans

F. Riccardo[1], E. Bolli[2], G. Barutello[1], V. Rolih[1], M. Arigoni[1], S. Occhipinti[2], S. Lanzardo[1], S. Ferrone[3], P. Buracco[4], F. Cavallo[1]

[1] Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences - University of Torino, Torino, Italy
[2] Città della Salute e della Scienza di Torino, Center for Experimental Research and Medical Studies CERMS, Torino, Italy
[3] Harvard Medical School - Massachusetts General Hospital, Department of Surgery, Boston, USA
[4] University of Torino, Department of Veterinary Sciences, Grugliasco, Italy

BACKGROUND: Malignant melanoma (MM) is the sixth cancer worldwide and 1/3 of MM patients will experience disease recurrence and metastasis often fatal. Due to the many similarities with its human counterpart, canine (c) MM offers an excellent opportunity for translational clinical investigations. The chondroitin sulfate proteoglycan (CSPG)4 is an attractive immunotherapy target for both human (Hu) and cMM. We have demonstrated the clinical relevance of a DNA vaccine against Hu-CSPG4 in a veterinary trial (Riccardo, Iussich et al., 2014; Piras, Riccardo et al., 2016). Hu-CSPG4 vaccination caused no side effects and resulted in significantly longer disease-free and overall survival in vaccinated dogs as compared to controls. Nevertheless, some vaccinated dogs eventually died because of metastasis. To increase the efficacy of our approach, we employed a hybrid plasmid coding for chimeric CSPG4 protein, expected to be effective in both veterinary and human settings.

METHODS: We generated a hybrid plasmid in part derived from the Hu- and in part from the dog (Do)-CSPG4 sequences. We tested the safety and immunogenicity of HuDo-CSPG4 intramuscular DNA vaccination followed by electroporation (electrovaccination) in mice, in dogs with stage II-III surgically resected CSPG4$^+$ oral MM, and in a human setting.

RESULTS AND DISCUSSION: The chimeric HuDo-CSPG4 is correctly folded and immunogenic in mice. In dogs HuDo-CSPG4 electrovaccination causes no side effects and induces antibodies (Ab) against both Hu- and Do-CSPG4. Moreover, HuDo-CSPG4 vaccine-induced Ab display a higher affinity against the Hu-CSPG4 protein and are more effective in inhibiting the proliferation of CSPG4$^+$ MM cells as compared to Hu-CSPG4. The mechanism of action of HuDo-CSPG4 induced Ab is under investigation. Interestingly, data obtained in vitro with T cells from human healthy donors also suggest HuDo-CSPG4 is more immunogenic than Hu-CSPG4 plasmid.

CONCLUSIONS: The employ of hybrid HuDo-CSPG4 plasmid is an effective way to break immune tolerance against the self antigen in dogs and humans and could be of impact for both veterinary and human clinical setting.

NO CONFLICT OF INTEREST

562 OMV platform: A synthetic biology approach for cancer immunotherapy

A. Grandi[1], L. Ganfini[2], M. Tomasi[2], I. Zanella[2], M. Parri[1], C. Irene[2], F. Zerbini[2], L. Fantappiè[2], E. Caproni[2], G. Grandi[2]

[1] Toscana life sciences, Toscana life sciences, Siena, Italy
[2] University of Trento, CIBIO-Center for Integrative Biology, Trento, Italy

BACKGROUND: Vaccination is one of the most effective interventions in the history of medicine, capable of completely or almost completely eliminating devastating diseases caused by various infectious agents. Chronic diseases such as cancer are still awaiting valid solutions to turn vaccination into an effective intervention. Bacterial Outer Membrane Vesicles (OMVs) are naturally produced by all Gram-negative bacteria and contain several Pathogen-Associated-Molecular Patterns, known to play a key role in effectively stimulating innate immunity. The ability to engineer OMVs by delivering recombinant proteins to the bacterial periplasm and outer membrane, together with their inherent adjuvanticity, make them a particularly interesting vaccine platform

MATERIAL AND METHODS: An OMV overproducing E.coli strain was engineered to express a number of cancer antigens, including epidermal growth factor receptor variant III or FAT1, either in the lumen or at the surface. The presence of the epitopes was checked by flow cytometry and by western blot. Mice were immunized with the antigen decorated OMVs and B cell response was analyzed by ELISA. Syngeneic mouse models of tumor protection were generated by injecting cancer cells (B16 and CT26) subcutaneously then immunizing with cancer-antigen decorated OMVs

RESULTS: OMVs were successfully engineered with cancer antigens by fusing them to different periplasmic and membrane-associated proteins. Fusion proteins represented a substantial fraction of total OMV proteins, allowing the vesicles to be efficiently decorated with heterologous antigens. Recombinant vesicles were able to induce an antigen specific immune responses when injected in mice. Moreover, OMV immunization conferred robust protection against tumor growth both in the prophylactic and therapeutic modality. Interestingly, immunization with a combination of different antigens worked synergistically leading to full protection against B16 and CT26 challenge

CONCLUSIONS: We demonstrated that it is possible to deliver a high amount of cancer antigens as fusion proteins either in the lumen or at the surface of bacterial vesicles. These decorated OMVs elicit antigen-specific immune responses in mice which confer solid protection against tumors. Considering the flexibility of the platform, which allows rapid decoration of OMVs with several antigens, as well as the potent adjuvanticity of the vesicles, OMVs have a great potential to be used in personalized anti-cancer medicine

NO CONFLICT OF INTEREST

563 CAF-released lactate induces an immunesuppressive environment which sustains prostate carcinoma progression via TLR8/miR21 axis

G. Comito[1], A. Iscaro[1], M. Bacci[1], A. Morandi[1], L. Ippolito[1], P. Chiarugi[1], E. Giannoni[1]

[1] Univeristy of Florence, Department of Experimental and Clinical Biomedical Sciences, Florence, Italy

BACKGROUND: Leukocyte infiltration plays a role in controlling tumor development: in the early stages of carcinogenesis, T cells counteract tumor growth, whereas in advanced stages, they acquire functional defects, resulting in the establishment of an immunosuppressive microenvironment that sustains tumor progression. Our aim is to investigate the role of cancer-associated fibroblasts (CAFs) in immune modulation and the impact on prostate carcinoma (PCa) progression.

MATERIAL AND METHODS: CAFs were isolated from PCa patients, peripheral blood mononuclear cells (PBMCs) from blood of healthy donors. Lymphocytes subclasses were isolated and characterized by FACS analysis.

RESULTS: Our data reveal that after cocolture of PBMCs with CAFs, there is a significant increase in the percentage of the protumoral Treg subset and a concomitant decrease in the antitumoral Th1 pool. We show that lactate released by CAFs, which convert to Warburg metabolism upon PCa cell conditioning, is responsible for this immunesuppression, since exogenous lactate administration to PBMC strictly resembles CAF-induced modulation of both Treg and Th1. The role of lactate in immunediting was confirmed by the treatment with the inhibitor of MCT1 (carrier mediating lactate influx), which dramatically impairs stromal-mediated immunosuppression. We highlight a dual role for lactate: i) when uploaded by Th1 cells, it promotes SIRT1 activation and deacetylation of the transcriptional factor Tbet, thereby impairing Th1 stability; ii) if uploaded by naïve T cells, it is responsible for Treg polarization, via NF-kB activation and FoxP3 expression. The exposure of PCa cells to the CAF-induced immunosuppressive environment result in the induction of epithelial-mesenchymal transition (EMT) and in the acquisition of invasive features. Our data suggest a key role of the miR21/TLR8/NF-kB axis in controlling this phenomenon. Indeed, in PCa cells cultured with immunosuppressed PBMC, we observed the ex-novo expression of the endosomal TLR8 and the upregulation of its ligand miR21, leading to the activation of a NF-kB-mediated pro-inflammatory pathway. In keeping, the silencing of miR21 or TLR8 almost completely abrogates PCa cells aggressive features.

CONCLUSION: Our data reveal the establishment of a metabolic-based relationship among cancer, stromal and immune cells in order to promote an immunosuppressive environment that sustain cancer malignancy, through a previously unexplored miR21/TLR8 axis.

NO CONFLICT OF INTEREST

564 Overcoming immune suppression in cervical cancer by local PD-(L)1 checkpoint inhibition

A. Stam[1], *M. Heeren*[1], *J. Rotman*[1], *S. Mom*[2], *G. Kenter*[2], *E. Jordanova*[2], *T. De Gruijl*[1]

[1] *VU University Medical Center-Cancer Center Amsterdam, Medical Oncology, Amsterdam, Netherlands*
[2] *VU University Medical Center, Centre for Gynecologic Oncology Amsterdam, Amsterdam, Netherlands*

INTRODUCTION: Cervical cancer initially spreads to regional lymph nodes, where the induction of immune suppression may facilitate further metastatic spread. We observed high and interrelated levels of regulatory T cells (Tregs) and PD-L1 positive macrophages in metastatic tumor-draining lymph nodes (TDLN) of cervical cancer patients. In addition, we have reported an immune suppressive field effect (defined by low CD8+ T cell/Treg ratios) surrounding tumor positive lymph nodes with high densities of PD-L1+ M2-like macrophages, which apparently preceded actual metastasis, creating metastatic niches in the tumor-draining lymphatic catchment area. We aim to break this immunosuppressive cycle by local PD-(L)1 blockade.

MATERIALS AND METHODS: We investigated the effect of PD1 or PD-L1 blocking antibodies on the T cell reactivity against the HPV16 E6 oncoprotein in tumor-draining lymph nodes (TDLN) of cervical cancer patients. Antigen-specific responses of TDLN single-cell suspensions were measured by IFN-γ Elispot either directly ex vivo , or after a 10-day in vitro culture with an HPV16 E6 overlapping long peptide pool. In addition, we are assessing the effects of PD-(L)1 blockade on different T cell and myeloid subsets by multi-parametric flow cytometry.

RESULTS AND DISCUSSION: We found HPV16-E6 specific T cell responses in 4/5 tumor-containing TDLN suspensions. After PD1 or PD-L1 blockade HPV16 E6 specific frequencies increased in 4/4 of the reactive TDLN. In contrast, only 1/4 tumor-negative TDLN suspensions showed a minor HPV16 E6 specific T cell response but no increase of specific T cell numbers was found following PD1/PD-L1 blockade. Detectable T cell responses and increases were much more pronounced after a 10-day in vitro culture period than directly ex vivo, with detected increases in tumor positive TDLN reaching statistical significance over tumor negative TDLN ($p<0.04$).

CONCLUSION: PD-(L)1 blockade counteracts prevailing immune suppressive conditions in the microenvironment of cervical tumor containing TDLN and reactivates effector T cells. These data clearly support earlier reports of a "poised" HPV-specific T cell repertoire present in TDLN, ready to be exploited for cancer immunotherapy after effective stimulation and provide important supportive evidence for the use of PD-(L)1 blockade in lifting loco-regional immune suppression in cervical cancer.

NO CONFLICT OF INTEREST

565 The pro-angiogenic phenotype and functions of colorectal cancer Tumour infiltrating (TINKs) and tumour associated (TANKs) Natural Killer cells

A. Bruno[1], *B. Bassani*[2], *G.D. D'Urso*[2], *S. Zanellato*[3], *I. Aida*[4], *L. Boni*[4], *L. Mortara*[3], *A. Albini*[2], *D. Noonan*[3]

[1] *IRCSS MultiMedica- Scientific and Technological Pole PST, Oncology Research, Milan, Italy*
[2] *IRCSS MultiMedica- Scientific and Technological Pole PST, Vascular Biology and angionenesis Laboratory, Milan, Italy*
[3] *University of Insubria- Varese- Italy, Department of Biotechnology and Life Sciences, Varese, Italy*
[4] *University of Insubria- Varese- Italy, Department of Surgical and Morphological Sciences, Varese, Italy*

INTRODUCTION: Natural Killer (NK) cells are effectors lymphocytes of innate immunity, primarily involved in immunosurveillance against tumors through their cytotoxic activity. We previously reported that NKs from Non Small Cell Lung Cancer (NSCLC) can acquire the decidual-like $CD56^{bright}CD16\text{-}VEGF^{high}PlGF^{high}IL\text{-}8^{+}IFNg^{low}$ phenotype and that $TGFb_1$ is a relevant orchestrator in NK angiogenic switch. Here, we investigated whether tumor associated (TANKs; peripheral blood NK cells) and tumor infiltrating (TINKs) NK, isolated from patients with colorectal cancer (CRC), are subjected to angiogenic-switch.

MATERIAL AND METHODS: NK subset distribution and cytokine profiling were performed by multicolor flow cytometry, using peripheral blood and tissue samples from CRC patients, for surface antigen and cytokine profiling characterization. Conditioned media (CM) from FACS-sorted NKs were used either for secretomic profiling, using antibody membrane array or in functional in vitro angiogenesis assay. Biochemical approaches were used to determine molecular pathways modulated in different CRC TINK/TANK phenotype and function.

RESULT AND DISCUSSION: We found that TINK/TANKs from CRC patients show impaired degranulation activities, which associates with NKG2D decreased level, and up-regulation of AMPK and PTEN at protein level. CRC TINK/TANKs express the decidual NK markers CD9 and CD49a and induced endothelial cell proliferation, migration, adhesion and formation of capillary-like structures on Human Umbilical Vein Endothelial Cells (HUVEC) in vitro. Secretome and flow cytometry analysis on CRC peripheral blood NK cells showed up-regulation of several pro-angiogenic factors, such VEGF, Angiogenin, Angiopoietin-1, Timp1-2, MMP-9. Molecularly, we observed p-STAT3 and p-STAT5 up-regulation in CRC TANKs and chemical inhibition of those molecules by pymozide resulted in both inhibited pro-angiogenic factor production and formation of capillary-like structures in vitro.

CONCLUSIONS: Our data demonstrate that TINK/TANKS from CRC patients are switched toward a pro-angiogenic/pro-tumor phenotype and function. Therefore, we propose TINK/TANKs as a new hallmark ad relevant target in CRC inflammation.

NO CONFLICT OF INTEREST

566 Resistance mechanisms to anti-CSF1R therapy in tumor-associated macrophages

L. Pradel[1], *C.H. Ooi*[2], *A. Franke*[3], *S. Romagnoli*[4], *M. Cannarile*[1], *H. Sade*[1], *D. Ruettinger*[1], *C. Ries*[1]

[1] *Roche Innovation Center Munich, Discovery Oncology, Penzberg, Germany*
[2] *Roche Innovation Center Basel, Pharmaceutical Sciences, Basel, Switzerland*
[3] *Roche Innovation Center Munich, Large Molecule Research, Penzberg, Germany*
[4] *Roche Innovation Center Basel, Biomarker Programs, Basel, Switzerland*

Targeting the colony stimulating factor 1 receptor (CSF1R) opens novel paths for therapeutic intervention for tumor-associated macrophages (TAM) in cancer patients. TAM represent an abundant cell component of the immune infiltrate in solid tumors and orchestrate escape from immune surveillance. Macrophages (MΦ) display a very plastic phenotype, recapitulated in vitro by exposure with e.g. T_h2 response IL-4/-13 or immunosuppressive IL-10 cytokines. As various CSF1R monoclonal antibodies and inhibitors are currently evaluated in clinical trials, it is crucial to know which MΦ are responsive to therapy and which factors can limit response. Hence, main focus of this study was to identify factors which mediate resistance to a CSF1R-blocking antibody, emactuzumab. Therefore, impact of these cytokines, hypoxia and genetic alterations within CSF1R and beyond were investigated.

Tumor-promoting MΦ were polarized in vitro by the respective cytokines solely or combined and analyzed for phenotype and function in the context of emactuzumab. In silico analysis of cancer patients was additionally used to highlight the result a priori gained in vitro. Translational relevance was shown by incorporating transcriptome data of clinical tumor biopsies.

Among all investigated factors – T_h2 cytokines rescued viability of emactuzumab treated MΦ. Furthermore, addition of IL-10 to IL-4 and -13 MΦ was not able to restore emactuzumab susceptibility but was characterized by activation of distinct pathways, namely NFκB or Wnt/β-catenin signaling. In silico analysis of cancer patients revealed distinct IL-4 or -13 overexpression patterns in a subset of patients with partial co-expression of IL-10. Using two surrogate molecules, CD163 and CD206 allowed for discrimination of distinct MΦ populations according to localization and frequency. In emactuzumab-treated patients, we found a significant reduction of CSF1R and CD163 mRNA levels in contrast to a less pronounced decrease of CD206 expression by transcriptome analysis of tumor biopsies. In addition, some of the genetic polymorphisms engaged, interfered with the treatment and were set into a spatial and functional context. In contrast, culturing of cells under hypoxia did increase killing in the emactuzumab-responsive MΦ.

Taking into account the result gained in this effort will help to explain why some patients may have less TAM depletion under therapy and in turn can help identifying those most likely to benefit from anti-CSF1R agents.

CONFLICT OF INTEREST

Corporate-sponsored Research: The current project was funded by Roche and sponsorship has been conducted in form of salaries given to the respective employees involved in this project Nevertheless, Roche had no influence on design and interpretation of the current project and results, which were exclusively handled by the respective authors themselves.

Other Substantive Relationships: All authors are or were employees of Roche. Carola Ries, Michael Cannarile and Dominik Rüttinger are inventors of granted and pending patent applications relating to emactuzmab and stockholders in F. Hoffman La Roche

567 Identification of chemotherapy-induced antigens suitable for immunotherapy in pancreatic cancer patients

S. Bulfamante[1], G. Mandili[1], M. Principe[1], E. Mazza[1], L. Follia[1,2], G. Ferrero[2], A. Evangelista[3], P. Cappello[1,4], F. Novelli[1,4]

[1] University of Turin, Department of Molecular Biotechnology and Health Sciences, Turin, Italy
[2] University of Turin, Computer Science Department, Turin, Italy
[3] Hospital Città della Salute e della Scienza di Torino, Unit of Clinical Epidemiology, Turin, Italy
[4] Hospital Città della Salute e della Scienza di Torino, Center for Experimental Research and Medical Studies, Turin, Italy

BACKGROUND: Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal cancer, both for lack of effective screening method and for resistance to chemotherapy (CTX) and radiotherapy. At present surgical resection is the only potentially curative option. Once diagnosed, chemotherapy , radiation or combination therapy regimens are used to treat patients, but responses remain poor. However, some chemotherapeutic agents, such as Gemcitabine (GEM) have an immune modulating effect and a combination of CTX and immunotherapy could increase therapeutic efficacy. Thus, more immunogenic antigens can be induced by CTX and targeted by passive or active immunotherapy. To discover TAAs that might be selected for immunotherapy, antibody response in PDA patients' sera were analyzed before and after CTX. TAAs selected on the basis of their increased recognition after CTX were used to evaluated whether PDA patient autologous T cells have an increased TAAs specific response after chemotherapy.

MATERIAL AND METHODS: Antibody response in sera of PDA patients, before and after CTX treatments, has been analyzed by Serological Proteome Analysis (SERPA) on 2-dimensional gel electrophoresis proteome map of CFPAC PDA cell line and the antigens recognized were identified by mass spectrometry. T cell proliferation was evaluated by ^{3}H-Thymidine incorporation assay on patients' PBMC stimulated with TAAs.

RESULTS: Several recognized antigens correlate with over-expressed genes in PDA. The increased antibody recognition of four of these antigens correlates with longer survival. The antigens recognized more frequently by patients have been selected for the analysis of T cell response. In most cases PDA patients' PBMC obtained from the draw blood after one or two cycle of CTX showed higher T cell proliferation than before CTX.

CONCLUSIONS: Data indicated that in PDA patients CTX induces an increase of antibody and T cell response to a series of TAAs whose expression is up regulated in PDA. The identification of these antigens forms a platform for ongoing immunological studies aimed to assess the ability of these TAAs, to induce specific helper and cytotoxic response to PDA. Validation of these TAAs will allow to develop specific immunotherapy combined with CTX.

NO CONFLICT OF INTEREST

568 Exploiting RNA profiling of activated dendritic cells to improve vaccine potency assessment

J. Dal Col[1,2], B. Montico[2], D. Martorelli[2], C. Lapenta[3], R. Tarallo[4], G. Giurato[4,5], F. Belardelli[3], A. Weisz[4], R. Dolcetti[2,6], M. Ravo[4]

[1] University of Salerno, Dept. of Medicine- Surgery and Dentistry "Scuola Madica Salernitana", Baronissi, Italy
[2] C.R.O. National Cancer Institute - IRCCS- Aviano, Cancer Bio-Immunotherapy Unit, Aviano, Italy
[3] Istituto Superiore di Sanità, Department of Hematology- Oncology and Molecular Medicine, Roma, Italy
[4] University of Salerno, Laboratory of Molecular Medicine and Genomics- Department of Medicine- Surgery and Dentistry "Scuola Medica Salernitana", Baronissi, Italy
[5] University of Salerno, Genomix4 Life Srl- Department of Medicine- Surgery and Dentistry 'Scuola Medica Salernitana', Baronissi, Italy
[6] The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia

INTRODUCTION: The definition of an 'appropriately activated' dendritic cell (DC) population that exhibits optimum stimulatory capacities is crucial to assess the potency of DC-based vaccines before transplantation back into patients. A novel strategy for harnessing DCs for vaccination consists in exploiting cancer cells undergoing immunogenic cell death (ICD) as broad tumor antigen source. Taking advantage of this approach, we developed a new DC-based vaccination protocol that combines the unique features of interferon-conditioned DC (IFN-DC), with highly immunogenic tumor cell lysates (TCL). Coding and noncoding-RNA profiling of IFN-DCs was performed to identify potential molecular predictors of DC functionality.

MATERIALS AND METHODS: DCs were generated from human monocytes in the presence of GM-CSF and IFN-α. IFN-DCs were loaded with TCL obtained from viable or apoptotic cells. DC maturation markers were analyzed by flow cytometry and cytokine release by ELISA assay. Small and long RNA profiling was performed by NGS. DC-based vaccine efficacy was evaluated in vitro and in vivo using a hu-PBL-SCID mouse model.

RESULTS: ICD was induced by treatment with retinoic acid plus IFN-a (RA/IFN), a highly effective modality to induce ICD ex vivo that we recently developed. IFN-DCs loaded with TCLs from RA/IFN-treated cells proved to be more efficient in eliciting tumor and antigen-specific cytotoxic T lymphocytes in vitro and in mediating tumor growth inhibition in vivo, compared with IFN-DCs loaded with TCLs from untreated or γ-irradiated cells. The improved immunogenicity was associated with an increased release of pro-inflammatory cytokines. RNA-Seq analysis identified 1,711 mRNAs showing different levels in RA/IFN-treated TCL-loaded IFN-DCs compared to INF-DCs pulsed with untreated lysates (FDR ≤0.05 and |FC|>1.5). Functional annotation analysis of the differentially expressed transcripts indicates an enrichment of several canonical pathways most significantly affected by the treatment, including Dendritic Cell Maturation, Toll-like Receptor Signaling and IL-6 Signaling. Interestingly, small RNA sequencing showed several differentially expressed miRNAs targeting deregulated transcripts involved in these pathways.

CONCLUSIONS: These result suggest that defining a transcriptional signature capable of predicting DC stimulatory ability and functions might represent a useful way to assess DC-based vaccine potency.

Supported by MOH (GR-2011-02350476)

AIRC (IG-17426)

NO CONFLICT OF INTEREST

569 Inhibitory immune checkpoint molecules in primary breast tumors

C. Solinas[1], S. Garaud[1], P. De Silva[1], J. Rodriguez Vitória[2], H. Duvillier[1], K. Willard-Gallo[1]

[1] Institut Jules Bordet, Molecular Immunology Unit, Bruxelles, Belgium
[2] Université Libre de Bruxelles, Bioinformatics Laboratory, Bruxelles, Belgium

BACKGROUND: There is a raising interest in identifying novel targets for immunotherapy in breast cancer. Aim of the present study was to investigate the expression of targetable inhibitory immune checkpoint molecules in primary breast cancer.

MATERIAL AND METHODS: We analyzed gene expression from public microarray data on PDCD1 (PD-1), LAG3, HAVCR2 (TIM-3), CTLA4, PDCD1LG1 (PD-L1) and PDCD1LG2 (PD-L2) (on METABRIC dataset). By flow cytometry, we analyzed the expression of immune checkpoint molecules on fresh tumor tissues from untreated primary breast tumors through a multi-color stain for CD45 (pan leukocyte), CD3 (pan T lymphocytes), CD4 (T helper), CD8 (cytotoxic T lymphocytes, CTL), CD19 (B lymphocytes), PD-1, LAG-3, TIM-3, intracellular CTLA-4 (iCTLA-4), PD-L1 and PD-L2.

RESULTS: By gene expression, PDCD1, LAG3, HAVCR2 and CTLA4 were more expressed by basal-like tumors versus luminal A subtype (as per PAM50 molecular definition). Eighty-six untreated fresh primary tumors were available for flow cytometry analysis; 35% were luminal A, 29% luminal B, 19% HER2-positive and 13% triple negative (TNBC), based on immunohistochemical (IHC) classification. PD-1, LAG-3 and TIM-3 were mostly expressed by T helper and CTL, iCTLA-4 by T helper, PD-L1 and PD-L2 by B and T cells. The proportion of PD-1$^+$ lymphocytes was significantly higher than LAG-3$^+$ and TIM-3$^+$ cells. These three markers were frequently expressed in the same tumor sample, particularly by CTL subset and in TNBC. IHC analysis revealed the expression of PD-1, LAG-3, TIM-3 and PD-L1 by tumor infiltrating lymphocytes and within organized tertiary lymphoid structures. LAG-3, TIM-3 and PD-L1 were also found expressed by immune cells in the tumor stroma.

CONCLUSION: Our result reveal that PD-1 is most frequently expressed by T lymphocytes, followed by iCTLA-4, TIM-3 and LAG-3. The expression of these markers might vary according to breast subtype, with LAG-3 expression by CTL being dominant in TNBC. These inhibitory molecules might be novel candidate targets for immunotherapy in TNBC.

NO CONFLICT OF INTEREST

570 In vivo osteosarcomagenesis and tumor immunoediting: From early to late stage disease

S. Gambera[1], A. Morales-Molina[1], D. Kones[1], T. Cejalvo Goyanes[1], A.J. Perisé-Barrios[1], M.A. Rodriguez Milla[1], I. Cubillo Moreno[1], A. Alfranca Gonzalez[2], J. Garcia-Castro[1]

[1] Instituto de Salud Carlos III - ISCIII, Instituto de Investigacion en Enfermedades Raras - IIER, Majadahonda, Spain
[2] Hospital Universitario La Princesa, Fundacion para la Investigacion Biomedica, Madrid, Spain

Osteosarcoma (OS) is the most common malignant primary tumor of bones in children and young adults. Over the past few decades the 5-years event free survival (EFS) of localized primary tumor has not changed (60-75% of cases) and only 15%-30% of patients presenting metastatic disease survive. Emerging evidence on the role of the antitumor activity of the immune system has generated great interest in immunotherapy. Current treatment of OS is based on chemotherapy (MAP: methotrexate, cisplatin, doxorubicin) and surgery but little is known about its immunobiology and if OS can be good candidates for therapies based on the immune system.

In order to explore OS immunology, OS formation was induced in FVB mice and tumor associated immune cells were analyzed at different time points. OS formation was achieved by the inoculation of in vitro transformed murine mesenchymal stem cells knock-out for p53 and RB genes. Cells were transformed in vitro and so still did not undergo immunoedition process. Immune cell infiltration was analyzed by immunohistochemistry and flow cytometry.

Data revealed many intriguing dynamics of tumor-immune system interactions in OS. Tumors presented a strong infiltration of CD45+ hematopoietic cells after tumor cells implantation and during early stage disease, but this feature was lost with disease progression. Tumor infiltrating lymphocytes (TILs) also seem to be relevant in the pathogeny. A strong TILs infiltration is observed in early stage tumors and unexpectedly frequent cell population (CD4$^+$CD8$^+$ T cells) were detected and further lost. Disease progression also seems to be associated with changes in phenotypic markers of neutrophil and macrophage cells. Early stage tumors are characterized by proinflammatory phenotype (M1&N1) that shifted to anti-inflammatory phenotype (N2&M2) with disease progression. Dendritic cells are the

most frequent cell types with a changing frequency during the disease that seems to correlate with tumor progression.

These result indicate that OS could be a potential candidate for immunotherapies and, depending on disease stage, immunomodulatory drugs could represent a new strategy for OS defeat.

NO CONFLICT OF INTEREST

571 Anti-tumor immunization of mothers delays tumor development in cancer prone offspring

G. Barutello[1], C. Voena[2], F. Riccardo[3], M. Arigoni[3], R. Chairle[4], F. Cavallo[3]

[1] Molecular Biotechnolgy Center, Dept of Molecular Biotechnology and Health Sciences, Torino, Italy
[2] Center for Experimental Research and Medical Studies CeRMS, Department of Molecular Biotechnology and Health Sciences, Torino, Italy
[3] Molecular Biotechnolgy Center, Department of Molecular Biotechnology and Health Sciences, Torino, Italy
[4] Children's Hospital Boston, Department of Pathology, Boston, USA

BACKGROUND: Childhood cancer is an issue of real urgency in oncology. Neuroblastoma (NB) is classified as the most common malignancy diagnosed within the first year of life and is frequently associated with mutations in the anaplastic lymphoma receptor tyrosine kinase (ALK) gene. The immune-based therapies are the most promising strategies currently studied to manage NB; however, the prevention of pediatric NB is a not yet reached reality. Recently, we demonstrated that maternal immunization (MI) against Her2/neu oncoantigen (neu) is effective in hampering tumor onset in offspring prone to develop neu-positive mammary cancer, because of the passive transfer of maternal immunity and immune-complexes to the pups, eliciting their active immunization against neu. Thus, we hypothesize a successful application of MI approach against the ALK oncoantigen to a transgenic mouse model of spontaneous NB, driven by the overexpression of a mutated form of ALK (ALK^{F1174L}) and MYCN oncogene in neural crest-derived cells: the ALK^{F1174L}/MYCN mice.

MATERIALS AND METHODS: Female BALB/c mice hemizygous for the MYCN oncogene underwent DNA electrovaccination with a prime-boost immunization schedule by intramuscular injection of a plasmid that codes for the extracellular and transmembrane domains of the human ALK protein (ALK-ECTM) or a control empty vector prior to be mated with BALB/c males hemizygous for the ALK^{F1174L} oncogene (ALK^{+} mice). Magnetic Resonance Imaging (MRI) technique has been exploited to determine the effect of anti-ALK MI in hampering NB progression in ALK^{F1174L}/MYCN double transgenic offspring born from ALK-ECTM or control mothers. Immunofluorescence, Western blot and cytofluorimetric analysis were performed in order to assess the immune response elicited by anti-ALK immunization in mothers and their offspring.

RESULTS: The fertility, the number and the size of the newborns resulted unaffected in the group of mice vaccinated with ALK-ECTM compared to control mothers. A significant reduction of tumor growth kinetic, together with a significantly enhanced overall survival, were shown in ALK^{F1174L}/MYCN offspring born from ALK-ECTM mothers compared to control offspring. Moreover, we detected specific anti-ALK vaccine-induced IgG antibodies in the sera of vaccinated mothers and their offspring.

CONCLUSIONS: These data are consistent with our recent findings about the role of DNA MI against specific oncoantigens as a weapon to hamper cancer development in genetically predestinated offspring, paving the way for the potential application of this groundbreaking approach to neonatal malignancies that may have a substantial impact on clinical practice.

NO CONFLICT OF INTEREST

572 Mouse breast tumor growth and metastasis inhibition via immunotargeting of the cancer stem cell antigen xCT

S. Lanzardo[1], L. Conti[1], E. Bolli[2], V. Rolih[1], A. Ballatore[1], R. Ruiu[1], G. Donofrio[2], O. John[3], F. Pericle[3], F. Cavallo[1]

[1] University of Turin, Department of Molecular Biotechnology and Health Sciences, Turin, Italy
[2] University of Parma, Department of Medical Veterinary Science, Parma, Italy
[3] Agilvax, Indian School Rd, Albuquerque, Mexico

INTRODUCTION: The several unsuccessful treatments in metastatic cancers might miss cancer stem cells (CSC), a sub-population of cells with a critical role in cancer. By contrast, a vaccine-elicited immune response against CSC might be particularly effective. The identification of oncoantigens (OA) expressed by CSC may provide new targets for effective anticancer vaccines.

MATERIAL AND METHODS: The $ErbB2^{+}$ TUBO cell line was plated in differentiative conditions to obtain epithelial cells or under specific conditions to generate mammospheres, and a transcription profiling analysis was performed. Integrating data obtained with meta-analyses of 7 independent human breast tumor data sets we identified xCT, a channel that supports glutathione synthesis, as a new CSC mammary OA that were validated both in vitro and in vivo. To set up immunotherapies targeting xCT, we used an approach based on Agilvax's innovative bacteriophage MS2 virus-like particle (VLP) or Bovine herpesvirus 4 based (BoHV4) technologies. Using genetic approaches, we produced VLPs that displayed different xCT extracellular domains (ECD) on an exposed loop of the VLP or BoHV4 coding for the full xCT protein.

RESULTS AND DISCUSSION: xCT expression increases over mammospheres passages; its silencing significantly reduces TUBO cells ability to generate mammospheres. The DNA vaccination-based in vivo immunotargeting of xCT slows established subcutaneous tumor growth and strongly impairs pulmonary metastasis formation in mice that have been challenged with syngeneic tumorsphere-derived cells. This effect depends on the generation of anti-xCT antibodies, which are able to alter CSC self-renewal and redox balance and was improved when combined with cytotoxic drugs. BoHV4 and VLP based anti-xCT vaccines were evaluated to improve the in vivo anticancer efficacy of xCT targeting.

Starting from the analysis of breast CSC transcriptoma, we developed a new DNA vaccine targeting a freshly identified breast CSC OA, xCT, whose inhibition strongly impairs mammary tumor development and metastases.

CONCLUSIONS: This study provides a genomic characterization of mammary CSC and identifies fresh targets for new and potentially effective anticancer vaccines, thus providing a new tool for the design of combined therapeutic approaches that efficaciously target both CSC and more differentiated cells in breast cancers, leading to both cancer treatment and prevention of metastases and recurrence.

NO CONFLICT OF INTEREST

573 Immunotherapy against Non Small Cell Lung Cancer: Exploiting DNA vaccination against ROS1

F. Riccardo[1], G. Barutello[1], M. Arigoni[1], L. Conti[1], C. Musiu[1], D.L. Longo[2], R. Calogero[1], M. Volante[3], M. Papotti[3], F. Cavallo[1], E. Quaglino[1]

[1] Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences, Torino, Italy
[2] Center for Molecular Imaging, Department of Chemistry IFM, Torino, Italy
[3] Pathology Unit, Department of Oncology, Torino, Italy

BACKGROUND: Non small cell lung cancer (NSCLC) is the leading cause of cancer death in men and women. The development of new therapeutic strategies is essential for improving the prognosis and treatment of patients. An interesting experimental model to study human lung adenocarcinoma is represented by $Kras^{G12D}$ mice. We previously characterized the lung cancer progression in $Kras^{G12D}$ mice that develop aggressive NSCLC, displaying a stepwise, age-related progression, mimicking several features observed in lung cancer patients. To identify targetable oncoantigens expressed during NSCLC development, we performed a gene expression profile analysis: among the genes found over-expressed in $Kras^{G12D}$ mice, the tyrosine kinase receptor ROS1 was identified as a potential candidate to be further investigated.

METHODS: Lungs and organs of 10, 20 and 30 week-old wild type (wt) and $Kras^{G12D}$ mice were collected for RNA extraction and further analysis. The expression of ROS1 in $Kras^{G12D}$ mice was validated at mRNA (qPCR) and protein (IHC) levels. Moreover, a $ROS1^{+}$ cell line (KL-ROS1) was generated from a $Kras^{G12D}$ mouse lung tumor. The efficacy of ROS1-immunotargeting was evaluated against both KL-ROS1 subcutaneously injected cells and spontaneous lung tumors using a mouse or a human anti-ROS1 DNA vaccines. Finally, to characterize the immune infiltration during lung cancer progression, tumor infiltrating-lymphocyte (TIL) analysis was performed by flow cytometry in $Kras^{G12D}$ mice at 10 and 30 weeks of age.

RESULTS AND DISCUSSION: While its expression was absent in wt mice, ROS1 over-expression was evident at mRNA and protein levels in both primary lung tumors and metastasis from $Kras^{G12D}$ mice. Interestingly, cancer stem cell (CSC) enriched-lung spheres, derived from KL-ROS1 cells, were also $ROS1^{+}$. These result suggest that ROS1 could be involved in the early and late stages of NSCLC progression and metastatization. Anti-ROS1 DNA vaccination against both subcutaneous and spontaneous lung tumors was quite effective. However, to increase the anti-tumor efficacy and to identify the potential immunosuppressive mechanism that could affect the success of DNA vaccines against NSCLC, we evaluated the evolving TIL during lung cancer progression in $Kras^{G12D}$ mice. A prominent $CD3^{+}$ infiltration characterized the early stage of tumor progression while immunosuppressive cells, i.e. potentially pro-tumorigenic gd T cells, tumor-associated macrophages and myeloid-derived suppressor cells, dominated the late response.

CONCLUSION: ROS1 overexpression not only in primary lung tumors but also in metastasis and in CSC-enriched lung spheres make it an even more interesting immunotherapeutic target. The combination of anti-ROS DNA vaccination with the modulation of the immunosuppressive microenvironment in the lung lesions could result in an effective strategy to fight against $ROS1^{+}$ tumors.

NO CONFLICT OF INTEREST

574 High affinity bispecific EGFR/CD16A antibodies specifically recruit NK-cells to target EGFR-expressing tumors

M. Kluge[1], U. Reusch[1], K. Ellwanger[1], I. Fucek[1], M. Weichel[1], T. Haneke[1], S. Knackmuss[1], E. Rajkovic[1], M. Treder[1]

[1] Affimed GmbH, Affimed GmbH, Heidelberg, Germany

BACKGROUND: The epidermal growth factor receptor (EGFR) is an important and established target for the treatment of several solid tumors, including colorectal, head and neck and lung cancer. Treatment with tyrosine kinase inhibitors and monoclonal antibodies targeting EGFR is dependent on mutation status of the receptor which may cause resistance to the treatments. An EGFR targeting therapy which is effective independent of the mutation status offers a differentiated treatment approach. Natural killer cells (NK-cells) play a central role in the innate immune system, have the capacity to destroy neoplastic cells and can be effectively utilized for effective anti-tumor engagement.

MATERIAL AND METHODS: To specifically utilize the cytotoxic potential of NK-cells for the elimination of EGFR-expressing cancer cells, we developed tetravalent bispecific EGFR/CD16A NK-cell engagers with two binding sites for EGFR and two binding sites for CD16A. CD16A is an isoform of CD16 which is specifically expressed by NK-cells and macrophages but not by neutrophils. The antibodies were generated using proprietary human anti-EGFR and anti-CD16A variable domains and characterized regarding binding, stability, manufacturability, efficacy and safety in a wide range of biophysical and functional assays in vitro and in vivo.

RESULTS: We identified high affinity antibodies recognizing epitopes in the extracellular domain 21 of EGFR, a domain that is not targeted by other therapeutic antibodies. We engineered a set of EGFR/CD16A antibodies and analyzed their characteristics. Antibodies containing domain 21 showed single digit picomolar or sub-picomolar EC_{50} values and were more potent than a control antibodies containing the variable domain from cetuximab. In addition, the EGFR/CD16A antibodies showed excellent biophysical properties and the lead candidate AFM24 is being characterized in in vivo pharmacology studies.

CONCLUSION: In summary, AFM24 is a novel, highly potent drug candidates suitable for the treatment of EGFR-expressing malignancies with the potential to overcome resistance to other EGFR targeting agents.

CONFLICT OF INTEREST

Other Substantive Relationships: All authors are employees of Affimed GmbH

575 AFM26: A first-in-class, high affinity bispecific NK-cell engager targeting BCMA to treat multiple myeloma

T. Gantke[1], U. Reusch[1], C. Kellner[2], K. Ellwanger[1], I. Fucek[2], M. Weichel[1], M. Peipp[2], M. Treder[1]

[1] Affimed GmbH, Affimed GmbH, Heidelberg, Germany
[2] Kiel Uniuersity- 2 nd Department of Medicine, Diuision of Stem Cell Transplantation and Immunotherapy, Kiel, Germany

INTRODUCTION:

Although current treatments for newly-diagnosed multiple myeloma (MM) including autologous stem cell transplantation (ASCT) achieve high remission rates, minimal residual disease (MRD) represents an unmet medical need in most patients. Consequently, a cure has remained elusive and there is a need for new treatments to induce deeper and longer lasting responses. Immunotherapies targeting the MM-expressed surface antigen BCMA hold the potential for potent efficacy due to BCMA's near universal expression on myeloma cells. AFM26 is a novel, tetravalent, bispecific antibody targeting BCMA and CD16A (FcγRIIIa) to selectively redirect natural killer (NK-) cell lysis to MM. NK-cells are thought to contribute to the efficacy of current treatments and are the first lymphocyte population to reappear following ASCT. Consequently, enhancing NK-cell activity against MM using antibodies, in particular in transplant-eligible MM, may be a promising intervention to increase rates of MRD negativity. However, antibody-mediated engagement of NK-cells may be hampered by high-level production of monoclonal immunoglobulin in MM, which competes for CD16A-binding. In this study, we analyzed the impact of high level IgG on antibody-induced NK-cell cytotoxicity towards MM.

MATERIAL AND METHODS: NK-cell engagement and activity of BCMA-targeting antibodies, including AFM26, to induce target cell lysis were tested in vitro using a panel of cell lines expressing different levels of BCMA. Further, cytokine release associated with antibody-induced target cell lysis in PBMC cultures was assessed.

RESULTS AND DISCUSSION: AFM26 was found to interact with NK-cells bivalently, resulting in high avidity and slow dissociation kinetics. Unlike IgG-based formats, NK-cell-binding of AFM26 was largely unaffected by competing IgG, suggesting a unique mode of binding to CD16A. AFM26 potently induced lysis of BCMA+ target cells in vitro even in presence of competing IgG. Of note, AFM26-induced release of pro-inflammatory cytokines in PBMC cultures was comparable with IgGs and NK-cell activation was strictly dependent on target cell contact suggesting a well-manageable safety profile.

CONCLUSIONS: AFM26 is a high-affinity and highly potent drug candidate for MM with a unique mode of NK-cell engagement that differentiates it from classical IgG-based approaches to redirect effector cell lysis to myeloma cells. Consequently, AFM26 is the first BCMA-targeting antibody to fully exploit the lytic potential of NK-cells in MM.

CONFLICT OF INTEREST

Other Substantive Relationships: TGA, URE, KEL, IFU, MWE and MTR are employees of Affimed GmbH

576 Superior safety profile and comparable efficacy of a localized CD137-activating DARPin protein compared to urelumab

A. Link[1], J. Hepp[1], U. Fiedler[1], C. Reichen[1], C. Metz[1], A. Titz[1], I. Tosevski[1], L. Juglair[1], V. Levitsky[1], M.T. Stumpp[1], D. Snell[1]

[1] Molecular Partners AG, Biology, Schlieren, Switzerland

BACKGROUND: Urelumab (BMS-663513) is a humanized monoclonal antibody binding to CD137 which, upon Fc-clustering, leads to activation of T-cells. Urelumab is currently in Phase 2 clinical development and has been reported to cause significant hepatotoxicities (around 15% Grade ≥2 ALT and AST elevation) when given as infusion every 3 weeks at doses ≥0.3 mg/kg. Currently ongoing clinical trials report decreased systemic toxicity but limited efficacy at lower doses of urelumab. We hypothesized that more effective triggering of CD137 without associated systemic toxicity may be achieved by targeting a CD137 agonistic engager without Fc to fibroblast activation protein (FAP) which is abundantly expressed in the stroma of many solid tumors. To achieve this, a targeted molecule belonging to the DARPin® family of binding proteins was composed of one FAP- and two CD137-binding domains in a 'beads on a string" format. The present study evaluated this hypothesis of an enlarged therapeutic window in a mouse model reconstituted with human PBMCs.

MATERIAL AND METHODS: Human PBMCs were used to reconstitute the immune system in NOG mice implanted subcutaneously with HT-29 human colon cancer cells. The CD137 antibody (8 mg/kg, n=10) or FAP-binding CD137-agonistic DARPin® drug candidates (at either 8 mg/kg, 1.6 mg/kg, or 0.3 mg/kg, n=10 each) were administered intravenously during 2 weeks. Mice were monitored for survival, body weight, and tumor size.

RESULTS: None of the mice in the control group died and no significant body weight loss was observed. Six of ten (60%) mice in the CD137 antibody group showed strong signs of graft vs. host disease and either died or reached the termination criterion of ≥20% body weight loss and were sacrificed. One of 30 (3%) mice died in the DARPin® drug candidate groups but none of the animals showed body weight loss of greater than 20% ($p < 0.001$, Log-rank test). Tumor growth inhibition was comparable for all treatment groups (around 20-30% at Day 18) and reached significant p-values of <0.05 (Mann Whitney Test).

CONCLUSION: This study confirms the hypothesis that systemic toxicities caused by the urelumab mode of action can be circumvented by FAP-targeting of a CD137 agonistic DARPin® drug candidate while achieving comparable tumor growth inhibition. Consequently, higher clinical doses of tumor stroma-targeted agonistic DARPin® drug candidates might be possible, resulting in stronger tumor growth inhibition.

CONFLICT OF INTEREST

Ownership: The authors are shareholders of Molecular Partners AG, Switzerland.

Corporate-sponsored Research: The authors are employees of Molecular Partners AG, Switzerland.

577 Local p53 activation in the tumor microenvironment with effective immune cell recruitment improves antitumor immunity and controls tumor progression

Y. Cui[1], M. Yu[1], S. Lu[1], H. Sultan[1], W. Xiao[1], E. Celis[1], G. Guo[1]

[1] Georgia Cancer Center- Augusta Uniuersity, Biochemistry and Molecular Biology, Augusta, USA

BACKGROUND: Trp53 dysfunction is considered a major cause of cancer and targeting p53 pathway in the tumor microenvironment (TME) represents an attractive strategy. Small molecules that activate wild type p53 or revert p53 mutants to wild type configuration have been developed and some been tested for in clinical trials as tumoricidal agents. However, hematopoietic toxicity following systemic delivery has hampered its application. Recent studies suggest that p53 inactivation/dysfunction in the TME skews the immunological landscape towards pro-tumor/inflammation and likely represents the immunological consequence for tumorigenesis. We propose that local activation of the p53 pathway in the TME represents a novel strategy to reverse immune suppression and harness antitumor immunity.

MATERIAL AND METHOD: We employed nutlin-3a, a pharmacological activator of p53 via inhibiting MDM2, and murine transplantable B16 melanoma and performed in vitro and in vivo experiments to examine the efficacy of nutlin-3a in killing tumor cells. We further investigated the effects of nutlin-3a-induced p53 activation in enhancing antitumor immunity and tumor control.

RESULTS AND DISCUSSION: Nutlin-3a treatment of B16 tumors in culture led to their immunogenic cell death, characterized as cell surface exposure of calreticulin, release of HMGB1 and ATP to extracellular space. However, intratumoral nutlin-3a injection into the B16 TME did not result in tumor regression in vivo. Further analysis revealed that immune cell infiltration, including myeloid cells and T cells, to the B16 TME was limited, despite nutlin-3a-induced localized tumor death. To improve the recruitment of tumor infiltrating immune cells, we injected poly-IC, a TLR3 agonist, intratumorally. A single injection of poly-IC significantly improved both myeloid and T cell infiltration to the B16 TME, but only modestly reduced tumor progression. On the other hand, nutlin-3a injections following poly-IC injection not only significantly enhanced the activation of tumor infiltrating myeloid cells and CD8 T cells, but also greatly improved effector cytokine production among tumor infiltrating CD8 T cells, resulting in marked tumor regression, with limited toxicity.

CONCLUSION: Local nutlin-3a-induced p53 activation in the TME represents a novel strategy that repurposed for both direct tumor killing and, more importantly, subsequent activation of antitumor immunity with improved therapeutic efficacy and limited toxicity.

NO CONFLICT OF INTEREST

578 Vascular responses to pembrolizumab and ipilimumab in patients with metastases to the brain receiving stereotactic radiosurgery

I. Digernes[1], E. Grøvig[2], L.B. Nilsen[1], C. Saxhaug[2], O.M. Geier[1], D.O. Sætre[3], B. Breivik[4], K.D. Jacobsen[5], A. Helland[5], K. Emblem[1]

[1] *Oslo University Hospital, Dept. of Diagnostic Physics, Oslo, Norway*
[2] *Oslo University Hospital, Dept. of Radiology and Nuclear Medicine, Oslo, Norway*
[3] *Østfold Hospital Trust-, Dept. of Radiology, Kalnes, Norway*
[4] *Hospital of Southern Norway, Dept. of Radiology, Kristiansand, Norway*
[5] *Oslo University Hospital, Dept. of Oncology, Oslo, Norway*

BACKGROUND: The clinical benefits of adding immunotherapy to stereotactic radiosurgery (SRS) in patients with metastases to the brain is conflicting [1]. Unlike traditional chemo-radiation, a tumor responding to immunotherapy may not result in a simple reduction in radiographic size. We here go beyond imaging-based volumetric assessments and present our initial observations of vascular responses to combined SRS and pembrolizumab or ipilimumab in patients with brain metastases from malignant melanomas and non-small cell lung cancer.

METHODS: Twenty-four patients with metastases to the brain from lung cancer (N=13) and malignant melanomas (N=11) have so far been included on the basis of MRI exams at pre-SRS, and then repeated every 3 month up until 9 months post-SRS [2]. Six patients (5 melanomas, 1 lung) received pembrolizumab (2mg/kg/3rd week) or ipilimumab (3mg/kg/3rd week) at 3-9 months post-SRS. SRS was delivered to the tumor bed (+2mm margin) with a peripheral dose of 18-25Gy, depending on tumor size. Voxel-wise estimations of blood volume and vessel calibers were acquired from perfusion MRI and Vessel Architectural Imaging [3]. Enhancing tumors receiving SRS were identified on contrast-enhanced MRIs, with peri-tumoral regions defined by a 2mm wide dilation outside the SRS target area.

RESULTS: After SRS, 4/6 (67%) of the patients on immunotherapy had an increase in the contrast enhancing volume within the 9 month period. The corresponding number for patients on SRS monotherapy was 7/18 (39%). At 6 months, the relative vessel calibre in the peri-tumoral area was higher (median value 1.79) in patients receiving immunotherapy compared to patients on SRS monotherapy (median value 0.77; $P<0.05$). All patients taken together, higher relative peri-tumoral blood volume values at 3-9 months were found in patients with overall survival >18 months ($P<0.05$). There was no observed difference in the vascular response to pembrolizumab compared to ipilimumab.

CONCLUSION: The increase in mean vessel calibers suggests pembrolizumab and ipilimumab cleans up the vascular bed by removing small caliber vessels and effectively reducing the capillary vessel density. Vascular MRI may help shed light on the clinical response to immunotherapy and identify patients with metastatic disease that benefit by prolonged survival.

REFERENCES:

[1] Patel et al, Am J Clin Oncol 2015;16:[Epub];

[2] Digernes et al, Can Res 2016;76(14 Suppl.):1471;

[3] Emblem et al, Nat Med 2013;19(9):1178-83

CONFLICT OF INTEREST

Other Substantive Relationships: Kyrre E. Emblem: Intellectual property rights, NordicNeuroLab AS, Bergen, Norway

579 Novel domain-targeted anti–Trop-2 monoclonal antibodies exhibit complementary binding and synergic anti-tumor efficacy in multiple human cancers

E. Guerra[1], M. Trerotola[1], V. Relli[1], C. Pedicone[1], A. D'Amore[1], F. Dini[1], S. Fratarcangeli[1], S. Alberti[1]

[1] *"G. d'Annunzio" University of Chieti-Pescara, Unit of Cancer Pathology- CeSI-MeT, Chieti, Italy*

BACKGROUND: The transmembrane glycoprotein Trop-2 is an epithelial calcium signal transducer that is overexpressed in most human carcinomas, where it is associated with worse prognosis. Trop-2 drives tumor cell proliferation though a pro-growth signaling network that converges on AKT as major hub. The Trop-2 extracellular region is organized into an N-terminal cysteine-rich globular domain (GD) and a stem-like cysteine-less domain (SD) that connects to the plasma membrane. Most of the existing anti–Trop-2 monoclonal antibodies (mAbs) target a single immunodominant epitope between GD and SD, and show limited or no anticancer efficacy. Target accessibility might be severely limited by homophilic binding between Trop-2 molecules on adjacent cells and/or multimeric molecular interactions with junctional proteins. To exploit the potential of Trop-2–targeted anti-cancer therapy, we have generated novel domain-targeted anti–Trop-2 mAbs.

METHODS: Hybridomas were generated through immunization with the recombinant Trop-2 extracellular region produced in human 293 and murine L cell lines and baculovirus expression system. Specific anti–Trop-2 hybridomas were identified and selected by differential binding to live 293 cells expressing GS or SD, using flow cytometry. The therapeutic efficacies of these domain-targeted anti–Trop-2 mAbs were determined in vivo on human cancer xenografts in nude and NSG mice. mAb binding/ internalization and induction of signaling and ADCC were determined in vitro using live-cell imaging, confocal microscopy, Western blotting, and reporter assays.

RESULTS: Tailored immunization and screening approaches provided two classes of novel anti–Trop-2 mAbs distinctly specific for GD or SR. The naked anti-GD OXG64 and anti-SD OXS55 mAbs were most effective for inhibition of in-vivo growth of multiple tumor types, including colon, ovary, and prostate cancers. OXG64 and OXS55 showed differential efficacies toward established tumors versus isolated-cell models of metastatic dissemination. This supports our strategy of maximizing the differential accessibility of Trop-2 according to growth mode. OXG64 and OXS55 showed anti-tumor synergy when co-administered in vivo. They also show efficient mediation of ADCC and inhibit Trop-2 pro-growth signaling. Efficient internalization was also shown for ADC development.

CONCLUSIONS: The novel OXG64 and OXS55 anti–Trop-2 mAbs are domain-targeted synergic therapeutic mAbs for game-changing anticancer immunotherapy.

CONFLICT OF INTEREST

Corporate-sponsored Research: Research was partially supported by funds from Oncoxx Biotech s.r.l.

580 Inhibition of tumor growth by cancer vaccine combined with metronomic chemotherapy and anti-PD-1 in a pre-clinical setting

A. Petrizzo[1], A. Mauriello[1], A. Luciano[1], C. Arra[1], M. Tornesello[1], G. Botti[1], G. Ciliberto[1], F.M. Buonaguro[1], M. Tagliamonte[1], L. Buonaguro[1]

[1] *National Cancer Institute Pascale, Biologia Molecolare e oncogenesi uirale, Naples, Italy*

BACKGROUND: The efficacy of cancer immunotherapies is considerably limited by the immune suppressive tumor microenvironment. In order to design strategies to improve the efficacy of cancer vaccines, a novel combinatorial approach was assessed in a mouse melanoma model.

MATERIAL AND METHODS: An aggressive therapeutic setting based on sub-cutaneous ectopic implantation of B16F10 melanoma cells was used as proof of concept. The tumor microenvironment was addressed combining a novel mix of chemotherapy agents, administered in a metronomic fashion (MCT), and an anti-PD-1 checkpoint inhibitor (CI). The vaccine (PEPT) was a multi-peptide cocktail, including melanoma-specific tumor-associated antigens.

RESULTS: The newly designed strategy was shown to be well tolerated and highly effective. Animals treated with the multi-peptide vaccine combined with MCT or CI showed remarkable delay in tumor growth and prolonged survival as compared to control groups. Remarkably, the multi-pronged combination including PEPT+weekly MCT+CI was able to prolong survival in all six mice (100%) and inhibit tumor growth in 66.6% of mice (4/6). Moreover, even without further administration of the combinatorial immunotherapy, the re-challenged tumor-negative mice showed prolonged survival and tumor growth inhibition in 1/4 mice (25%). The anti-tumor effect was associated with a strong T cell immune response to vaccine mutated peptides and a significant reduction of regulatory T cells.

CONCLUSIONS: Overall, the result showed that combination of a vaccine with multiple therapies targeting the tumor microenvironment, and in particular the one including both MCT and CI, was highly efficient in potentiating the vaccine's anti-tumor effects. The described approach is highly promising to be moved into human clinical trial.

NO CONFLICT OF INTEREST

581 IL-33 inhibits melanoma growth and pulmonary metastasis in mice through recruitment and activation of eosinophils

V. Lucarini[1], G. Zicchedda[1], I. Macchia[1], V. La Sorsa[1], F. Peschiaroli[1], C. Buccione[2], A. Sistigu[2], C. Afferni[3], F. Mattei[1], G. Schiavoni[1]

[1] *Istituto Superiore di Sanità, Oncology and Molecular Medicine, Rome, Italy*
[2] *Regina Elena National Cancer Institute, Rome, Rome, Italy*
[3] *Istituto Superiore di Sanità, Infectious- Parasitic and Immune-mediated Diseases, Rome, Italy*

BACKGROUND: The alarmin IL-33 is an IL-1 family-member that plays pleiotropic roles in allergy, autoimmunity and inflammation through binding to its specific receptor ST2. Emerging evidences suggest an involvement of this cytokine also in cancer immunity, although its function remains ill-defined. In this study, we have investigated the role of IL-33/ST2 axis in anti-tumor response to melanoma.

MATERIAL AND METHODS: C57Bl/6 mice were implanted subcutaneously with B16.F10 melanoma cells and injected with intraperitoneal IL-33. Tumor growth was monitored and tumor-immune infiltrates were examined by flow cytometry and qPCR. ST2-deficient or control mice received intranasal instillations of IL-33 prior to intravenous transfer of B16.F10. Pulmonary metastases were enumerated and lung immune infiltrates were analysed by flow cytometry and qPCR. Depletion of eosinophils in vivo was carried out by repeated injections with intraperitoneal anti-Siglec-F antibody. Finally, bone marrow-derived eosinophils were exposed to IL-33 in vitro and assayed for direct cytotoxic activity against B16 melanoma cells by flow cytometry.

RESULTS: Injection of IL-33 in melanoma-bearing mice resulted in significant tumor growth delay. This effect was associated with intratumoral accumulation of CD8 T cells and eosinophils, decrease of immunosuppressive myeloid cells, and a mixed Th1/Th2 cytokine expression pattern with local and systemic activation of CD8 T and NK cells. Intranasal administration of IL-33 determined ST2-dependent eosinophil recruitment in the lung that prevented the onset of pulmonary metastasis after intravenous injection of melanoma cells. In addition,

ST2-deficient mice developed pulmonary metastasis at higher extent than wild-type counterparts, associated with lower eosinophil frequencies in the lung. In vivo depletion of eosinophils abolished the ability of IL-33 to both restrict primary tumor growth and metastasis formation. Finally, IL-33 was able to activate eosinophils resulting in efficient killing of target melanoma cells, suggesting a direct anti-tumor activity of eosinophils following IL-33 treatment.

CONCLUSIONS: Our result identify a previously unrecognized eosinophil-dependent anti-tumor mechanism triggered by IL-33/ST2 axis that restricts melanoma growth and metastasis. Our findings may open perspectives for novel combined cancer immunotherapy strategies.

NO CONFLICT OF INTEREST

582 Pharmacodynamic (PD) Biomarkers in Syngeneic Tumor Models Treated by a GITR Agonist (GITRL-Fc) and Prevalence of GITR Expression

F. Cattaruzza[1], P. Yeung[1], M. Wang[1], A. Brunner[1], E. LeScolan[1], Y. Liu[1], G. O'Young[1], B. Cancilla[1], G. Argast[1], A.M. Kapoun[1]

[1] Oncomed Pharmaceuticals Inc., Redwood City, CA, USA

INTRODUCTION: GITRL (Glucocorticoid-Induced Tumor Necrosis Factor Receptor Ligand, TNFSF18) is a member of the tumor necrosis factor (TNF) ligand superfamily. GITRL binds and activates the co-stimulatory surface receptor GITR, which promotes proliferation and activation of effector T cells (Teff) and inhibits suppressive activity of regulatory T cells (Treg), both important to promote anti-tumor immunity. We generated a novel single-gene GITRL trimer fused to an immunoglobulin Fc domain (GITRL-Fc) that shows robust single agent antitumor efficacy and immune effects in multiple syngeneic mouse models, suggesting its potential benefit in cancer immunotherapy

MATERIAL AND METHOD: A multi-platform approach was taken to investigate GITRL-Fc pharmacodynamic (PD) biomarkers in tumors and in matched whole blood samples from mice bearing CT26 colon, 4T1 breast, and B16F10 melanoma carcinoma models. Global gene expression levels were profiled by microarray, while changes in immune cell populations and cytokine secretion were measured by flow cytometry, Luminex and immunohistochemistry (IHC). To investigate the prevalence of GITR expression, a GITR IHC assay was developed and used to screen a panel of human tumors. In addition, RNA-Seq data from 33 tumor types in TCGA and 24 patient-derived xenograft (PDX) samples were used for prevalence analysis.

RESULTS AND DISCUSSION: In tumor samples, GITRL-Fc increased the gene expression associated with T cells, CD8 T cells, cytotoxicity, Th1 cells, interferon gamma (IFN-γ), natural killer cells, Teff cells, and T cell activation markers. These gene changes were validated by quantitative real-time PCR. Similarly, flow cytometry analysis showed that GITRL-Fc promoted activation of CD4+ effector cells, decreased Treg frequency, and increased the ratio of CD8+ T cell/Treg in the tumor. GITRL-Fc also modulated secretion of cytokines in splenocytes, including an increase in IFN-γ.

To investigate the prevalence of GITR expression in human tumors, RNA-Seq data analyses of 33 tumor types in TCGA showed GITR is highly expressed in a subset of solid tumors, including head & neck, lung, breast, esophageal, and bladder cancers. In most solid tumors, GITR expression correlated poorly with T cell markers, implying that GITR may not be exclusive to immune cells and may be expressed in tumor cells as well. Similar findings emerged from RNA-Seq data analysis of patient-derived xenograft (PDX) samples from 24 tumor types. The gene expression data was corroborated by IHC analysis of GITR expression in 17 tumor types, which showed that in addition to immune cells, GITR was expressed on tumor cell membranes.

CONCLUSIONS: Taken together, the PD biomarker changes in immune-related gene expression, immune cell populations, and cytokine secretions observed in these preclinical tumor models are consistent with GITRL-Fc mechanism of action and demonstrated target engagement of GITRL-Fc.

CONFLICT OF INTEREST

All abstract authors are Oncomed Pharmaceuticals employees

583 Vangl2 overexpression/silencing increases apoptosis and effects inflammation in Jurkat t-cells

M. Lindqvist[1], D. Lindholm[2], T. Sejersen[1]

[1] Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden
[2] Institute of Biomedicine/Biochemistry and Deuelopmental Biology, University of Helsinki, Helsinki, Finland

AIMS: We had earlier by TUNEL staining established that overexpression of Wnt7a which is a ligand of Vangl2 receptor increases apoptosis compared to controle. We now wanted to see how overexpression/silencing of Vangl2 affects apoptosis and inflammation.

METHODS: Jurkat T-cells were cultured on coverslips in 6-well plates until they became 80% confluent. They were transfected with a commercially bought Vangl2-GFP construct for 24 or 48 hours while the controls where transfected with GFP. result The frequency of TUNEL stained cells were higher among the Jurkat T-cells that were transfected with Vangl2 compared to controle cells. We could also see a rearrangment of p53 and Th17 expression that are important in inflammation signaling.

CONCLUSION: We conclude that Vangl2 overexpression/silencing affects apoptosis and hypothise that this is done via caspase-8 signaling. We also suggest that the rearrangement of p53 and Th17 expression is a result of actin translocation from the cytoplasm into the nucleus.

NO CONFLICT OF INTEREST

584 A pipeline for studying neoantigen landscapes in tumors

M. Punta[1], S. Lise[1]

[1] Centre for Evolution and Cancer, The Institute of Cancer Research, London UK

INTRODUCTION: Tumor-specific neoantigens originate from mutations that occur in the DNA of cancer cells. Recent studies have suggested that the neoantigen landscape and its evolution are important for determining patients' response to checkpoint-blockade anti-cancer treatments[1-3]. In our group, we have developed a pipeline for the annotation of neoantigens from next generation sequencing data with the goal to further investigate the link between neoantigens, cancer evolution and response to treatment.

Material and Method. Given a patient's list of annotated somatic variants and their corresponding germline variants, neopeptides k-mers of length 8 to 11 are generated by taking into consideration missense, inframe insertion/deletion and frameshift variants. The publicly available programs olysolver[4] and NetMHCpan-3.0[5] are used for HLA-typing and HLA-neopeptide affinity prediction, respectively. Our pipeline generates several lists of putative neoantigens including strong binders (rank<0.5%), weak binders (rank<2.0%) and binders with predicted affinity <500nM.

RESULTS AND DISCUSSION: We recently applied our pipeline to a unique colorectal cancer clinical case [6] asking if tumor immunogenicity could at least partially explain the observed indolent course of the patient's disease. We are currently applying the pipeline to larger-scale datasets taken from the literature across different tumor types (including melanoma, colorectal and ovarian cancer). At the same time, we are also looking into other genomic variations with a potential as biomarkers in cancer immunotherapy (e.g. HLA copy number variation).

CONCLUSION: We have developed a pipeline that will be of help for investigating the effects of the tumor neoantigen landscape on clinical outcomes in cancer patients and, in particular, on response to immunotherapy. Work is in progress to apply neoantigen analysis across many cancer types.

REFERENCES:

[1] Van Allen et al. Science; 350:207-11 (2015)
[2] McGranahan et al. Science 25; 351:1463-9 (2016)
[3] Anagnostou et al. Cancer Discov; doi: 10.1158/2159-8290.CD-16-0828(2016)
[4] Shukla et al. Nat Biotechnol.; 33:1152-8 (2015)
[5] Nielsen and Andreatta Genome Med.; 8:33 (2016)
[6] Lote et al. Annals of Oncology (2017) (accepted)

NO CONFLICT OF INTEREST

585 Prime-boost immunization by both DNA vaccine and oncolytic adenovirus expressing GM-CSF and shRNA of TGF-β2 induces anti-tumor immune activation

Y. Joo[1], S. Kim[1], D. Kang[1], H. Choi[2], J. Kim[3], J. Song[1],

[1] Institute for Cancer Research, Brain Korea 21 PLUS Project for Medical Science, Yonsei University College of Medicine, Seoul, Korea
[2] Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea
[3] CHA Bundang Medical Center, CHA University, Seongnam, Korea

A successful DNA vaccine for the treatment of tumors should break established immune tolerance to tumor antigen. However, due to the relatively low immunogenicity of DNA vaccines, compared to other kinds of vaccines using live virus or protein, a recombinant viral vector was used to enhance humoral and cellular immunity. In the current study, we sought to develop a novel anti-cancer agent as a complex of DNA and oncolytic adenovirus for the treatment of malignant melanoma in the C57BL/6 mouse model. MART1, a human melanoma-specific tumor antigen, was used to induce an increased immune reaction, since a MART1-protective response is required to overcome immune tolerance to the melanoma antigen MelanA. Because GM-CSF is a potent inducer of anti-tumor immunity and TGF-β2 is involved in tumor survival and host immune suppression, mouse GM-CSF (mGM-CSF) and shRNA of mouse TGF-β2 (shmTGF-β2) genes were delivered together with MART1 via oncolytic adenovirus. MART1 plasmid was also used for antigen-priming. To compare the anti-tumor effect of oncolytic adenovirus expressing both mGM-CSF and shmTGF-β2 (AdGshT) with that of oncolytic adenovirus expressing mGM-CSF only (AdG), each virus was intratumorally injected into melanoma-bearing C57BL/6 mice. As a result, mice that received AdGshT showed delayed tumor growth than those that received AdG. Heterologous prime-boost immunization was combined with oncolytic AdGshT and MART1 expression to result in further delayed tumor growth. This regression is likely due to the following 4 combinations: MART1-derived mouse melanoma antigen-specific immune reaction, immune stimulation by mGM-CSF/shmTGF-β2, tumor growth inhibition by shmTGF-β2, and tumor cell-specific lysis via an oncolytic adenovirus. Immune activation was mainly induced by mature tumor-infiltrating dendritic cell (TIDC) and lowered regulatory T cells in tumor-infiltrating lymphocytes (TIL). Taken together, these findings demonstrate that human MART1 induces a mouse

melanoma antigen-specific immune reaction. In addition, the result also indicate that combination therapy of MART1 plasmid, together with an oncolytic adenovirus expressing MART1, mGM-CSF, and shmTGF-β2, is a promising candidate for the treatment of malignant melanoma.

NO CONFLICT OF INTEREST

586 Balance between different subsets of helper T cells in human squamous cell carcinoma

G. Perri[1], V.G. Vilas Boas[1], K.P. Siqueira[1], M.R.S. Nogueira[2], K. Cavassani[3], A.P. Campanelli[1]

[1] Department of Biological Sciences, Bauru School of Dentistry, University of São Paulo
[2] Lauro de Souza Lima Institute, Research Division, Bauru, Brazil
[3] Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles

CD4 T helper (Th) cells regulates important aspects of the adaptative immune response. Th cells are divided into functionally distinct subsets, and different cytokines have been shown to either promote or inhibit tumor development and progression. Studies have indicated that there is a direct association between Th1-related immune responses and a better prognosis in patients with squamous cell carcinoma (SCC). In this study, we analyzed the balance between different subsets of helper T cells in human squamous cell carcinoma. A panel of immune transcription factors and cytokines were analyzed in peripheral blood lymphocytes (PBL) and tumor-associated lymphocytes (TILs) from patients with SCC. Compared to healthy volunteers, we identified increased expression of transcription factors and cytokines associated with Th17 (RORC, IL17A), Th2 (Gata3, IL4), Th1 (Tbet, FN-γ), Th9 (IL-9, PU.1), and Th22 (IL22, AHR) in PBL and TILS from patients with SCC. Our data support the notion that different frequencies of CD4 Th cells populations are detected in the blood and tumor tissues of squamous cell carcinoma patients.

Financial support: FAPESP (grant number 2014/06215-1) and CNPq

NO CONFLICT OF INTEREST

POSTER SESSION: OTHER

588 Post-operative pain management for elective gastrointestinal surgeries in shaukat khanum memorial cancer hospital

R. Tasleem[1], A.W. Khan[1]

[1] Shaukat Khanum Memorial Cancer Hospital & Research Centre, Department of Anesthesiology and Pain Management, LAHORE, Pakistan

BACKGROUND: In SKMCH&RC, we deal mostly with cancer surgeries. Pain management is an important issue during intraoperative and postoperative period. Post-operative pain control depends on different kind and severity of surgery. In patients who undergo major surgeries and experience severe postoperative pain are provided with multimodal analgesia that includes patient control analgesia (PCA) and epidural analgesia.

Purpose: To compare the effectiveness of multimodal pain regimes provided in SKMCH& RC for postoperative pain management in gastrointestinal surgery population.

METHOD: Performa based survey conducted to assess effectiveness of multimodal analgesia provided in elective gastrointestinal surgeries. The audit period included data of patients provided with multimodal analgesia from **1st June, 2016** to **31st August 2016**. Post-operative pain score and overall patient satisfaction level was recorded up till day 4 postoperatively.

RESULTS: Total number of patients audited in period of 3 months were **178**. Out of **178,** 23 **(12.9%)** patients were provided with PCA and 155 **(87.0%)** patients were provided with Epidural analgesia. Patients underwent major gastrointestinal surgeries were included in this audit. Total patients provided with PCA were 23 **(12.9%),** patients fully satisfied were 18 **(78.2%)**,patients partially satisfied were 5 **(21.7%).**Total patients provided with epidural analgesia were 155 **(87.0%),** patients fully satisfied were 119 **(76.7%),** patients partially satisfied were 36 **(23.2%).**

CONCLUSION: Our audit concluded that patient population was generally satisfied with postoperative analgesia. Although overall numbers are small, but patient satisfaction was more in patient with PCA over those who received epidural analgesia.

NO CONFLICT OF INTEREST

589 Assessment of patient's understanding of PCA & E-PCA usage at the time of discharge, a comparative analysis

R. Tasleem[1], A.W. Khan[2]

[1] Pakistan Nursing Council, Deapartment of Anesthesiology & Pain Management, LAHORE, Pakistan
[2] Shaukat Khanum Memorial Cancer Hospital & Research Centre Lahore- Pakistan, Department of Anesthesiology & Pain Management, Lahore, Pakistan

BACKGROUND: Pain control remains a major concern for patients undergoing surgery. Proper education for the patients regarding PCA and E-PCA usage can minimize the surgical complications, post-operative morbidity, un-planned admissions of day case patients, prolonged hospital stay and increases their quality of life.

AIM: The goal of this study is to define the level of patient's understanding regarding the proper use of PCA vs E-PCA at the time of discharge from the hospital, in post-operative population at SKMCH & RC.

METHODS: This was a retrospective survey over a period of 3 months from 1st June 2016 to 31st August 2016. A Performa-based survey was carried out for the assessment of patient's education level in relation with PCA and E-PCA usage at the time of discharge. Survey was conducted during 3 months period from June to August 2016. 36 patients aged 16–86 years were included. The patient's understanding regarding PCA & E-PCA use was recorded for 72-hours postoperatively or at the time of discharge from hospital. Different factors used for correlation were gender, language and type of surgery etc.

RESULTS: In PACU & then in IPD, the level of understanding regarding use of PCA & E-PCA in our patients was 97%. Level of understanding in female and male patients were same. And similarly the type of surgery had no bearing on the level of understanding. The minor gap of 3% was attributed to language barrier (Persian, Pashto etc.).

CONCLUSION: Overall understanding level of the patients regarding PCA & E-PCA usage is 97% in our institution. We need to improve departmental clinical practice by optimising the PCA & E-PCA education for both staff and patients on regular basis to provide quality patient care.

NO CONFLICT OF INTEREST

590 Postoperative pain management for elective surgeries in SKMCH & RC

R. Tasleem[1], A.W. Khan[2]

[1] Shaukat Khanum Memorial Cancer Hospital & Research Centre, OR Department, LAHORE, Pakistan
[2] Shaukat Khanum Memorial Cancer Hospital & Research Centre, Department of Anesthesiology & Pain Management, LAHORE, Pakistan

BACKGROUND: In SKMCH&RC, we deal mostly with cancer surgeries. Pain management is an important issue during intraoperative and postoperative period. Post operative pain control depends on different kind and severity of surgery. In patients who undergo major surgeries and experience severe postoperative pain are provided with multimodal analgesia that includes patient control analgesia(PCA) and epidural analgesia.

Purpose: To compare the effectiveness of multimodal pain regimes provided in SKMCH& RC for postoperative pain management.

METHOD: Performa based survey conducted to assess effectiveness of multimodal analgesia provided in elective surgeries. The audit period included data of patients provided with multimodal analgesia from **1st June,2016** to **31st July 2016**.post operative pain score and overall patient satisfaction level was recorded up till day 4 postoperatively.

RESULTS: Total number of patients audited in period of 2 months were **138**. Out of 138**, 19(13.7%)** patients were provided with PCA and **119(86.2%)** patients were provided with Epidural analgesia. Patients underwent major surgeries like gastrointestinal **101(73.1%)**, urology **22(15.9%)**,thoracic **7(5%),** Hepatobiliary **6(4.3%)** & orthopedic **2(1.4%)** surgeries were included in this audit. Total patients provided with PCA were **19(13.7%),**patients fully satisfied were **17(89.4%)**,patients partially satisfied were **2(10.5%).**Total patients provided with epidural analgesia were **119(86.2%),** patients fully satisfied were **99(83.1%),** patients partially satisfied were **20(16.8%).**

CONCLUSION: Our audit concluded that patient population was generally satisfied with postoperative analgesia .Overall PCA seems to be more satisfactory analgesic modality than Epidural.

NO CONFLICT OF INTEREST

591 Inadvertant epidural catheter removal and the effect of tunnelling

R. Tasleem[1]

[1] Pakistan Nursing Council, OR Department- SKMCH & RC, Lahore, Pakistan

BACKGROUND: Accidental epidural catheter migration and unscheduled removal is one of the most hazardous problems encountered by pain management team as it has a lasting impact on patient's pain optimization, overall recovery and may result in increased patient morbidity. One of the methods employed to address this issue is through tunneling epidural catheters or suturing it to the skin, along with different dressing techniques. These methods have shown to have variable amount of success and failure largely depending on the technique, skills and early recognition of the pending threat by the attending health care provider.

AIM: To audit the incidence of accidental migration and unscheduled removal of epidural catheters placed for postoperative analgesia, and to examine the effect of epidural catheter tunneling (under the skin) on the accidental catheter migration.

METHODS: An audit of all the epidural catheters inserted for analgesia during and after surgery, and looked after by pain nurse in Operation theatre, Post Anaesthesia Care Unit and In-patient department under the supervision of anesthetist. Some of the catheters were tunneled, decision purely at the discretion of attending anaesthetist, while other catheters were not. All the data collected on the performa, from the notes of the patients. The data has been transferred to and compiled after the audit duration to the MS Excel.

RESULTS: 343 epidural catheters were placed in the 5 months period, between 1st April 2016 to 31st August 2016. Out of 343 epidural catheters, 90 were tunneled (26%) and 253 were non-tunneled (74%). Overall incidence for accidental catheter dislodgement and unscheduled removal remained 32 (35.5%) out of 343. In tunneled group the incidence of accidental dislodgement was less, 7 (7.7%) out of 90, as compared of non-tunneled which remained almost four times more i.e. 25 (9.8%) out of 253.

CONCLUSION: Tunneling epidural catheters under skin had a remarkable effect on avoiding accidental removal. Our data has discrepant number in tunneled versus non-tunneled group which may have affected the overall results. Better documentation and further evaluation in different groups may yield more elaborate results. Further study needs to be conducted to determine the effect of tunneling and overall impact on catheter migration and related complications.

NO CONFLICT OF INTEREST

594 Evaluation of the effect of Glycyrrhiza glabra extract on T47D breast cancer cell line and study of influences on nm23 gene expression

A. Salehzadeh[1]

[1] Department of Biology- Rasht Branch- Islamic Azad University- Rasht- Iran., Biology, Rasht, Iran

BACKGROUND: Licorice has long been used as medicinal plant. So far, about 30 species of the genus Glycyrrhiza been identified as one of the plants widely used in traditional medicine. Some of the uses that can be noted are anti-inflammatory, anti- flatulence and allergy treatment agent. In recent years the use of botanicals to prevent and intervene in various stages of carcinogenesis has received more attention. The increased prevalence of deaths caused by cancer and defect in chemotherapy and radio therapy methods, the new ways to control of cancer is felt. Among the cancer inhibitor compounds that have been identified in recent years, natural polyphenolic antioxidant due to high efficiency and less systemic effects are one of the most effective cancer inhibitor.

MATERIAL AND METHODS: In this study to isolation of the Glycyrrhiza glabra extract the percolation method was used and to investigate toxicity effects on T47D breast cancer cell line MTT assay was used. The GC-mass analysis was used to identified all the chemicals in the Glycyrrhiza glabra leaf extract.

RESULTS: MTT assay result showed that concentrations of 0 , 50 , 100 , 200 , 400 , 600 , 800 and 1000 microgram per ml for 24 hours resulted in decreased cell survival(P>0.05)92.35± 54, (P>0.05)85.06± 0.04, (P<0.05)80.29± 1.14, (P>0.05)58.73± 0.05, (P<0.01)43.33± 1.18, (P<0.001)7.43± 0.74,and (P<0.001)5.03± 0.04 respectively. The result of the NM23 gene expression showed that the relative gene expression levels of NM23 in the group who received IC50 of licorice extract compared to control cells and those that received sub-IC50 concentration have the greatest increase of gene expression (30.33, P<0.001).While this amount in the sub-IC50 concentration was 5.06, (P<0.01), which reflects the positive effect of licorice extract on the expression of the NM23 gene.

CONCLUSION: G. glabra extract displayed great antioxidant activity with a less IC_{50} value and high phenolic content. More findings in this study showed that G. glabra extract can induce up regulation of nm23 gene expression in T47D cells. Moreover, the anti-cancer effects of G. glabra were showed through modulation of apoptosis rather than necrosis in T47D cells. Therefore, G. glabra remains a good candidate for utilization as an inhibitor of the human breast carcinoma in the future.

NO CONFLICT OF INTEREST

595 S-1-induced lacrimal drainage obstruction and its association with ingredients/metabolites of S-1 in tears and plasma: A prospective multi-institutional study

N. Kim[1], J.W. Kim[2], J.H. Baek[3], J.S. Kim[4], H.K. Choung[5], Y.J. Bang[6], S.I. Khwarg[7], D.J. Park[8], H.H. Kim[8], K.W. Lee[2]

[1] Seoul National University Bundang Hospital- Seoul National University College of Medicine, Ophthalmology, Seongnam, Korea
[2] Seoul National University Bundang Hospital- Seoul National University College of Medicine, Internal Medicine, Seongnam, Korea
[3] Diatech Korea Co., Center of Biomedical Mass Spectrometry, Seoul, Korea
[4] Seoul National University Boramae Medical Center, Internal Medicine, Seoul, Korea
[5] Seoul National University Boramae Medical Center, Ophthalmology, Seoul, Korea
[6] Seoul National University College of Medicine- Seoul National University Hospital, Internal Medicine, Seoul, Korea
[7] Seoul National University College of Medicine- Seoul National University Hospital, Ophthalmology, Seoul, Korea
[8] Seoul National University Bundang Hospital- Seoul National University College of Medicine, Surgery, Seongnam, Korea

BACKGROUND: This prospective study was conducted to determine the incidence of lacrimal drainage obstruction (LDO) during S-1 chemotherapy and to evaluate the association between the development of LDO and the concentrations of ingredients/metabolites of S-1 in tears and plasma.

MATERIALS AND METHODS: One-hundred forty-five patients with gastric cancer, receiving adjuvant S-1 therapy, were enrolled. Ophthalmologic examinations were regularly performed during S-1 chemotherapy. Concentrations of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and 5-fluorouracil at steady-state trough level were measured in both tears and plasma.

RESULTS: Fifty-three patients (37%) developed LDO. The median time to the onset of LDO was 10.9 weeks, and LDO developed most frequently in the nasolacrimal duct. In univariable analyses, an older age (≥ 70 years), creatinine clearance rate (Ccr) < 80 mL/min, 5-fluorouracil concentration in plasma ≥ 22.3 ng/mL (median), CDHP concentration in plasma ≥ 42.0 ng/mL (median), and tegafur concentration in tears ≥ 479.2 ng/mL (median) were related to the increased development of LDO. In multivariable analysis, a high plasma 5-fluorouracil concentration was predictive of the increased development of LDO (hazard ratio: 2.02; P = 0.040), along with the older age and decreased Ccr. The patients with LDO also developed S-1-related non-hematologic toxicity more frequently than those without LDO did (P = 0.016).

CONCLUSIONS: LDO is a frequent adverse event during S-1 chemotherapy. An older age, decreased Ccr, and high plasma 5-fluorouracil concentration were found to be independent risk factors for LDO. The high incidence of LDO warrants regular ophthalmologic examination and early intervention in patients receiving S-1 therapy

NO CONFLICT OF INTEREST

596 Comparative transcriptomics of triple-negative breast cancer stem cells and differentiated tumor cells identifies Teneurin-4 as a potential therapeutic target

R. Ruiu[1], M. Arigoni[1], F. Riccardo[1], L. Conti[1], S. Lanzardo[1], R.A. Calogero[1], F. Cavallo[1], E. Quaglino[1]

[1] University of Turin, Department of Molecular Biotechnology and Health Sciences, Turin, Italy

BACKGROUND: Triple-negative breast cancer (TNBC) is insensitive to some of the most effective therapies for other breast cancers, including endocrine and HER2-directed therapies. The lack of specific treatments prompted us to search for new TNBC-associated molecules to be used as targets for cancer therapy. As patients with TNBC usually experience a quicker relapse and metastatic progression compared to those with other breast cancer subtypes, we hypothesized that cancer stem cells (CSC) could play a central role in TNBC. We thus directed our efforts on the identification of differentially expressed genes between CSC and non-CSC of TNBC cell lines.

MATERIAL AND METHODS: We established tumorsphere cultures from mammary cancer cell lines 4T1 and HCC1806 to enrich the CSC population. Flow cytometry analysis was used to determine changes in CSC markers. RNA-Seq was used to identify differences in gene expression between tumorspheres and their monolayer counterparts. Real Time PCR (RT-PCR) and Western Blot (WB) were used to validate gene expression.

RESULTS AND DISCUSSION: 74 genes were upregulated in the tumorspheres of both the cell lines, while 42 genes were downregulated. Enrichment analysis of biological processes showed in tumorspheres an upregulation in genes involved in 'regulation of apoptosis" and a down-regulation in genes involved in 'lipid metabolism" and 'cell cycle regulation". By focusing on upregulated genes coding for cell membrane-associated proteins, we selected Teneurin-4 (TENM4) as a candidate target for further studies. TENM4 upregulation was confirmed by both RT-PCR and WB.

Initial result seem thus to indicate that the stem-like status of TNBC cells is accompanied by altered regulation of apoptosis, cell cycle and lipid metabolism pathways. The biologic significance of these findings has still to be explored. Poor literature information regarding TENM4 exists, and to our knowledge this is the first time that a link between TENM4 and CSC is established. Further experiments will help us to better define the role TENM4 as a potential therapeutic target.

CONCLUSION: TENM4 may have a role in CSC biology, thus its targeting could help to improve the outcome of TNBC patients in the future.

NO CONFLICT OF INTEREST

597 Debulking surgery for adrenocortical carcinoma with multiple metastases for reducing chemoresistance: A case report

P. Kwang Yeol[1]

[1] Yeouido St.Mary's Hospital, HBP Surgery, Seoul, Korea

BACKGROUND: Adrenocortical carcinoma (ACC) with multiple metastasis (MM) only require palliative therapies. Debulking surgery is sometimes considered for patients with large, symptomatic tumors or for patients with secretion uncontrolled by usual medical therapy. Response to preoperative chemotherapy has been shown to be an important prognostic factor in the surgical treatment of liver metastasis from a variety of primary sites, and some have suggested that mitotane could be used in the neoadjuvant setting to select patients for surgery. We herein report the patient with ACC and MM underwent liver resection and adrenalectomy for debulking tumor burden when partial response was obtained by primary chemotherapy.

MATERIAL AND METHODS: We report the case of a 35-year-old man who was diagnosed with stage 4 ACC with MM.

RESULTS: Given that he had unresectable stage 4 ACC with both lung and liver, M-EDP (Mitotane+etoposide+doxorubicin+cisplatin) was started three years ago. After six cycles, multiple metastatic lesions in lung were disappeared and liver metastasis showed stable status. One year after chemotherapy, metastases of liver and adrenal gland slightly progressed. Although radiotherapy and salvage second line chemotherapy with gemcitabine+capecitabine applied to patient, progression

of liver metastasis and ACC continued. The patient underwent debulking resection for adrenal gland and liver metastases successfully for the purpose of reducing chemoresistance and showed stable disease after surgery.

CONCLUSIONS: In highly selected patients with ACC with MM shown response to preoperative chemotherapy, debulking resection could be chosen to obtain progression free time by reducing chemoresistance.

NO CONFLICT OF INTEREST

598 New ifosfamide analogs for immunotherapy and nanomedicine against cancer

J. Delahousse[1,2], C. Skarbek[2], M. Desbois[3], T. Martens[4], M. Rivard[4], D. Desmaële[5], P. Couvreur[5], N. Chaput[3], A. Paci[1,2]

[1] Gustave Roussy, Pharmacology, Villejuif, France
[2] UMR CNRS 8 2 0 3 , Université de Paris-Saclay, Villejuif, France
[3] Gustave Roussy, Immunomonitoring lab-LIO- UMS 3 6 5 5 CNRS & US 2 3 Inserm, Villejuif, France
[4] ICMPE, Université Paris Est, Thiais, France
[5] Institut Galien Paris Sud, Université de Paris Saclay, Châtenay-Malabry, France

Oxazaphosphorines (cyclophosphamide-CPA, ifosfamide-IFO) are antineoplasic agents widely used in the treatment of malignancies. They are prodrugs requiring cytochrome P450 (CYP) bioactivation. Regarding IFO, bioactivation produces the active nitrogen mustards and releases metabolites responsible for toxicities. Increasing the therapeutic index of IFO could be of major interest. Moreover, CPA is known for its antitumor immune based mechanism to treat cancer. As CPA is an isomeric form of IFO, we investigated its effect on the immune response. The combination of cytotoxicity and immunomodulation in a dual-targeting drug delivery system (DDS) strategy may increase specificity to the tumor and hereby the response to the antitumor treatment.

We have designed pre-activated IFO analogs coupling a poly-isoprenoyl chains (geranyloxy-IFO, farnesyloxy-IFO and squalenyloxy-IFO). These analogs can self-assemble into nano-assemblies (NAs). Their cytotoxic activity was studied in vitro on various tumor cells lines. Then, NAs PEGylation was studied by co-nanoprecipitation of the pre-activated analog with PEGylated moieties. Concomitantly, the immunomodulatory properties of IFO were explored on non-tumor bearing mice and on murine fibrosarcoma model after a single intraperitoneal injection of escalating dose of IFO. The distribution and phenotype of the immune cells were studied by flow cytometry and ELISA.

The pre-activated IFO analogs show a cytotoxic activity in absence of CYP on a large panel of human cancer cell lines validating the proof of concept of pre-activation. Studies on immune cells, showed a dose-dependent effect of IFO towards a modulation of the secretion of cytokines reflecting a Th1/Th17 polarization. The dose of 150 mg/kg of IFO has been found as the immunomodulatory dose. In addition, the T-cells mediated antitumor efficacy of IFO was demonstrated in mice depleted CD4 and CD8 T-cells.

The proof of concept of pre-activation of IFO was established. The designed analogs can be formulated as NAs. These DDS would take advantage of passive and active targeting in order to improve the response selectivity of highly active new pre-activated drug. The result on immunomodulatory effect of IFO display its significant effect on the cellular immune response.

Developing new antitumor strategies using cytotoxicity and immunomodulatory properties of new designs from conventional treatment could offer synergistic effects and hereby increase specificity to the tumor.

NO CONFLICT OF INTEREST

599 Mechanisms of telomere maintenance in brain tumors: Is ALT the key to treatment?

A.I. Idilli[1], M. Cayuela[2], M. Mione[1]

[1] CIBIO- Centre for Integrative Biology, University of Trento, Trento, Italy
[2] IMIB-Arrixaca, University Hospital "Virgen de la Arrixaca", Murcia, Spain

INTRODUCTION: Glioblastoma (GBM) is the most aggressive tumor of the central nervous system with a median survival of 12-15 months post-diagnosis. Approximately 30% of GBMs employ a strategy called Alternative Lengthening of Telomeres (ALT) to attain cellular immortality. ALT is a telomerase-independent pathway that maintains telomere length, which may involve telomere extension using telomeric DNA as template, instead of telomerase RNA and still uncharacterized mechanisms.

Since ALT molecular mechanisms are still largely unknown, currently there are no target therapies, with an increase of deaths due to ALT cancers every year.

MATERIAL AND METHOD: We have developed zebrafish models of glioblastoma based on the conditional expression of human-relevant oncogenes in brain progenitor cells in Tert-proficient and Tert-deficient backgrounds. We investigated whether tumor progression in the models are dependent on telomere maintenance and through which mechanism (telomerase or ALT pathway). Using telomere QPCR we provides parameters of mean lenght of telomeres distribution that we correlated with FLOW and Q-FISH techniques to verify the presence of irregular telomere length in function of ALT. We check telomerase activity with Q-Trap assay and the presence of extrachromosomal telomeric DNA was evaluated with C-circle assay.

RESULTS AND DISCUSSION: We found that brain tumors develop in the absence of functional telomerase, albeit smaller and less invasive. Analysis of telomere maintenance in brain tumors suggests that ALT mechanisms may be important even in the presence of functional telomerase. In tert mutants very low telomerase activity can be detected, however telomeres are maintained, albeit at half of the control levels. Furthermore, in telomerase null zebrafish, brain tumors develop with the same frequency, although with a less aggressive phenotype. Remarkably, zebrafish GBM cells show high telomere content and strong and irregular telomere FISH signals, typical of telomeres with variable length found in ALT cells. Finally we confirmed that in these GBM models, telomere maintenance is due to ALT, as c-circle assay showed that ALT mechanisms are predominant in the zebrafish brain tumor model.

CONCLUSION: We describe here a new model of zebrafish brain tumor to the study ALT mechanisms in vivo. The model allows the molecular dissection of ALT mechanisms during cancer development and is ideal for preclinical studies of drug screening for ALT-targeting therapies.

NO CONFLICT OF INTEREST

600 Chemopreventive activity of hydroxytyrosol and a purified extract from olive mill wastewaters (OMWW) on prostate cancer cell lines

D. Noonan[1], D. De Stefano[2], A. Bruno[3], T. Rossi[4], D. Pizzichini[5], A. Albini[2]

[1] University of Insubria, Clinical and Biological Sciences, Varese, Italy
[2] Scientific and Technology Park- IRCCS MultiMedica- Milano- Italy, Vascular Biology and angionenesis Laboratory, Milano, Italy
[3] Scientific and Technology Park- IRCCS MultiMedica- Milano- Italy, Vascular biology and angiogenesis laboratory, Milano, Italy
[4] IRCCS "Istituto in Tecnologie Avanzate e Modelli Assistenziali in Oncologia" Arcispedale S. Maria Nuova-Reggio Emilia- Italy, Translational oncology Lab, Reggio Emilia, Italy
[5] ENEA- Casaccia Research Center- Rome- Italy, Research, Roma, Italy

INTRODUCTION: Cancer chemoprevention by dietary phytochemicals is particularly attractive for their potential low toxicity and for their ability to modulate a plethora of signal transduction pathways in biological processes associated with cancer. Olive oil, a major component of the Mediterranean diet, is an abundant source of phenolic compounds. Olive oil production is associated with the generation of waste material, termed 'olive mill wastewaters' (OMWW), that have been reported to be enriched in polyphenols as well. Given the known beneficial effects of polyphenols in different complex diseases, here we investigated whether the use of purified extracts from OMWW, defined A009, might be effective in exerting chemopreventive activities in three different prostate cancer (PCa) cell lines (PC-3, DU-145, LNCap) in vitro.

MATERIAL AND METHODS: The SANIST platform, based on the Surface-Activated Chemical Ionization/Electrospray Ionization mass spectrometry (SACI/ESI-MS) was used to determine polyphenol content in our extracts. Chemopreventive activity of A009 were tested by proliferation assays and functional study for cell adhesion, migration and invasion. **result and Discussion:** Mass spectrometry analysis revealed that hydroxytyrosol, resulted the major component of A009 our extracts and vas used as a reference compound to test A009 chemopreventive properties in vitro. A009 significantly reduced PCa cell viability up to 96 hours in all cell lines investigated, in a similar manner that hydroxytyrosol (HyT), the major polyphenols present in our extract. A009 inhibited CRC PCa cell adhesion, migration, invasion and sprouting. Therefore, in LNCap cell line, A009 strongly interfere with their ability to form colonies/islets in vitro.

CONCLUSIONS: Our result suggest that A009 extracts show promising chemopreventive properties on PCa in vitro, suggesting that different polyphenols act synergistically, improving their single component effects in PCa cell lines. Finally, our result support the idea of repositioning a waste derived material for nutraceutical employment, with environmental and industrial cost management benefits.

NO CONFLICT OF INTEREST

601 Microbial contamination of ulcerated surface and depth of melanoma invasion

I. Galaychuk[1], O. Pokryshko[1], I. Danylkiv[1]

[1] Ternopil State Medical University, Department of Oncology and Radiology, Ternopil, Ukraine

INTRODUCTION: Recent studies showed the host microbiome has come to the forefront as a potential modulator of cancer metabolism, and the bacterial biofilms might act as direct triggering factors contributing to cancer [Johnson et al., 2016; Li et al., 2017].

Aim: to compare the microbial contamination of ulcerated melanoma with depth of melanoma cells invasion into derma and postoperative wound complications.

MATERIAL AND METHODS: 23 patients (aged 25–70 yr.) with primary ulcerated melanoma T3b-T4b were included in study. All patients underwent radical surgery. The postop wounds were closed by local flaps rotation in 15 pts, in other 5 pts the open wound was treated with hydrogel bandages, and in three cases we used vacuum-assisted technique. For these 8 pts the split-thickness skin grafting of granulation wound was performed at 21-36 days after melanoma removing.

Materials for microbiological examination were taken from the ulcerated surface of melanoma (3-4 days before surgery), then from the postop wound on 3rd-4th, 6th-7th and 14th day, and also from the intact skin at a distance of 5-6 cm from

the tumor or wound. Microorganisms were identified by bacterial analyzer Vitek2 Compact (bioMerieux, France).

RESULTS AND DISCUSSION: The gram-positive cocci S. aureus, S. epidermidis and gram-negative rods such as E. coli, P. vulgaris were presented on ulcerated surface of melanoma. Microbial spectrum was more diverse on the intact skin: S. lentus, S. haemolyticus, Kocuria kristinae, Enterococcus faecalis, Actinomicetes spp., Propionibacterium spp., Klebsiella spp., Bacillus megaterium, Pseudomonas putida. The concentration of microorganisms was significantly higher on melanoma ulcer (S. aureus: 5.84±1.39 lg CFU/cm^2) than on intact skin (3.41±0.65 lg CFU/cm^2). The concentration of gram-negative bacilli was 5.49±0.31 lg CFU/cm^2 on ulcerated melanoma, which in 1.7 times more compared to intact skin. Morphological exam of removed melanomas revealed the IV and V level of tumor invasion (by Clark). The inflammatory postop wound complications were minimized due to usage the target antibiotics according to the preoperative antibiograms.

CONCLUSION: There are qualitative and quantitative differences of microflora on ulcerated surface of melanoma and surface of intact skin. The concentration of microorganisms is significantly higher on ulcer tumor's surface than intact skin. Microbiota in chronic melanoma ulcer may increase local pathogenicity, leading to enhanced tissue degradation, and may be essential for melanoma spreading into derma.

NO CONFLICT OF INTEREST

602 The role of epigenetic mark profile, cell cycle alteration and DNA repair in resistance of glioblastoma cells to photodynamic therapy (PDT)

S. Shahmoradi Ghahe[1], A. Ciuba[1], K. Kopania[1], M. Foksinski[2], R. Olinski[2], B. Tudek[1,3]

[1] Institute of Genetics and Biotechnology, Faculty of Biology- University of Warsaw, Warsaw, Poland
[2] Department of Clinical Biochemistry, Collegium Medicum- Nicolaus Copernicus University, Bydgoszcz, Poland
[3] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland

Glioblastoma is the most aggressive primary brain tumor. Treatment regimens for glioblastoma tumors, such as surgery, radiotherapy, and chemotherapy, are very invasive and can only prolong the median patient life to several months. It was shown that 5-aminolevulinic acid (5-ALA) -based photodynamic therapy (PDT) is a promising and less aggressive adjuvant modality for diagnosis and treatment of glioblastoma. Limiting point of PDT outcome is appearing of cells with intrinsic or acquired PDT resistance that finally result in repopulating the tumor and short-term survival of glioma patients. Therefore, our study was designed to determine therapy resistance markers in PDT resistant glioblastoma cell line.

Glioblastoma (U-87) cell line resistant to PDT (U-87R) was isolated from parental, sensitive line (U-87P) by applying several cycles of ALA-PDT. U-87R cells subsequently were characterized from different aspects such as epigenetic markers, cell cycle events, oxidative stress and DNA repair capacity.

Assessment of nucleotide modifications and epigenetic marks in PDT resistant glioblastoma and its parental cell line showed significant higher level of two epigenetic marks including 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) in U-87R compare to U-87P. Cell cycle analysis showed that G1 phase is longer in U-87R cells compare to U-87P. Moreover, accumulation in G2 phase following PDT was observed earlier in resistant cells than in parental line. Resistant glioblastoma cells were then sensitized to PDT by applying inhibitor of one of the main kinases of DNA damage response, ATM kinase. In comparison with parental cells, U-87R cells also showed higher activity of some DNA base excision repair (BER) enzymes including glycosylases and AP-endonuclease1 (APE1). Studies of the whole protein profile of PDT resistant and parental cells demonstrated that the level of superoxide dismutase (SOD) was considerably higher in U-87R than in U-87P. That data was then confirmed by detecting lower oxidative stress in U-87R following PDT.

PDT resistant glioblastoma cells as a model of resistant cells in glioblastoma tumor population demonstrated the significant role of epigenetic mark profile, cell cycle alteration, higher DNA repair capacity, and antioxidant defense in conferring resistance to photodynamic therapy.

This work was supported by the Polish National Science Centre (NCN) grants: DEC-2012/07/B/NZ1/00008, UMO-2014/15/B/NZ5/01444, UMO-2014/13/N/NZ3 /00863

NO CONFLICT OF INTEREST

603 Anti-angiogenic and angiopreventive activities of beer hop xanthohumol-derivatives in vitro

A. Albini[1], B. Bassani[2], A. Bruno[2], C. Gallo[3], D. Noonan[4], A. Rossello[5], A. Cantelmo[2]

[1] Multimedica ONWS, Vascular Biology Laboratory, Milan, Italy
[2] Scientific and Technology Park- IRCCS MultiMedica- Milano- Italy, Vascular Biology and angiogenesis Laboratory, Milan, Italy
[3] IRCCS "Istituto in Tecnologie Avanzate e Modelli Assistenziali in Oncologia" Arcispedale S. Maria Nuova-Reggio Emilia- Italy, Translational Oncology Lab., Reggio Emilia, Italy
[4] Univeersity of Insubria- Varese- Italy-, Department of Biotechnology and Life Sciences-, Varese, Italy
[5] Univeersity of Pisa, Department of Pharmacy-, Pisa, Italy

BACKGROUND: Angiogenesis, a process characterized by the formation of new blood vessels from pre-existing ones, is a crucial step in tumor growth and dissemination. Recently, increased attention has been addressed to the ability of flavonoids to prevent cancer by suppressing angiogenesis, strategy that we named "angioprevention". During the last decades, the prenylated chalcone Xanthohumol (XN), from the hop plant, has emerged as cancer chemopreventive agent and we recently published that XN exhibits anti-angiogenic/angiopreventive properties via AMPK activation. Here we tested the anti-angiogenic/angiopreventive potential of XN - derivatives on human umbilical-vein endothelial cells (HUVE) in vitro, as compared with XN alone.

MATERIAL AND METHODS: Cell proliferation assay was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on treated HUVE cell by, while detection of apoptosis was assessed by flow cytometry analysis. Functional angiogenesis assays included adhesion assays on fibronectin, migration and invasion in Boyden chambers and capillary-like structure formation on matrigel,.

RESULTS AND DISCUSSION: We found that selected XN derivatives can inhibit HUVEC proliferation in a time and dose dependent manner. Flow cytometry analysis showed that 24h treatment with 20μM XN and some XN derivatives resulted in increased rates of apoptotic endothelial cell (AnnexinV+7-AAD-/+) cells. Only one selected derivative at the intermediate concentration (10μM) was shown to increase apoptosis on HUVECs. Selected XN derivatives decrease HUVEC adhesion, migration and invasion. They also interfered with HUVEC ability to form capillary-like networks and some of them exert more potent effect as compared with XN alone.

CONCLUSION: Together, our result allowed the identification of XN derivatives with promising anti-angiogenic/angiopreventive effects for further in vivo studies and molecular pathway characterization.

NO CONFLICT OF INTEREST

604 Targeted therapy with sorafenib in kidney cancer with bone metastases

V. Protsenko[1], V. Ilnitskyi[1]

[1] Institute of Traumatology and Orthopedics of NAMS of Ukraine, Onco-orthopedics, Kyiv, Ukraine

BACKGROUND: Renal cell carcinoma amounts 2-3% in the structure of all malignancies in adults, wherein kidney cancer is increasing annually by 1,5-5,9%. Metastases usually develop in more than half of patients with advanced renal cell carcinoma after the surgery. Usually there is a poor prognosis of the disease concerning the development of metastatic disease in patients with renal cell carcinoma: the average survival period after detection of metastases amounts from 10 to 13 months. Treatment of metastatic renal cell carcinoma is a difficult task, because kidney cancer is almost not sensitive to cytotoxic chemotherapy drugs.

MATERIAL AND METHODS: From 2009 to 2016 years 27 patients received surgical treatment of metastatic renal cell carcinoma in long bones. Patients received the following treatment: segmental bone resection with total hip reinforced metalosteosynthesis, transosseus extrafocal osteosynthesis using external fixation devices. After surgical treatment phase, patients received radiotherapy treatment, bisphosphonates, and targeted therapy in the form of sorafenib.

RESULTS: Continuous treatment with sorafenib significantly increased progression-free survival period (average 30 weeks). 18 (66.7%) patients treated with sorafenib had stabilization of the disease for the past 12-week period of treatment. 4 (14.8%) patients had a partial reduction in the size of metastatic lesions in the bones, and 5 (18.5%) showed a single disappearance of metastatic lesions in the bones. Tolerability of sorafenib was satisfactory, the toxicity of sorafenib was low. Analysis of disease-free survival showed that treatment with sorafenib amounted 4,5 months and resulted in a statistically significant increase in the average period until progression, regardless of age, the group forecast MSKCC criteria, the presence or absence of prior cytokine therapy, and localization of metastases. Average survival of patients with metastatic kidney cancer in bones treated with sorafenib amounted 18.2 months.

CONCLUSIONS: The drug sorafenib, which is a multi-kinase inhibitor that affects angiogenesis and proliferation of tumor cells, result in a significant improvement in progression-free survival in patients with metastatic kidney cancer in the bone and is well tolerated, without compromising the quality of life of patients.

NO CONFLICT OF INTEREST

Notes

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