UNIVERSITÀ DEL PIEMONTE ORIENTALE

Novara (Italy) October 29th-30th, 2017

International workshop NO-CANCER 2017

From Cancerogenesis to Therapy:

new paradigms, new opportunities

ABSTRACT BOOK

INTERNATIONAL WORKSHOP

From Cancerogenesis to Therapy:

new paradigms, new opportunities

October 29th - 30th, 2017

University of Piemonte Orientale

School of Medicine

Novara (NO), Italy

CONFERENCE VENUE

Aula Magna, Azienda Ospedaliera – Universitaria "*Maggiore della Carità*" di Novara.

Corso G. Mazzini 18

INDEX

ACKNOWLEDGEMENTS

First, we thank all the Speakers for having accepted the invitation, despite the short notice time and their busy schedule. We are particularly grateful for their dedication. A special Thank to those speakers who have travelled long distances. We hope that they have enjoyed their stay in Novara.

We thank the School of Medicine of Università del Piemonte Orientale and the University-Hospital "Maggiore della Carità" for hosting the event.

Special Thanks are due to the sponsoring Charities "Associazione italiana per la lotta contro Leucemie, Linfomi e Mielomi" di Novara (AIL) and "Lega Italiana per la Lotta contro i Tumori" di Novara (LILT) for their collaboration and support.

The kind support from Istituto Professionale G. Ravizza and from Tipografia Italgrafica di Novara is gratefully acknowledged.

I am personally in debt to the Institutions that offered their kind support in the organization of the social events, and more in particular with the Municipality of Novara, the Agenzia per il Turismo Locale, the Conservatorio Musicale, and the Fondazione Castello.

A list of the Academic and Institutional Partners with their logo is provided in the following pages.

Almost hundred and eighty participants have attended the Conference. We are grateful to the Molecular Pathology Lab staff for their invaluable help in the practical management of all the organizational issues that such enthusiastic participation has inevitably posed.

Ciro Isidoro,

On behalf of the local organizing committee.

My personal thanking to the many persons who have dedicated time and energy in the organization of the Conference is reported (in Italian) at the end of this book.

PARTNERS

ACADEMIC AND INSTITUTIONAL PARTNERS

UPO – Università del Piemonte Orientale

Comune di Novara

Stephenson Cancer Center – The University of Oklahoma

Columbia University Medical Center

Azienda Ospedaliero-Universitaria Maggiore della Carità di Novara

NOVARAIL – Associazione Italiana contro le Leucemie-Linfomi e Mieloma – Onlus

LILT – Lega Italiana per la Lotta contro i Tumori – Sezione provinciale di Novara – prevenire è vivere

ASSOCIAZIONE PER LA RICERCA MEDICA 'IPPOCRATE-RHAZI'

Istituto Superiore di Studi Musicali – Conservatorio Guido Cantelli – Novara

Ordine Provinciale dei Medici Chirurghi e degli Odontoiatri di Novara

IN COLLABORATION WITH

NOVARAIL – Associazione Italiana contro le Leucemie-Linfomi e Mieloma – Onlus

LILT – Lega Italiana per la Lotta contro i Tumori – Sezione provinciale di Novara – prevenire è vivere

ORGANIZATIONAL AND TECHNICAL SUPPORTERS

GR

Atl Turismo – Provincia di Novara

italgrafica Novara

il Castello di Novara

WELCOME ADDRESS

Dear Students, dear Colleagues, dear distinguished Guests,

On behalf of the organizing committee, I am pleased to welcome you to the International Workshop NO-CANCER2017 "From cancerogenesis to therapy: new pardigms, new opportunities", hosted by the Università del Piemonte Orientale in collaboration with the University Hospital in Novara.

The Conference ideally follows that of last year ("Basic to Translational Medicine 2016: focus on cancer"), to which eighteen speakers and over three hundred scholars participated in two days of scientific sessions.

This year, the workshop is condensed in a one-day scientific marathon, in which ten internationally renowned speakers coming from France, Germany, Spain, Switzerland, United States, and Italy will present their recent research.

The main objective of the workshop is to offer a critical view of the current models of cancerogenesis, and to propose novel paradigms that may pave the way for new strategies to combat Cancer.

In this respect, particular emphasis will be given to the role of the stroma and of the immune response in Cancer, the peculiar metabolism of cancer cells, the role of oncogenes and tumor suppressor genes and of Non Coding RNAs in Cancer development, and to the emerging technologies for cancer diagnosis.

The invited lectures will focus particularly on hemato-oncology, mesothelioma, breast cancer, ovary cancer, prostate cancer, and pancreatic cancer.

The conference represents a unique opportunity for the students and the scientists working in the Universities, Hospitals and Research centers to meet up with colleagues from various parts of the world.

More importantly, it will be an occasion to confront the different perspectives in cancer research.

The conference has attracted many students and scientists from Italian research centers from Alessandria, Biella, Brescia, Milan, Padua, Rome, Turin, Varese, Vercelli and Verona, besides Novara.

On top, we have many participants coming from abroad (Poland, Slovenia, Spain, and Thailand) and Visiting fellows and students representing various Countries, among which Ghana, India, Iran, Lebanon, Libia, Marocco, Pakistan.

We regret we had to close the registration at the Conference six days before the deadline, because of over-booking. To now, more than 160 attendees have registered (in spite of the initial limitation to 120), and up to 37 Abstracts have been submitted.

To stimulate the active participation of Young researches, five short communications and six flash communications have been selected from the submitted abstracts.

With this Conference, we wish to promote the interactions between Young and Senjor scientists, having in mind that only from the confrontation and complementation of different expertise we may succeed in the battle against Cancer.

We hope that you will benefit from the Scientific lecturing and will enjoy the friendly atmosphere.

We encourage you to bring your enthusiasm into new collaborations.

Thanks for coming, and for sharing with us your knowledge.

Ciro Isidoro

CIRO ISIDORO

ciro.isidoro@med.uniupo.it

Associate Professor of General Pathology

Qualified Full Professor of Clinical Biochemistry and Clinical Molecular Biology

Qualified Full Professor of Medical Oncology

Università del Piemonte Orientale, Department of Health Sciences; via Paolo Solaroli 17 – 28100, Novara (Italy)

http://www.isidorolab.com/

Education:

1983 – Laurea *Summa cum Laude* - Doctor in Biological Sciences (D.Sc.), Università di Torino (Italy)

1984 – National License for Board of Doctor Biologists

1999 - Laurea *Summa cum Laude* - Doctor in Medicine and Surgery (M.D.), Università del Piemonte Orientale (Novara, Italy)

2000 – National License for Board of Medical Doctors and Surgeons.

Representative Careers and affiliations:

1986-1989 PhD fellow-Assistant Researcher at the Institut fuer Pathobiochemie, Westfaelish Wilhems Universitaet Muenster (Germany)

1989-1992 Post-doc-Assistant Researcher at Università di Torino, Dipartimento di Medicina e Oncologia Sperimentale (Italy)

1993-1999 Assistant Professor of General Pathology, University of Turin (Italy)

2000 to date: Associate Professor of Cell Pathology and of Experimental Oncology, School of Medicine, Università de Piemonte Orientale (Novara, Italy).

2000-2000: Visiting Professor at Institute fuer Physiologische Chemie (Prof. A. Hasilik), Klinikum der Philipps-Universitaet Marburg (D)

2002-2005: Visiting Professor at Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, University of Bristol (UK).

2005 to date: overseas advisor of PhD students at Mahidol University, at Chulalongkorn University, Bangkok; at Khon Kaen University (Thailand)

2013 to date: Visiting Professor, Siriraj Faculty of Medicine, Mahidol University of Bangkok (Thailand).

2016 to date: Visiting Professor, Department of Cell Biology, Oklahoma University Health Science Center (OKC, US).

Representative Awards:

2014: Professor Honoris Causa Faculty of Medicine and Pharmacy, Université de la Franche-Comté of Besançon (France).

2015: member of the Scientific board of « Integrative Cancer Research Center of the Georgia Institute of Technology », Georgia Tech University, Georgia (Atlanta, US)

2015: Executive Vice-President of the International Association of Traditional and Complementary Medicine.

EDITORIAL BOARD (Associate Editor of):

Autophagy, Molecular Carcinogenesis, BMC Cancer, Genes and Cancer, J Traditional and Complementary Medicine (*Co-Editor-in-Chief*), J. Ovarian Research, J. Molecular Signaling, Frontiers in Endocrinology and Ageing, Am J Cancer Biol, (others)

Interesting Research Areas:

Autophagy in Cancer and in Neurodegeneration. Organelle biogenesis, vesicular traffic and diseases. Epigenetic regulation of Autophagy and cell death. Nanotheranostics ('in cellulo' imaging).

Selected Publications:

1. Phadngam S, Castiglioni A, Ferraresi A, Morani F, Follo C, Isidoro C. PTEN dephosphorylates AKT to prevent the expression of GLUT1 on plasmamembrane and to limit glucose consumption in cancer cells. Oncotarget. 2016 Dec 20;7(51):84999-85020. doi: 10.18632/oncotarget.13113.
2. Ferraresi A, Phadngam S, Morani F, Galetto A, Alabiso O, Chiorino G, Isidoro C. Resveratrol inhibits IL-6-induced ovarian cancer cell migration through epigenetic up-regulation of autophagy. Mol Carcinog. 2017 Mar;56(3):1164-1181.doi: 10.1002/mc.22582.
3. Klionsky DJ, et al., Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy. 2016 Jan 2;12(1):1-222.
4. Janda E, Lascala A, Carresi C, Parafati M, Aprigliano S, Russo V, Savoia C, Ziviani E, Musolino V, Morani F, Isidoro C, Mollace V. Parkinsonian toxin-induced oxidative stress inhibits basal autophagy in astrocytes via NQO2/quinone oxidoreductase 2: Implications for neuroprotection. Autophagy. 2015 Jul3;11(7):1063-80. doi: 10.1080/15548627.2015.1058683.
5. Tang H, Sebti S, Titone R, Zhou Y, Isidoro C, Ross TS, Hibshoosh H, Xiao G, Packer M, Xie Y, Levine B. Decreased BECN1 mRNA Expression in Human Breast Cancer is Associated with Estrogen Receptor-Negative Subtypes and Poor Prognosis. EBioMedicine. 2015 Mar;2(3):255-263.

ORGANIZERS

Ciro Isidoro – *President. Head of Laboratory of molecular Patology and Nanobioimaging- University of Piemonte Orientale*

Gianluca Gaidano (Vice President) – *Head of Hematology (University of Piemonte Orientale)*

INTERNATIONAL SCIENTIFIC COMMITEE

Ciro Isidoro, Chair

Danny Dhanasekaran

Gianluca Gaidano

Gloria Su

INVITED SPEAKERS

- **Cedric Gaggioli –** gaggioli@nice.fr
- **Walter Birchmeier –** wbirch@mdc-berlin.de
- **Paola Chiarugi –** paola.chiarugi@unifi.it
- **Lizzia Raffaghello –** lizziaraffaghello@ospedale-gaslini.ge.it
- **Salvatore Pece –** salvatore.pece@ieo.it
- **Matilde LLeonart –** matilde.lleonart@vhir.org
- **Danny Dhanasekaran –** danny.dhanasekaran@ouhsc.edu
- **Gloria Su –** gs2157@columbia.edu
- **Carlo Follo –** carlo.follo@ucsf.edu
- **Davide Rossi –** davide.rossi@ior.iosi.ch

SCIENTIFIC and SOCIAL PROGRAMME

Day 1 - SUNDAY OCTOBER 29th

9.30-12.30	Arrival and accommodation at Hotel Europa
12.30-14.00	LUNCH WITH INVITED SPEAKERS
14.30-17.00	**CITY TOUR OF NOVARA**. (Courtesy of the Municipality of Novara, thanks to the Lord Mayor Dr. Alessandro Canelli). Visit of Cupola Antonelliana, Basilica di San Gaudenzio, Complesso del Broletto, Galleria Giannoni, Quadriportico della Canonica, Castello Visconteo-Sforzesco, Palazzo Gallarini. (Organized by Dott.ssa Maria Rosa Fagnoni)
18-45-20.00	**CLASSICAL MUSIC CONCERT (By invitation)** Conservatorio musicale "Guido Cantelli", Via Solaroli (Novara) (See map at www.consno.it). Courtesy of the Conservatorio (thanks to Dr. Anna Belfiore) Meet in the lobby at 18.15
20.00-22.00	WELCOME APERICENA **(By invitation)**

Day 2- MONDAY OCTOBER 30th

8.30-9.30	REGISTRATION of Attendees
9.40-10.00	OPENING CERIMONY Welcome address:

Dr. Alessandro Canelli, Lord Mayor of the City of Novara

Dr. Mario Minola, General Manager of the University-Hospital of Novara

Prof. Giorgio Bellomo, President of the School of Medicine

Prof. Umberto Dianzani, Director of the Department of Health Sciences

Prof. Giancarlo Avanzi, Director of Department of Translational Medicine

Prof. Marco Krengli, President of the Master Degree Course in Medicine and Surgery

10.00-10.15 INTRODUCTION TO THE WORKSHOP (by Ciro Isidoro)

10.15-12.30 **FIRST SESSION** (4 Lectures + 1 short communication)

Chairpersons: Mathilde Lleonart, Salvatore Pece.

Cedric Gaggioli, Nice (France): A journey into the tumor microenvironment: a LIF(e) story.

Walter Birchmeier, Berlin (Germany): Cancer Stem Cells regulate Cancer-Associated Fibroblasts via activation of Hedgehog signaling in Mammary Gland tumors.

Paola Chiarugi, Florence (Italy): Mitochondria at the crossroad: metabolic symbiosis within cancer microenvironment.

Lizzia Raffaghello, Genoa (Italy): Cancer metabolism and T Cell response.

One short communication: Giuseppe Taurino, University of Parma, Parma (Italy)

12.40-13.40 LUNCH BREAK (Only registered participants)

13.45-15.45 ***SECOND SESSION*** (3 Lectures + 1 short communication)

Chairpersons: Paola Chiarugi, Walter Birchmeier.

Salvatore Pece, Milan (Italy): Loss of Numb in breast carcinogenesis: a paradigm for a mechanism-based selective anti-cancer stem cell therapy

Matilde LLeonart, Barcelona (Spain): MicroRNAs in tumorigenesis: from bench to the bed-side.

Danny Dhanasekaran, Oklahoma City (US): Long Non-coding RNA codes in Ovarian cancer.

Two short communications:

Aurelia Spina**,** University of Padua, Padua (Italy)

Paolo Porporato**,** University of Turin, Turin (Italy)

15.45-16.00 COFFEE BREAK

16.00-17.45 ***THIRD SESSION*** (3 Lectures + 1 short communication)

Chairperson: Lizzia Raffaghello, Gianluca Gaidano.

Gloria Su, New York (US): The impact of tumor-suppressor genes on kras-induced pancreatic carcinogenesis.

Carlo Follo, San Francisco (US): Inhibition of autophagy initiation potentiates chemosensitivity in mesothelioma.

Davide Rossi, Bellinzona (Switzerland): Genotyping of classical Hodking lymphoma on the liquid biopsy.

One short communication: Fary Diop, University of Piemonte Orientale, Novara (Italy)

17.45-18.00 Overview of submitted Abstracts (by Ciro Isidoro)

SIX FLASH COMMUNICATIONS, selected from Abstracts:

Elena Darra**,** University of Verona, Verona (Italy)

Simona Nanni**,** Università Cattolica, Rome (Italy)

Eleonora Secomandi**,** University of Piemonte Orientale, Novara (Italy)

Monika Szeliga**,** Mossakowski Medical Research Center, Warsaw (Poland)

Olga Tarasiuk**,** University of Piemonte Orientale, Novara (Italy)

Suresh Velnati**,** University of Piemonte Orientale, Novara (Italy)

18.00-18.15 CONCLUDING REMARKS (Gloria Su, Danny Dhanasekaran, Gianluca Gaidano)

---------- **CLOSING CERIMONY** (Acknowledgements by Ciro Isidoro) ----------

18.15-19.00 **SOCIAL EVENT** at LOGGIONE D'ONORE of Ospedale Maggiore della Carità: Farewell cocktail party **(ALL registered partecipants)**

20.00-22.30 **GALA DINNER** at Castello Visconteo-Sforzesco **(by invitation)** (Courtesy of the Municipality of Novara and of Fondazione del Castello; thanks to dott.ssa Laura Bianchi)

First Session

10.15 – 12.30 A.M.

Chairpersons:

Mathilde LLeonart, Salvatore Pece

CEDRIC GAGGIOLI

cedric.gaggioli@unice.fr; cedric.gaggioli.org

Appointed Principal Investigator

Qualified Full Professor of Cellular and Molecular Biology

Research Institute on Cancer and Ageing, Nice (France)

Education:

2015: HDR, Life Sciences (UNSA, Nice, France.)

2005: PhD (Paris VII, France)

2000 – 2001: Master (Paris VII, France)

1996 – 2000: Under-graduation (UNSA, Nice, France)

Representative Careers and affiliations:

2015: Team leader at IRCAN, Nice, France. « Tumor-Stroma Interactions».

2012 – 2015: Junior team leader at IRCAN, Nice, France. « Tumor-Stroma Interactions».

2008- 2011: CDD INSERM: INSERM U634, Nice, France

2005- 2008: Post-Doc Tumor Cell Biology laboratory. London Research Institute, Cancer Research UK, London, UK.

2000-2005: PhD INSERM U597, Nice, France.

Representative Awards :

2005 Foundation Bettencourt-Schueller, young Reseacher of the Year award.

Selected Publications:

1. Bonan S, Albrengues J, Grasset E, Kuzet SE, Nottet N, Bourget I, Bertero T, Mari B, Meneguzzi G, Gaggioli C. Membrane-bound ICAM-1 contributes to the onset of proinvasive tumor stroma by controlling acto-myosin contractility in carcinoma-associated fibroblasts. Oncotarget. 2016 Nov 25
2. Bertero T, Oldham WM, Cottrill KA, Pisano S, Vanderpool RR, Yu Q, Zhao J, Tai Y, Tang Y, Zhang YY, Rehman S, Sugahara M, Qi Z, Gorcsan J 3rd, Vargas SO, Saggar R, Saggar R, Wallace WD, Ross DJ, Haley KJ, Waxman AB, Parikh VN, De Marco T, Hsue PY, Morris A, Simon MA, Norris KA, Gaggioli C, Loscalzo J, Fessel J, Chan SY. Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. J Clin Invest. 2016 Sep 1;126(9):3313-35.
3. Albrengues J, Bertero T, Grasset E, Bonan S, Maiel M, Bourget I, Philippe C, Herraiz Serrano C, Benamar S, Croce O, Sanz-Moreno V, Meneguzzi G, Feral CC, Cristofari G, Gaggioli C. Epigenetic

switch drives the conversion of fibroblasts into pro-invasive cancer-associated fibroblasts. Nature Communications. 2015

4. Albrengues J, Bourget I, Pons C, Butet V, Hofman P, Tartare-Deckert S, Feral CC, Meneguzzi G, Gaggioli C. LIF mediates proinvasive activation of stromal fibroblasts in cancer. Cell Reports. 2014 Jun 12;7(5):1664-78.
5. Sanz-Moreno V[†], Gaggioli C[†], Yeo M, Albrengues J, Wallberg F, Hooper S, Viros A, Mitter R, Féral C, Marais R, Meneguzzi G, Sahai E, Marshall C. ROCK and JAK1 signalling cooperate to control actomyosin contractility in tumour cells and stroma. Cancer cell. 2011 Aug 16;20(2):229-45.

ABSTRACT

A JOURNEY INTO THE TUMOR MICROENVIRONMENT ; A LIF(E) STORY.

Cédric Gaggioli[1]

[1]IRCAN,INSERM, France

Squamous Cell Carcinomas (SCC) are solid tumours arising from epithelial cells. SCC cell expansion is influenced by the multiple cell types present in the tumour stroma, which includes fibroblasts. The role of stroma fibroblasts, also called Carcinoma-Associated Fibroblast (CAF) in cancer cell proliferation, survival, angiogenesis and invasion, notably by producing a wide range of growth factors and cytokines besides Extra-Cellular Matrix (ECM) components, has been widely documented. However, the molecular and cellular mechanisms that govern CAF conversion from normal resident fibroblasts (human Dermal Fibroblasts "hDF") remain unclear. We have demonstrated that the Leukemia Inhibitory Factor (LIF) controls specific pro-invasive fibroblast activation in cancer; this independently from expression of alpha-Smooth Muscle Actin (αSMA). We showed that a pulse of TGFβ establishes stable pro-invasive fibroblast activation by inducing LIF production in both fibroblasts and tumour cells. In fibroblasts, LIF mediates acto-myosin contractility and extracellular matrix remodeling, which results in collective carcinoma cell invasion. Along with these investigations, we noticed that in fibroblasts, LIF also induces sustained pro-invasive activity. In this context, we unveiled the involvement of epigenetic modifications that underlie the sustained fibroblast activation. Indeed, we demonstrated that post-transcriptional modifications of the STAT3 transcription factor that leads to methylation of the Shp-1 phosphatase promoter result in constitutive JAK phosphorylation and activity. These findings make LIF a pro-invasive fibroblast mediator that is dissociated from αSMA expression and they may open novel therapeutic perspectives for patients with aggressive primary tumours. Interestingly, we provide evidence that an inhibitory treatment of CAF combining Ruxolitinib and 5'-Azacytidine results in a constitutive molecular and phenotypic reversion of activated fibroblasts to hDF.

REFERENCES

1) Albrengues J, et al., **Cell Rep**. 2014.
2) Albrengues J, et al., **Nat Commun.** 2015.
3) Sanz-Moreno V*, Gaggioli C*, et al., **Cancer Cell** 2011.

WALTER BIRCHMEIER

wbirch@mdc-berlin.de

Research Group Leader

Max Delbrueck Center for Molecular Medicine

(MDC), Robert -Roessle-Str. 10, 13125 Berlin

Education:

1973: PhD from the University of Zürich

1970-1973: PhD Studies at the Institute of Biochemistry, Univ. of Zürich (with P. Christen)

1966-1970: Studies of Biology and Biochemistry at the Univ. of Zürich, Diploma obtained in 1970

Representative Careers:

2004-2008: Scientific Director at the Max Delbrück Center for Molecular Medicine (MDC), Berlin

Since 1996: University Professor at the Humboldt University/Charité, Berlin

Since 1993: Senior Research Group Leader at the MDC, Berlin

1988-1993: Full Professor at the University Clinic Essen

1982-1988: Independent Group Leader at the Max Planck Institute (FML), Tübingen

1978-1980: Junior Group Leader at ETH Zürich

1974-1977: Postdoc at Cornell University, the Biocenter Basel, and the University of California, San Diego (with Jeff Schatz, Jon Singer)

Representative Awards:

Membership 2005: EMBO Member; Swiss Acad. Medical Sciences (SAMW).

Awards: 1999: German Cancer Award (Deutscher Krebspreis), German Cancer Society; 1992: Meyenburg-Award for Cancer Research, Heidelberg; 1990: Award for Cancer Res., Wilhelm-Warner-Stiftung, Hamburg.

Honorus: 1973-1977: PhD with Honours from the University of Zürich; 1973: PhD with Honours from the University of Zürich

Interesting Research Areas:

Role of Wnt/β-catenin and Met/Shp2 in Development and Cancer, Metastasis, Epigenetic control of Cancer Stem Cells, use in Therapy approaches

Selected Publications:

1. Valenti G, Quinn HM, Heynen GJJE, Lan L, Holland JD, Vogel R, Wulf-Goldenberg A, Birchmeier W. Cancer Stem Cells Regulate Cancer-Associated Fibroblasts via Activation of Hedgehog Signaling in Mammary Gland Tumors. Cancer Res. 2017 Apr 15;77(8):2134-2147.
2. Holland JD, Györffy B, Vogel R, Eckert K, Valenti G, Fang L, Lohneis P, Elezkurtaj S, Ziebold U, Birchmeier W. Combined Wnt/β-catenin, Met, and CXCL12/CXCR4 signals characterize basal breast cancer and predict disease outcome. Cell Rep. 2013 Dec 12;5(5):1214-27.
3. Wend P, Fang L, Zhu Q, Schipper JH, Loddenkemper C, Kosel F, Brinkmann V, Eckert K, Hindersin S, Holland JD, Lehr S, Kahn M, Ziebold U, Birchmeier W. Wnt/β-catenin signalling induces Mll1 to create epigenetic changes in salivary gland tumours. EMBO J. 2013 Jul 17;32(14):1977-89.
4. Grigoryan T, Wend P, Klaus A, Birchmeier W. Deciphering the function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. Genes Dev. 2008 Sep 1;22(17):2308-41.
5. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer. 2008 May;8(5):387-98.
6. Soshnikova N, Zechner D, Huelsken J, Mishina Y, Behringer RR, Taketo MM, Crenshaw EB 3rd, Birchmeier W. Genetic interaction between Wnt/beta-catenin and BMP receptor signaling during formation of the AER and the dorsal-ventral axis in the limb. Genes Dev. 2003 Aug 15;17(16):1963-8.
7. Huelsken J, Vogel R, Erdmann B, Cotsarelis G, Birchmeier W. Catenin controls hair follicle morphogenesis and stem cell differentiation in the skin. Cell. 2001 May 18;105(4):533-45.
8. Behrens J, Jerchow BA, Würtele M, Grimm J, Asbrand C, Wirtz R, Kühl M, Wedlich D, Birchmeier W. Functional interaction of an axin homolog, conductin, with beta-catenin, APC, and GSK3beta. Science. 1998 Apr 24;280(5363):596-9.
9. Weidner KM, Di Cesare S, Sachs M, Brinkmann V, Behrens J, Birchmeier W. Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. Nature. 1996 Nov 14;384(6605):173-6.
10. Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R, Birchmeier W. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature. 1996 Aug 15;382(6592):638-42.
11. Imhof BA, Vollmers HP, Goodman SL, Birchmeier W. Cell-cell interaction and polarity of epithelial cells: specific perturbation using a monoclonal antibody. Cell. 1983 Dec;35(3 Pt 2):667

ABSTRACT

CSCS AND THEIR SYNERGY WITH CAFS IN BASAL-LIKE MAMMARY GLAND TUMORS

Walter Birchmeier[1], Hazel Quinn[1].

[1]Max-Delbrück Center for Molecular Medicine (MDC), Berlin

We expressed in mice HGF that activates the Met receptor and gain-of-function beta-catenin under control of the milk component Whey Acidic Protein (WAP). Upon stimulation via pregnancy, mice developed mammary gland tumors in as little as 2 weeks post-partum, and these tumors had a basal-like phenotype. We then studied the synergy between CSCs and CAFs in these tumors. Isolating CAFs was possible using a combination of the cell surface markers CD24-, CD90+ and CD140b+ via FACS. In co-culture, we grew CSCs in non-adherent conditions as mammospheres and either control fibroblasts or CAFs in transwell plates, so that only secreted factors could be exchanged. Co-culture with CAFs but not control fibroblasts enhanced growth of each cell type. We conducted transcriptome analysis of these cells and saw a significant difference in gene expression. One pathway that stood out were genes associated with HH signalling such as Gli1 and Patched1. We also looked at HH ligands, and SHH was expressed in the CSCs and not in CAFs. We then treated CAFs with increasing concentrations of recombinant SHH, which strongly increased their growth. We also used the HH inhibitor vismodegib, which delayed tumor growth in concentration-dependent manners. We then did microarray analyses and filtered out genes for ligands that were expressed in CAFs and had their corresponding receptors in CSCs. We identified ligands whose expression decreased upon inhibition of HH signaling by vismodegib, showing that HH signaling in CAFs controls ligands acting on CSCs, Activin A, IGF-1, LIF and NOV. We showed that Activin A and NOV increased sphere size significantly, and Activin A also increased secondary sphere formation. In order to check if YAP was activated in our mammary gland tumor model, we stained Wnt-Met tumor sections for YAP. Indeed, we observed nuclear YAP staining and activated YAP targets such as CTGF, CYR61 and IGFBP3. Since both YAP activity and Wnt activity are important for CSCs, we utilized an inhibitor of each of these pathways for tumor interference; the Wnt inhibitor ICG-001 and the YAP inhibitor Verteporfin indeed produced effective knockdown of both Wnt and YAP target genes. We now have obtained mice with floxed SHH and with floxed YAP alleles, and will examine whether we can obtain effects on tumor growth in vivo.

REFERENCES

1. Valenti G, Quinn HM, Heynen GJJE, Lan L, Holland JD, Vogel R, Wulf-Goldenberg A, Birchmeier W. Cancer Stem Cells Regulate Cancer-Associated Fibroblasts via Activation of Hedgehog Signaling in Mammary Gland Tumors. Cancer Research 2017; 77:2134-2147.
2. Holland JD, Györffy B, Vogel R, Eckert K, Valenti G, Fang L, Lohneis P, Elezkurtaj S, Ziebold U, Birchmeier W. Combined Wnt/β-catenin, Met, and CXCL12/CXCR4 signals characterize basal breast cancer and predict disease outcome. Cell Reports 2013; 5:1214-27.

PAOLA CHIARUGI

paola.chiarugi@unifi.it

Full Professor of Biochemistry

Department of Clinical and Experimental Biomedical Sciences, Viale Morgagni 50, Florence

Education and Representative Careers

1998- Specialist in Biochemistry and Clinical Chemistry

1994- PhD in Biochemistry

1989- Magistral Degree in Biological Sciences

Representative Careers

2005-Full Professor in Biochemistry, Faculty of Medicine and Surgery, University of Florence

2001-Associate Professor in Biochemistry, Faculty of Medicine and Surgery, University of Florence

1998-Assistant Professor of Molecular Biology, Faculty of Medicine and Surgery, University of Florence

Representative Awards:

She is member of:

- The Excellence and Research Centre for Transfer and High Formation DENOThe',
- The Italian Society of Biochemistry and Molecular Biology,
- The Italian Cancer Society,
- The Advisory Board of Breakthrough Breast Cancer Research Unit of the University of Manchester,
- The Advisory Board of Institute Marie Curie, Paris,
- The Assessment Review Panel of Tuscany Tumor Institute

Editorial activity: She is member of the editorial board of Journal of Molecular Medicine, Frontiers in Cancer Molecular Targets e Therapeutics and Cell Communications and Signalling

Interesting Research Areas:

Prof. Chiarugi has studied for up to 15 years the structure-function relationship and the redox regulation of oxidant-sensitive proteins during cancer cell proliferation and cell adhesion to extracellular matrix, particularly focusing on the role of anchorage proteins (integrin and Src/FAK signaling) as well as cytoskeleton and motility factors (repulsive ephrin receptor tyrosine kinases).

She contributed studies on motility and anchorage independence of cancer cells, their achievement of a phenotype resistant to anoikis, as well as studies on plasticity of motility in cancer cells, as epithelial mesenchymal transition or mesenchymal amoeboid transition. She studies tumor microenvironment since 10 years, particularly focusing on cancer associated fibroblasts, endothelial precursor cells and hypoxia, and the relationship with tumor metabolic deregulation.

Publications of the last five years:

1. Ippolito L, Marini A, Cavallini L, Morandi A, Pietrovito L, Pintus G, Giannoni E, Schrader T, Puhr M, **Chiarugi P**, Taddei ML. Metabolic shift toward oxidative phosphorylation in docetaxel resistant prostate cancer cells. Oncotarget. 2016 Sep 20;7(38):61890-61904.
2. Denise C, Paoli P, Calvani M, Taddei ML, Giannoni E, Kopetz S, Kazmi SM, Pia MM, Pettazzoni P, Sacco E, Caselli A, Vanoni M, Landriscina M, Cirri P, **Chiarugi P**. 5-fluorouracil resistant colon cancer cells are addicted to OXPHOS to survive and enhance stem-like traits. Oncotarget. 2015 Dec 8;6(39):41706-21.
3. Giannoni E, Taddei ML, Morandi A, Comito G, Calvani M, Bianchini F, Richichi B, Raugei G, Wong N, Tang D, **Chiarugi P**. Targeting stromal-induced pyruvate kinase M2 nuclear translocation impairs oxphos and prostate cancer metastatic spread. Oncotarget. 2015 Sep 15;6(27):24061-74.
4. Comito G, Giannoni E, Segura CP, Barcellos-de-Souza P, Raspollini MR, Baroni G, Lanciotti M, Serni S, **Chiarugi P**. Cancer-associated fibroblasts and M2-polarized macrophages synergize during prostate carcinoma progression. Oncogene. 2013 Jun 3. 1223
5. Fiaschi T, Marini A, Giannoni E, Taddei ML, Gandellini P, De Donatis A, Lanciotti M, Serni S, Cirri P, **Chiarugi P**. Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. Cancer Res. 2012 Oct 1;72(19):5130-40. doi: 10.1158/0008-5472.CAN-12-1949.

ABSTRACT

MITOCHONDRIA AT THE CROSSROAD: METABOLIC SYMBIOSIS WITHIN CANCER MICROENVIRONMENT

Chiarugi P, Ippolito L, Morandi A, Masquelier J, Sonveaux P, Giannoni E.

Department of Experimental and Clinical Biomedical Sciences 'Mario Serio', University of Florence, viale Morgagni 50, I-50134 Florence, Italy. Louvain Drug Research Institute (LDRI), Université catholique de Louvain (UCL), B-1200, Brussels, Belgium.

The acquisition of malignant traits during tumorigenesis is strongly influenced by the surrounding microenvironment and cancer associated fibroblasts (CAFs), key players of cancer progression, have also been correlated with resistance to therapy. CAFs induce epithelial mesenchymal transition (EMT), metabolic reprogramming towards oxidative phosphorylation (OXPHOS) and activation of a lactate shuttle, promoting tumor growth and metastatic dissemination. The effect of stromal cells on EMT undergoing cancer cells is mainly mediated by the metabolic regulator pyruvate kinase M2 (PKM2). EMT-driven oxidative signaling leads to PKM2 oxidation and Src-mediated phosphorylation, nuclear migration, association with hypoxia inducible factor-1, down-regulation of miR205 and activation of OXPHOS. PKM2 and OXPHOS targeting in vivo confirms the relevance of the pathway for stromal reprogramming of tumor cells. CAFs induced a lactate-dependent activation of the SIRT1/PGC-1α axis in PCa cells, consequently increasing the mitochondrial mass and stimulating mitochondrial respiration. Overactivation of mitochondrial metabolism first stimulated the generation of mitochondrial reactive oxygen species (mtROS) in CAF-exposed PCa cells, which is closely related to EMT and invasion. CAFs-induced TCA cycle activation in PCa cells further resulted in the accumulation of succinate and fumarate, two oncometabolites responsible for the activation of transcription factor hypoxia-inducible factor-1 (HIF-1). Succinate was also released by CAFs and uploaded by PCa cells, further raising its intracellular content and suggesting a potential non-metabolic function. Interestingly, increased OXPHOS in PCa cells was associated to their ability to steal functional mitochondria from nearby CAFs through the formation of tunneling nanotubes *in vitro* and *in vivo*. Analysis of CAF-transferred mitochondria revealed their functionality and their capability to generate mtROS in PCa cells, thus promoting their metastatic potential. Our data reveal that CAFs overactivate mitochondrial metabolism in PCa cells by providing mitochondrial fuels and functional mitochondria in order to foster tumor malignancy.

LIZZIA RAFFAGHELLO

lizziaraffaghello@gaslini.org

PhD Senior researcher Affiliation

Laboratorio Cellule Staminali post-natali e Terapie Cellulari, IRCCS - G. Gaslini Institute, Genoa, Italy

Education

1984-1989 10/1989-11/1995 01/1996-06/1999

High School (Liceo Classico A. D'Oria Genoa) BS and Master in Biological Sciences at the University of Genoa.

Specialization in Biochemistry and Clinical Chemistry at the University of Pavia.

Representative Careers and awards:

09/1992-12/1995: Graduated and post graduated student researcher at the Laboratory of Oncology of G. Gaslini Institute, Genova

01/1996- 12/1998: Fellowship from the Italian Association for Cancer Research at the Laboratory of Oncology of G. Gaslini Institute of Genova.

10/1997- 10/1998: Visiting scholar at the Hematology/Oncology Laboratory of the Childrens' Hospital of Los Angeles.

05/2000: AACR-Pezcoller Foundation Young Investigator Scholar Award at 91st Annual Meeting AACR, 2000, San Francisco.

02/1999- 09/2011: Fellow at the Laboratory of Oncology of G. Gaslini Institute

10/2008- 10/2008: Fellowship "Marie Curie Host Fellowships for the Transfer of Knowledge At the University of Crackow.

2013-09/2017: Senior researcher with permanent position at the Laboratory of Oncology of G. Gaslini Institute, Genoa.

09/2017-up today: Senior researcher with permanent position at the Laboratory of Oncology of G. Gaslini Institute, Genova.

Interesting Research Areas

Neuroblastoma, tumor microenvironment and mechanisms of tumor escape, cancer metabolism and calorie restriction as an antitumor strategy, adoptive cell therapies, Chimeric-Antigen-Receptors T cells, immune metabolism.

Selected Publications:

1. Metabolic Alterations at the Crossroad of Aging and Oncogenesis. Raffaghello L, Longo V. Int Rev Cell Mol Biol. 2017;332:1-42.
2. Fasting induces anti-Warburg effect that increases respiration but reduces ATP-synthesis to promote apoptosis in colon cancer models. Bianchi G, Martella R, Ravera S, Marini C, Capitanio S, Orengo A, Emionite L, Lavarello C, Amaro A, Petretto A, Pfeffer U, Sambuceti G, Pistoia V, Raffaghello L, Longo VD. Oncotarget. 2015 May 20;6(14):11806- 19.
3. Divergent targets of glycolysis and oxidative phosphorylation result in additive effects of metformin and starvation in colon and breast cancer. Marini C, Bianchi G, Buschiazzo A, Ravera S, Martella R, Bottoni G, Petretto A, Emionite L, Monteverde E, Capitanio S, Inglese E, Fabbi M, Bongioanni F, Garaboldi L, Bruzzi P, Orengo AM, Raffaghello L, Sambuceti G. Sci Rep. 2016 Jan 22;6:19569.
4. Fasting cycles retard growth of tumors and sensitize a range of cancer cell types to chemotherapy. Lee C,Raffaghello L, Brandhorst S, Safdie FM, Bianchi G, Martin- Montalvo A, Pistoia V, Wei M, Hwang S, Merlino A, Emionite L, de Cabo R, Longo VD. Sci Transl Med. 2012 Mar 7;4(124):124ra27.
5. Starvation-dependent differential stress resistance protects normal but not cancer cells against high-dose chemotherapy. Raffaghello L, Lee C, Safdie FM, Wei M, Madia F, Bianchi G, Longo VD. Proc Natl Acad Sci U S A. 2008 Jun 17;105(24):8215-20

ABSTRACT

CANCER METABOLISM AND T CELL RESPONSE

Lizzia Raffaghello[1], Giovanna Bianchi[1], Vito Pistoia[2], Valter D Longo[3].

[1]Laboratorio Cellule Staminali post-natali e Terapie Cellulari, IRCCS - G. Gaslini Institute, Genoa, Italy; [2] Immunology Area, IRCCS Bambino Gesù, Rome, Italy; [3] Longevity Institute, Davis School of Gerontology, University of Southern California, Los Angeles, CA, United States; IFOM, FIRC Institute of Molecular Oncology, Milano, Italy.

Tumor metabolism is defined as an emerging hallmark of cancer cells which are characterized by high glucose uptake and lactate production regardless of oxygen concentration, a phenomenon known as the "Warburg effect"[1]. Therefore, strategies inhibiting glycolysis in order to delay tumor growth and overcome drug resistance are being investigated. Fasting/fasting mimicking diet causes a generalized glucose and amino acid reduction/deficiency which protects normal but not tumor cells against chemotherapy-mediated cytotoxicity and induces potent chemosensitizing effects in many tumors [2-4]. In addition to protecting haematopoietic cells from chemotoxicity, multiple cycles of fasting promote hematopoietic stem cell self-renewal to alleviate or reverse the immunosuppression or immunosenescence caused by chemotherapy treatment [5]. More recently, fasting has also been shown to enhance the therapeutic index of chemo-treatments by exerting an anti-Warburg effect. In this way cancer cells are shifted from a glycolytic mode into an uncoupled oxidative phosphorylation which promotes increased reactive oxygen specie generation and apoptosis [6]. Targeting T cell metabolism is another promising approach in order to improve the efficacy of adoptive cell therapy [7]. Strategies able to inhibit glycolysis block $CD8^+$ T lymphocyte differentiation toward T effector cells and increase the T stem central memory counterpart, thus improving the anti-tumor activity efficacy of cytotoxic T cells (8). Moreover, fatty acid oxidation (FAO) catabolism increases CD8 T memory cells, and considerably improves the efficacy of an experimental anti-cancer vaccine (9). In this line, we are investigating strategies promoting FAO in order to enhance the *in vivo* persistence and the efficacy of Chimeric-Antigen-Receptors T cells which *per se* do not exert significant efficacy against neuroblastoma cells (10). Taken together, these studies provide evidence that strategies targeting both tumor and immune metabolism may be promising against cancer.

Short communication

GIUSEPPE TAURINO

giuseppe.taurino@studenti.unipr.it

PhD Student of Molecular Medicine

Laboratory of General Pathology, Department of Medicine and Surgery, University of Parma, Via Gramsci 14 - 43126 Parma (Italy)

Education:

2014- Degree in Biology (110/110 with honors), University of Parma (Italy) "Determinants of toxicity of carbon nanotubes on macrophages and airway epithelial cells"

2016 Master's Degree in Biology and Biomedical Applications (110/110 with honors), University of Parma (Italy) " Glutamine Synthetase negative Human Oligodendrioglioma Cells are Auxotroph for Glutamine: rationale for novel therapeutic approaches based on nano-forms of L-asparaginase"

Representative Awards:

Erasmus Traineeship Grant: March-May 2016, University of Angers, MINT laboratory UMR-S1066 4 rue Larrey, 49933 Angers (France). Development of drug-loaded nanocarriers for cancer treatment

Interesting Research Areas:

Role of glutamine metabolism in cancer, metabolic interactions of cancer cells with stroma, characterization of amino acid transport, use of nanostructures for drug delivery

Selected Publications:

1. Chiu M, Sabino C, **Taurino G**, Bianchi MG, Andreoli R, Giuliani N, Bussolati O. GPNA inhibits the sodium-independent transport system L for neutral amino acids. Amino Acids. 2017 Aug;49(8):1365-1372.

ABSTRACT

MESENCHYMAL STROMAL CELLS FEED ASPARAGINASE-TREATED BCP-ALL BLASTS: KEY ROLES OF GLUTAMINE SYNTHETASE AND THE GLUTAMINE TRANSPORTER SNAT5

Giuseppe Taurino[1], Martina Chiu[1], Erica Dander[2], Donatella Bardelli[2], Prisco Mirandola[3], Cecilia Carubbi[3], Roberta Andreoli[4], Carmelo Rizzari[5], Giovanna D'Amico[2] and Ovidio Bussolati[1]

[1]Laboratory of General Pathology, [3]Human Anatomy and [4]Industrial Toxicology, Department of Medicine and Surgery, University of Parma, Parma; [2]"M. Tettamanti" Research Center, Department of Pediatrics, University of Milano Bicocca, MBBM Foundation, Maria Letizia Verga Center, Monza; [5]Pediatric Hematology Oncology Unit, Department of Pediatrics, San Gerardo Hospital, University of Milano Bicocca, Monza

INTRODUCTION: Due to the low expression of Asparagine Synthetase, Acute Lymphoblastic Leukemia (ALL) blasts are extremely sensitive to L-Asparaginase (ASNase), which depletes blood asparagine (Asn) and glutamine (Gln). It has been proposed that bone marrow Mesenchymal Stromal Cells (MSC) support ALL blast viability during antileukemic therapy through Asn secretion[1]. However, the mechanisms involved have remained thus far elusive.

EXPERIMENTAL MODEL: MSC from ALL patients were cultured in DMEM+10% FBS, 2mM Gln and antibiotics as monocultures or co-cultures with the BCP-ALL cell line RS4;11 (ratio 1:4). ASNase from *Erwinia chrysanthemi* (1U/ml) was used to deplete extracellular Asn and Gln. Glutamine Synthetase (GS) was inhibited by L-methionine sulfoximine (MSO, 1mM).

RESULTS: ASNase-treated MSC, after a transient proliferative arrest associated with CHOP induction, adapted to the nutritional stress, increasing GS expression, and showing a partial recovery of intracellular Gln. Under these conditions, MSC exerted a clear cut protective effect on ASNase-treated RS4;11 cells. The GS inhibitor MSO suppressed MSC adaptation, prevented MSC cell Gln recovery and hindered ALL cell protection. Even under nutritional stress conditions, MSC show a high efflux rate of Gln and Asn into the extracellular medium. Compared with MSC from healthy donors, MSC from ALL patients had a significantly larger Gln efflux and express higher levels of SNAT5, a bi-directional transporter for Gln and Asn.

CONCLUSION: MSC support the viability of ASNase-treated ALL blasts through (a) metabolic adaptation due to a partial, GS-dependent restoration of cell Gln, and (b) the secretion of Gln and Asn through the transporter SNAT5.

REFERENCES

1. Iwamoto S, Mihara K, Downing JR, Pui CH, Campana D. Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to asparaginase. J Clin Invest. 2007 Apr;117(4):1049-57.

Second Session

13.45 – 15.45 P.M.

Chairpersons:

Paola Chiarugi, Walter Birchmeier

SALVATORE PECE

salvatore.pece@ieo.it

Appointed Associate Professor of General Pathology, University of Milan Medical School. Deputy Director, Program of Molecular Medicine, European Institute of Oncology, Milan

European Institute of Oncology, Via Ripamonti 435, 20141 Milan, Italy

Education

1998 - Specialty Board in Microbiology and Virology, *summa cum laude*, University of Bari Medical School, Bari, Italy.

1993 - Ph.D., Infectious Diseases, University of Bari Medical School, Bari, Italy.

1989 - M.D., *summa cum laude*, University of Bari Medical School, Bari, Italy.

Representative Careers and affiliations:

2006 to date. Associate Professor, Chair of General Pathology, Milan University School of Medicine, Milan, Italy.

2001 – 2006. University Researcher, Chair of General Pathology, Milan University School of Medicine, Milan, Italy.

1998-2000. *Visiting Associate*, Oral and Pharyngeal Cancer Branch, NIDCR, National Institutes of Health (NIH), Bethesda, USA.

1996 - 2001. University Researcher, Department of General Pathology, Bari University School of Medicine, Bari, Italy.

1992. *Visiting Scientist,* Laboratoire de Pharmacologie Cellulaire et Moleculaire, Faculté de Pharmacie, University “Louis Pasteur”, Strasbourg, France.

Representative Awards:

2005. ‘Giancarla Vollaro’ Foundation award – Start up of a Stem Cell Biology Unit at the European Institute of Oncology in Milan.

2000. Technology Transfer Award, National Institutes of Health (NIH), Bethesda, USA.

1998-2000. Fogarty Fellowship, National Institutes of Health (NIH), Bethesda, USA.

Major Research Areas:

Molecular mechanisms of breast and lung tumorigenesis. Normal and tumor stem cell biology. Preclinical studies for the translation of targeted therapies in breast and prostate cancer. Bioethical aspects related to informed participation of cancer patients to research studies.

Selected Publications:

1. Pre-clinical validation of a selective anti-cancer stem cell therapy for Numb-deficient human breast cancers. Tosoni, D., Pambianco, S., Ekalle-Soppo, B., Zecchini, S., Bertalot, G., Pruneri, G., Viale, G., Di Fiore, P.P., and **Pece, S**. EMBO Mol. Med., Mar 15, 2017.
2. The scaffold protein p140Cap limits ERBB2-mediated breast cancer progression interfering with Rac GTPase-controlled circuitries. Grasso, S., Chapelle, J., Salemme, V. et al. *Nature Commun*., 8: 14797, 2017.
3. Modelling TFE renal cell carcinoma in mice reveals a critical role of WNT signaling. Calcagnì, A., Kors, L., Verschuren, E., De Cegli, R., Zampelli, N., Nusco, E., Confalonieri, S., Bertalot, G., **Pece, S.,** Settembre, C., Malouf, G.G., Leemans, J.C., de Heer, E., Salvatore, M., Peters, D.J., Di Fiore, P.P., Ballabio, A. *Elife*, 26; 5. pii: e17047, 2016.
4. The Numb/p53 circuitry couples replicative self-renewal and tumor suppression in mammary epithelial cells. Tosoni D, Zecchini S, Coazzoli M, Colaluca I, Mazzarol G, Rubio A, Caccia M, Villa E, Zilian O, Di Fiore PP, **Pece S.** *J Cell Biol*., 23; 211 (4): 845-62, 2015.
5. HMGA1 silencing restores normal stem cell characteristics in colon cancer stem cells by increasing p53 levels. F. Puca, M. Colamaio, A. Federico, M. Gemei, N. Tosti, A.U. Bastos, L. Del Vecchio, S. Pece, S. Battista, A. Fusco. *Oncotarget*, 30; 5(10): 3234-45, 2014.
6. flowFit: a Bioconductor package to estimate proliferation in cell-tracking dye studies.D. Rambaldi, **S. Pece**, P.P. Di Fiore. *Bioinformatics*, 15; 30 (14): 2060-5, 2014.
7. Trusted consent and research biobanks: towards a 'new alliance' between researchers and donors. G. Boniolo, P.P. Di Fiore, S. Pece. *Bioethics*, 26 (2): 93-100, 2012.
8. Reciprocal repression between P53 and TCTP. R. Amson*, **S. Pece*,** A. Lespagnol, R. Vyas, G. Mazzarol, D. Tosoni, I. Colaluca, G. Viale, S. Rodriguez-Ferreira, J. Wynendaele, O. Chaloin, J. Hoebeke, J.C. Marine, P.P. Di Fiore, A. Telerman. *Nat. Medicine*, 18 (1): 91- 9, 2012. **Equal contribution as 1st author*.
9. Biological and molecular heterogeneity of breast cancers correlates with their cancer stem cell content. **S. Pece*,** D. Tosoni, S. Confalonieri, G. Mazzarol, M. Vecchi, S. Ronzoni, L. Bernard, G. Viale, P.G. Pelicci, P.P. Di Fiore. *Cell*, 140 (1): 62-73, 2010. **Co- corresponding author*.
10. Alterations of the Notch pathway in lung cancer. B. Westhoff, I.N. Colaluca, G. D'Ario, M. Donzelli, D. Tosoni, S. Volorio, G. Pelosi, L. Spaggiari, G. Mazzarol, G. Viale, **S. Pece*,** P.P. Di Fiore. *PNAS*, 106 (52): 22293-8, 2009. **Co-corresponding author*.
11. Alterations of ubiquitin ligases in human cancer and their association with the natural history of the tumor. S. Confalonieri, M. Quarto, G. Goisis, P. Nuciforo, M. Donzelli, G. Jodice, G. Pelosi, G. Viale, **S. Pece**, P.P. Di Fiore. *Oncogene*, (33): 2959-6, 2009.
12. The prolyl-isomerase Pin1 is a Notch1 target that enhances Notch1 activation in cancer. Rustighi, L. Tiberi, A. Soldano, M. Napoli, P. Nuciforo, A. Rosato, F. Kaplan, A. Capobianco**, S. Pece**, P.P. Di Fiore, G. Del Sal. *Nat. Cell Biol*., 11 (2): 133-42, 2009.
13. Numb controls p53 tumour suppressor activity. I.N. Colaluca, D. Tosoni, P. Nuciforo, F. Senic-Matuglia V. Galimberti, G. Viale, **S. Pece*,** P.P. Di Fiore. *Nature*, 3 (7174):76-80, 2008. **Co-corresponding author*.

ABSTRACT

LOSS OF NUMB IN BREAST CARCINOGENESIS: A PARADIGM FOR A MECHANISM-BASED SELECTIVE ANTI-CANCER STEM CELL THERAPY

Salvatore Pece[1]

[1]Program of Molecular Medicine, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy. Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, 20142 Milano, Italy.

The cell fate determinant Numb is a tumor suppressor in the mammary gland and is frequently downregulated in human breast cancers, resulting in p53 inactivation and an aggressive disease course. In the mouse mammary gland, Numb/p53 downregulation leads to aberrant tissue morphogenesis, expansion of the stem cell compartment, and emergence of cancer stem cells. Numb-deficient cancer stem cells show unlimited self-renewal and proliferative potential, which is a function of their ability to execute unchecked self-renewing symmetric divisions. These phenotypes that can be reverted by Numb/p53 restoration in a Numb-knockout mouse model, arguing that targeting Numb/p53 dysfunction in Numb-deficient human breast cancer could represent a novel anti-cancer stem cell therapy. Using patient-derived xenografts, we have recently demonstrated that expansion of the cancer stem cell pool, due to altered self-renewing divisions, is also a distinguishing feature of naturally occurring Numb-deficient human breast cancers. In these cancers, using the inhibitor Nutlin-3 to restore p53, we corrected the defective self-renewal properties of Numb-deficient cancer stem cells and inhibited cancer stem cell expansion, with a marked effect on tumorigenicity and metastasis. Remarkably, a regimen combining Nutlin-3 and chemotherapy induced persistent tumor growth inhibition, or even regression, and prevented cancer stem cell-driven tumor relapse after removal of chemotherapy. We therefore provided a pre-clinical proof-of-concept that targeting Numb/p53 dysfunction results in a specific anti-cancer stem cell therapy in Numb-deficient human breast cancers. We will discuss how functional assays based on the biology of cancer stem cells should complement the currently used RECIST criteria for the evaluation of the efficacy of novel anti-cancer therapeutics, in the ultimate perspective of developing effective mechanism-based therapies to target cancer stem cells.

MATILDE ESTHER LLEONART PAJARÍN

matilde.lleonart@vhir.org

Laboratory Head "Biomedical Research in Cancer Stem Cells" Group, Vall de Hebron Institut de Recerca (VHIR)

Pg. Vall de Hebron 119-129, 08035 Barcelona, Spain

http://es.vhir.org/portal1/grup-equip.asp?t=investigacion-biomedica-con-celulas-madre-de-cancer&s=recerca&contentid=186741

Education

1998- *cum Laude* - Doctor in Biological Sciences

2000- Marie Curie Senior Reseacher Fellowship (Wolfson Institute for Biomedical Research, London, UK)

2003- Marie Curie Reincorporation Fellowship

2004- Laboratory Head at the VHIR

2010- Recognition and search accreditation by the Autonoma´s University of Barcerlona (UAB). Master lecturer

2014- Consolidated Research Group (Generalitat de Cataluña)

Representative Careers

1998-2000: Senior Scientist at the World Health Organization (International Agency for Research on Cancer), Lyon, France

2000-2003: Senior Scientist at the Wolfson Institute for Biomedical Research (WIBR), London, UK

2008: Senior Scientist at the Institute of Cell and Biological Sciences (ICMS), London, UK

2004 to date: Laboratory Head at the VHIR

Representative Awards

Two Marie Curie Fellowships (2000 and 2003)

2016- AECC (Spanish Association for Cancer Research) Award

Regular reviewer:

Carcinogenesis, Molecular and cellular biology, Nucleics acid research, Biological reviews, etc.

Interesting Research Areas

microRNAs, oncogenes, tumor suppressor genes, autophagy, oxidative stress

Selected Publications

1. Lorente J, Velandia C, Leal JA, Garcia-Mayea Y, Lyakhovich A, Kondoh H, **LLeonart ME**. The interplay between autophagy and tumorigenesis: exploiting autophagy as a means of anticancer therapy. Biol Rev Camb Philos Soc. 2017 May 2. doi: 10.1111/brv.12337
2. Esner M, Graifer D, **Lleonart ME**, Lyakhovich A. Targeting cancer cells through antibiotics-induced mitochondrial dysfunction requires autophagy inhibition. Cancer Lett. 2017 Jan 1;384:60-69. doi: 10.1016/j.canlet.2016.09.023.
3. Carnero A, Garcia-Mayea Y, Mir C, Lorente J, Rubio IT, **LLeonart ME**. The cancer stem-cell signaling network and resistance to therapy. Cancer Treat Rev. 2016 Sep;49:25-36. doi: 10.1016/j.ctrv.2016.07.001.
4. Artero-Castro A, Perez-Alea M, Feliciano A, Leal JA, Genestar M, Castellvi J, Peg V, Ramón Y Cajal S, **Lleonart ME**. Disruption of the ribosomal P complex leads to stress-induced autophagy. Autophagy. 2015;11(9):1499-519. doi: 10.1080/15548627.2015.1063764.
5. Feliciano A, Garcia-Mayea Y, Jubierre L, Mir C, Hummel M, Castellvi J, Hernández-Losa J, Paciucci R, Sansano I, Sun Y, Ramón y Cajal S, Kondon H, Soriano A, Segura M, Lyakhovich A, **LLeonart ME**. miR-99a reveals two novel oncogenic proteins E2F2 and EMR2 and represses stemness in lung cancer. (*In press* CDDis).

ABSTRACT

MIR-99A REVEALS TWO NOVEL ONCOGENIC PROTEINS E2F2 AND EMR2 AND REPRESSES STEMNESS IN LUNG CANCER

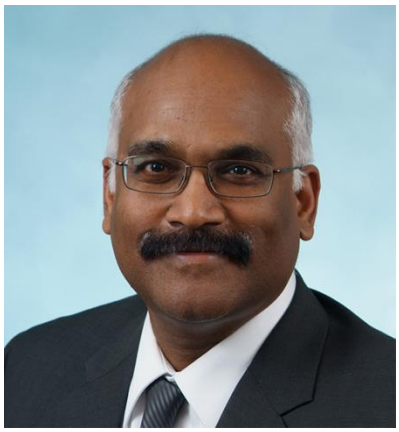
LLeonart ME, Garcia-Mayea, Mir C, Abad E, Sun Y, Masson F, Lorente J, Lyakhovich A

Vall de Hebron Institut de Recerca (VHIR)

Lung cancer is one of the most aggressive tumours with very low life expectancy. Altered microRNA expression is found in human tumours because it is involved in tumour growth, progression and metastasis. In this study, we analyzed microRNA expression in 47 lung cancer biopsies and one of the most de-regulated microRNA, miR-99a was extensively characterized. Among the most downregulated microRNAs we focused on the miR-99a characterization. In vitro experiments showed that miR-99a expression decreases the proliferation of H1650, H1975 and H1299 lung cancer cells causing cell cycle arrest and apoptosis. We identified two novel proteins, E2F transcription factor 2 (E2F2) and egf-like module containing, mucin-like, hormone receptor-like 2 (EMR2) downregulated by miR-99a by its direct binding to their 3´-UTR. Moreover, miR-99a expression prevented cancer cells epithelial to mesenchymal transition (EMT) and repressed the tumorigenic potential of the cancer stem cell (CSC) population both in these cell lines and mice tumours originated from H1975 cells. The expression of E2F2 and EMR2 at protein level was studied in 119 lung cancer biopsies. E2F2 and EMR2 are preferentially expressed in adenocarcinomas subtypes *versus* other tumour types (squamous and others). Interestingly, the expression of E2F2 correlates with the presence of vimentin and both E2F2 and EMR2 correlate with the presence of β-catenin. Moreover, miR-99a expression correlates inversely with E2F2 and directly with β-catenin expression in lung cancer biopsies. miR-99a reveals two novel targets, E2F2 and EMR2that play a key role in lung tumorigenesis. By inhibiting E2F2 and EMR2, miR-99a represses in vivo the transition of epithelial cells through an EMT process concomitantly with the inhibition of stemness features and consequently decreasing the CSC population.

REFERENCES

1)Lu J, Getz G, Miska EA, Alvarez-Saavedra, Lamb J, Peck D et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-838. 2)Nguyen DX, Chiang AC, Zhang XH, Kim JY, Kris MG, Ladanyi M et al. WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. Cell 2009; 138:51-62. 3)Yang Z, Han Y, Cheng K, Zhang G, Wang X. mir-99a directly targets the mTOR signaling pathway in breast cancer side population cells. 4)Wrighton KH. Cell migration: EMT promotes contact inhibition of locomotion. Nat Rev Mol Cell Biol 2015.



DANNY N DHANASEKARAN

danny-dhanasekaran@ouhsc.edu

Samuel Noble Foundation Endowed Chair in Cancer Biology & Professor of Cell Biology, Director, SCC-COBRE & Center for Basic Cancer Research, Deputy Director for Basic Research

Stephenson Cancer Center, University of Oklahoma Health Sciences Center, 975 NE 10th Street, Oklahoma City, OK 73104, USA

Education

1985-Ph.D in Biochemistry, Indian Institute of Science, Bangalore, India

Representative Careers

1985-1988 Research Associate, Dept. of Pharmacology, University of Wisconsin Medical School, Madison, WI

1988-1990 Senior Research Associate, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO, USA

1990-1992 Assistant Scientist, Dept. of Pharmacology, University of Wisconsin Medical School, Madison, WI, USA

1992-1998 Assistant Professor, Department of Biochemistry, Fels Institute for Cancer Research and Molecular Biology, Temple University, Philadelphia, PA, USA

1998 - 2008 Associate Professor, Department of Biochemistry, Fels Institute for Cancer Research and Molecular Biology, Temple University, Philadelphia, PA

2008-2009 Professor, Department of Biochemistry, Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA

2009-2012 WCU Visiting Professor, Seoul National University, Seoul, S. Korea

2009-present Director, Center for Basic Cancer Research; Deputy Director for Basic Research, Stephenson Cancer Center, Professor, Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104

Representative Awards

1998 Acres of Diamond award, Temple University , Philadelphia, PA, USA

1998-2010 Million Dollar Research Awards Club, Temple University, PA, USA

2010-2014 WCU Professor, Seoul National University, Seoul, Korea

2016-present Visiting Professor, Università del Piemonte Orientale, Via Solaroli, 17 - 28100 Novara, Italy

Editorial Board

Founding Editor-in-Chief: Journal of Molecular Signaling
Associate Editor: Genes & Cancer; Molecular carcinogenesis.

Interested Research Areas

Oncogenes, Cell Signaling, Biomarkers, lncRNAs, miRNAs, Tumor Micro-environment

Selected Publications

1. Ha JH, Ward JD, Radhakrishnan R, Jayaraman M, Song YS, **Dhanasekaran DN**. Lysophosphatidic acid stimulates epithelial to mesenchymal transition marker Slug/Snail2 in ovarian cancer cells via Gαi2, Src, and HIF1α signaling nexus. Oncotarget. 2016; 7: 72845-72859. PMID: 27166196
2. Kim S, Gwak H, Kim HS, Kim B**, Dhanasekaran DN**, Song YS. Malignant ascites enhances migratory and invasive properties of ovarian cancer cells with membrane bound IL-6R in vitro. Oncotarget. 2016; 7:83148-83159. PMID: 27825119
3. Jayaraman M, Radhakrishnan R, Mathews CA, Yan M, Husain S, Moxley KM, Song YS, **Dhanasekaran DN**. Identification of novel diagnostic and prognostic miRNA signatures in endometrial cancer. Genes Cancer. 2017; 8:566-576. PMID: 28740575
4. Kim B, Jung JW, Jung J, Han Y, Suh DH, Kim HS, **Dhanasekaran D**N, Song YS. PGC1α induced by reactive oxygen species contributes to chemoresistance of ovarian cancer cells. Oncotarget. 2017; 8:60299-60311. PMID: 28947972.
5. Ferraresi A, Titone R, Follo C, Castiglioni A, Chiorino G, **Dhanasekaran DN**, Isidoro C. The protein restriction mimetic Resveratrol is an autophagy inducer stronger than amino acid starvation in ovarian cancer cells. Mol Carcinog. 2017; doi: 10.1002/mc.22711. [Epub ahead of print] PMID: 28856729

ABSTRACT

LONG NON-CODING RNA CODES IN OVARIAN CANCER

[1]**Danny N. Dhanasekaran**, [1]Muralidharan Jayaraman, [1]Ji Hee Ha, [1]Rangasudhagar Radhakrishnan, [2]Ciro Isidoro

[1]Stephenson Cancer Center, University of Oklahoma Health Sciences Center Oklahoma City, OK 73104, USA; [2] Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100 - Novara (Italy).

Cancer is the second leading cause of death globally with nearly 1 in 6 deaths due to cancer. The mortality rate of cancer is more acutely felt in ovarian cancer, which has the lowest survival rate among all female cancers. Ovarian cancer is diagnosed annually in nearly 250,000 women globally, and is responsible for 140,000 deaths each year. Statistics show that only 45% of women with ovarian cancer survive for five years compared to up to 89% with breast cancer. This high mortality rate is in large part due to its diagnosis at late stage compounded by the lack of a targeted therapy. Focusing on identifying critical molecules involved in ovarian cancer genesis and progression, we have identified a family of long non-coding RNAs (lncRNAs) that are overexpressed in ovarian cancer. Using patient derived ovarian cancer cells, we made an expression network anlysis of lncRNAs and mRNAs. Our Results, using this approach, have identified a distinct set of lncRNA-signatures associated with ovarian cancer. These findings also unravel the presence of a central hub of lncRNAs, which is involved in the regulation of multiple, pro-tumorigenic, protein coding genes. Our results indicate that one such hub lncRNA, UCA1, is overexpressed in ovarian cancer and its expression is associated with poor prognosis, disease recurrence and therapy resistance. Consistent with these observations, silencing of UCA1 overcomes cisplatin- resistance in ovarian cancer cells. It can also be demonstrated that the intratumoral injection of UCA1-siRNA suppresses ovarian cancer xenograft growth in nude mice , thus establishing UCA1 as a therapeutic target in ovarian cancer. In addition to establishing UCA1 as a potential therapeutic target in ovarian cancer, our studies point to atypical signaling codes through which dysregulated lncRNAs can promote tumorigenesis and tumor progression.

Short communication

AURELIA SPINA

aurelia.spina@studenti.unipd.it

Fellow

University of Padua, Department of Biomedical Sciences, Italy

Education:

2017: Laurea Summa cum Laude- Master Degree in Sanitary Biology, University of Padua, Italy

2015: Laurea Summa cum Laude- Bachelor Degree in Molecular Biology, University of Padua, Italy

Interesting Research Areas:

Stem cells application, cancer therapy, molecular mechanisms of cancerogenesis, intercellular communication strategies and mitochondria.

ABSTRACT

EXTRACELLULAR VESICLES FROM HUMAN ADIPOSE DERIVED STEM CELLS AS A POSSIBLE NOVEL ANTICANCER STRATEGY

Aurelia Spina[1], Chiara Gardin[1], Letizia Ferroni[1], Paolo Pinton[2] and Barbara Zavan[1]

[1]Department of Biomedical Sciences, University of Padua, Italy; [2]Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Italy

INTRODUCTION Colorectal cancer is the third most common malignant disease in the world (1361000 cases/year). Despite a significant improvement in the outcome of these patients in the last decades, the prognosis remains dismal especially for those with metastatic disease. Therefore, the discovery of new therapeutic approaches is an important unmet medical need. Basing on this consideration, we designed an experimental project aiming to investigate whether Extracellular Vesicles (EVs) isolated from Human Adipose Derived Stem Cells (hADSCs) could alter colorectal cancer cells' (HCT-116) physiology in vitro, acting as antitumor modulators.

EXPERIMENTAL MODEL hADSCs were cultured in complete DMEM/F12 with 10% exosome-free FBS and 1% P/S (cDMEM@). EVs were harvested from hADSCs conditioned medium (CM) by ultracentrifugation. Different hADSCs secreted components were tested on HCT-116: CM, soluble molecules and EVs (for both cells in cDMEM@ and cDMEM). We performed: MTT test, Scratch test, PDT test on HCT-116 after 24, 48, 72 hours of different cultural conditions; RT-PCR analysis after 72 hours; mitochondrial [Ca2+] measurement after 90' and 180' of hADSCs-EVs incubation. RESULTS We showed that hADSCs secreted components reduce HCT-116 vitality, migration and proliferation with time depending effects and impair mRNA levels of genes involved in Epithelial to Mesenchymal Transition, immune responses, growth and metabolism (mTOR). In particular, EVs induce a reduction in HCT-116 viability, migration and proliferation clearly detectable after 72 hours of treatments. No evident [Ca2+]mit changes were observed after 90' or 180' on HCT-116 incubated with EVs, suggesting that EVs may exert a tardive anticancer action on HCT-116. We also observed that EVs effects on HCT-116 behaviour may vary with cells cultural condition (cDMEM@ or cDMEM).

CONCLUSION Our promising preliminary in vitro results open to the opportunity to develop new therapeutic anticancer strategies based on hADSCs-EVs.

REFERENCES

1. Arvelo F., Sojo F., Cotte C. Biology of colorectal cancer. Ecancermedicalscience. 2015 Apr 9;9:520. doi: 10.3332/ecancer.2015.520.
2. György B., Hung M.E., Breakefield X.O., Leonard J.N. Therapeutic Applications of Extracellular Vesicles: Clinical Promise and Open Questions. Annu Rev Pharmacol Toxicol. 2015;55:439-464. doi: 10.1146/annurev-pharmtox-010814-124630. 3. Yu B., Zhang X., Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci. 2014 Mar 7;15(3):4142-57. doi: 10.3390/ijms15034142.

Short communication

PAOLO E. PORPORATO

paolo.porporato@unito.it

Assistant Professor of Experimental Biology

University of Torino

Molecular Biotechnology Center – MBC. Dept. of Molecular Biotechnology and Health Sciences

Education:

2009: PhD in Molecular Medicine in the laboratory of Prof. A.Graziani at the Dept. of Medical Sciences of University of Piemonte Orientale, Novara, Italy.

2004: Master Degree in Medical Biotechnologies at the University of Torino, Italy, 110/110 cum laude.

Representative Careers:

2016-: as Assistant Professor in Experimental Biology at the Molecular Biotechnology Center, Research group "Cancer and Cachexia Metabolism", University of Torino, Italy.

2016- 2013: Research Associate (chargé de recherche) at the Unit of Pharmacology & Therapeutics, Research group 'Cancer and Metabolism, University of Louvain (UCL) Medical School, Brussels, Belgium.

2010- 2013 as post-doctoral fellow at the Unit of Pharmacology & Therapeutics, Research group 'Cancer and Metabolism, University of Louvain (UCL) Medical School, Brussels, Belgium.

Representative Awards:

Board member and treasurer (2016) of the International Society of Cancer Metabolism (ISCAM)

Fondo Rita Levi-Montalcini (2016) "Understanding and targeting cancer-related muscle atrophy "

Prix d'Alvarenga, de Piauhy (2015) "Role of mitochondrial superoxide targeting on tumor signaling and metastasis prevention".

Fond Joseph Maisin (2014) « Characterization of the contribution of monocarboxylate transporter 1 (MCT1) to tumor metastasis and of its therapeutic inhibition for metastasis prevention".

Interesting Research Areas:

Cancer metabolism, mitochondrial ROS, wound healing skeletal muscle atrophy and cancer cachexia

Selected Publications:

1. Payen VL, Hsu MY, Rädecke KS, Wyart E, Vazeille T, Bouzin C, **Porporato PE***, Sonveaux P*. Monocarboxylate transporter MCT1 promotes tumor metastasis independently of its activity as a lactate transporter. Cancer Res. 2017 Aug 21. * co-last
2. **Porporato PE**, Payen VL, Pérez-Escuredo J, De Saedeleer CJ, Danhier P, Copetti T, Dhup S, Tardy M, Vazeille T, Bouzin C, Feron O, Michiels C, Gallez B, Sonveaux P. A mitochondrial switch promotes tumor metastasis. Cell Reports. 2014 Aug 7;8(3):754-66.
3. De Saedeleer CJ, **Porporato PE**, Copetti T, Pérez-Escuredo J, Payen VL, Brisson L, Feron O, Sonveaux P. Glucose deprivation increases monocarboxylate transporter 1 (MCT1) expression and MCT1-dependent tumor cell migration. Oncogene. 2014 Jul31;33(31):4060-8
4. **Porporato PE**, Filigheddu N, Reano S, Ferrara M, Angelino E, Gnocchi V, Prodam F, Ronchi G, Fagoone S, Fornaro M, Chianale F, Chianale F, Baldanzi G, Sinigaglia F, Surico N, Perroteau I, Smith R, Sun Y, Geuna S, Graziani A. Acylated and UnAcylated Ghrelin impair skeletal muscle atrophy in Mice. J Clin Invest. 2013 Jan 2
5. **Porporato PE**, Payen VL, De Saedeleer CJ, Préat V, Thissen JP, Feron O, Sonveaux P. Lactate stimulates angiogenesis and accelerates the healing of superficial and ischemic wounds in mice. Angiogenesis. 2012 Jun 3.

ABSTRACT

TARGETING MITOCHONDRIAL ROS GENERATION PREVENTS CHEMOTHERAPY-INDUCED TUMOR CELL AGGRESSIVENESS

Paolo E. Porporato [1,2], P. Sonveaux [1]

[1]Pole of Pharmacology, Inst. of Experimental and Clinical Research (IREC), Univ. of Louvain (UCL) Medical School, Brussels, Belgium [2] Dept of Molecular Biotechnology and Health Sciences, Univ. of Turin, Turin, Italy

INTRODUCTION Low levels of mitochondrial ROS (mtROS) promote cancer metastasis in breast and melanoma by inducing Src and Pyk2. Comparatively, high levels of mtROS are a common pro-apoptotic signal. Because several antitumor drugs, including anthracyclines, can trigger mtROS production, we decided to evaluate the effects of antimetastatic drugs targeting mtROS on the doxorubicin-mediated cell death of breast cancer cells.

EXPERIMENTAL MODEL Breast cancer cell lines were treated with different combinations of mtROS scavenger and doxorubicin, and cell death, proliferation, and clonogenicity were assessed by various methods.

RESULTS No protective effects of mtROS scavengers on doxorubicin-induced cell death were detectable, suggesting that there is no contraindication to combine antimetastatic mtROS scavengers with a normal doxorubicin chemotherapy regimen. Interestingly, we further found that, compared to untreated cells, breast cancer cells treated with doxorubicin consumed more oxygen, resulting in increased ROS generation. This metabolic reprogramming promoted Pyk2 activation and SNAIL expression, along with morphological changes typical of an epithelial-to-mesenchymal transition, increased migration, and invasion.

CONCLUSIONS: mtROS scavengers prevent the pro-metastatic activity of chemoterapy and reduced metastatic colonization of the lung. Altogether, this study provides a strong rationale for combining anthracycline-based chemotherapy with drugs lowering mtROS production in breast cancer.

Third Session

16.00 – 17.45 P.M.

Chairpersons:

Lizzia Raffaghello, Gianluca Gaidano

GLORIA SU, PHD

gs2157@columbia.edu

Professor

Columbia University Medical Center

Education

Ph.D. Committee on Immunology, University of Chicago, Chicago, IL, USA

B.A. Dept. of Biological Sciences, Northwestern University, Evanston, IL, USA

Representative Careers

2017-present: Professor, Columbia University, Dept. of Pathology & Cell Biology

2012-2017: Associate Professor, Columbia University, Dept. of Pathology & Cell Biology

2003-2012: Assistant Professor, Columbia University, Dept. of OTO/HNS

2000-2003: Assistant Professor, The Johns Hopkins Medical Institutes, Dept. of Pathology

Representative Awards

2014: Ruth Leff Siegel Award for Excellence in Pancreatic Cancer Research

2015:Distinguished Achievement Award from Shanghai Tongji University East Hospital fo exceptional contribution to pancreatic cancer research and clinical management.

Interesting Research Areas

-Novel mouse models for pancreatic diseases
-Potential role of wild-type KRAS in pancreatic tumorigenesis
-The PTEN-PI3K axis in IPMN/PDA and head and neck cancer
-Activin signaling in tumorigenesis

Selected Publications

1. W. Qiu, F. Schönleben, X. Li, D. J. Ho, L. G. Close, S. Manolidis, B. P. Bennett, **G. H. Su**. *PIK3CA* mutations in head and neck squamous cell carcinoma. *Clinical Cancer Research* 2006, 12:1441-6. PMID: 16533766
2. W. Qiu, F. Sahin, C.A. Iacobuzio-Donahue, Dario Garcia-Carracedo, W. M. Wang, Chia-Yu Kuo, Dan E. Arking, A. M. Lowy, R. H. Hruban, H. E. Remotti, **G. H. Su**. Disruption of *p16* and Activation of *Kras* in Pancreas Increases Ductal Adenocarcinoma Formation and Metastasis in vivo. *Oncotarget 2011,* 2:862-873. PMID:22113502, PMCID: PMC3259996.

3. D. Garcia-Carracedo, A. T. Turk, S. A. Fine, N. Akhavan, B. C. Tweel, R. Parsons, J. A. Chabot, J. D. Allendorf, J. M. Genkinger, H. E. Remotti, **G. H. Su**. Loss of PTEN expression is associated with poor prognosis in patients with intraductal papillary mucinous neoplasms of the pancreas. *Clin Cancer Research* 2013; 19(24):6830-6841. PMID: 24132918, PMCID: PMC3915026.
4. D. Garcia-Carracedo, C. C. Yu, N. Akhavan, S. A. Fine, F. Schönleben, N. Maehara, D. C. Karg, W. Qiu, R. L. Fine, H. E. Remotti, **G. H. Su**. Smad4 loss synergizes with TGFalpha overexpression in promoting metaplasia, PanIN development, and fibrosis. *PLoS One* 2015, 10(3):e0120851. PMID: 25803032, PMCID: PMC4372593
5. W. Qiu, S. M. Tang, S. Lee, A. T. Turk, A. N. Sireci, A. Qiu, C. Rose, C. Xie, J. Kitajewski, H.-J. Wen, H. C. Crawford, P. A. Sims, R. H. Hruban, H. E. Remotti, **G. H. Su**. Loss of Activin Receptor Type 1B Promotes Development of Intraductal Papillary Mucinous Neoplasms in Mice with Activated KRAS. *Gastroenterology* 2016, 150(1):218-228. PMID: 26408346, PMCID: PMC4860725.

ABSTRACT

THE IMPACT OF TUMOR-SUPPRESSOR GENES ON KRAS-INDUCED PANCREATIC TUMORIGENESIS

Su, Gloria H.

Columbia University Medical Center

Genetically engineered animal models (GEMMs) have established robust platforms for exploring the molecular mechanisms underlying the progression of pancreatic precancerous lesions to invasive PDA (pancreatic ductal adenocarcinoma). For example, using Pdx1-Cre to activate mutant $Kras^{G12D}$ allele in the pancreatic progenitor cells induces full spectrum of premalignant PanIN (pancreatic intraepithelial neoplasias) lesions that can eventually progress to invasive PDA. Others and we have reported that concomitant inactivation of the *tumor suppressors p16, p19*, *p53, or* TGF-β receptor type 2 (*TgfβR2)* can synergize with oncogenic $Kras^{G12D}$ *in promoting* the progression of the non-invasive PanINs to invasive cancer *in vivo*. In contrast, the inactivation of *Smad4* or *Acvr1b* in the context of mutant $Kras^{G12D}$ preferentially promotes the development of pancreatic IPMNs (intraductal papillary mucinous neoplasms) but not PanINs.

These GEMMs are powerful tools for interrogating the processes of pancreatic tumorigenesis and metastasis, and platforms for biomarker and drug discoveries.

REFERENCES

1. W. Qiu, F. Sahin, C.A. Iacobuzio-Donahue, Dario Garcia-Carracedo, W. M. Wang, Chia-Yu Kuo, Dan E. Arking, A. M. Lowy, R. H. Hruban, H. E. Remotti, G. H. Su. Disruption of *p16* and Activation of *Kras* in Pancreas Increases Ductal Adenocarcinoma Formation and Metastasis in vivo. *Oncotarget 2011,* 2:862-873. PMID:22113502, PMCID: PMC3259996.
2. W. Qiu, S. M. Tang, S. Lee, A. T. Turk, A. N. Sireci, A. Qiu, C. Rose, C. Xie, J. Kitajewski, H.-J. Wen, H. C. Crawford, P. A. Sims, R. H. Hruban, H. E. Remotti, G. H. Su. Loss of Activin Receptor Type 1B Promotes Development of Intraductal Papillary Mucinous Neoplasms in Mice with Activated KRAS. *Gastroenterology* 2016, 150(1):218-228. PMID: 26408346, PMCID: PMC4860725.

CARLO FOLLO

Carlo.Follo@ucsf.edu

Associate Specialist

Zuckerberg San Francisco General Hospital, University of California San Francisco, San Francisco, CA, USA.

Education:

2003 – M.S. in Industrial Biotechnology, University of Milano-Bicocca, Milano (Italy)

2009 – Ph.D. in Biotechnologies for Human Health, University of Piemonte Orientale 'Amedeo Avogadro', Novara (Italy)

Representative Careers:

2004-2009: Graduate researcher, Dipartimento di Scienze della Salute, University of Piemonte Orientale 'Amedeo Avogadro', Novara (Italy)

2009-2010: Postdoctoral Researcher, Dipartimento di Scienze della Salute, University of Piemonte Orientale 'Amedeo Avogadro', Novara (Italy)

2010-2011: Postdoctoral Investigator, Department of Pathology, Albert Einstein College of Medicine of Yeshiva University, New York (USA)

2011-2013: Postdoctoral Research Assistant, Dipartimento di Scienze della Salute, University of Piemonte Orientale 'Amedeo Avogadro', Novara (Italy)

2014-present: Associate Specialist, Department of Medicine, Pulmonary, Zuckerberg San Francisco General Hospital, University of California, San Francisco, California (USA)

Representative Awards:

2014 - Inaugural Awardee - Simmons Fellow in Mesothelioma Research, The Simmons Mesothelioma Foundation

Interesting Research Areas:

Autophagy and chemoresistance in three-dimensional tumor spheroids models. Role and regulation of autophagy in cancer. Autophagy-apoptosis crosstalk. Endosomal/lysosomal proteases.

Selected Publications:

1. **Follo C**, Barbone D, Richards WG, Bueno R, Broaddus VC. Autophagy initiation correlates with the autophagic flux in 3D models of mesothelioma and with patient outcome. Autophagy. 2016 Jul 2;12(7):1180-94. doi: 10.1080/15548627.2016.1173799. Epub 2016 Apr 20. PubMed PMID: 27097020.

2. Barbone D, Van Dam L, **Follo C**, Jithesh PV, Zhang SD, Richards WG, Bueno R, Fennell DA, Broaddus VC. Analysis of Gene Expression in 3D Spheroids Highlights a Survival Role for ASS1 in Mesothelioma. PLoS One. 2016 Mar 16;11(3):e0150044. doi: 10.1371/journal.pone.0150044. eCollection 2016. PubMed PMID: 26982031; PubMed Central PMCID: PMC4794185.
3. Klionsky DJ, et al., Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016;12(1):1-222. doi: 10.1080/15548627.2015.1100356. PubMed PMID: 26799652; PubMed Central PMCID: PMC4835977.
4. Barbone D, **Follo C**, Echeverry N, Gerbaudo VH, Klabatsa A, Bueno R, Felley-Bosco E, Broaddus VC. Autophagy Correlates with the Therapeutic Responsiveness of Malignant Pleural Mesothelioma in 3D Models. PLoS One. 2015 Aug 18;10(8):e0134825. doi: 10.1371/journal.pone.0134825. eCollection 2015. PubMed PMID: 26284517; PubMed Central PMCID: PMC4540424.
5. **Follo C**, Castino R, Nicotra G, Trincheri NF, Isidoro C. Folding, activity and targeting of mutated human cathepsin D that cannot be processed into the double-chain form. Int J Biochem Cell Biol. 2007;39(3):638-49. Epub 2006 Nov 25. PubMed PMID: 17188016.

ABSTRACT

INHIBITION OF AUTOPHAGY INITIATION POTENTIATES CHEMOSENSITIVITY IN MESOTHELIOMA

Carlo Follo[1*], Yao Cheng[1], William G. Richards[2], Raphael Bueno[2], V. Courtney Broaddus[1]

[1]Zuckerberg San Francisco General Hospital and Trauma Center, University of California San Francisco, San Francisco, CA, 94110, USA. [2]Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, 02115, USA.

The role of autophagy (ATG) and the benefits of its inhibition in cancer still remain unclear, with different roles and outcomes depending on the tumor, context, and stage of inhibition. Limitations include a poor availability of models and static markers reflecting ATG of the actual tumor, and of studies comparing ATG inhibition at different stages. In mesothelioma, we found that autophagic flux correlates with ATG initiation, measured as ATG13 puncta, in 3D models, but not in 2D cultures. *Ex vivo* 3D models maintain the ATG initiation of the original tumor from which they are generated. We concluded that ATG is better represented in 3D than in 2D and that ATG13 is a static marker of ATG.[1] Here, we investigated the impact of inhibiting ATG at different stages on chemosensitivity. ATG was studied in mesothelioma cell lines grown as 3D multicellular spheroids, and in 3D *ex vivo* tumor fragment spheroids generated from mesothelioma samples. ATG was measured as autophagic flux by detection of accumulated LC3 after lysosomal inhibition and as ATG initiation by detection of ATG13 puncta. ATG was successfully inhibited in 3D models using either an early stage inhibitor that acts at the initiation stage (MRT 68921), or a late stage inhibitor (hydroxychloroquine). Inhibition of ATG at the early stage, but not at late stage, potentiated chemosensitivity. High ATG initiation identified tumors that are more chemosensitive at baseline and after ATG inhibition. Our results highlight a potential role of ATG initiation in supporting mesothelioma cells during chemotherapy. Our work also shows the importance of testing the inhibition of different stages in order to uncover the role of ATG in cancer.

REFERENCES

1. Follo C, Barbone D, Richards WG, Bueno R, Broaddus VC. Autophagy initiation correlates with the autophagic flux in 3D models of mesothelioma and with patient outcome. Autophagy. 2016 Jul 2;12(7):1180-94.

DAVIDE ROSSI

Senior Consultant, Hematology, IOSI - Oncology Institute of Southern Switzerland, Bellinzona, Switzerland
Head, Laboratory of Experimental Hematology, IOR - Institute of Oncology Research, Bellinzona, Switzerland

Hematology, IOSI - Oncology Institute of Southern Switzerland, Bellinzona, Switzerland, Laboratory of Experimental Hematology, IOR - Institute of Oncology Research, Bellinzona, Switzerland

Education

27-10-2000: M.D., School of Medicine, University of Eastern Piedmont, Novara, Italy
07-11-2005: Residency in Internal Medicine, School of Medicine, University of Eastern Piedmont, Novara, Italy
14-12-2009: Ph.D. in Clinical and Experimental Medicine, University of Eastern Piedmont, Novara, Italy (Ph.D. Supervisor: Prof. Gianluca Gaidano)

Representative Careers

- **PREVIOUS POSITIONS**

2005–2008: Staff Clinician, Division of Hematology, Department of Oncology, Maggiore della Carità Hospital, Novara, Italy
2008–2015: Assistant Professor of Hematology, Department of Translational Medicine, University of Eastern Piedmont, Novara, Italy
2008–2015: Consultant, Division of Hematology, Department of Oncology, Maggiore della Carità Hospital, Novara, Italy
2014–2015: Head, Clinical Program in Lymphoproliferative Disorders, Division of Hematology, Department of Oncology, Maggiore della Carità Hospital, Novara, Italy

- TEACHING ACTIVITIES

2008–2016: Assistant Professor of Hematology, Biothecnology School, University of Eastern Piedmont, Novara, Italy
2014–2016: Assistant Professor of Hematology, Medical School, University of Eastern Piedmont, Novara, Italy

- ORGANISATION OF SCIENTIFIC MEETINGS

2017 Member of the International Program Committee, XVII International Workshop on Chronic Lymphocytic Leukaemia (iwCLL), New York, NY
2017 Member of the Organizing Committee, 14 - International Conference on Malignant Lymphoma (ICML), Lugano, Switzerland

- INSTITUTIONAL RESPONSIBILITIES

2008–2015: Faculty member, School of Medicine, University of Eastern Piedmont, Novara, Italy
2013–2015: President, University Library Committee, University of Eastern Piedmont, Novara, Italy
2011–2013: Ph.D. Student Advisor, Ph.D. program in Clinical and Experimental Medicine, University of Eastern Piedmont, Novara, Italy
2013: Ph.D. Student Advisor, Ph.D. program in Sciences and Medical Biotechnology, University of Eastern Piedmont, Novara, Italy.

2013: External Ph.D. Student Advisor, Ph.D. program in Biomedical Sciences and Biotechnology, University of Udine, Udine, Italy
2016: Organizer of the Internal Seminars, Hematology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland
2016: Chair of the Hematology Tumor Board, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland
2016: Chair of the Laboratory of Molecular Diagnostics in Hematology, Ente Ospedaliero Cantonale, Bellinzona, Switzerland

Representative Awards

2009: Italian Society of Hematology young investigator prize
2011: Accademia Nazionale dei Lincei - "Silvia Fiocco" young investigator prize for the research in the field of lymphomas and leukemias
2011: Federico Calabresi Foundation prize for the best hematologic paper in 2011
2012: "Under 40 Hematology" young investigator prize for the research in the field of hematology

Interesting Research Areas

The research topic of the applicant is the molecular pathogenesis and diagnosis of B-cell tumors and translation of biological information into markers for disease diagnosis and prognostication. As documented by the list of publications, original and ground-breaking contributions of the proponent include: i) identification of *NOTCH1*, *SF3B1* and *BIRC3* mutations in chronic lymphocytic leukemia (CLL) and characterization of their clinical role; ii) definition of the molecular bases of high risk CLL, including refractory and transformed disease; iii) definition of the genetic profile of very low risk CLL patients, including highly stable/non-progressing patients and patients who gain durable remission after chemoimmunotherapy; iv) first description of the clinical relevance of small *TP53* mutated subclones in CLL; v) seminal characterization of the splenic and nodal marginal zone lymphoma genome, including the identification of *NOTCH2*, *PTPRD* and non-canonical NF-κB gene mutations; and vi) development of circulating tumor DNA as a tool to inform on tumor genetics in lymphomas.

Selected Publications:

1. **Rossi D**, Bruscaggin A, et al. Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness. *Blood*, 118:6904-6908, 2011.
2. **Rossi D**, Fangazio M, Rasi S, et al. Disruption of BIRC3 associates with fludarabine chemorefractoriness in TP53 wild-type chronic lymphocytic leukemia. *Blood*, 119:2854-2862, 2012.
3. **Rossi D**, Rasi S, Fabbri G et al. Mutations of NOTCH1 are an independent predictor of survival in chronic lymphocytic leukemia. *Blood*, 119:521-529, 2012.
4. **Rossi D**, Trifonov V, Fangazio M, et al. The coding genome of splenic marginal zone lymphoma: activation of NOTCH2 and other pathways regulating marginal zone development. *J Exp Med*, 209:1537-1551, 2012.
5. Spina V, Khiabanian H, Messina M, et al. The genetics of nodal marginal zone lymphoma. *Blood*, 128:1362-73, 2016.

ABSTRACT

GENOTYPING OF CLASSICAL HODGKIN LYMPHOMA ON THE LIQUID BIOPSY

Spina V[1], Bruscaggin A[1], Cuccaro A[2], Martini M[3], Di Trani M[4], Forestieri G[1], Manzoni M[5], Condoluci A[1,6], Arribas A[1], Locatelli S[4], Cupelli E[2], Ceriani L[6], Moccia A[6], Stathis A[6], Deambrogi C[7], Diop F[7], Guidetti F[1], Neri N[5,8], Gerber B[6], Bertoni F[1,6], Ghielmini M[6], Stüssi G[6], Santoro A[4], Cavalli F[6], Zucca E[6], Larocca LM[3], Gaidano G[7], Hohaus S[2], Carlo-Stella C[4], **Rossi D[1,6]**

[1]Institute of Oncology Research, Bellinzona, Switzerland; [2]Institute of Hematology, Catholic University of the Sacred Heart, Rome, Italy; [3]Division of Pathology and Histology, Catholic University of the Sacred Heart, Rome, Italy; [4]Department of Oncology and Haematology, Humanitas Cancer Center, Humanitas Clinical and Research Center, Milan, Italy; [5]Department of Oncology and Hemato-oncology, University of Milano, Milan Italy; [6]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [7]Division of Hematology, Department of Translational Medicine, University of Eastern Piedmont, Novara, Italy; [8]Hematology Unit, Foundation Ca' Granda IRCCS, Ospedale Maggiore Policlinico, Milan, Italy

In classical Hodgkin lymphoma (cHL) the low representation (~5%) of Hodgkin-Reed-Sternberg cells (HRS) challenged tumor genotyping on the tissue biopsy. Cell free DNA (cfDNA) is shed into the blood by tumor cells and can be used as source of tumor DNA for the identification of somatic mutations, track clonal evolution of tumors and detect minimal residual disease during therapy. AIMS. The study aimed at: i) showing that cfDNA mirrors the genetics of HRS cells in cHL patients; ii) characterizing the mutational profile of a large cohort of newly diagnosed and chemorefractory cHL; iii) identifying molecular prognostic subtypes; iv) early detecting residual disease during therapy; and v) longitudinally tracking tumor clonal evolution under different treatment modalities. METHODS. The study included 80 newly diagnosed cHL and 32 chemorefractory cHL. The following biological material was analyzed: i) cfDNA from plasma collected at diagnosis, during ABVD courses, at refractory progression, before and during therapy with brentuximab or nivolumab; and ii) normal germline genomic DNA (gDNA) from granulocytes. For comparative purposes, paired tumor gDNA from microdissected HRS cells of 13 cases was also analyzed. A targeted resequencing panel optimized to include the coding exons and splice sites of 77 genes recurrently mutated in B-cell lymphomas was used for genotyping. Ultra-deep next-generation sequencing (NGS) of the gene panel was performed on NexSeq 500 (Illumina) using the CAPP-seq library preparation strategy (NimbleGen). RESULTS. In cHL patients, cfDNA surrogated gDNA from HRS cells, since it harbored 87.5% of the tumor confirmed mutations. Genes recurrently affected by non-synonymous somatic mutations in >20% of cHL included *STAT6* (37.5%), *TNFAIP3* (35%), and *ITPKB* (27.5%). Mutations clustered in major pathways, including NF-κB, PI3K-AKT, cytokine and NOTCH signaling, and immune evasion. *ITPKB* mutations: i) were quite specific for cHL, being rare or absent in other lymphomas; ii) caused the subcellular delocalization of the protein in primary HRS cells of mutated patients; iii) correlated with clues of PI3K-AKT signaling activation both at gene expression and protein levels; and iv) consistent with the positioning of ITPKB downstream PI3K in the pathway, associate with resistance to PI3K inhibitors. Mutations of *CD58* and *TNFRSF14*, encoding co-stimulatory molecules for T-cells, associated with short PFS independent of interim PET/CT results, pointing to immune escape genetic lesions as biomarkers of aggressive disease. Newly diagnosed and chemorafractory cHL shared a largely overlapping mutational landscape. *TP53* mutations were not enriched in refractory cHL as instead commonly found in other types of refractory B-cell tumors.

Conversely, more *TET2* mutations were documented in refractory cHL, including newly acquired ones, thus signaling towards aberrant DNA methylation programming as a mechanism of resistance in cHL with potential therapeutic implications. By longitudinal analysis, in patients relapsing under/after chemotherapy or brentuximab vedotin, pre-treatment/relapse tumor pairs branched through the acquisition of phase specific mutations from an ancestral clone, that always persisted. Conversely, in patients maintained under nivolumab, clones were cyclically suppressed and replaced by completely novel clones. We utilized the change in circulating tumor cfDNA load from baseline to interim timepoint to predict the best response to ABVD and to complement interim PET/CT in anticipating cure. A drop of 100-fold or 2-log drop in tumor cfDNA after 2 ABVD courses associated with an eventual complete response and cure. All cured patients that were inconsistently judged as interim PET/CT positive turned out to have a >2 log drop in tumor cfDNA. A drop of less than 2-log in tumor cfDNA after 2 ABVD courses associated with an eventual progression. All relapsed patients that were inconsistently judged as interim PET/CT negative turned out to have a <2 log drop in tumor cfDNA. Circulating tumor cfDNA allows to noninvasively detect tumor-specific mutations; ii) identify prognostic subtypes; iii) early detect residual disease during therapy; and iv) longitudinally track tumor clonal evolution under different treatment modalities.

Short communication

FARY DIOP

fary.diop@med.uniupo.it

Ph.D student in Science and Medical Biotechnologies – Laboratory of Hematology

Università del Piemonte Orientale, Department of Translational Medicine, Laboratory of Hematology; Via Paolo Solaroli 17, 28100, Novara (Italy)

Education:

2015: Master Degree in Medical Biotechnology (MSc), Amedeo Avogadro University of Eastern Piedmont, Novara, Italy. Thesis title: *"Genotipization on liquid biopsy of diffuse large B cell lymphoma"*

2013: Bachelor Degree in Medical Biotechnology, Amedeo Avogadro University of Eastern Piedmont, Alessandria, Italy. Thesis title: *"Monitoring of patients treated with oral anticoagulants in a chemical-clinical analysis base laboratory"*

Representative Career:

2015-ongoing: PhD student in Onco-Hematology, Laboratory of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont

Representative Awards:

- 2016: Winner of third prize of UNDER40 in hematology 2016, held in Villanfranca di Verona (VN), 17th-18th November 2016.
- 2016: Winner of Travel grant for XIV congresso nazionale SIES (Società Italiana di Ematologia Sperimentale), 19th -21st October 2016, in Rimini.
- 2016: Winner of silver award special prize at "Basic to Translational Medicine 2016: Focus on Cancer" congress, held in Novara, 6th -7th October 2016.

Interesting Research Areas:

Next generation Sequencing applied in hematological diseases. Molecular Biology and diagnosis of lymphoproliferative diseases. Detection of mutations in genomic DNA, RNA and circulating plasma Cell-free DNA (cfDNA).

Selected Publications:

1. **Fary Diop*,** Davide Rossi*, Elisa Spaccarotella, Sara Monti, Manuela Zanni, Silvia Rasi, Clara Deambrogi, Valeria Spina, Alessio Bruscaggin, Chiara Favini, Roberto Serra, Antonio Ramponi, Renzo Boldorini, Robin Foá, and Gianluca Gaidano. Diffuse Large B-cell lymphoma Genotyping on the Liquid Biopsy. Blood, 2017. *Equally contributed.

2. Valeria Spina, Alessio Bruscaggin, Annarosa Cuccaro, Maurizio Martini, Martina Di Trani, Gabriela Forestieri, Martina Manzoni, Adalgisa Condoluci, Alberto Arribas, Silvia Locatelli, Elisa Cupelli, Luca Ceriani, Alden Moccia, Anastasios Stathis, Clara Deambrogi, **Fary Diop**, Francesca Guidetti, Salvatore Annunziata, Vittoria Ruffini, Alessandro Giordano, Antonino Neri, Bernhard Gerber, Francesco Bertoni, Michele Ghielmini, Georg Stüssi, Armando Santoro, Franco Cavalli, Emanuele Zucca, Luigi Maria Larocca, Gianluca Gaidano, Stefan Hohaus, Carmelo Carlo-Stella, Davide Rossi. Genotyping of classical hodgkin lymphoma on the liquid biopsy. SUBMITTED

3. Sara Raponi, Ilaria Del Giudice, Caterina Ilari, Luciana Cafforio, Alfonso Piciocchi, Marilisa Marinelli, Monica Messina, Sabina Chiaretti, Silvia Bonina, Francesca R. Mauro, Gian Matteo Rigolin, Francesca Rossi, Riccardo Bomben, Michele Dal Bo, **Fary Diop**, Davide Rossi, Gianluca Gaidano, Antonio Cuneo, Valter Gattei, Anna Guarini and Robin Foá. Biallelic BIRC3 inactivation significantly reduces time-to-first treatment in chronic lymphocytic leukemia patients with 11q deletion, independently of ATM mutation. SUBMITTED

4. Francesca Arruga, Branimir Gizdic, Cinzia Bologna, Simona Cignetto, Roberta Buonincontri, Sara Serra, Tiziana Vaisitti, Katiuscia Gizzi, Nicoletta Vitale, Giulia Garaffo, Elisabetta Mereu, **Fary Diop**, Francesco Neri, Marta Coscia, John Allan, Roberto Piva, Salvatore Oliviero, Richard R. Furman, Davide Rossi, Gianluca Gaidano, Silvia Deaglio. Mutations in NOTCH1 PEST domain orchestrate CCL19-driven homing of leukemic cells by modulating the tumor suppressor gene DUSP22. Leukemia, 2016.

5. Valeria Spina, Hossein Khiabanian, Monica Messina, Sara Monti, Luciano Cascione, Alessio Bruscaggin, Elisa Spaccarotella, Antony B Holmes, Luca Arcaini, Marco Lucioni, Fabrizio Tabbò, Sakellarios Zairis, **Fary Diop**, Michaela Cerri, Sabina Chiaretti, Roberto Marasca, Maurilio Ponzoni, Silvia Deaglio, Antonio Ramponi, Enrico Tiacci, Laura Pasqualucci, Marco Paulli, Brunangelo Falini, Giorgio Inghirami, Francesco Bertoni, Robin Foá, Raul Rabadan, Gianluca Gaidano and Davide Rossi. The Genetics of Nodal Marginal Zone Lymphoma. Blood, 2016.

ABSTRACT

BRAF AND *BIRC3* MUTATIONS STRATIFY A POOR PROGNOSTIC SUBGROUP IN FCR TREATED CHRONIC LYMPHOCYTIC LEUKEMIA

Fary Diop ,[1] Riccardo Moia,[1] Chiara Favini,[1] Elisa Spaccarotella,[1] Lorenzo De Paoli,[1] Alessio Bruscaggin,[2] Valeria Spina,[2] Michaela Cerri,[1] Simone Favini,[1] Ahad Ahmed Kodipad,[1] Sruthi Sagiraju,[1] Clive Jabangwe,[1] Francesca R. Mauro,[3] Ilaria Del Giudice,[3] Francesco Forconi,[4,5] Agostino Cortelezzi,[6] Francesco Zaja,[7] Carlo Visco,[8] Annalisa Chiarenza,[9] Gian Matteo Rigolin,[10] Roberto Marasca,[11] Marta Coscia,[12] Omar Perbellini,[13] Alessandra Tedeschi,[14] Luca Laurenti,[15] Marina Motta,[16] Giovanni Del Poeta,[17] Antonio Cuneo,[10] Valter Gattei,[18] Robin Foà,[3] Gianluca Gaidano,[1] Davide Rossi[2]

[1]Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy; [2]Institute of Oncology Research and Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [3]Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy; [4]Cancer Sciences Unit,Southampton Cancer Research UK and National Institute for Health Research Experimental Cancer Medicine Centre, University of Southampton, Southampton, United Kingdom; [5]Division of Hematology, University of Siena, Siena, Italy; [6]Department of Hematology Oncology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, Italy; [7]Clinica Ematologica, Centro Trapianti e Terapie Cellulari "Carlo Melzi" Department of Experimental and Clinical Medical Sciences (DISM), Azienda Ospedaliera Universitaria S. Maria Misericordia, Udine, Italy; [8]Department of Cell Therapy and Hematology, Ospedale San Bortolo, Vicenza, Italy; [9]Hematology, Department of Clinical and Molecular Biomedicine, University of Catania, Catania, Italy; [10]Hematology Section, Azienda Ospedaliero Universitaria Arcispedale S. Anna, University of Ferrara, Ferrara, Italy; [11]Division of Hematology, Department of Oncology and Hematology, University of Modena and Reggio Emilia, Modena, Italy; [12] Division of Hematology, Azienda Ospedaliero Universitaria Città della Salute e della Scienza and University of Turin,Italy; [13]Section of Hematology, Department of Medicine, University of Verona, Verona, Italy; [14]Department of Oncology/Haematology, Niguarda Cancer Center, Niguarda Ca Granda Hospital, Milan, Italy; [15]Institute of Hematology, Catholic University of the Sacred Heart, Rome, Italy; [16]Department of Hematology, Spedali Civili, Brescia, Italy; [17]Department of Hematology, Tor Vergata University, Rome, Italy; [18]Clinical and Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Aviano, Italy.

INTRODUCTION: The current therapeutic landscape of chronic lymphocytic leukemia (CLL) mandates the identification of molecular predictors to optimize treatment choices. We aimed at refining the genetic-based prognostic stratification of FCR (fludarabine, cyclophosphamide, rituximab) treated CLL patients by integrating the mutational profile with *IGHV* mutation status and FISH cytogenetics. **METHODS:** Tumor genomic DNA has been analysed with next-generation-sequencing (NGS) to identify clonal mutations in frequently mutated genes in CLL. The primary outcome was progression free survival (PFS). **RESULTS:** Among patients categorized as low-risk by *IGHV* and FISH status, mutations of genes associated with poor prognosis were virtually absent. Among patients categorized as intermediate-risk, in multivariate analysis, mutations of *BIRC3* (HR: 7.46; 95% CI 2.60-21.4; p < .001) and *BRAF* (HR: 6.76; 95% CI: 1.75-26; p = .005) maintained independent association with an increased risk of progression. Consistently, intermediate-risk patients according to *IGHV* and FISH were further stratified in two groups represented by those harboring *BIRC3* or *BRAF* mutations, and those wild type for both these genes. Two high-risk groups emerged sharing a similarly poor PFS, defined by; *i) TP53* abnormalities (median PFS 2.1 years), and *ii) BIRC3* or *BRAF* mutations (median PFS 0.3 years). The new intermediate-risk group comprised patients with unmutated *IGHV* genes but lacking alterations of *TP53*, *BIRC3* and *BRAF* lesions (median PFS 5.3 years).

The low-risk group comprised patients with mutated *IGHV* genes, and lacking *TP53*, *BIRC3* and *BRAF* lesions (median PFS not reached, 50.0% at 8 years being progression free). **CONCLUSIONS:** *BIRC3* and *BRAF* mutations identify a very poor prognostic subgroup independent of *TP53* status. Mutations of *BIRC3* and *BRAF* mutations might be used as molecular predictors to select high-risk patients for novel therapeutic approaches.

REFERENCES

1. Zhang S, Kipps TJ. The pathogenesis of chronic lymphocytic leukemia. *Annu Rev Pathol*. 2014;9:103-18.

2. Rossi D, Gaidano G. The clinical implications of gene mutations in chronic lymphocytic leukaemia. *Br J Cancer*. 2016;114(8):849-54.

3. Rossi D, Terzi-di-Bergamo L, De Paoli L, et al. Molecular prediction of durable remission after first-line fludarabine-cyclophosphamide-rituximab in chronic lymphocytic leukemia. *Blood*. 2015;126(16):1921-4.

4. Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in *IGHV*-mutated chronic lymphocytic leukemia. *Blood*. 2016;127(3):303-9.

5. Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: updated results of the CLL8 trial. *Blood*. 2016;127(2):208-15.

6. Brown JR, Kay NE. Chemoimmunotherapy Is Not Dead Yet in Chronic Lymphocytic Leukemia. *J Clin Oncol*. 2017;35(26):2989-2992.

7. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature*. 2015;526(7574):525-30.

8. Puente XS, Beà S, Valdés-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2015;526(7574):519-24.

Flash Communications

17.45 – 18.00 P.M.

Chairperson: Ciro Isidoro

ELENA DARRA

elena.darra@univr.it

PostDoc researcher

Department of Medicine, Geriatrics Section, University of Verona, Verona, Italy

Education:

2004 - Degree in Biotechnology at the Faculty of Mathematical Physical and Natural Science of the University of Verona.

2008 - Ph.D. in Bioscience curriculum Biochemistry University of Verona.

Representative Careers:

2009-2010 February: Post doctoral fellowship provided by Banca Popolare di Verona at the Department of Morphological and Biomedical Sciences, Biological Chemistry Section of University of Verona to perform an experimental work on the study molecular mechanism of inhibitory action of different natural compounds on STAT1 and STAT3 activation.

2010-2012 February- 2011 october: Post doctoral fellowship provided by "Cariverona foudation" by title: "Functional changes and changes in gene and protein expression resulting from the use of cardiac circulatory support devices (artificial heart)"

2012-2014 February: Post doctoral fellowship provided by "Verona Nano-Medicine Initiative".

2014-to now: Post doctoral fellowship at Department of Medicine, Geriatrics Section, University of Verona the research group of Professor Mauro Zamboni to perform an experimental work on the characterization of peritumoral adipose tissue and its role in carcinogenesis and tumor progression in pancreatic and breast cancer.

Representative Awards:

- 2007: **"**Young researcher awards" at the International Conference of Polyphenols and Health. Kyoto 2007 Nov 25-28,

- 2007: **"**Young researcher awards" at the International Conference of Foods Factors for Health Promotion. 2007 Nov 27-Dec.1, "Study of molecular mechanism of antitumor actions of alfa-bisabolol"

Interesting Research Areas:

- Study of the molecular mechanism of action of α-bisabolol pro-apoptotic activity
- Study of the modulation of the activation of some transcription factors involved in the inflammatory response
- *in vitro* analysis of the tumor microenvironment using cocolture cell system and assessing the molecular mechanisms behind the creation of crosstalk .

Selected Publications:

1. Zoico E, **Darra E**, Rizzatti V, Tebon M, Franceschetti G, Mazzali G, Rossi AP,Fantin F, Zamboni M. Role of Adipose tissue in melanoma cancer microenvironmentand progression. Int J Obes (Lond). 2017 Sep 8. doi: 10.1038/ijo.2017.218.

2. Zoico E, **Darra E**, Rizzatti V, Budui S, Franceschetti G, Mazzali G, Rossi AP,Fantin F, Menegazzi M, Cinti S, Zamboni M. Adipocytes WNT5a mediateddedifferentiation: a possible target in pancreatic cancer microenvironment.Oncotarget. 2016 Apr 12;7(15):20223-35.

3. Butturini E, **Darra E**, Chiavegato G, Cellini B, Cozzolino F, Monti M, Pucci P, Dell'Orco D, Mariotto S. S-Glutathionylation at Cys328 and Cys542 impairs STAT3phosphorylation. ACS Chem Biol. 2014 Aug 15;9(8):1885-93. doi: 10.1021/cb500407d.

4. Cavalieri E, Bergamini C, Mariotto S, Leoni S, Perbellini L, **Darra E**, Suzuki H, Fato R, Lenaz G.Involvement of mitochondrial permeability transition poreopening in alpha-bisabolol induced apoptosis. FEBS J. 2009 Aug;276(15):3990-4000.doi: 10.1111/j.1742-4658.2009.07108.x.

5. **Darra E**, Abdel-Azeim S, Manara A, Shoji K, Maréchal JD, Mariotto S, CavalieriE, Perbellini L, Pizza C, Perahia D, Crimi M, Suzuki H. Insight into theapoptosis-inducing action of alpha-bisabolol towards malignant tumor cells: involvement of lipid rafts and Bid. Arch Biochem Biophys. 2008 Aug15;476(2):113-23. doi: 10.1016/j.abb.2008.02.004.

ABSTRACT

EFFECTS OF ALPHA BISABOLOL IN THE CROSS-TALK BETWEEN MELANOMA AND ADIPOCYTES IN AN *IN VITRO* CO-CULTURE STUDY: PRELIMINARY RESULTS

Darra E.[1], Rizzatti V.[1], Tebon Maela[1], Policastro G.[1], Carcereri De Prati A.[2], Mariotto S.[2], Zoico E.[1] and Zamboni[1].

[1]Department of Medicine, Geriatrics Section, University of Verona, Verona, Italy.[2]Department of Neuroscience, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Verona, Italy

INTRODUCTION: In melanoma an epidemiological association between excess weight and increased risk of cancer has been described. Moreover, the relation between adipocytes in tumor microenvironment and cancer development, progression and metastasis has received increasing relevance.

EXPERIMENTAL MODEL: We investigated the morphological and molecular mechanism involved in the cross-talk between adipocytes (3T3-L1) and melanoma cells (A375) using a co-culture system and assessing the role of adipocytes on melanoma cell migration. In this model we tested the effect of □-Bisabolol that might influence the de-differentiation of adipocytes and induce apoptosis on melanoma cancer cells**.**

RESULTS: Morphological analysis shows that 3T3-L1 adipocytes in co-culture with A375 cells were reduced in number and size and show a fibroblast like phenotype, with a reduction of adipocytes markers and increased expression of collagen, metalloproteinases and genes typical of de-differentiation processes. Furthermore, we show an increased migratory capacity of co-cultured melanoma cells. These phenomena were suppressed after the addition of the protein SFRP-5, supporting the involvement of the Wnt-5a pathway was further characterized by the activation of pathways linked to AKT correlated with migration processes, neo-angiogenesis and metastasis. After treatment with □-Bisabolol adipocytes appear less de-differentiated and the amount of □-Bisabolol needed to induce apoptosis in A375 is halved compared to controls.

CONCLUSION: These preliminary data allow us to hypothesize that in tumor microenvironment □-Bisabolol could interfere with de-differentiation process of adipocytes toward fibroblast-like cells possibly blocking downstream phenomena like migration and metastasis of melanoma cells.

REFERENCE : Zoico E, Darra E, Rizzatti V, Tebon M, Franceschetti G, Mazzali G, Zamboni M. Role of Adipose tissue in melanoma cancer microenvironment and progression. Int J Obes (Lond). 2017 Sep 8.

SIMONA NANNI

simona.nanni@unicatt.it

Assistant Professor of Applied Technical and Medical Sciences, Faculty of Medicine and Surgery - Fondazione Policlinico Gemelli and Università Cattolica, Rome, Italy Medical Pathology Institute, Università Cattolica, Rome, Largo F. Vito 1-00168 Rome

Education:

1998: Laurea *summa cum laude*, Doctor in Biological Sciences, "La Sapienza" University, Rome, Italy.

1999: National License for Board of Doctor Biologists

2002: PhD in Endocrinology and Metabolic Sciences, University of Messina and Catholic University.

2006: Board certification in Clinical Pathology, "La Sapienza" University, Rome, Italy.

Representative Careers:

1996-1998: Student Fellow, Experimental Oncogenesis Laboratory, Regina Elena Cancer Institute - Centro Ricerca Sperimentale, Rome, Italy. Main research interests: Thyroid tumor and p53 onco-suppressor gene. 1998-1999 "Tirocinio" fellowship, Experimental Oncogenesis Laboratory, Regina Elena Cancer Institute - Centro Ricerca Sperimentale, Rome, Italy. Main research interests: primary cultures, molecular and cellular biology.

1999: Fellowship, University of Messina, (Decreto Rettorale n° 169 23/02/1999) Main research interests: production and use of recombinant adenoviral and retroviral vectors. 2000-2002 Student Fellow, Experimental Oncogenesis Laboratory, Regina Elena Cancer Institute -Centro Ricerca Sperimentale, Rome, Italy. Main research interests: transcriptional regulation of gene expression and Estrogen receptor action

2003-2005: Fondazione Italiana per la Ricerca sul Cancro (FIRC) Fellowship, Experimental Oncogenesis Laboratory, Regina Elena Cancer Institute -Centro Ricerca Sperimentale, Rome, Italy. Main research interests: gene expression profiling (Affymetrix) and Real Time PCR. 2002-2006 Student Fellow, Experimental Oncogenesis Laboratory, Regina Elena Cancer Institute -Centro Ricerca Sperimentale, Rome, Italy. Main research interests: establishment and characterization of prostate primary cultures.

2006: Research Scolar, International Union against Cancer (UICC) Fellowship, Department of Medical Oncology, Director Prof. Massimo Loda, Dana Farber Cancer Institute and Harvard Medical School, Boston (MA),USA. Main research interests: Laser Capture Microdissection (LCM) technology. 2006 Co.Co.Pro Istituto Dermopatico dell'Immacolata (IDI), Rome, Italy Main research interests: Chromatin Immunoprecipitation in vivo by quantitative Real-Time PCR and Ideation of ChIP-on-chip methodology.

2007-2008 Co.Co.Pro MIUR (FIRB), Centro Cardiologico Monzino, Milan, Italy. Main research interests: Ideation, construction and functioning of home-made Microarray Chips and TaqMan Low Density Arrays.

Biographical sketch SIMONA NANNI Conference Book – CV Page

2008- Assistant Professor of Applied Technical and Medical Sciences, Medical Pathology Institute, Università Cattolica, Rome, Italy

Representative Awards:

2006 Award of the International Union against Cancer for the UICC fellowship (2006) at the Department of Medical Oncology (Director Prof. Massimo Loda), Dana Farber Cancer Institute, Harvard Medical School, Boston (MA),USA.

2008 CNR Highlights 2008-2009 of the National Reasearch Council for the article "Estrogen Receptor-α and Endothelial Nitric Oxide Synthase Nuclear Complex Regulates Transcription of Human Telomerase" Annalisa Grasselli, Simona Nanni, et al. Circulation Research 2008;103:34-4.

2014 Carocci Editore Selection as Medical Writer for the book of V Forum of Fondazione IBSA "Aging: is it a disease?", September 27th 2014, Frankfurt (Germany).

Interesting Research Areas:

Estrogen signaling in normal and transformed hormone-dependent cells and tissues, regulation of transcription by estrogen receptors, nitric oxide synthases and lncRNAs in hormone dependent cancer.

Selected Publications:

1. Aiello A et al. MALAT1 and HOTAIR Long Non-Coding RNAs Play Opposite Role in Estrogen-Mediated Transcriptional Regulation in Prostate Cancer Cells. Sci Rep. 2016 Dec 6;6:38414.
2. **Nanni S** et al. Estrogen-dependent dynamic profile of eNOS-DNA associations in prostate cancer. PLoS One. 2013 May 3;8(5):e62522.
3. **Nanni S** et al. Endothelial NOS, estrogen receptor beta, and HIFs cooperate in the activation of a prognostic transcriptional pattern in aggressive human prostate cancer. J Clin Invest. 2009
4. **Nanni S** et al. Epithelial-restricted gene profile of primary cultures from human prostate tumors: a molecular approach to predict clinical behavior of prostate cancer. Mol Cancer Res. 2006 Feb;4(2):79-92.
5. **Nanni S** et al. Signaling through estrogen receptors modulates telomerase activity in human prostate cancer. J Clin Invest. 2002 Jul;110(2):219-27.

ABSTRACT

MALAT1 AS A MASTER REGULATOR OF CELL METABOLISM IN PROSTATE CANCER

S. Nanni[2], A. Aiello[1,2], L. Bacci[2], A. Re[1], C. Ripoli[2], F. Pierconti[2i], F. Pinto[2], D. Pugliese[2], C. Grassi[2], C. Gaetano[3], PF. Bassi[2], M. Mello-Grand[4], P. Ostano[4], A. Pontecorvi[2], G. Chiorino[4] and A. Farsetti[1]

[1]CNR-IBCN, Rome, Italy, [2]Universita´Cattolica, Rome, Italy, [3]Goethe University, Frankfurt, Germany, [4]Fondazione Edo ed Elvo Tempia, Biella, Italy

This study stems from our recent finding that in prostate cancer (PCa) the long non-coding RNA MALAT1 is capable of repressing basal transcription of hormone-responsive genes relevant for the disease, such as hTERT, PSA and pS2 (1). Our aim is investigating MALAT1 as an active target for PCa inhibition *at single patient level*. Three advanced/metastatic PCa cell lines (C27IM, DU145 and PC3) and Organotypic Slice Cultures (OSCs) derived from freshly explanted tissue obtained upon surgery were analysed upon MALAT1 depletion by gapmers. Gene profiling of PCa cell lines and 3 OSCs showed that, upon MALAT1 silencing, about 200 genes were concordantly modulated in PCa cells and in OSCs, either up- or down-regulated (e.g. Malic enzyme, RNA Polymerase II and DNA polymerase subunit gamma). Transcriptomic data were validated by *q*RT-PCR in our *ex-vivo* experimental model of 30 independent OSCs from PCa patients with diverse outcome.

MALAT1 depletion induces the expression of genes involved in RNA metabolic processes and impairs that of key enzymes involved in Glycolysis, Kreb's cycle and Oxidative phosphorylation, suggesting MALAT1 as a metabolic active target for PCa.

REFERENCES

1. Aiello A, Bacci L, Re A, Ripoli C, Pierconti F, Pinto F, Masetti R, Grassi C, Gaetano C, Bassi PF, Pontecorvi A, Nanni S, Farsetti A. MALAT1 and HOTAIR Long Non-Coding RNAs Play Opposite Role in Estrogen-Mediated Transcriptional Regulation in Prostate Cancer Cells. Sci Rep. 2016 Dec 6;6:38414. doi:10.1038/srep38414.

ELEONORA SECOMANDI

eleonora.secomandi@uniupo.it

Master student

Laboratory of Molecular Pathology, Department of Health Sciences,

Università del Piemonte Orientale, Novara, Via Paolo Solaroli 17 – 28100, Novara (Italy)

Education:

October 2016 – present: Master student in Medical Biotechnologies, Università del Piemonte Orientale, Department of Health Sciences, Novara, Italy.

March 31st, 2016: Bachelor Degree in Biology, Università del Piemonte Orientale, Department of Science and Technology Innovation, Alessandria, Italy. Graduation mark: 105/110. Representative Careers:

August – September 2017: Summership at Clinical Investigative Center, Inserm 1431, University of Franche-Comté, Besançon, France.

6-7 October 2016: International Congress "Basic to Translational Medicine 2016: Focus on cancer"; Novara, Italy.

March 2016 – October 2016: Volunteer attendant, Laboratory of Molecular Pathology and Nanobioimaging, School of Medicine, Department of Health Sciences, Novara, Italy. Supervisor Prof. Ciro Isidoro.

September 2015 – March 2016: Internship for Bachelor Degree, Laboratory of Molecular Pathology and Nanobioimaging, School of Medicine, Department of Health Sciences, Novara, Italy. Supervisor Prof. Ciro Isidoro.

Interesting Research Areas:

Molecular mechanisms involved in neurodegenerative disorders and cancer – autophagy – cancer-keloids –3D models- epigenetics- cell migration – biogenesis and function of lysosomes, lysosomal cathepsins and lysosome-related organelles.

Selected Publications:

1. Chiara Vidoni, **Eleonora Secomandi**, Andrea Castiglioni, Mariarosa A.B. Melone and Ciro Isidoro. Resveratrol protects neuronal-like cells expressing mutant Huntingtin from Dopamine toxicity by rescuing ATG4-mediated autophagosome formation. Neurochem Int. 2017 May 19. pii: S0197-0186(17)30243-7. doi: 10.1016/j.neuint.2017.05.013.
2. Chiara Vidoni, Andrea Castiglioni, Christian Seca, **Eleonora Secomandi**, Mariarosa A.B. Melone and Ciro Isidoro. Dopamine exacerbates mutant Huntingtin toxicity via oxidative-mediated inhibition of autophagy in SH-SY5Y neuroblastoma cells: Beneficial effects of anti-oxidant therapeutics. Neurochem Int. 2016 Dec;101:132-143.

ABSTRACT

RESVERATROL AMELIORATES TGF-B-INDUCED FIBROSIS BY RESCUING AUTOPHAGY IN KELOID FIBROBLASTS

Eleonora Secomandi[1], Sami Ibazizen[2], Gwenael Rolin[2], Ciro Isidoro[1]

[1]Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100 - Novara (Italy). [2]Inserm UMR 1098, Engineering and Cutaneous Biology Team, Besancon, France

INTRODUCTION: Keloids are pathological scars derived from an altered skin wound healing process. One of the main clinical feature of keloids is their progressive growth due to fibroblast hyperproliferation and consequently extracellular matrix (ECM) protein deposition, leading to fibrosis (1). Transforming growth factor-β (TGF-β) is a pro-fibrotic cytokine involved in cell proliferation and myofibroblast differentiation. The common hallmark of myofibroblasts is the increase of α-SMA and COL1 expression (2). Resveratrol (RV) is a naturally polyphenolic compound, which inhibits oxidation, inflammation and collagen deposition (3). Cancer can be viewed as a wound that never heals (4). In this respect, keloids can be considered as the 'benign' counterparts of epithelioid cancers.

RESULTS: Fkc 316 keloid fibroblasts showed a reduced cell migration in both 2D and 3D models upon RV plus TGF-β co-treatment; in addition, in the same experimental conditions it was observed an increase of autophagic flux, suggesting autophagy as a putative mechanism responsible for slowing down cell proliferation. In particular, here we show that RV also attenuates α-SMA and COL1 synthesis and represses TGF-β-mediated myofibroblast phenoconversion.

CONCLUSION: Taken together, these preliminary data suggest that RV could ameliorate fibrosis by suppressing TGF-β activity and slow down cell proliferation by up-regulating autophagy.

REFERENCES:

1. Suttho D, Mankhetkorn S, Binda D, Pazart L, Humbert P, Rolin G. 3D modeling of keloid scars in vitro by cell and tissue engineering. Arch Dermatol Res. 2017 Jan;309(1):55-62. doi: 10.1007/s00403-016-1703-2.
2. Biernacka A, Dobaczewski M, Frangogiannis NG. TGF-β signaling in fibrosis. Growth Factors. 2011 Oct;29(5):196-202. doi: 10.3109/08977194.2011.595714.
3. Gharaee-Kermani M, Moore BB, Macoska JA. Resveratrol-Mediated Repression and Reversion of Prostatic Myofibroblast Phenoconversion. PLoS One. 2016 Jul 1;11(7):e0158357. doi: 10.1371/journal.pone.0158357.
4. Dvorak HF. Tumors: wounds that do not heal-redux. Cancer immunology research. 2015;3(1):1-11. doi: 10.1158/2326-6066.

MONIKA SZELIGA

mszeliga@imdik.pan.pl

Assistant professor

Mossakowski Medical Research Centre, PAS, Department of Neurotoxicology, Pawinskiego 5, 02-106 Warsaw, Poland

Education:

2008 – PhD, Mossakowski Medical Research Centre, PAS, Poland

2001 – MSc, Faculty of Biology, University of Warsaw, Poland

Representative Careers:

2001 – 2008 Research Assistant, Department of Pathomorphology, Institute of Mother and Child, Warsaw, Poland

2008 – 2009 Research Associate, Department of Neurotoxicology, Mossakowski Medical Research Centre, PAS, Poland

2009 – pres. Assistant Professor, Department of Neurotoxicology, Mossakowski Medical Research Centre, PAS, Poland

Interesting Research Areas:

Brain tumors, targeted therapy, cancer cell signaling and metabolism, epigenetic and genetic alterations in carcinogenesis.

Selected publications:

1. Majewska E, Rola R, Barczewska M, Marquez J, Albrecht J, **Szeliga M**. Transcription factor GATA3 expression is induced by GLS2 overexpression in a glioblastoma cell line but is GLS2-independent in patient-derived glioblastoma. J Physiol Pharmacol. 2017 Apr;68(2):209-214.
2. **Szeliga M**, Bogacińska-Karaś M, Kuźmicz K, Rola R, Albrecht J. Downregulation of GLS2 in glioblastoma cells is related to DNA hypermethylation but not to the p53 status. Mol Carcinog. 2016 Sep;55(9):1309-16. doi: 10.1002/mc.22372. Epub 2015 Aug 10.
3. Martín-Rufián M, Nascimento-Gomes R, Higuero A, Crisma AR, Campos-Sandoval JA, Gómez-García MC, Cardona C, Cheng T, Lobo C, Segura JA, Alonso FJ, **Szeliga M**, Albrecht J, Curi R, Márquez J, Colquhoun A, Deberardinis RJ, Matés JM. Both GLS silencing and GLS2 overexpression synergize with oxidative stress against proliferation of glioma cells. J Mol Med (Berl). 2014 Mar;92(3):277-90. doi: 10.1007/s00109-013-1105-2. Epub 2013 Nov 26.

4. **Szeliga M**, Zgrzywa A, Obara-Michlewska M, Albrecht J. Transfection of a human glioblastoma cell line with liver-type glutaminase (LGA) down-regulates the expression of DNA-repair gene MGMT and sensitizes the cells to alkylating agents. J Neurochem. 2012 Nov;123(3):428-36. doi: 10.1111/j.1471-4159.2012.07917.x. Epub 2012 Sep 21.
5. **Szeliga M**, Obara-Michlewska M, Matyja E, Łazarczyk M, Lobo C, Hilgier W, Alonso FJ, Márquez J, Albrecht J. Transfection with liver-type glutaminase cDNA alters gene expression and reduces survival, migration and proliferation of T98G glioma cells. Glia. 2009 Jul;57(9):1014-23. doi: 10.1002/glia.20825

ABSTRACT

ANTICANCER ACTIVITY OF 1,3,4-THIADIAZOLE DERIVATIVES IN HUMAN GLIOBLASTOMA CELLS

Monika Szeliga[1], Małgorzata Bogacińska-Karaś[1], Radosław Rola[2], Monika Karpińska[3], Andrzej Niewiadomy[3,4], Jan Albrecht[1]

[1]Mossakowski Medical Research Centre, Polish Academy of Sciences, Poland; [2]Department of Neurosurgery and Paediatric Neurosurgery of the Lublin Medical University, Poland; [3]Institute of Industrial Organic Chemistry, Poland; [4]University of Life Sciences in Lublin, Poland

Glioblastoma (GBM, WHO IV) is the most frequent and aggressive primary brain tumor in adults. The clinical outcome of patients with GBM remains poor: median survival time is less than one year. Side effects of treatment cause significant decrease in quality of life. Therefore, continuous efforts should be made to find new molecular targets and effective therapies of GBM. The key premise is that potential anti-glioma drug should display antiproliferative activity in tumor cells without affecting normal tissues. *Thiadiazole* derivatives exhibit a wide range of biological properties including anticancer activity [1]. The molecular mechanism underlying this activity most likely depends on the type of modification of thiadiazole ring. In this study we evaluated the anti-glioblastoma activity of six 1,3,4-thiadiazole derivatives. Human GBM cell lines, T98G, U251 and U87-MG were treated with thiadiazole derivatives and the antitumor efficacy was evaluated with MTT, BrdU and colony forming assays. The influence of tested compounds on GBM cells isolated from tumor samples and commercially available cultured human astrocytes was assessed by MTT assay. Treatment with *thiadiazole* derivatives containing different substitutes in the ring inhibited viability, proliferation and ability to form colonies of all GBM cell lines in a dose-dependent manner. All tested compounds inhibited also survival of GBM cells isolated from tumor tissues. In antiproliferative concentrations, three of thiadiazole derivatives showed only slight – if any - toxicity towards human astrocytes. These results may constitute a robust basis for the design of the new anti-glioma compounds with improved therapeutic potential (supported by National Science Centre grant no: 2013/11/D/NZ7/00925).

REFERENCES:

[1]. Matysiak J. Biological and pharmacological activities of 1,3,4-thiadiazole based compounds. Mini Rev Med Chem. 2015;15(9):762-75

OLGA TARASIUK

olga.tarasiuk@med.uniupo.it

PhD student

Università del Piemonte Orientale, Department of Health Sciences; via Paolo Solaroli 17 28100, Novara (Italy)

Education:

2014 – Laurea Summa cum Laude – Master degree in Biomedical Sciences, University of Hasselt (Belgium)

2012 - Laurea – Bachelor degree in Biomedical Sciences, University of Hasselt (Belgium)

2007 – Laurea Summa cum Laude - Professional education in care and nursing, Kiev, Ukraine

Representative careers and forming:

August 2017 – Poster presentation “Phage display selection and development of monoclonal antibodies against Protein Disulfide Isomerase, a novel tumor associated antigen and potential target for cancer immunotherapy”

April 2016 - Poster presentation “Protein Disulfide Isomerase in ovarian cancer: a novel tumor associated antigen and a potential target for cancer immunotherapy”. National PhD. meeting, Salerno, Italy.

July 2014 – Poster presentation “Monitoring and modelling the dynamic and pathogenicity of airborne bacteria communities at the places of potential harm”. University of Hasselt, Belgium.

2013-2014 special course of Laboratory Animal Science (certificate Felasa A), University of Hasselt, Belgium.

Interesting Research Areas:

Ovarian cancer. Cancerogenesis. Biomarkers discovery. Cancer immunotherapy

ABSTRACT

PHAGE DISPLAY SELECTION AND DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST PROTEIN DISULFIDE ISOMERASE: A NOVEL TUMOR ASSOCIATED ANTIGEN AND POTENTIAL TARGET FOR CANCER IMMUNOTHERAPY

Olga Tarasiuk[1], Frank Antony[1], Delia Mezzanzanica[3], Paolo Macor[2], Francesco Novelli[4], Serena Rizzo[2], Diego Cotella[1], Daniele Sblattero[2], Claudio Santoro[1]

[1]Dept. of Health Sciences, University of Eastern Piedmont, Novara, Italy; [2]Dept. of Life Sciences, University of Trieste, Italy; [3]Dep. of Experimental Oncology and Molecular Medicine, National institute of cancer, Milan, Italy; [4]Dep. of Molecular Biotechnology and Heath Sciences, University of Torino, Torino, Italy.

INTRODUCTION: Ovarian cancer (OC) is the fifth most common cancer in women worldwide. About 70% of cases are diagnosed at a late stage and therefore are poorly treatable (1). Despite progressive development of medical treatment last decades, the main treatment for OC remains chemotherapy and surgery (2). It's obviously that new therapeutic approaches have to be developed instantly. One of the promising alternative is immunotherapy (3).

EXPERIMENTAL MODEL: Ascites of OC patients were analyzed to identify antibodies against tumor associated antigens (TAA) for future diagnose and therapy. SERPA was conducted to identify which proteins trigger antibody production. Antibodies from OC patient ascites were purified to test it ability to activate Complement Dependent Cytotoxicity (CDC) in vitro. Recombinant monoclonal humanized antibodies were engineered and tested for it activity in standard laboratory assays.

RESULTS: Immunofluorescent (IF) staining showed that OC patient ascites have a high titer of antibodies against OVCAR3 membrane proteins. SERPA identified that anti-PDIA1 is the most abundant in ascites antibodies pool. ELISA screenings on ascites confirmed PDIA1 as a common TAA. OC patients with high anti-PDIA1 titers showed a better survival rate. Further experiments showed that anti-PDIA1 purified from OC ascites activate CDC pathway leading to OC cells killing. Using phage display library selection, specific for PDIA1 scFv's were fused to human Fc. CHO cells were used for recombinant antibodies production. Following, they were tested and showed it specificity and functionality in ELISA, IF, WB.

CONCLUSION: PDIA1 protein was identified as TAA that trigger immune system to produce specific antibodies. Purified from ascites anti-PDIA1 showed it ability to activate CDC and lead to OC cells killing. Based on hypothesis that anti-PDIA1 can be a potential OC immunotherapy, we engineered recombinant anti-PDIA1 antibodies, which represent it functionality in main laboratory assays.

REFERENCES:

1. S.Xua, N.Alexey, B.Butkevich, Y.Roppei. Discovery of an orally active small-molecule irreversible inhibitor of protein disulfide isomerase for ovarian cancer treatment. PNAS. 2012;109(40):16348–53. 2. N.Di Santo, J.Ehrisman. Research Perspective: Potential Role of Nitazoxanide in Ovarian Cancer Treatment. Old Drug, New Purpose? . Cancers. 2013;5:1163-76. 3. J.E.Ang, S.B.Kaye. Molecular targeted therapies in the treatment of ovarian cancer. Global science book. 2007.

SURESH VELNATI

suresh.velnati@med.uniupo.it

PhD Student

Università del Piemonte Orientale, Department of Translational Medicine; via Paolo Solaroli 17 –28100, Novara, Italy

Education:

2016: Master Degree (Summa cum Laude) in Medical Biotechnologies; Università del Piemonte Orientale, Novara**, Italy**

2012: Bachelor of Pharmacy; Acharya Nagarjuna University, **India**

Representative Careers:

11/2016 - to date: PhD in Autoimmune and Allergic diseases under Prof. Gianluca Baldanzi, laboratory of Biochemistry, department of translational medicine, Università del Piemonte Orientale, Novara, **Italy**

08/2016 - 11/2016: Post-graduate fellow in the laboratory of Biochemistry, department of translational medicine, Università del Piemonte Orientale, Novara, **Italy**

06/2015 - 09/2015: Academic visitor, INTERNSHIP title: "Role of BAFF receptors in CLL patients" under Prof. Francesco Forconi, Cancer Science Unit, Cancer Research UK Centre, Hematology Dept., Southampton University Hospital, Southampton, **UK**.

12/2014 – 07/2016: Master trainee at laboratory of Biochemistry, Università del Piemonte Orientale, Novara, **Italy**

08/2011 – 05/2012: Bachelor trainee at laboratory of Pharmaceutics, Acharya Nagarjuna University, **India**

Interesting Research Areas:

Diacylglycerol kinase α, cell signaling, XLP1 therapy, and T-cell malignancies.

ABSTRACT

NOVEL DIACYLGLYCEROL KINASE ALPHA INHIBITORS FOR X-LINKED LYMPHOPROLIFERATIVE DISEASE 1 THERAPY

Suresh Velnati[1,2], Alberto Massarotti[3], Elisa Ruffo[1,4], Giancesare Tron[3], Andrew Snow[4], Andrea Graziani[1,5], Gianluca Baldanzi[1,2]

[1]Department of Translational Medicine, University of Piemonte Orientale, via Solaroli 17 28100, Novara, Italy; [2]Institute for Research and Cure of Autoimmune Diseases, 28100, Novara, Italy; [3]Department of Pharmaceutical Sciences, University of Piemonte Orientale, largo Donegani 12, 28100, Novara, Italy; [4]Department of Pharmacology and Molecular Therapeutics, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, USA. [5]Division of Experimental Oncology, School of Medicine, University Vita e Salute San Raffaele, Milan, Italy

INTRODUCTION: X-linked lymphoproliferative disease 1 (XLP1) is a primary immunodeficiency due to mutations in the SH2D1a gene, encoding the SAP adaptor protein [1]. SAP deficiency perturbs TCR signalling and results in a constitutive diacylglycerol kinase alpha (DGKα) activity that impairs CD8+ T cell restimulation induced cell death. Indeed, pharmacological inhibition of DGKα in XLP1 animal models limits CD8+ T cell expansion and interferon-γ production, suggesting the development of DGKα inhibitors for XLP1 therapy [2].

EXPERIMENTAL MODEL: To find new inhibitors of DGKα suitable for clinical trials in XLP1, we selected a library of 150 compounds based on chemical homology with the two commercially available DGKα inhibitors. This library comprised uncharacterized molecules, several compounds already in clinical use or development and some specifically synthetized molecules. The library was screened for inhibitory activity at 100μM concentration on DGKα overexpressing homogenates, using the two commercial inhibitors as positive controls. Active compounds (≥50% inhibition at 100μM) were tested at concentration from 0.1 to 100 μM in order to estimate the IC50. Furthermore, we tested the most active compounds in restimulation induced cell death assay using SAP silenced lymphocytes as XLP1 model.

RESULTS: In the primary screen 20 compounds inhibit DGKα at least by 25%. Of those, compound01 and ritanserin showed a potency equal or superior to the two commercial inhibitors. Based on the results of the first screen we created a pharmacological model by which we synthetized a second wave of 22 compounds, among which compound02 resulted active. Compound01, compound02 and ritanserin restored apoptosis in SAP deficient lymphocytes at concentrations lower than the two previously available inhibitors supporting their potential for XLP1 therapy.

CONCLUSION: Concluding our work allow us to propose a pharmacological model for the rational design or DGKα inhibitors and to select three active compounds. Of those, Ritanserin is highly attractive for drug repurposing as it was previously tested in clinical trials, as it is safe in humans at doses sufficient to inhibit DGKα [3].

REFERENCES:

1. Baldanzi, G., et al. (2011). "SAP-mediated inhibition of diacylglycerol kinase alpha regulates TCR-induced diacylglycerol signaling." J Immunol **187**(11): 5941-5951.

2. Ruffo, E., et al. (2016). "Inhibition of diacylglycerol kinase alpha restores restimulation-induced cell death and reduces immunopathology in XLP-1." Sci Transl Med 8(321): 321ra327.

3. Baroda, S. et al. (2017) . “Dual activities of ritanserin and R59002 as DGKalpha inhibitors and serotonin receptor atagonist”. Biochem Pharmacol 123: 29-39.

Abstracts

SALAH EDDINE AYOUBI

ayoubisalaheddine2015@gmail.com

Ph.D. researcher in Genetics and Molecular Pathology

Cancer Genomics Lab, Fondazione “Edo ed Elvo Tempia”, Via Malta 3, 13900 Biella Italy. Genetics and Molecular Pathology Lab, Medical School, University Hassan II, 19, Tarik Ibnou Ziad street, 9154 Casablanca, Morocco.

Education:

2014 to date: PH.D. In Genetics and Molecular Pathology, University Hassan II of Casablanca, Medical School, Casablanca (Morocco), Laboratory of Genetics and Molecular Pathology.

2013: Master's degree in Biology and Health, University Hassan II Casablanca, Faculty of Sciences Ben M'sik, Casablanca (Morocco).

2011: Bachelor's degree in Biology, University Hassan II Casablanca, Faculty of Sciences Ben M'sik, Casablanca (Morocco)

2008: Baccalaureate of Experimental Sciences option ''Life Sciences and Earth'' High school Ouad Eddahab, Casablanca (Morocco)

Representative Careers:

2016 to date: Research internship, Dr. Giovanna CHIORINO, “Edo and Elvo Tempia” Foundation, Laboratory of Cancer Genomics, Biella (Italy). Participation in the "Andromeda" project for the personalization of mammography screening.

2014 to date: Research internship, Pr. Sellama NADIFI, Casablanca (Morocco), University Hassan II Casablanca, Medical school, Genetics and Molecular Pathology department.

2013: Research internship, Dr. Aziz HICHAMI, Dijon (France) University of Bourgogne, National Institute of Health and Medical research (INSERM), Unit 866, Laboratory "Lipid, Nutrition, Cancer", Chemotherapy team, lipid, metabolism and anti-tumor immune response.

2011: Research internship, Louis Pasteur Institute of Morocco, Casablanca (Morocco); Internship at the department of Cellular Immunology and Molecular Biology.

Interesting Research Areas:

Transcriptomic, cell signaling, breast carcinogenesis, genetic and epigenetic, apoptosis.

Representative Awards:

2016-2017: PH.D. Assistant researcher at the Cancer Genomics Laboratory, “Edo and Elvo Tempia” Foundation (Biella, Italy).

Selected Publications:

1. **Salah Eddine A**, Mohamed E, Meryam E, Hind HI, Simohamed E, Mounia A, Sellama N. Involvement of TP53 Arg72Pro polymorphism in breast cancer risk in Moroccan population **(Article submitted)**.
2. **Salah Eddine A**, Mohamed E, Meryam E, , Sellama N. Association of TP53 codon72 polymorphism with breast cancer risk: An updated Meta-Analysis **(Article submitted)**.

ABSTRACT

LONG NON-CODING RNA EXPRESSION PROFILING OF BREAST CANCER IN MOROCCAN WOMEN

Salah Eddine Ayoubi[1,2,3] , Paola Ostano[1] , Maurizia Mello-Grand[1] , Ilaria Gregnanin[1] , Alessandro Bollo[1] Mohamed Elkarroumi[4] , Sellama Nadifi[2,3] and Giovanna Chiorino[1].

[1]Cancer Genomics Lab, Fondazione "Edo ed Elvo Tempia" per la lotta contro i tumori, Biella, Italy.[2] Genetics and Molecular Pathology Lab, Medical School, University Hassan II, Casablanca, Morocco. [3] Mohammed VI Center for Cancer Treatment, University Hospital Center Ibn Rochd, Casablanca, Morocco.

INTRODUCTION : Long non coding RNAs (lncRNAs) are non protein coding genomic transcripts longer than 200 nucleotides. LncRNAs were recently emerged as a new heterogeneous class of non coding RNAs, involved in gene regulation either at the transcriptional or the post-transcriptional level [1]. The role of lncRNAs in breast carcinogenesis has been widely studied throughout the world, but there is no study performed in developing countries like Morocco regarding the involvement of lncRNAs in breast cancer. Hence, we conducted this transcriptomic study to analyze the implication of lncRNAs in breast cancer molecular subtypes in Moroccan population.

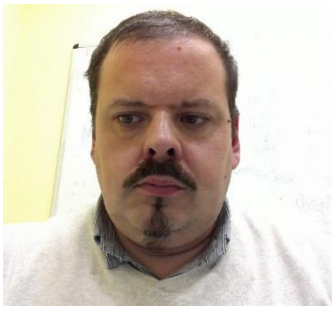
EXPERIMENTAL MODEL: We analyzed lncRNAs differential gene expressions of 30 fresh breast tumors and 13 normal matched breast tissues by Agilent Human Genome Microarrays. Functional analysis of differentially expressed lncRNAs was carried out for the four molecular subtypes (luminal A, luminal B, HER2 over-expressed, and triple negative breast cancer).

RESULTS: A total of 159 lncRNAs were differentially expressed in all molecular subtypes combined together (logFC>1; *P* value<0.01). Interestingly, HER2 and luminal A subtypes involved the highest number of differentially expressed lncRNAs , followed by luminal B and triple negative subtypes. MALAT1 lncRNA was strongly up-regulated in all molecular subtypes. While, MIR497HG lncRNA was commonly under-expressed in the four breast cancer types.

CONCLUSION: This study is to our knowledge the first to analyze the lncRNAs differential expression in Moroccan women. Focusing on the lncRNAs expression machinery may help to better understand the mRNAs-lncRNAs interactions and to explore new possibilities for breast cancer targeted therapy.

REFERENCES:

1. J. Wang *et al.*, "Dysregulation of long non-coding RNA in breast cancer: an overview of mechanism and clinical implication," *Oncotarget*, vol. 8, no. 3, pp. 5508–5522, Jan. 2017.



GIANLUCA BALDANZI

gianluca.baldanzi@med.uniupo.it

Associate Professor in Biochemistry

Università del Piemonte Orientale, Dep. Medicina Traslazionale, Via Solaroli 17, 28100, Novara, Italy

Education:

2004-10 to 2008-04-23 PhD in Molecular Medicine (School of Medicine), Università degli Studi del Piemonte Orientale Amedeo Avogadro: Vercelli, Piemonte, Italy.

1998-10 to 2002-11-06 Clinical biochemistry / Laboratory medicine, Università degli Studi di Torino: Torino, Piemonte, Italy.

1993-09 to 1998-07-09 Biological Sciences (School of Science), Università degli Studi di Torino: Torino, Piemonte, Italy.

Representative Careers:

2016-03 to present. Associate Professor in Biochemistry (Translational Medicine). Università degli Studi del Piemonte Orientale Amedeo Avogadro: Vercelli, Piemonte, Italy

2008-04 to 2016-03-02. Assistant Professor in Biochemistry (Translational Medicine). Università degli Studi del Piemonte Orientale: Vercelli, Italy

2004-03 to 2007-02. Graduate fellow. "E. Menni" Scientific Park: Brescia, Italy

2000 to 2003 Graduate Fellow. Associazione Italiana per la Ricerca sul Cancro: Milano, Lombardia, Italy

Representative Awards:

- Terapie Innovative per la PSOriasi- TIPSO. Regione Piemonte (Torino, Piemonte, Italy). 2014-12 to 2017-11
- DGKα inhibition restores restimulation induced apoptosis in XLP patient's lymphocytes. Università degli Studi del Piemonte Orientale (Vercelli, Piemonte, Italy). 2013-01 to 2014-12

Interesting Research Areas:

1. Signal transduction
2. Lipid metabolism
3. Immunity

Selected Publications:

1: Ruffo E, Malacarne V, Larsen SE, Das R, Patrussi L, Wülfing C, Biskup C, Kapnick SM, Verbist K, Tedrick P, Schwartzberg PL, Baldari CT, Rubio I, Nichols KE, Snow AL, **Baldanzi G**, Graziani A. Inhibition of diacylglycerol kinase α restores restimulation-induced cell death and reduces immunopathology in XLP-1. Sci Transl Med. 2016 Jan 13;8(321):321ra7.

2: Rainero E, Cianflone C, Porporato PE, Chianale F, Malacarne V, Bettio V, Ruffo E, Ferrara M, Benecchia F, Capello D, Paster W, Locatelli I, Bertoni A, Filigheddu N, Sinigaglia F, Norman JC, **Baldanzi G**, Graziani A. The diacylglycerol kinase α/atypical PKC/β1 integrin pathway in SDF-1α mammary carcinoma invasiveness. PLoS One. 2014 Jun 2;9(6):e97144.

3: Porporato PE, Filigheddu N, Reano S, Ferrara M, Angelino E, Gnocchi VF, Prodam F, Ronchi G, Fagoonee S, Fornaro M, Chianale F, **Baldanzi G**, Surico N, Sinigaglia F, Perroteau I, Smith RG, Sun Y, Geuna S, Graziani A. Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest. 2013 Feb;123(2):611-22.

4: **Baldanzi G**, Pighini A, Bettio V, Rainero E, Traini S, Chianale F, Porporato PE, Filigheddu N, Mesturini R, Song S, Schweighoffer T, Patrussi L, Baldari CT, Zhong XP, van Blitterswijk WJ, Sinigaglia F, Nichols KE, Rubio I, Parolini O, Graziani A. SAP-mediated inhibition of diacylglycerol kinase α regulates TCR-induced diacylglycerol signaling. J Immunol. 2011 Dec 1;187(11):5941-51. doi: 10.4049/jimmunol.1002476. Epub 2011 Nov 2.

5: Chianale F, Rainero E, Cianflone C, Bettio V, Pighini A, Porporato PE, Filigheddu N, Serini G, Sinigaglia F, **Baldanzi G**, Graziani A. Diacylglycerol kinase alpha mediates HGF-induced Rac activation and membrane ruffling by regulating atypical PKC and RhoGDI. Proc Natl Acad Sci U S A. 2010 Mar 2;107(9):4182-7.

ABSTRACT

NUCLEAR LOCALIZATION OF DGKα IN K562 CELLS IS INVOLVED IN CELL CYCLE PROGRESSION

Gianluca Baldanzi[4], Alessandro Poli[1,2,3], Roberta Fiume[1], Daniela Capello[4], Stefano Ratti[1], Marco Gesi[5], Lucia Manzoli[1], Andrea Graziani[4,6], Pann-Ghill Suh[7], Lucio Cocco[1], Matilde Y. Follo[1].

[1] Cellular Signalling Laboratory, Department of Biomedical and Neuromotor Sciences, University of Bologna; [2] Istituto Nazionale Genetica Molecolare "Romeo e Enrica Invernizzi", Milano. Italy; [3] Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Milan, Italy; [4] Department of Translational Medicine and Institute for Research and Cure of Autoimmune Diseases, University of Piemonte Orientale, Novara, Italy; [5]Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy; [6]University Vita e Salute San Raffaele, Milan, Italy; [7]School of Life Sciences, Ulsan National Institute of Science and Technology, Ulsan, Republic of Korea.

INTRODUCTION: Phosphatidylinositol (PI) signaling is an essential regulator of cell motility and proliferation. A portion of PI metabolism and signaling takes place in the nuclear compartment of eukaryotic cells, where an array of kinases and phosphatases localize and modulate PI. Among these, Diacylglycerol Kinases (DGKs) are a class of phosphotransferases that phosphorylate diacylglycerol and induce the synthesis of phosphatidic acid. Nuclear DGKalpha modulates cell cycle progression, and its activity or expression can lead to changes in the phosphorylated status of the Retinoblastoma protein, thus, impairing G1/S transition and, subsequently, inducing cell cycle arrest, which is often uncoupled with apoptosis or autophagy induction.

EXPERIMENTAL MODEL: Human erythroleukemia cell line K562.

RESULTS: Here we report for the first time not only that the DGKalpha isoform is highly expressed in the nuclei of K562, but also that its nuclear activity drives K562 cells through the G1/S transition during cell cycle progression by control of pRb phosphorylation.

CONCLUSION: The evidences reported in this study may therefore contribute to consider DGKalpha as part of the important machinery regulating PI metabolism in the nuclear compartment of K562 cells and, consequently, fundamental processes affecting cell cycle and cell proliferation.

REFERENCES:

1. Poli A, Fiume R, Baldanzi G, Capello D, Ratti S, Gesi M, Manzoli L, Graziani A, Suh PG, Cocco L, Follo MY. Nuclear Localization of Diacylglycerol Kinase Alpha in K562 Cells Is Involved in Cell Cycle Progression. J Cell Physiol. 2017 Sep;232(9):2550-2557. 2) Poli A, Fiume R, Baldanzi G, Capello D, Ratti S, Gesi M, Manzoli L, Graziani A, Suh PG, Cocco L, Follo MY. Cover Image, Volume 232, Number 9, September 2017. J Cell Physiol. 2017 Sep;232(9):i.

RICCARDO BALLARO'

riccardo.ballaro@unito.it

PhD student

University of Torino, Department of Clinical and Biological Sciences, Corso Raffaello, 30, 10125 Torino (Italy)

Education:

2012 - 1st level degree – Bachelor in Biology (L-13), University of Perugia (Italy), Faculty of Mathematical, Physical and Natural Sciences.

2014 - 2nd level degree – Master in Cellular and Molecular Biology (LM-6), University of Torino (Italy), School of Natural Sciences – Department of Life Sciences and Systems Biology.

Representative Careers:

January-April 2016: Research project as PhD student: "*Oxidative stress and muscle wasting in cancer and chemotherapy-associated muscle wasting*"- effort 3 month, at the Prof. J. Viña's laboratory, Department of Physiology, University of Valencia, Fundacion Investigacion Hospital Clinico Universitario/INCLIVA.

November 2014 to date: PhD student in Experimental Medicine and Therapy at the Department of Clinical and Biological Sciences (University of Torino, Italy).

October 2014: Student in Cellular and Molecular Biology at the Prof. G. Barrera's laboratory, Department of Clinical and Biological Sciences (University of Torino, Italy). Sperimental thesis entitled: *GSH-responsive nanoparticles in the treatment of chemoresistant tumor cells* – effort 6 months.

July 2012: Student in Biology at the Prof. C.Arcuri's laboratory, Department of Sperimental Medicine (University of Perugia, Italy). Sperimental thesis entitled: : *Neurospheres and nanotubes: different structures for different biological functions* – effort 6 months.

Interesting Research Areas:

Cancer cachexia, particularly focused on skeletal muscle wasting, muscle protein breakdown and myogenesis; exercise metabolism; mithocondria; oxidative stress; chemotherapy.

Publications:

Penna F, Pin F, **Ballarò R**, Baccino FM, Costelli P. *Novel investigational drugs mimicking exercise for the treatment of cachexia*. Expert Opin Investig Drugs. 2015 Nov 26:1-10.

Ballarò R, Costelli P, Penna F. *Animal models for cancer cachexia.* Curr Opin Support Palliat Care. Review in press. 2016 Dec;10(4):281-287.

ABSTRACT

EFFECTS OF MODERATE EXERCISE TRAINING ON MUSCLE WASTING IN TUMOR-BEARING MICE EXPOSED TO CHEMOTHERAPY

R. Ballarò[1,2], M. Beltrà[1,2], F. Pin[1,2], P. Costelli[1,2] and F. Penna[1,2].

[1]Department of Clinical and Biological Sciences, Experimental Medicine and Clinical Pathology Unit, University of Turin, Italy; [2]Interuniversity Institute of Myology, Italy.

Cachexia is a multifactorial syndrome characterized by body weight loss, muscle wasting and metabolic abnormalities, accounting for more than 20% of cancer-related deaths.
The present study aimed at evaluating the effects of moderate exercise training on muscle wasting in C26-bearing mice treated with chemotherapy.
As preclinical model of cancer cachexia, Balb/c mice were subcutaneously injected with C26 colon carcinoma cells. Animals were randomized and divided into two groups, namely C26-bearing mice under unrestricted tumor growth (C26; 14 days of tumor growth) or treated with chemotherapy (oxaliplatin+5-fluorouracil; C26 OXFU; 28 days of tumor growth). C26-bearing mice treated with chemotherapy were divided into two sub-groups, sedentary or exercised C26 OXFU mice.
OXFU administration was able to extend the lifespan of C26-bearing mice. C26-OXFU mice displayed more severe cachexia than untreated mice and exercise partially protected from the loss of muscle mass and function. Chemotherapy exacerbated tumor-associated autophagy/mitophagy dysregulation and decreased myofiber oxidative capacity and protein synthesis. Conversely, moderate exercise training was able to partially counteract muscle wasting and to increase autophagy and the clearance of damaged mitochondria. Moreover, exercise increased mitochondrial content and rescued muscle oxidative capacity in C26-OXFU mice but did not improve protein synthesis.
In conclusion, exercise training exerts beneficial effects potentially exploitable in the management of cancer patients receiving chemotherapy.

REFERENCES

1. Argilés JM, Busquets S, Stemmler B, López-Soriano FJ. Cancer cachexia: understanding the molecular basis. Nat Rev Cancer. 2014 Nov;14(11):754-62. 2) Pin F, Busquets S, Toledo M, Camperi A, Lopez-Soriano FJ, Costelli P, Argilés JM, Penna F. Combination of exercise training and erythropoietin prevents cancer-induced muscle alterations. Oncotarget. 2015 Dec 22;6(41):43202-15.

MARTA BORCHIELLINI

marta.borchiellini@med.uniupo.it

PhD student

University of Eastern Piedmont,

Department of Health Sciences,

via Solaroli 17, 28100, Novara (Italy)

Education:

2015- Laura *Summa cum Laude* in Chemistry and Pharmaceutical Technologies, Università Degli Studi di Perugia, Perugia (Italy)

2015- Qualification as Pharmacist, Università Degli Studi di Perugia

Representative Careers:

January 2015 –March 2015- Student worker and assistant in the Department of Pharmacology, Goëthe Universität, Frankfurt am Main (Germany)

July 2015- December 2015- Scholarship holder, Department of Experimental Medicine, Università Degli Studi di Perugia, Perugia (Italy)

January 2016 - October 2016- Research fellow, Department of Experimental Medicine, Università Degli Studi di Perugia, Perugia (Italy)

November 2016 to date- PhD student at the Department of Health Sciences, University of Eastern Piedmont, Novara (Italy)

Interesting Research Areas:

Epigenetic regulation of gene expression; RNA-based approach for gene reactivation; G-quadruplexes structures in nucleic acids

Publication:

“Effects of grape skin extract on age-related mitochondrial dysfunction, memory and life span in C57BL/6J mice”, Heike Asseburg, Madeleine Müller, Stephanie Hagl, Carmina Schäfer, Maximilian Pohland, Dirk Berressem, Marta Borchiellini, Christina Plank, Gunter P. Eckert, *Neuromolecular Medicine*, 2016

ABSTRACT

RNA-MEDIATED CORRECTION OF ABERRANT DNA METHYLATION AT THE *P15* LOCUS

Marta Borchiellini[1], Simone Ummarino[1], Mailin Li[1], Andy Joe Seelam[1], Nagy Habib[2], Claudio Santoro[1], Annalisa Di Ruscio[1]

[1]University of Eastern Piedmont, Novara, Italy; [2]Imperial College London, Hammersmith Campus, London, United Kingdom

INTRODUCTION: *P15* is a methylation sensitive gene located on chromosome 9p21 and commonly found silenced by aberrant DNA promoter methylation during Myelodysplastic Syndrome (MDS) progression to Acute Myeloid Leukemia (AML). *P15* encodes for a cyclin-dependent kinase inhibitor increasingly expressed during granulomonocytic maturation(1). As MDS evolution to AML includes both myeloid proliferation and blocked differentiation stages, restoration of the natural P15 transcript may alleviate some characteristic symptoms of the disease. Currently available demethylating agents, e.g. 5-azacytidine, have major side effects of high toxicity and non-specific DNA methylation. Therefore, the aim of this study is to achieve RNA-mediated correction of the aberrantly methylated *P15* locus using small activating RNAs (saRNAs)(2).

EXPERIMENTAL MODEL: SaRNAs were designed against the proximal promoter, first exon, and intron regions of the *P15* gene body. SaRNAs were introduced to KG1a myeloid cell line through electroporation, and re-activation of the locus was measured at the transcript level by qRT-PCR. Changes in *P15* promoter methylation were determined by Combined Bisulfite Restriction Analysis (COBRA) and Bisulfite Sequencing (BS).

RESULTS: Delivery of saRNAs into the KG1a cell line showed upregulated p15 expression 72 hr post-transfection by qRT-PCR. Moreover, preliminary data from BS analysis showed statistically significant changes in DNA methylation pattern within the 5'-untranslated region of *P15* gene.

CONCLUSION: There is much interest in using RNA molecules as a therapeutic tool(3). Introduction of such an approach offers greater advantages over existing hypomethylating based protocols, including high gene specificity, lower cytotoxicity and absence of drug based off-target side-effects. In conclusion, this research might pave the way for development of RNA-based gene demethylating agents for cancer treatment.

REFERENCES:

1. Teofili L, Morosetti R, Martini M, Urbano R, Putzulu R, Rutella S, et al. Expression of cyclin-dependent kinase inhibitor p15(INK4B) during normal and leukemic myeloid differentiation. Exp Hematol. 2000;28(5):519–26.

2. Li L-C, Okino ST, Zhao H, Pookot D, Place RF, Urakami S, et al. Small dsRNAs induce transcriptional activation in human cells. Proc Natl Acad Sci [Internet]. 2006;103(46):17337–42. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0607015103

3. Reebye V, Sætrom P, Mintz PJ, Huang KW, Swiderski P, Peng L, et al. Novel RNA oligonucleotide improves liver function and inhibits liver carcinogenesis in vivo. Hepatology. 2014;59(1):216–27.

ANDREA CASTIGLIONI

andrea.castiglioni@ieo.it

Post-graduate fellow in Molecular Medicine Programme

Department of Experimental Oncology,

European Institute of Oncology (IEO), 20139, Milan (Italy)

Education

2016- Master Degree in Medical Biotechnologies, *summa cum laude and honorable mention,* University of Piemonte Orientale (Novara, Italy)

2014- Bachelor Degree in Biotechnology, University of Piemonte Orientale (Novara, Italy)

Representative Careers:

October 2017-present: Post-graduate fellowship with Professor Salvatore Pece, Istituto Oncologico Europeo (IEO), (Milano, Italy).

September 2016-September 2017: Post-graduation in the laboratory of Molecular Pathology with Professor Ciro Isidoro, University of Piemonte Orientale, Department of Health Sciences (Novara, Italy).

2013-2016: Trainee in the laboratory of Molecular Pathology with Professor Ciro Isidoro, University of Piemonte Orientale, Department of Health Sciences (Novara, Italy).

November 2012- May 2013: Trainee in the laboratory of Hematology with Professor Gianluca Gaidano and Dr. Davide Rossi, University of Piemonte Orientale, Department of Translational Medicine (Novara, Italy).

Representative Awards:

- **2017-** Scholarship award, Associazione per la ricerca medica 'Ippocrate-Rhazi')
- **2017**- Award for the best scientific publication in the year 2016 (University of Piemonte Orientale, School of Medicine)
- **2017**- Premio di studio ad personam, for the highest impact scientific publication in the field of molecular oncology in the Department of Health of Sciences (Associazione per la ricerca medica 'Ippocrate-Rhazi')
- **2016**- Gold award for young investigator in Cancer Research (oral presentation at the International Conference "Basic to Translational Medicine 2016: focus on cancer")
- **2016-** Fellowship for the tutoring of Medical Biotechnologies students

Insteresting Reasearch Areas:

Cancer, Autophagy, Interactomics, Cell metabolism, Cancer Stem Cells, EMT.

Publications:

1. **Castiglioni A*** - Phadngam S*, Ferraresi A, Morani F, Follo C, Isidoro C. PTEN dephosphorylates AKT to prevent the expression of GLUT1 on plasmamembrane and limit glucose consumption in cancer cells. *These authors have equally contributed and each should be regarded as first author. Oncotarget. 2016 Dec 20;7(51):84999-85020.
2. Vidoni C, **Castiglioni A**, Seca C, Secomandi E, Melone M.A.B., Isidoro C. Dopamine kills neuroblastoma cells expressing transgenic mutant Huntington following ROS-dependent inhibition of autophagy. Neurochem Int. 2016 Dec; 101:132-143.
3. Duangtum N, Junking M, Phadngam S, Sawasdee N, **Castiglioni A**, Charngkaew, Limjindaporn T, Isidoro C. γCOPI mediates the retention of mutant kAE1 protein in Golgi apparatus – a mechanistic explanation of distal renal tubular acidosis. Biochem J. 2017 Jul 17; 474(15):2573-2584.
4. Vidoni C, Secomandi E, **Castiglioni A**, Melone M.A.B, Isidoro C. Resveratrol protects neuronal-like cells expressing mutant Huntingtin from Dopamine toxicity by rescuing ATG4-mediated autophagosome formation. Neurochem Int. 2017 May 19; S0197-0186(17)30243-7.
5. Ferraresi A, Titone R, Follo C, **Castiglioni A**, Chiorino G, Dhanasekaran DN, Isidoro C. The protein restriction mimetic Resveratrol is an autophagy inducer stronger than amino acid starvation in ovarian cancer cells. Mol Carcinog. 2017 Aug 30. [Epub ahead of print].

ABSTRACT

THE ROLE OF PTEN IN THE REGULATION OF GLUCOSE UPTAKE IN CANCER CELLS: IDENTIFICATION OF AKT AS NOVEL SUBSTRATE.

Castiglioni A*, Phadngam S, Ferraresi A, Follo C, Morani F, Vidoni C, Isidoro C.

Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100 - Novara (Italy). * Present adress: Program of Molecular Medicine, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy. Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, 20142 Milano, Italy.

Cancer cells consume glucose up to 200 times more than their normal counterparts do, and the glycolytic rate is even higher in cancer stem cells. The GLUT1-dependent glucose internalization sustains growth and survival for cancer stem cells and is essential for the maintenance of the stemness properties. Furthermore, the expression of GLUT1 has been linked functionally to chemoresistance, cell proliferation and metastasis formation. The PI3KCI-AKT pathway plays a major role in driving the translocation of GLUT1 on the plasmamembrane, and this pathway is frequently up-regulated in cancer. The phosphorylation of the lipid PIP2 in PIP3, mediated by PI3KCI, allows the activating phosphorylation ok AKT at Thr308. PTEN, through its lipid phosphatase activity, counteracts the PIP_2-PIP_3 conversion. PTEN possesses also a protein phosphatase activity. Yet, only few substrates of PTEN protein phosphatase are known. PTEN is a tumor suppressor gene very frequently mutated, silenced or deleted in human cancers; the most common mutations are C124S, G129E, K128_R130del and Y155C, which involve the exon 5, coding for the phosphatase domain. In this work, we demonstrated firstly that the Y155C mutant was expressed in the cell line OVCAR-3 and that this mutation leads to loss of both the lipid and protein phosphatase activities. We also found that PTEN is epigenetically silenced through histone de-acetylation in OAW42 cells. The VPA-mediated inhibition of histone de-acetylase could rescue PTEN expression and, consequently, down-regulated the AKT pathway and glucose uptake in these cells. More importantly, we found that AKT phosphorylated at Thr308 is a novel target of the PTEN protein phosphatase domain. By co-immunoprecipitation and in vitro de-phosphorylation essays we demonstrated that PTEN physically interacts with AKT and drives its phosphorylation. In this manner, PTEN controls the plasmamembrane expression of GLUT1 and the uptake of glucose, in absence of growth factors. In conclusion, our data emphasize the fact that PTEN acts in two distinct steps of the PI3K/AKT pathway to control the expression of GLUT1 at the plasmamembrane and, further, revealed AKT as novel substrate of PTEN protein phosphatase (6).

REFERENCES

1. Cairns RA et al. Regulation of cancer cell metabolism. Nat Rev Cancer. 2011; 11: 85-95. 2
2. Shibuya K et al. Targeting the facilitative glucose transporter GLUT1 inhibits the self-renewal and tumor-initiating capacity of cancer stem cells. Oncotarget. 2015; 6: 651-661.
3. Salmena L et al. Tenets of PTEN tumor suppression. Cell. 2008; 133: 403-144.
4. Saito M et al. Allelic imbalance and mutations of the PTEN gene in ovarian cancer. Int J Cancer. 2000; 85: 160-165.
5. Kato H et al. Functional evaluation of p53 and PTEN gene mutations in gliomas. Clin Cancer Res. 2000; 6:3973-3943.
6. Phadngam S, Castiglioni A et al. PTEN dephosphorylates AKT to prevent the expression of GLUT1 on plasmamembrane and to limit glucose consumption in cancer cells. Oncotarget. 2016; 7: 84999-85020.

RAFFAELLA COLELLA

raffaella.colella@studenti.unimi.it

Master student

Università degli Studi di Milano, Department of Health Science; Via Antonio di Rudinì, 8 - 20100 Milano

Education:

2015-present: Master degree in Medical Biotechnology and Molecular Medicine, University of Milan, Milan,

07/2014: Bachelor degree in Biotechnology, University of Verona , Verona, Italy.

Representative Careers:

02/2016- PRESENT: Master degree in Medical Biotechnology and Molecular Medicine, University of Milan, Milan,

01/2013- 07/2013: Bachelor trainee in Biotechnology at Policlinico G.B. Rossi, Verona, Italy.

09/2008-06/2010: Stage at research centre Biogem, Ariano Irpino (AV), Italy

Interesting Research Areas:

Multiple myeloma, Notch pathway, bone marrow microenvironment, cell cross-talk, extracellular vesicles, RNA editing

ABSTRACT

THE NOTCH PATHWAY DRIVES THE ABILITY OF THE BONE MARROW NICHE TO PROMOTE RNA EDITING IN MULTIPLE MYELOMA

Colella R.[1], Colombo M.[1], Barbieri M.[2], Baccianti F.[1], Lazzari E.[1,3], Todoerti K.[4], Crews L.A.[3], Jamieson C.H.M.[3], Neri A.[2], Chiaramonte R.[1]

[1]Department of Health Sciences, Università degli Studi di Milano, 20142 Milano, Italy. [2]Department of Oncology and Hemato-Oncology, Università degli Studi di Milano; Hematology Unit, Fondazione Cà Granda IRCCS Policlinico, 20122 Milano, Italy. [3]Division of Regenerative Medicine, Department of Medicine, Moores Cancer Center at University of California, San Diego, La Jolla, CA 92093, USA. [4]Laboratory of Pre-Clinical and Translational Research, IRCCS-CROB, Referral Cancer Center of Basilicata, Rionero in Vulture, Italy

INTRODUCTION Multiple myeloma (MM) is the second most frequently diagnosed hematological malignancy. MM is characterized by the accumulation of malignant plasma cells in the bone marrow. Here, the interaction with the normal cells of the surrounding microenvironment promotes tumor progression. Recently, RNA editing has emerged as one important mechanism which determines expression variability and therefore may promote myeloma cells growth and survival.

EXPERIMENTAL MODEL The MM cell line H929 was co-cultured with the BMSC line HS5. Notch1 signaling was inhibited in HS5 using anti-Notch1 specific siRNAs or 50uM DAPT. The use of GFP+ HS5 cell line allowed us to discriminate the two cell types in flow cytometry, while molecular analysis of H929 cells was carried out after immunomagnetic separation. ADAR1 expression in MM cells was analyzed by qRT-PCR and WB. RNA editing levels of ADAR1 target genes in H929 cells were determined by RESSqPCR assay.

RESULTS Our results indicate that BMSCs are able to upregulate ADAR1 in MM cells through the IL6/IL6R pathway and that this effect is reverted by Notch1 inhibition. RESSqPCR analysis shown that ADAR1 expression correlate with an increase in the RNA editing of genes involved in generation of molecular diversity such as APOBEC3D and AZIN1 and GLI1.

CONCLUSION These data provides the first evidence that Notch dysregulation in the bone marrow is responsible of the increased ADAR1 activity in MM cells. Further studies will allow us to better elucidate the effect of RNA editing on the ability of targeted mRNAs to promote tumor progression.

REFERENCES

1. Kyle RA, (2008) Blood 111: 2962-2972; 2. Colombo M, (2013) Leukemia 27: 1009-1018.; 3.Jiang Q, (2013) Proc Natl Acad Sci U S A 110: 1041-1046.; 4.Colombo M, (2016). Oncotarget 7(35):56013-29.; 5. Crews LA, J Transl Med (2015) 13: 52.

PAOLA D'ANTONA

p.dantona@uninsubria.it; paodantona@hotmail.it

PhD student in Biotechnology, Bioscience and Surgical Techniques, XXXI cycle

Department of Biotechnology and Life Sciences, DBSV, University of Insubria, Via J.H. Dunant 3, Varese, Italy

http://www.dbsm.uninsubria.it/dbsv/?page_id=695

Education:

10/2014-to date: PhD in Biotechnologies and Surgical Techniques. Dept of Biotechnology and Life Sciences, University of Insubria, Varese, Italy. Project title: "Serum microRNAs as biomarkers for early diagnosis of non small cell lung cancer".

04/2014-09/2015 Fellowship at the Invertebrate biology laboratory, University of Insubria, Varese, Italy

01/2011-10/2013 Master Degree in Biology (Biologia Applicata alla Ricerca Biomedica) at University of Insubria, Varese, Italy. Thesis title: "Caratterizzazione morfo-funzionale di sferoidi dalla linea cellulare di carcinoma mammario umano MCF7".

12/2009 Bachelor Degree in Biology (Biologia Cellulare-Molecolare) at University of Catania, Catania, Italy. Thesis title: "L'evoluzione dei cromosomi nei Primati: il cromosoma HSA-2".

Representative Careers:

10/2015-current: PhD student. Supervisor: Prof. Lorenzo Dominioni, tutors: Dr. P. Campomenosi and DM. Noonan, Dept of Biotechnology and Life Sciences. University of Insubria, Varese.

09/2014-10/2015 Laboratory Research. Supervisor: Prof. M de Eguileor. Dept of Biotechnology and Life Sciences. University of Insubria, via Dunant 3, 2100, Varese. 03/2012-03/2013 Laboratory Research. Supervisor: Prof. D. Noonan and Prof. M. de Eguileor. Dept of Biotechnology and Life Sciences. University of Insubria, Varese.

12/2012-12/2013 Laboratory Research. Supervisor: Prof. S. Saccone. Genetic Lab, via Androne 81, 95100, Catania (CT).

Interesting Research areas:

Circulating miRNA quantification. Lung cancer biology. Lung cancer biomarkers. Control of cancer cell growth in 3 D cultures.

Selected Publications:

1. Moretti F, **D'Antona P**, Finardi E, Barbetta M, Dominioni L, Poli A, Gini E, Noonan DM, Imperatori A, Rotolo N, Cattoni M, Campomenosi P. Systematic review and critique of circulating miRNAs as biomarkers of stage I-II non-small cell lung cancer Oncotarget. 2017.

2. Romoli O, Saviane A, Bozzato A, **D'Antona P**, Tettamanti G, Squartini A, Cappellozza S, Sandrelli F. Differential sensitivity to infections and antimicrobial peptide-mediated immune response in four silkworm strains with different geographical origin. Sci Rep. 2017 Apr 21;7(1):1048.
3. Campomenosi P, Gini E, Noonan DM, Poli A, **D'Antona P**, Rotolo N, Dominioni L, Imperatori A. A comparison between quantitative PCR and droplet digital PCR technologies for circulating microRNA quantification in human lung cancer. BMC Biotechnol. 2016 Aug 18;16(1):60.
4. Franzetti E, Casartelli M, **D'Antona P**, Montali A, Romanelli D, Cappellozza S, Caccia S, Grimaldi A, de Eguileor M, Tettamanti G. Midgut epithelium in molting silkworm: A fine balance among cell growth, differentiation, and survival. Arthropod Struct Dev. 2016 Jul;45(4):368-79.
5. Pulze L, Bassani B, Gini E, **D'Antona P**, Grimaldi A, Luini A, Marino F, Noonan DM, Tettamanti G, Valvassori R, de Eguileor M. NET amyloidogenic backbone in human activated neutrophils.

ABSTRACT

SERUM MIRNA AS BIOMARKERS FOR EARLY DIAGNOSIS OF NON SMALL CELL LUNG CANCER

Paola D'Antona[1], Elena Lombardi[1], Elisabetta Gini[1], Francesca Moretti[4], Emanuele Finardi[4], Marco Barbetta[4], Sarah Grossi[1], Lorenzo Dominioni[2], Douglas M. Noonan[3], Albino Poli[4], Paola Campomenosi[1,5]

[1]Department of Biotechnology and Life Science, DBSV, University of Insubria, Varese, Italy; [2]Department of Medicine and Surgery, DMS, Center for Thoracic Surgery, University of Insubria, Italy; [3]Scientific and Technological Pole, IRCCS MultiMedica, Milan, Italy; [4]Department of Diagnostic and Public Health, University of Verona, Verona, Italy; [5]The Protein Factory, Centro Interuniversitario di Ricerca in Biotecnologie Proteiche, Politecnico di Milano, ICRM-CNR Milano and University of Insubria, Varese, Italy

Lung cancer is the main cause of cancer-related mortality worldwide. Patients with early stage (I-II) non-small cell lung cancer (NSCLC), have a much better prognosis than those diagnosed at late stage. Thus, the development of sensitive and non-invasive methods for screening individuals at high risk for NSCLC is needed. microRNAs (miRNAs) have been suggested as a novel class of tumor biomarkers; their stability in biofluids and their change in levels in disease suggest their potential application as circulating biomarkers. Upon a critical review of the literature, we selected 8 miRNAs for a two-step screening for early lung cancer, based on their reported sensitivity and specificity and the fact that are not influenced by hemolysis[a]. Since smoking habit or inflammatory conditions may influence miRNA levels in serum, we quantified miRNAs of our panels in three groups of controls (non-smokers, smokers and COPD patients) and in stage I-II NSCLC patients. Droplet digital PCR was applied for quantification of miRNAs. The two-step screening is composed of a panel of 4 miRNAs endowed with high sensitivity and a second panel at high specificity. The scan. For 3 of the 6 miRNAs analyzed there was no significant difference among control subgroups (non-smokers, smokers, and subject with Chronic Obstructive Pulmonary Disease), whereas miRNA levels were expressed at significantly different levels in tumor and control groups, confirming their possible role as biomarkers previously proposed in the literature.

The selected miRNAs may help to identify high risk subjects who need further investigation for the presence of early NSCLC; in particular, miR-223 showed a good accuracy in distinguishing tumor samples from controls.

REFERENCES

1. Moretti F, D'Antona P, Finardi E, Barbetta M, Dominioni L, Poli A, Gini E, Noonan DM, Imperatori A, Rotolo N, Cattoni M, Campomenosi P. Systematic review and critique of circulating miRNAs as biomarkers of stage I-II non-small cell lung cancer Oncotarget. 2017.

SHARMILA FAGOONEE

sharmila.fagoonee@unito.it

CNR researcher

Institute of Biostructure and Bioimaging, CNR, Department of Molecular Biotechnology and Health Sciences, University of Turin, Italy

Education:

2013: M.Sc. in Molecular Biotechnology (University of Turin, Italy)

2005-2013: Post-doctorate at Molecular Biotechnology Center (Department of Genetics, Biology and Biochemistry, University of Turin)

2006: Master in Bioinformatics, first level (University of Turin)

2004: Ph.D. in Human Biology: Cell and Molecular biology (Department of Genetics, Biology and Biochemistry, University of Turin)

2002: Diploma (Honours) in Freelance and Feature Writing (London School of Journalism, London, UK)

1999: M.Sc. in Cellular Biology and Physiology: Molecular Biology and Genetics (University Bordeaux 2, France); Scholarship offered by the French Government.

1997: B.Sc. (Honours) degree in Biology with First Class, First Division (University of Mauritius)

1993: Cambridge Higher School Certificate (Dr Maurice Cure S.S.S): Subjects: General Paper; French; Biology; Chemistry; Mathematics (A level)

1991: Cambridge School Certificate (Loreto Convent St. Pierre): Subjects: English, Literature in English, French, Mathematics, Additional Mathematics, Biology and Chemistry (O level)

Representative Careers:

2014-present: Researcher at the Institute of Biostructure and Bioimaging (IBB) of the Italian National Research Council at Molecular Biotechnology Center in Turin

2015-present: assistant lecturer in Biology and Genetics (INT0643), M.Sc. in Medical Biotechnology, University of Turin

2011-2013 Research Fellowship, Institute of Biostructure and Bioimaging (IBB) of the Italian National Research Council at Molecular Biotechnology Center in Turin

2005-2011 Research Fellowship, Molecular Biotechnology Center, University of Turin

2008: Post-doc training: pluripotent spermatogonial stem cells derivation, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA.

2003: PhD training: Microarray technique and data analysis, Department of Pathology, Faculty of Medicine and Surgery, University of Naples II, Italy.

2002: Visiting scientist, Department of Medical Biochemistry, University of Aarhus, Denmark.

2002-2004: Doctorate Fellowship, Department of Genetics, Biology, and Biochemistry, University of Turin.

2000: Lecturer in Immunology, Department of Health and Medical Sciences, University of Mauritius, Mauritius.

Representative Awards:

- PRIN 2015 grantee as CNR research team leader; project: Regenerative potential of extracellular vesicles-derived from mesenchymal stem cells on epithelial wound healing (Prot. 201572SHXJ)

Interesting Research Areas:

Regenerative medicine: in vitro differentiation of adult stem cells in functional hepatocytes and assessment of their engraftment in vivo in animal models; correction of metabolic diseases of liver with stem cells as platform for gene therapy; Liver fibrosis: search for new therapies and biomarkers.

Cancer: study the role of RNA binding proteins in the progression of human gastrointestinal tumors

Selected Publications:

1. The RNA-binding protein ESRP1 promotes human colorectal cancer progression. **Fagoonee S**, Picco G, Orso F, Arrigoni A, Longo DL, Forni M, Scarfò I, Cassenti A, Piva R, Cassoni P, Silengo L, Tolosano E, Aime S, Taverna D, Pandolfi PP, Brancaccio M, Medico E, Altruda F. Oncotarget. 2017 Feb 7;8(6):10007-10024.

2. Prospects for Adult Stem Cells in the Treatment of Liver Diseases. **Fagoonee S**, Famulari ES, Silengo L, Camussi G, Altruda F. Stem Cells Dev. 2016 Sep 7. [Epub ahead of print]

3. Oncogenic ALK regulates EMT in non-small cell lung carcinoma through repression of the epithelial splicing regulatory protein 1. Voena C, Varesio LM, Zhang L, Menotti M, Poggio T, Panizza E, Wang Q, Minero VG, **Fagoonee S**, Compagno M, Altruda F, Monti S, Chiarle R. Oncotarget. 2016 May 31;7(22):33316-30.

4. Long Term Liver Engraftment of Functional Hepatocytes Obtained from Germline Cell-Derived Pluripotent Stem Cells. **Fagoonee S**, Famulari ES, Silengo L, Tolosano E, Altruda F. PLoS One. 2015 Aug 31;10(8).

5. The RNA binding protein ESRP1 fine-tunes the expression of pluripotency-related factors in mouse embryonic stem cells. **Fagoonee S**, Bearzi C, Di Cunto F, Clohessy JG, Rizzi R, Reschke M, Tolosano E, Provero P, Pandolfi PP, Silengo L, Altruda F. PLoS One. 2013 Aug 27;8(8)

ABSTRACT

THE RNA-BINDING PROTEIN ESRP1 PROMOTES HUMAN COLORECTAL CANCER PROGRESSION

Sharmila Fagoonee[1,2*], Gabriele Picco[3], Francesca Orso[2], Arrigo Arrigoni[4], Dario L. Longo[1,2], Marco Forni[5], Irene Scarfò[6], Adele Cassenti[7], Roberto Piva[6], Paola Cassoni[7], Lorenzo Silengo[2], Emanuela Tolosano[2], Silvio Aime[2], Daniela Taverna[2], Pier Paolo Pandolfi[2,8], Mara Brancaccio[2], Enzo Medico[3], Fiorella Altruda[2]

[1]IBB-CNR,Turin; [2]Molecular Biotechnology Center, University of Turin; [3] Candiolo Cancer Institute-IRCCS, University of Turin; [4] S.C. Gastroenterologia U, San Giovanni A.S., Turin; [5]EuroClone S.p.A Research Laboratory, Turin; [6]CERMS, University of Turin; [7]Department of Medical Sciences, University of Turin; [8]Cancer research institute, Harvard Medical School, Boston.

Epithelial splicing regulatory protein 1 (ESRP1) is an epithelial cell-specific RNA binding protein (RBP) that controls several key cellular processes. Previous studies have demonstrated a tumor suppressor role for this protein. Recently, however, a pro-metastatic function of ESRP1 has been reported. We thus aimed at clarifying the role of ESRP1 in Colorectal Cancer (CRC).

EXPERIMENTAL MODEL Loss- and gain-of-function studies were performed, and tumorigenesis and malignancy evaluated with *in vitro* and *in vivo* approaches.

RESULTS ESRP1-overexpression promoted anchorage-independent growth, enhanced FGFR1/2 signalling, Akt activation, and Snail upregulation in CRC cells. ESRP1 also promoted the ability of CRC cells to generate macrometastases in mice livers.

CONCLUSION High ESRP1 expression may stimulate growth of cancer epithelial cells and promote CRC progression, indicating that fine-tuning the level of this RBP could be relevant in modulating tumor growth in a subset of patients. We are now investigating the role of ESRP1 in other gastrointestinal cancers.

REFERENCES

1. The RNA-binding protein ESRP1 promotes human colorectal cancer progression. Oncotarget. 2017 Feb 7;8(6):10007-10024.

ALESSANDRA FERRARESI

alessandra.ferraresi@med.uniupo.it

PhD student in Medical Sciences and Biotechnology

Laboratory of Molecular Pathology and Nanobioimaging, Università del Piemonte Orientale, Department of Health Sciences, Via Solaroli 17, 28100 Novara (Italy)

Education:

June 2014 State Exam: Biologist Professional Qualification (section A), Università di Parma. Final mark: 175/200.

March 27th 2014 Master Degree in Pharmaceutical Biotechnology, Università di Bologna, Italy. Graduation mark: 110/110 *cum laude*.

September 29th 2011 Bachelor Degree in Biotechnology, Università di Parma, Italy. Graduation mark: 103/110.

2008 Scientific High School Diploma, ITGS "B. Pascal", Reggio nell'Emilia, Italy. Final mark: 94/100.

Representative Careers:

October 2017 Selected posters presentation at "22nd World Congress on Advances in Oncology and 20th International Symposium on Molecular Medicine", October 5th-7th 2017, Metropolitan Hotel, Athens, Greece

September 2017 Selected poster presentation at ABCD Congress 2017, September 21st-23rd 2017, Savoia Hotel Regency, Bologna, Italy

July 2017-August 2017 Research Internship at at Stephenson Cancer Center, The University of Oklahoma Health Sciences Center, 975 N.E. 10th Street, BRC West Oklahoma City, OK 73104, USA (Prof. Danny Dhanasekaran's Lab)

2016/2017 Lecturer in "Laboratorio di Fondamenti di Patologia generale e Immunologia", Università del Piemonte Orientale, Vercelli, Italy

October 2016 International Conference "Basic to Translational Medicine 2016: focus on cancer", October 6th-7th 2016, Università del Piemonte Orientale, Novara, Italy.

November 2015-to date PhD student in Medical Sciences and Biotechnology, Università del Piemonte Orientale, Department of Health Sciences, Laboratory of Molecular Pathology and Nanobioimaging, Novara, Italy (Mentor: Prof. Ciro Isidoro)

January 2015-October 2015 Fellowship recipient, Università del Piemonte Orientale, Department of Health Sciences, Laboratory of Molecular Pathology and Nanobioimaging, Novara, Italy (Mentor: Prof. Ciro Isidoro);

July 2014-December 2014 Stage, Università del Piemonte Orientale, Department of Health Sciences, Laboratory of Molecular Pathology and Nanobioimaging, (Mentor: Prof. Ciro Isidoro)

2013-2014 MS stage, Department of Pharmacology, University of Bologna (Mentor: Prof. Santi Mario Spampinato)

2011 BS stage, Department of Genetics, University of Parma (Mentor: Prof. Paola Goffrini)

Representative Awards:

- **2016** Gold award for young investigator in Cancer Research at International Conference "Basic to Translational Medicine 2016: focus on Cancer", Novara, October 7th-8th 2016
- **2015** Financial support of Comoli, Ferrari & C., Project title: "Ruolo e regolazione epigenetica dell'autofagia nella riprogrammazione genica delle cellule tumorali", Laboratory of Molecular Pathology and Nanobioimaging (Prof. Ciro Isidoro), Università del Piemonte Orientale, Novara, Italy

Interesting Research Areas:

Molecular mechanisms involved in cancer - autophagy - cancer stem cells biology - epigenetics - dormancy - cell migration - cancer microenvironment.

Selected Publications:

1. Thuwajit C, **Ferraresi A**, Titone R, Thuwajit P, Isidoro C. The metabolic cross-talk between epithelial cancer cells and stromal fibroblasts in ovarian cancer progression: Autophagy plays a role. Med Res Rev. 2017 Sep 19. doi: 10.1002/med.21473. [Epub ahead of print] Review. PubMed PMID: 28926101.
2. **Ferraresi A**, Titone R, Follo C, Castiglioni A, Chiorino G, Dhanasekaran DN, Isidoro C. The protein restriction mimetic Resveratrol is an autophagy inducer stronger than amino acid starvation in ovarian cancer cells. Mol Carcinog. 2017 Aug 30. doi: 10.1002/mc.22711. [Epub ahead of print] PubMed PMID: 28856729.
3. **Ferraresi A**, Phadngam S, Morani F, Galetto A, Alabiso O, Chiorino G, Isidoro C. Resveratrol inhibits IL-6-induced ovarian cancer cell migration through epigenetic up-regulation of autophagy. Mol Carcinog. 2017 Mar;56(3):1164-1181. doi: 10.1002/mc.22582. PubMed PMID: 27787915.
4. Phadngam S, Castiglioni A, **Ferraresi A**, Morani F, Follo C, Isidoro C. PTEN dephosphorylates AKT to prevent the expression of GLUT1 on plasmamembrane and to limit glucose consumption in cancer cells. Oncotarget. 2016 Dec 20;7(51):84999-85020. doi: 10.18632/oncotarget.13113. PubMed PMID: 27829222.

ABSTRACT

RESVERATROL COUNTERACTS THE PRO-INVASIVE ACTIVITY OF LYSOPHOSPHATIDIC ACID IN OVARIAN CANCER CELLS BY RESCUING AUTOPHAGY AND DOWN-REGULATING THE HEDGEHOG PATHWAY

Alessandra Ferraresi[1], Christian Seca[1], Andrea Castiglioni[1*], Suyanee Thongchot[1], Danny N Dhanasekaran[2], Giovanna Chiorino[3] and Ciro Isidoro[1#]

[1]Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", via Solaroli 17, 28100 - Novara (Italy). # E-mail: ciro.isidoro@med.uniupo.it ;[2]Stephenson Cancer Center and Department of Cell Biology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA. [3]Cancer Genomics Laboratory, Fondazione Edo ed Elvo Tempia, Biella, Italy. [*]Program of Molecular Medicine, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy. Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, 20142 Milano, Italy.

INTRODUCTION Ovarian cancer remains the most lethal gynecologic cancer with a five-year survival rate of 47% [1]. Lysophosphatidic acid (LPA) is a small bioactive phospholipid highly secreted by ovarian cancers that stimulates cell proliferation and tissue invasion of cancer cells [2]. Resveratrol is a dietary phytochemical with the potential to inhibit cancer cell migration through epigenetic up-regulation of autophagy [3].

RESULTS Here we show that LPA induces the Epithelial to Mesenchymal transition (EMT) through induction of the Hedgehog pathway and concomitant inhibition of autophagy in the cancer cells at the migration front. We found that Resveratrol and LPA regulate in an opposite fashion the expression of BMI-1, a polycomb transcriptional repressor belonging to the Hedgehog pathway, involved in cancer cell stemness and metastasis. Interestingly, BMI-1 silencing restores autophagy and halts ovarian cancer cell migration.

CONCLUSIONS Our data indicate that Resveratrol elicits its anti-tumour effect through induction of autophagy and down-regulation of BMI-1, and that this process is sufficient to inhibit cancer cell migration and invasion.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: a cancer journal for clinicians. 2016;66(1):7-30.
2. Jesionowska A, Cecerska-Heryc E, Matoszka N, Dolegowska B. Lysophosphatidic acid signaling in ovarian cancer. Journal of receptor and signal transduction research. 2015;35(6):578-584.
3. Ferraresi A, Phadngam S, Morani F, Galetto A, Alabiso O, Chiorino G, Isidoro C. Resveratrol inhibits IL-6-induced ovarian cancer cell migration through epigenetic up-regulation of autophagy. Mol Carcinog. 2017 Mar;56(3):1164-1181.

FOGLIA BEATRICE

beatrice.foglia@unito.it

PhD student

University of Torino, Department of Clinical and Biological Sciences, Corso Raffaello, 30, 10125 Torino (Italy)

Education:

2015: 2nd level Degree – Master in Cellular and Molecular Biology (LM-6), University of Torino (Italy), School of Natural Sciences – Department of Life Sciences and Systems Biology.

2012: 1st level Degree – Bachelor in Biological Sciences (L-13), University of Torino (Italy), Faculty of Science MFN

Representative Careers:

October 2015 to date: PhD student in Experimental Medicine and Therapy at the Department of Clinical and Biological Sciences (University of Torino, Italy).

October 2013: Student in Cellular and Molecular Biology at the Prof. M. Parola's laboratory, Department of Clinical and Biological Sciences (University of Torino, Italy). Sperimental thesis entitled: *Cross talk between SERPINB3 and HIF2α in human liver cancer cells: increased Neddylation versus decreased ubiquitination and proteasome degradation of HIF2α.*

Interesting Research Areas

The research activity is focused on the study of hypoxia and hypoxia-related mechanisms of liver pathogenesis and metabolic changes in "in vitro" and "in vivo" models of NAFLD/NASH, liver fibrosis, cirrhosis and HCC.

Selected Publications:

1. CannitoS, Morello E, Bocca C, **Foglia B**, Benetti E, Novo E, Chiazza F, Rogazzo M, Fantozzi R, Povero D, Sutti S, Feldstein AE, Albano E, Collino M, Parola M. Microvesicles Released from Fat-Laden Cells Promote Activation of Hepatocellular NLRP3 Inflammasome: a Pro-inflammatory Link between Lipotoxicity and Non-alcoholic Steatohepatitis. PLos ONE (2017), 2017 Mar 1;12(3):e0172575

ABSTRACT

ONCOSTATIN M INDUCES INCREASED INVASIVENESS AND ANGIOGENESIS IN HEPATIC CANCER CELLS THROUGH HIF1α-RELATED RELEASE OF VEGF-A.

Foglia B[1], Cannito S[1], Morello E[1], Turato C[2], Di Maira G[3], Novo E[1], Napione L[4,5], Alvaro M[4,5], Autelli R[1], Colombatto S[4], Bussolino F[4,5], Pontisso P[2], Marra F[3], Parola M[1].

[1]Dept. Clinical and Biological Sciences, University of Torino, Torino,Italy; [2]Dept. of Medicine, University of Padova, Padova,Italy; [3]Dept. Experimental and Clinical Medicine, University of Florence, Florence,Italy; [4]Dept of Oncology, University of Torino, Torino,Italy; [5]Dept. Of Oncology, Institute for Cancer Research and Treatment, University of Torino,Italy.

INTRODUCTION: Oncostatin M (OSM), a pleiotropic cytokine belonging to the interleukin-6 (IL-6) family, can modulate hypoxia-dependent liver processes contributing to chronic liver disease progression and hepatocellular carcinoma (HCC) development. Recently, hypoxia, as an independent signal operating through hypoxia-inducible factors (HIFs), has been shown to induce epithelial-to-mesenchymal transition (EMT) in cancer cells, including HepG2 cells. In this connection, OSM-related signaling pathway has been reported to up-regulate HIF1α and switch on EMT program. In this study we investigated in vivo and in vitro, the relationships between OSM, expression of vascular endothelial growth factor A (VEGF-A), and increased invasiveness.

EXPERIMENTAL MODEL. a) Cohort of HCC patients b) HepG2 cells exposed to human recombinant OSM (hrOSM) or stably transfected in order to overexpress OSM (H/OSM) or empty vector; c) murine xenograft.

RESULTS. OSM was expressed in HCC specimens and its expression correlates with early recurrence in HCC patients. HepG2 cells exposed to hrOSM or H/OSM cells show EMT-related changes, increased invasiveness and metallo-proteinase-2 activity within 48-72 hrs. Different experimental approaches revealed that 1) OSM-dependent invasiveness is due to HIF1α-dependent release of VEGF and involve activation of PI-3K, ERK1/2, and p38MAPK: 2) OSM affect cell proliferation blocking HepG2 cells in G0/G1 phase 3) OSM seems to promote angiogenesis in vivo (xenograft model) and in vitro (sprouting spheroid assay).

CONCLUSIONS OSM, expressed in human HCC, can induce EMT and increased invasiveness in human hepatic cancer cells through a mechanism involving HIF1α-dependent release of VEGF-A.

MARA GAGLIARDI

mara.gagliardi@uniupo.it

Ph.D student

University of Rome “Tor Vergata”

Education:

2009-2012: Bachelor Degree in Biology (100/110), University of Rome “Tor Vergata”

2013-2015: Master Degree in Cellular and Molecular Biology (110/110elode), University of Rome “Tor Vergata”

2016-2018: Ph.D student in Biology and Cellular Biology, University of Rome “Tor Vergata” and in collaboration with University of Eastern Piedmont (Novara)

Representative Careers:

2017: PKR and GCN2 promote ER stress-independent immunogenic cell death induction in melanoma cells

Interesting Research Areas:

To study new therapeutic approach of cell death to treat human cancer, in particular human skin melanoma

Selected Publications:

1. P. Giglio, **M. Gagliardi**, N. Tumino, F. Antunes, S. Smaili, M. Piacentini, M. Corazzari (OncoImmunology, SUBMITTED)

ABSTRACT

ALDO-KETO REDUCTASES CONFER RESISTANCE OF HUMAN SKIN MELANOMA TO FERROPTOSIS INDUCTION

Mara Gagliardi[1], Paola Giglio[1], Mauro Piacentini[1,2], Marco Corazzari[2,3]

[1]Dep of Biology, University of Rome 'Tor Vergata', Rome, IT. [2]Dep of Epidemiology and Translational Research, INMI-IRCCS 'L. Spallanzani', Rome, IT. [3]Dep of Health Science (DSS), University of 'Piemonte Orientale', Novara, IT

Cutaneous melanoma, the most aggressive form of skin cancer, remains one of the most difficult to treat human malignancies. Oncogenic mutations in the Ras/Raf pathway are mostly involved in melanoma, with $BRAF^{V600E}$ present in 40-60% of all melanomas. Notoriously unresponsive and resistant to therapy, metastatic melanoma is highly invasive and evolved a vast repertoire of molecular defences against immunological and cytotoxic attack. Hence, new and most effective therapies are urgently needed to treat this malignancy. Recently, a new form of cell death has been described with iron representing the main actor and lipid-ROS playing a pivotal role, named ferroptosis. Due to its peculiar signalling pathway and almost completely independence from classical apoptotic or related cell death programs, this pathway might represent a new valuable approach to treat resistant malignancies, such as human melanoma. We found that most melanoma cell lines are resistant to ferroptosis execution. However, all resistant cells are competent to induce the early stage of this cell death program as described by the early upregulation of the main ferroptotic marker CHAC1, indicating a downstream block of the signalling pathway. In fact, we identified the involvement of aldo-keto reductases (AKR1C1÷3) as potential detoxifying enzymes responsible for lipid-ROS reduction and, therefore, downstream inhibition of ferroptosis execution. Inhibiting the activity of these enzymes resulted in fact in the re-sensitization of all resistant cells to ferroptosis execution. Collectively our evidences indicate that the combined treatment of melanoma with a pro-ferroptotic agent together with an inhibitor of AKRs might represent a new valuable approach to treat this resistant human malignancy.

SILVIA GARAVELLI

silvia.garavelli@unimi.it

PhD Student

University of Milan, Dept. oh Health Science, Via di Rudinì 8 20142 Milano (Italy)

Education:

10/2014-present: Ph.D Student, University of Milan, Milan, Italy

07/2014: Ms in Medical Biotechnology and Molecular Medicine, University of Milan, Milan, Italy

06/2011: Bachelor Degree in Biotchnology, University of Milano-Bicocca, Milan, Italy

Representative Careers:

11/2010-02/2011: Bachelor trainee at Istituto Ortopedico Galeazzi and University of Milano-Bicocca, Milano, Italy. Research field: study of the differentiation of the human cell line Saos-2 into osteoblasts

09/2012-07/2014: Master trainee, Health Sciences Department, University of Milano, Milano, Italy. Research field: Evaluation of the role of Notch signaling in multiple myeloma induced osteoclastogenesis and osteolytic activity: molecular characterization of the signaling pathways involved.

04/2014-06/2014: Visiting student, Institute of Infection, Immununity and Inflammation, University of Glasgow, Glasgow. Research field: isolation, characterization and culturing of mesenchymal bone marrow stromal cells from myeloma patients.

10/2014- PRESENT: PhD student at the Molecular and Translational Medicine PhD Program, Department of Health Sciences, University of Milano, Milano, Italy. Research field: Evaluation of the role of Notch signaling in cross-talk of myeloma cells with bone marrow niche: focus on drug resistance induced by bone marrow stromal cells stromal cells.

Representative Awards:July/2015: Immunotools Special Award 2015

Interesting Research Areas: Multiple myeloma, Notch pathway, drug resistance, bone marrow microenvironment, cell cross-talk

Selected Publications:

1. Platonova N., Lesma E., Basile A., Bignotto M., **Garavelli S.**, Palano M. T., Moschini A., Neri A., Colombo M. and Chiaramonte R.. Targeting Notch as a therapeutic approach for human malignancies. Curr Pharm Des. 2016 Oct 6. PMID: 27719637.
2. Colombo M., Galletti S., **Garavelli S.**, Platonova N., Paoli A., Basile A., Taiana E., Neri A., Chiaramonte R.; "Notch signaling deregulation in multiple myeloma: A rational molecular target."Oncotarget. 2015 Sep 29;6(29):26826-40.
3. Chiaramonte R., Colombo M., Bulfamante G., Falleni M., Tosi D., **Garavelli S.**, De Simone D., Vigolo E., Todoerti K., Neri A., Platonova N.; "Notch pathway promotes ovarian cancer growth and migration via CXCR4/SDF1α chemokine system."Int J Biochem Cell Biol. 2015 Sep;66:134-40
4. Platonova N., Manzo T., Mirandola L., Colombo M., Calzavara E., Vigolo E., Cermisoni G.C., De Simone D., **Garavelli S.**, Cecchinato V., Lazzari E., Neri A., Chiaramonte R.; "PI3K/AKT signaling inhibits NOTCH1 lysosome-mediated degradation." Genes Chromosomes Cancer. 2015 Jun 6; 54:516–526
5. Colombo M.**,** Thümmler K., Mirandola L., **Garavelli S.**, Todoerti K., Apicella L., Lazzari E., Lancellotti M., Platonova N., Akbar M., Chiriva-Internati M., Soutar R., Neri A., Goodyear C.S., Chiaramonte R.; "Notch signaling drives multiple myeloma induced osteoclastogenesis". Oncotarget 2014 Nov; 5: 10393-406.

ABSTRACT

NOTCH PATHWAY AND MULTIPLE MYELOMA-ASSOCIATED DRUG RESISTANCE: THE ROLE OF BONE MARROW STROMAL CELLS

Garavelli S.[1], Lazzari E.[1,2], Colombo M.[1], Palano M. T.[1], Neri A.[3], Chiaramonte R.[1]

[1]Department of Health Sciences, University of Milano, via Di Rudinì 8, I-20142 Milano, Italy. [2]Division of Hematology/Oncology, Department of Medicine, University of California, San Diego, La Jolla, CA, USA. [3]Department of Oncology and Hemato-oncology, University of Milano; Hematology, Fondazione Cà Granda IRCCS Policlinico, Milano, Italy

Multiple myeloma (MM) is an incurable hematological cancer characterized by the localization of malignant plasma cells in the bone marrow (BM). In MM, the expression of two Notch ligands, Jag1 and 2, is dysregulated causing an aberrant activation of the pathway either in MM cells or in BM stromal cells (BMSCs). MM cells are dependent on the BMSCs, which sustain MM cells proliferation and mediate drug resistance (DR) through direct contact or the release of soluble factors. Our previous results point out that Notch pathway knock-down in MM cells results in: i) decreased expression of anti-apoptotic proteins, ii) increased sensitivity of MM cells to drugs. Here we aim to investigate if downstream mediators of Notch pathway may play a role in BMSCs supportive effect. Jag ligands expression was silenced in MM cell lines with Jag1/2 siRNAs while Notch1 expression was inhibited by transfecting Notch1 siRNAs in HS5 BM stromal cell line. Cell line transfected with scrambled siRNAs were used as control. MM cells silenced or not were co-cultured with NIH3T3 or HS5 cell lines. Protein and gene expression variations were assessed by flow cytometry and qRT-PCR, respectively. Co-culture experiments of MM cells and BMSCs demonstrate that MM cells are able to activate Notch pathway in BMSCs resulting in the release of SDF-1α and IL-6; MM cells silenced for Jagged1 are no more able to induce BMSCs to secrete SDF-1α and IL-6. The production of SDF-1α and IL-6 has been confirmed to be Notch dependent since Notch1 silencing in HS5 cells results in the lost of their ability to release soluble factors crucial for MM cells proliferation and DR. Our findings demonstrate that MM cells are able to “educate” BMSCs to release soluble factors crucial for malignant cell survival and DR in a Notch-dependent manner; it is crucial to find new therapies to selectively inhibit Notch-Jag interaction to overcome MM-associated DR.

REFERENCES:

Colombo M., Galletti S., Garavelli S., Platonova N., Paoli A., Basile A., Taiana E., Neri A., Chiaramonte R.; “Notch signaling deregulation in multiple myeloma: A rational molecular target.”Oncotarget. 2015 Sep 29;6(29):26826-40

SARAH GROSSI

s.grossi1@uninsubria.it, sarah.grossi89@gmail.com, paola.campomenosi@uninsubria.it

PhD student in Biotechnology, Bioscience and Surgical Techniques, XXXII cycle

Department of Biotechnology and Life Sciences, DBSV, University of Insubria, Via J.H. Dunant 3, Varese, Italy

http://www.dbsm.uninsubria.it/dbsv/?page_id=695

Education:

11/2016-to date: PhD in Molecular and Food Biotechnologies. Dept of Biotechnology and Life Sciences, University of Insubria, Varese, Italy. Project title: "Study of regulatory factors in Non Small Cell Lung Cancer: a role for Proline Dehydrogenase?".

04/2015-10/2016 Fellowship at the Molecular Genetics laboratory. Study title: "Expression of matrix degrading enzymes and other markers by different cell types grown on artificial or biological materials", University of Insubria, Varese, Italy

01/2014-12/2014 Master Degree in Molecular and Industrial Biotechnology at University of Insubria, Varese, Italy. Thesis title: "La suscettibilità alla rinosinusite cronica: studio genetico e funzionale del ruolo del recettore del gusto amaro T2R38".

02/2012-10/2012 Bachelor Degree in Biological Sciences at University of Insubria, Varese, Italy. Thesis title: "Caratterizzazione elettrofisiologica del ruolo del residuo Thr327 nel trasportatore di oligopeptidi PepT1".

Representative Careers:

11/2015-current: PhD student. Supervisor: Prof. Loredano Pollegioni, tutor: Dr. P. Campomenosi, Dept of Biotechnology and Life Sciences. University of Insubria, Varese.

01/2014-12/2014 Laboratory Research. Supervisor: Prof. Paola Campomenosi. Dept of Biotechnology and Life Sciences. University of Insubria, Varese.

02/2012-12/2013 Laboratory Research. Supervisor: Prof. E. Bossi. Cellular and Molecular Physiology Lab, University of Insubria, Varese.

Interesting Research areas:

Genes involved in cancer cell growth control; Proline dehydrogenase; lung cancer; gene expression regulation.

Selected Publications:

1. Guarino MP, Altomare A, Barera S et al. Effect of Inulin on Proteome Changes Induced by Pathogenic Lipopolysaccharide in Human Colon. PLoS One. 2017 Jan 9;12(1): e0169481.
2. Gallo S, **Grossi S**, Montrasio G et al. TAS2R38 taste receptor gene and chronic rhinosinusitis: new data from an Italian population. BMC Med Genet. 2016 Aug 11;17(1):54.

ABSTRACT

PROLINE DEHYDROGENASE EXPRESSION AND REGULATION IN NON-SMALL CELL LUNG CARCINOMA

Sarah Grossi[1], Arianna Parnigoni[1], Paola D'Antona[1], Alessandro Calicchio[1], Eleonora Nigro[1], Alessia Gasparini[1], Marco Giagnolini[1], Roberto Mainini[1], Anna Maria Chiaravalli[2], Fausto Sessa[2,3], Paola Campomenosi[1,4]

[1]Department of Biotechnology and Life Sciences, DBSV, University of Insubria, Varese, Italy; [2]Pathology Unit, Ospedale di Circolo e Fondazione Macchi, Varese, Italy; [3]Department of Surgical and Morphological Sciences, DSCM, University of Insubria, Varese, Italy; [4]The Protein Factory, Centro Interuniversitario di Ricerca in Biotecnologie Proteiche, Politecnico di Milano, ICRM-CNR Milano and University of Insubria, Varese, Italy

Non-Small Cell Lung Cancer (NSCLC) is one of the most frequent and deadliest 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. We investigated if proline dehydrogenase (PRODH), a mitochondrial flavoenzyme catalyzing the key step in proline degradation and involved in the regulation of cell survival, autophagy and apoptosis, may be one of such markers.

We characterized PRODH expression in NSCLC by immunohistochemistry and qPCR and tested if there was correlation between expression of PRODH and clinical features of the tumors or expression of other markers. We also tested the role of TTF-1 as transactivator of the *PRODH* gene in two NSCLC cell lines by transfection experiments and expression analyses. Moreover, putative TTF-1 response elements, bioinformatically predicted in the *PRODH* promoter, were cloned and tested in luciferase reporter assays.

We found PRODH immunostaining in the majority (70%) of lung ADCs. Patients with PRODH positive tumors had better overall survival than those with negative tumors. Protein staining was paralleled by high transcript levels. TTF-1, a transcription factor essential for thyroid and lung development and physiology, showed similar expression to PRODH and putative TTF-1 response elements were found in *PRODH* promoter. This prompted us to investigate if *PRODH* could be a target of TTF-1. Transfection of a TTF-1 expression construct into ADC cell lines led to an increase in PRODH transcript and luciferase reporter assays showed that one PRODH RE was transactivated by TTF-1.

Our data support a possible application of PRODH immunostaining as a prognostic marker and warrant further research aimed to investigate PRODH transactivation by TTF-1 and the role of PRODH in the biology of NSCLC.

ANGELICA LAPADULA

angelica.lapadula@hotmail.com

Master degree

Università del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze ed Innovazione Tecnologica, via Teresa Michael 11, Alessandria

Education:

2017 – Master Degree (MSc) in Biology, Biomolecular / Biomedical curriculum, Università del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze ed Innovazione Tecnologica, via Teresa Michael 11, Alessandria

2015 – Bachelor Degree (BSc) in Biological Sciences, Università del Piemonte Orientale "Amedeo Avogadro", Dipartimento di Scienze ed Innovazione Tecnologica, via Teresa Michael 11, Alessandria

2014 – Erasmus Student Universidad de Granada, Facultad de Ciencias, Avenida de la Fuente Nueva S/N, Granada (Spain)

Representative Careers:

2017- Laboratory of Molecular Imaging, Department of Molecular Biotechnology and Health Sciences, University of Turin, Molecular Biotechnology Center, via Nizza 52, Torino (TO)

2014/2015 –Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, Università del Piemonte Orientale "Amedeo Avogadro", via Solaroli 17, Novara (NO)

2014 – Laboratory of Immunology and Molecular Pathology, Department of Sciences, Universidad de Granada, Avenida de la Fuente Nueva S/N, Granada (Spain)

Interesting Research Areas:

Cancer, Authophagy, Nanoparticles, MRI reporter

ABSTRACT

BIOORTHOGONAL COPPER-FREE CLICK CHEMISTRY WITH LABELLED SILICA NANOPARTICLES AND GADOLINIUM COMPOUNDS FOR MRI ANALYSIS IN SIALIC ACID OVEREXPRESSING TUMOUR CELL LINES

Angelica Lapadula[1], Diego Alberti[2], Simonetta Geninatti Crich[2], Lorenzo Tei[3], Silvio Aime[2] and Ciro Isidoro[1]

[1]Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, University of Eastern Piedmont (Novara, Italy) [2]Department of Molecular Biotechnology and Health Sciences, University of Turin (Torino, Italy) [3]Laboratory of Chemistry, Department of Science and Technological Innovation, University of Eastern Piedmont (Alessandria, Italy)

Sialic acid residues are abnormally highly expressed in tumour cells, including human breast cancer MCF7, murine mammary adenocarcinoma TS/A, and mesothelioma ZL34 cells (1). We aimed to build Gadolinium (Gd)-containing silica nanoparticles (NPs) that could be targeted to cancer cells through the specific recognition of sialic acid residues. Using metabolic glycoengineering, cells can express unnatural sialic acid residues with azide groups by treatment with the tetraacetylated N-azidoacetyl-D-mannosamine (Ac4ManNAz). Azide groups can be selectively identified by a cyclooctyne attached to the surface of activated Gd silica NPs doped with Rhodamine. We first used ADIBO-FITC (azadibenzocyclooctyne-fluorescein) to confirm the selectivity of this click chemistry reaction.

Preliminary data show that treated cells exhibit an enhanced capability to bind ADIBO and NPs. Data were obtained by fluorimetry, fluorescence microscopy and mass spectrometry. Fluorescence microscopy revealed that both ADIBO and NPs could label the surface of the cells. Furthermore, it is possible to directly target sialic acid via phenylboronic acid vectors conjugated with an MRI reporter (2). Tumours can be discriminated by the highly selective binding between the diol function of sialic acid and a Gd-dimer-F_2-phenylboronic acid conjugated through an ethylenediamine (en) spacer. Sialic acid overexpressing tumour cell lines, such as MCF7 and ZL34 could strongly bind to Gd dimer compared to non-tumorigenic cell lines, as shown by MRI analysis.

This study demonstrates the potential of metabolic glycoengineering combined with click chemistry to enhance the targeting to cancer cells.

REFERENCES

1. Yoon HY et al., Molecular imaging based on metabolic glycoengineering and bioorthogonal click chemistry. Biomaterials. 2017; 132:28.
2. Geninatti Crich S et al., MRI visualization of melanoma cells by targeting overexpressed sialic acid with a Gd(III)-dota-en-pba imaging reporter. Angew Chem Int Ed Engl. 2013; 52:1161.

MIRIAM MARTINI

miriam.martini@unito.it

Postdoctoral Fellow

University of Torino, Dept. of Molecular Biotechnology and Health Sciences.

Education

26/07/2017-26/07/2023: Abilitazione Scientifica Nazionale alle funzioni di professore universitario di seconda fascia (Associate Professor) per il settore concorsuale 05/F1 – biologia applicata

January 2009: PhD degree in Cell Science and Technology. University of Torino, IRCCS, Molecular Genetics Laboratory, Dept. of Oncological Sciences, Candiolo (TO), Italy

July 2004: Master Degree in Medical Biotechnology. Final mark 110/100. University of Torino, Dept. of Anatomy, Pharmacology and Forensic Medicine, Laboratory of developmental Neuroanatomy, Turin, Italy; Score 110/110

Representive Careers

July 2017- present: Postdoctoral Fellow. Supervisor: prof. Emilio Hirsch. University of Torino, Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences, Torino, Italy

July 2016-2017: Postdoctoral Fellow (Post-Doctoral Fellowship-year 2016, Fondazione Umberto Veronesi). Supervisor: prof. Emilio Hirsch. University of Torino, Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences, Torino, Italy

June 2015-June 2016: Postdoctoral Fellow (Post-Doctoral Fellowship-year 2015, Fondazione Umberto Veronesi). Supervisor: prof. Emilio Hirsch. University of Torino, Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences, Torino, Italy.

October 2011-June 2015: Assegnista di Ricerca (BIO/13 05) (art. 22 della Legge 240/2010) Supervisor: prof. Emilio Hirsch. University of Torino, Molecular Biotechnology Center, Department of Molecular Biotechnology and Health Sciences, Torino, Italy

December 2009-September 2011: Assegnista di Ricerca (BIO/17 05) (art.51, comma 6 della Legge 27 /I 2/l 997 n. 449) Supervisor: prof. Alberto Bardelli. University of Torino, Dept. of Oncological Sciences, IRCCS, Candiolo (TO), Italy.

Representative Awards:

- SIC (Società Italiana Cancerologia) member since 2011
- EACR (European Association for Cancer Research) member since 2011
- Reviewer activity for international journals
- 2013: Premio Giovedì Scienza, Second edition, Finalist (3° Prize)
- 2014: Premio Giovani Ricercatori 2014 (Young Researchers Prize), Fondazione Guido Berlucchi

- 2015: Travel grant at the ABCD meeting, Cell Biology of Disease: Cancer, Parma, Italy
- 2015: Grant "Post-Doctoral Fellowship-year 2015, Fondazione Umberto Veronesi
- 2016: Grant "Post-Doctoral Fellowship-year 2016, Fondazione Umberto Veronesi
- 2017: Member of the Editorial Board of Biotechnology and SCIOLBiotechnology

Selected Pubblication

1. Gulluni F*, **Martini M***,#, De Santis MC*, Campa CC, Ghigo A, Margaria JP, Ciraolo E, Franco I, Ala U, Annaratone L, Di Salvatore D, Bertalot G, Viale G, Noatynska A, Compagno M, Sigismund S, Montemurro F, Thelen M, Fan F., Meraldi P, Marchiò C, Pece S, Sapino A, Chiarle R , Di Fiore PP and Hirsch E#. Mitotic spindle assembly and genomic stability in breast cancer require PI3K-C2 scaffolding function (2017). Cancer Cell *contributed equally this work; # corresponding author.
2. Costa C, Ebi H, **Martini M**, Beausoleil SA, Faber AC, Jakubik CT, Huang A, Wang Y, Nishtala M, Hall B, Rikova K, Zhao J, Hirsch E, Benes CH, Engelman JA. Measurement of PIP3 levels reveals an unexpected role for p110β in early adaptive responses to p110α-specific inhibitors in luminal breast cancer. Cancer Cell. 2015 Jan 12;27(1):97-108.
3. **Martini M**, Russo M, Lamba S, Vitiello E, Crowley EH, Sassi F, Romanelli D, Frattini M, Marchetti A, Bardelli A (2013). Mixed lineage kinase MLK4 is activated in colorectal cancers where it synergistically cooperates with activated RAS signaling in driving tumorigenesis. CANCER RESEARCH, vol. 73, p. 1912-1921.
4. Bottos A*, **Martini M*,** Di Nicolantonio F, Comunanza V, Maione F, Minassi A, Appendino G, Bussolino F, Bardelli A. (2012). Targeting oncogenic serine/threonine-protein kinase BRAF in cancer cells inhibits angiogenesis and abrogates hypoxia. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 109, p. E353-E359.*contributed equally this work.
5. Di Nicolantonio F*, **Martini M*,** Molinari F*, Sartore-Bianchi A, Arena S, Saletti P, De Dosso S, Mazzucchelli L, Frattini M, Siena S, Bardelli A (2008). Wild-Type BRAF Is Required for Response to Panitumumab or Cetuximab in Metastatic Colorectal Cancer. JOURNAL OF CLINICAL ONCOLOGY, vol. 26, p. 5705-5712.*contributed equally this work.

ABSTRACT

MITOTIC SPINDLE ASSEMBLY AND GENOMIC STABILITY IN BREAST CANCER REQUIRE PI3K-C2α SCAFFOLDING FUNCTION

Miriam Martini[1], Federico Gulluni[1], Maria Chiara De Santis[1], Laura Annaratone[2], Davide Disalvatore[3], Giovanni Bertalot[4], Salvatore Pece[4,5], Filippo Montemurro[6], Caterina Marchiò[2], Anna Sapino[7], Roberto Chiarle[1,8], Pier Paolo Di Fiore[3,4,5] and Emilio Hirsch[1]

[1]Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Torino, Italy. [2]Department of Medical Sciences, University of Turin, Turin, Italy; Pathology Unit, Department of Laboratory Medicine, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Turin, Italy. [3]IFOM, the FIRC Institute for Molecular Oncology Foundation, Milan, Italy. [4]Program Of Molecular Medicine, IEO, European Institute of Oncology, Milan, Italy. [5]Department of Oncology and Hemato-oncology (DIPO), University of Milan, Milan, Italy. [6]Unit of Investigative Oncology, IRCC Candiolo. [7]Department of Medical Sciences, University of Turin, Turin, Italy; Unit of Pathology, Fondazione Piemontese per l'Oncologia (FPO), IRCCs Candiolo, Italy. [8]Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, USA.

Proper organization of the mitotic spindle is key to genetic stability but molecular components of inter-microtubule bridges that crosslink kinetochore fibers (K-fibers) are still largely unknown.

We generated a mouse strain lacking PI3KC2A expression and found that the mutation is embryonic lethal. *Pik3c2a*$^{+/-}$ mice were intercrossed with a transgenic strain expressing the activated HER2/Neu oncogene in the mammary gland. We derived mouse embryonic fibroblast (MEF) and Primary Murine Mammary Epithelial Tumor cells (MMET). Effects of heterozygous loss of *Pik3c2a* were evaluated by biological, biochemical and immunofluorescence analysis. Here we identify a kinase-independent function of class II phosphoinositide 3-OH kinase α (PI3K-C2α) acting as scaffold protein organizing clathrin and TACC3 complex crosslinking K-fibers. Downregulation of PI3K-C2α causes spindle alterations, delayed anaphase onset and aneuploidy, indicating that PI3K-C2α expression is required for genomic stability. Reduced abundance of PI3K-C2α in breast cancer models initially impairs tumor growth but later leads to the convergent evolution of fast growing clones with mitotic checkpoint defects. As a consequence of altered spindle, loss of PI3K-C2α increases sensitivity to Taxane-based therapy in preclinical models and in neoadjuvant settings.

Our study shows that low PI3K-C2α associated with the loss of a scaffold function stabilizing K-fibers, the Taxanes targets. Overall, these observations will open the way to more accurate patient stratification in neoadjuvant regimens.

REFERENCES

1) PI3K class II α controls spatially restricted endosomal PtdIns3P and Rab11 activation to promote primary cilium function. Franco I, Gulluni F, Campa CC, Costa C, Margaria JP, Ciraolo E, Martini M, Monteyne D, De Luca E, Germena G, Posor Y, Maffucci T, Marengo S, Haucke V, Falasca M, Perez-Morga D, Boletta A, Merlo GR, Hirsch E. *Dev Cell*. 2014 Mar 31;28(6):647-58

JOYS AFRAKOMA OBENG

joyce.obeng@med.uniupo.it

Education

2014-present: PhD in Sciences and Medical Biotechnology (2014-Present). Università del Piemonte Orientale, Novara (Italy)

2012-2014: Università del Piemonte Orientale, Novara (Italy)

2009-2012: Bachelor in Biotechnology Università del Piemonte Orientale, Novara (Italy)

Representive Careers:

2015-present: West Virginia University, Morgantown, WV (USA): Visiting Research Scholar Desmoplakin expression induced by the novel prolyl hydroxylase-3 inhibitor AKB-6899 suppresses breast cancer cell migration and promotes aggregation. We are studying the effect of a novel small molecule inhibitor of oxygen-sensing prolyl hydroxylase-3 (PHD3), AKB-6899, on the expression of Desmoplakin in triple negative breast cancer cells

2014-2015: Pharmacology Laboratory, Università del Piemonte Orientale, Novara (Italy). Modulation of monocytes / macrophages by Abatacept, Etanercept and Tocilizumab, biotech drugs used in the treatment of Rheumatoid Arthritis (RA).

May 2013- August 2013: Erasmus Project, Institute of Infection and Immunity, Cardiff University School of Medicine, UK. Generation of a Vδ1 T Cell line. The project was focused on the Isolation, stimulation and expansion of Vδ1 T Cells from PBMCs obtained from healthy donors and assessment of their APC characteristics.

2009-2012: Immunology Laboratory, Università del Piemonte Orientale, Novara (Italy), (2009-2012). We studied the role of ICOS gene variants in ICOS costimulatory activity in Multiple Sclerosis.

Selected Publication

1. **Joyce Afrakoma Obeng**, Luigia Grazia Fresu, Daniele Sola, Angela Amoruso, Gian Luca Ermanno Camaschella, Sandra Brunelleschi "Modulation of human monocyte/macrophage activity by tocilizumab, abatacept and etanercept: an in vitro study" European Jounal of Pharmacology, S0014-2999(16)30151-0. doi: 10.1016/j.ejphar.2016.03.028 (2016)
2. Angela Amoruso, Daniele Sola, Luca Rossi, **Joyce Afrakoma Obeng**, Luigia Fresu, Pier Sainaghi, Mario Pirisi, "Relation among anti-rheumatic drug therapy, CD14+CD16+ blood monocytes and disease activity markers (DAS28 and US7 scores) in rheumatoid arthritis: a pilot study"
3. **Joyce Afrakoma Obeng,** Tyler Nelson, DuaaDakhlallah, Ivory L. Patterson, Amy Gross, Randall Evans, Clay B. Marsh, John J. Lannutti, Tim D. Eubank "Desmoplakin expression induced by the novel prolyl hydroxylase-3 inhibitor AKB-6899 suppresses breast cancer cell migration and promotes aggregation" *In preparation*
4. Tierra A. Ware, Duaa Dakhlallah, **Joyce Afrakoma Obeng**, Jonathan Kropski, Timothy Blackwell, Timothy D. Eubank, Clay B. Marsh. Examining Epigenetic Regulation of TERT in IPF. *In preparation*
5. Tierra A. Ware**,** Duaa Dakhlallah**, Joyce Afrakoma Obeng**, Timothy Blackwell, Timothy D. Eubank, Clay B. Marsh. TGFβ1 Augments DNA methylation in IPF. *In preparation*

ABSTRACT

INDUCTION OF DESMOPLAKIN EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER CELLS BY PROLYL HYDROXYLASE-3 INHIBITOR AKB-6899

Joyce A. Obeng[1,2], Tyler Nelson[3], Duaa Dakhlallah[1,] Ivory L. Patterson[3], Amy Gross[3], Randall Evans[3], Luigia G. Fresu[3], Clay B. Marsh[1], John J. Lannutti[3], Tim D. Eubank[1,3]

[1]West Virginia University, Morgantown, WV; [2]Università del Piemonte Orientale, Novara, Italy; [3]The Ohio State University Columbus, OH

INTRODUCTION. Desmoplakin *(DSP)* is a member of the plakin family and major protein component of the desmosome. It serves as the anchoring protein for keratin cytoskeletal filaments and cadherin proteins to maintain strong adhesive junctions between cells (1). Clinically, DSP expression is reduced as breast cancer progresses and its loss predicts a poor prognosis and increased risk of metastasis (1,2).

EXPERIMENTAL MODEL. Metastatic human breast tumor cells (MDA-MB231) were used to evaluate: 1) the ability of a novel small molecule inhibitor of prolyl hydroxylase-3, AKB-6899, to induce expression of DSP; 2) DSP function in regulating tumor cell migration on a 2D 96 well-radius plate and on 3D nanofiber coated plates, and aggregation by hanging drop assay; 3) toxicity on breast tumor cells; and 4) its potential as a treatment for metastatic breast cancer using patient-derived xenografts (PDX) in NOD/SCID/IL2Rγ−/− (NSG) mice.

RESULTS. We demonstrated, by qRT-PCR and by Microarray analysis, that AKB-6899 augments DSP mRNA expression in both mouse and human breast cancer cells followed by significant increase of tumor cell aggregation and decrease of cell migration. These effects were observed using concentrations of AKB-6899 that are not toxic to the cells as shown by Trypan Blue exclusion assays.

CONCLUSION. In summary, our pre-clinical data supports the potential for AKB-6899 as a novel therapy for metastatic breast cancers that lose DSP expression as they progress.

REFERENCES

1. Davies EL et al., Eur J Cancer 1999, 35:902-907; 2) Van Zijl F et al., Mutat Res 2011, 728:23-34; 3) Friedl P and Wolf K. Nat Rev Cancer 2003, 3:362-7

FRANCESCA OLTOLINA

francesca.oltolina@med.uniupo.it

Postdoctoral Research Fellow

Università del Piemonte Orientale "A. Avogadro", Department of Health Sciences; via Paolo Solaroli 17 – 28100, Novara (Italy)

Education:

2010 - Bachelor in Biotechnology (B.Sc.) with a score of 97/110, Scuola di Medicina, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100, Novara, Italy

2012 - Master in Medical Biotechnology (M.Sc.) with a score of 110/110 *cum laude*, Scuola di Medicina, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100, Novara, Italy

2016 – Doctor of Philosophy (PhD) in Biotechnologies for Human Health, Scuola di Medicina, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100, Novara, Italy

Representative Awards:

- Prof. Andrea Facchini Young Investigator Award for the Oral Presentation at VII Meeting Stem Cell Research Italy 2016
- ImmunoTools special Award 2014
- 1st Prize Poster Competition and accommodation grant at 4th International School on Biological Crystallization, Granada, Spain 2013.

Interesting Research Areas:

- Targeted nanoparticles for drug delivery to tumour cells.
- Adult stem cells and tissue engineering applied to myocardium.
- Tumour associated markers and oncoproteins in human tumors.
- Monoclonal antibodies as probes and biomimetic tools.

Selected Publications:

1. **Oltolina F**, Zamperone A, Colangelo D, Gregoletto L, Reano S, Pietronave S, Merlin S, Talmon M, Novelli E, Diena M, Nicoletti C, Musarò A, Filigheddu N, Follenzi A, Prat M. Human Cardiac Progenitor Spheroids Exhibit Enhanced Engraftment Potential. PLoS One. 2015 Oct 23;10(10):e0141632. doi:10.1371/journal.pone.0141632. eCollection 2015. PubMed PMID: 26495969; PubMed Central PMCID: PMC4619885.
2. **Oltolina F**, Gregoletto L, Colangelo D, Gómez-Morales J, Delgado-López JM, Prat M. Monoclonal antibody-targeted fluorescein-5-isothiocyanate-labeled biomimetic nanoapatites: a promising fluorescent probe for imaging applications. Langmuir. 2015 Feb 10;31(5):1766-75. doi: 10.1021/la503747s. Epub 2015 Jan 30. PubMed PMID:25602940.

3. Martínez-Casado FJ, Gómez Morales J, Delgado López JM, Iafisco M, Martínez Benito C, Ruiz Pérez C, Colangelo D, **Oltolina F**, Prat M. Bio-inspired citrate-apatite nanocrystals doped with divalent transition metal ions. Crystal Growth & Design 2016, 16 (1), pp 145–153. doi: 10.1021/acs.cgd.5b01045
4. Prat M, **Oltolina F**, Basilico C. Monoclonal Antibodies against the MET/HGF Receptor and Its Ligand: Multitask Tools with Applications from Basic Research to Therapy. *Biomedicines.* 2014 Dec 3; *2*(4), 359-383; doi:10.3390/biomedicines2040359
5. Pietronave S, Zamperone A, **Oltolina F**, Colangelo D, Follenzi A, Novelli E,Diena M, Pavesi A, Consolo F, Fiore GB, Soncini M, Prat M. Monophasic and biphasic electrical stimulation induces a precardiac differentiation in progenitor cells isolated from human heart. Stem Cells Dev. 2014 Apr 15;23(8):888-98. doi: 10.1089/scd.2013.0375. Epub 2014 Jan 24. PubMed PMID:24328510; PubMed Central PMCID: PMC3991992.

ABSTRACT

DEVELOPMENT OF MULTIFUNCTIONAL MAGNETIC NANOPARTICLES FUNCTIONALIZED WITH MONOCLONAL ANTIBODY AND DOXORUBICIN FOR CANCER THERAPY

Francesca Oltolina[1], Ester Borroni[1], Marta Miola[2], Enrica Vernè[2], Maria Prat[1] and Antonia Follenzi[1]

[1]Laboratory of Histology, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Novara (Italy). [2]Applied Science and Technology Department, Politecnico di Torino, Torino (Italy).

Nanosized drug carriers represent innovative and attractive tools in the field of cancer therapy. Magnetic nanoparticles (MNPs) are an appealing class of NPs because of their ability to be mobilized in response to a continuous magnetic field. Among different MNPs prepared as described by Borroni et al. [Acta Biomater, 2017], the Fe_3O_4 MNPs were considered the best in terms of biocompatibility, and were functionalized by isothermal adsorption with a monoclonal antibody (mAb) directed against the ectodomain of the tyrosine kinase receptor for HGF (HGFR/Met), which can be considered a tumor associated marker because of its overexpression in cancer cells, and with the chemotherapeutic drug Doxorubicin. Both naïve and mAb functionalized MNPs were highly cytocompatible (MTT assay and Annexin V/PI staining). MNPs functionalized with both mAbs and Doxo were highly stable up to at least 7days. The mAb adsorbed on MNPs maintained its immunocompetence and specificity being able to recognize Met β-chain in extracts from HGF-R/Met expressing cells (GTL-16 cells; immunoprecipitation & WB). mAb-functionalized MNPs specifically interacted with the cell surface of GTL-16 cells, and not with HGF-R/Met negative Huh7 cells (confocal microscopy experiments and Prussian Blue staining). The presence of the mAbs on Doxo-MNPs (mAb-Doxo-MNPs) increased significantly the toxic activity of Doxo-MNPs on GTL-16 cells, while no such effect was detectable on Huh7 cells (real time by XCelligence analysis). Fe_3O_4 MNPs were biocompatible when injected in mice and the presence of the mAb on MNPs enhanced their retention when injected in situ in GTL-16 cells induced xenograft tumors in γ-null mice. MNPs may be considered a new and promising carrier for targeted drug delivery opening new perspectives for *in vivo* studies, in which continuous and alternate magnetic fields can be applied to enhance targeting and inducing hyperthermia respectively.

PAOLA OSTANO

paola.ostano@fondazionetempia.org

Bioinformatician

Laboratory of Cancer Genomics, "Fondazione Edo ed Elvo Tempia Valenta", Biella

Education:

2003 - Bachelor-level degree in Biology at "Università degli Studi di Torino". Mark: 110/110.

2005 - Master-level degree in Molecular Cell Biology at "Università degli Studi di Milano". Mark: 110/110 cum laude.

2006 - 1st level Master degree in Bioinformatics at "Università degli Studi di Torino". Mark: 110/110.

2009 - Ph.D. in Complex Systems in Post-Genomic Biology at "Università degli Studi di Torino".

2011 - Italian Government Qualification for the Practice of Biologist

Representive Careers:

2003 – 2009: Internship/Ph.D. at Laboratory of Cancer Genomics, "Fondazione Edo ed Elvo Tempia Valenta", Biella.

2010 – 2014: Reasearch collaborator at Laboratory of Cancer Genomics, "Fondazione Edo ed Elvo Tempia Valenta", Biella.

2015 – Present Day: Permanent staff at Laboratory of Cancer Genomics, "Fondazione Edo ed Elvo Tempia Valenta", Biella.

Interesting Research Areas:

Bioinformatics and computational biology applied to cancer research. Gene expression (from microarray and RNA-Seq data), long non-coding RNAs, microRNAs, ChIP-Seq and array CGH data analysis. Prostate cancer, skin squamous cell carcinoma, breast cancer. Notch1 and CSL mechanisms in normal and cancer tissues from skin/dermal compartments.

Selected Publications:

1. Lefort K, **Ostano P**, Mello-Grand M, Calpini V, Scatolini M, Farsetti A, Dotto GP, Chiorino G. Dual tumor suppressing and promoting function of Notch1 signaling in human prostate cancer. Oncotarget. 2016 Jul 26;7(30):48011-48026.

2. Dallavalle C, Albino D, Civenni G, Merulla J, **Ostano P**, Mello-Grand M, Rossi S, Losa M, D'Ambrosio G, Sessa F, Thalmann GN, Garcia-Escudero R, Zitella A, Chiorino G, Catapano CV, Carbone GM. MicroRNA-424 impairs ubiquitination to activate STAT3 and promote prostate tumor progression. J Clin Invest. 2016 Dec 1;126(12):4585-4602.

3. Brooks YS, **Ostano P**, Jo SH, Dai J, Getsios S, Dziunycz P, Hofbauer GF, Cerveny K, Chiorino G, Lefort K, Dotto GP. Multifactorial ERβ and NOTCH1 control of squamous differentiation and cancer. J Clin Invest. 2014 May 1;124(5):2260-76. doi: 10.1172/JCI72718. Epub 2014 Apr 17.

4. Hu B, Castillo E, Harewood L, **Ostano P**, Reymond A, Dummer R, Raffoul W, Hoetzenecker W, Hofbauer GF, Dotto GP. Multifocal epithelial tumors and field cancerization from loss of mesenchymal CSL signaling. Cell. 2012 Jun 8;149(6):1207-20.

5. **Ostano P**, Bione S, Belgiovine C, Chiodi I, Ghimenti C, Scovassi AI, Chiorino G, Mondello C. Cross-analysis of gene and miRNA genome-wide expression profiles in human fibroblasts at different stages of transformation. OMICS. 2012 Jan-Feb;16(1-2):24-36.

ABSTRACT

GENOME EXPRESSION PROFILING OF PATIENT-DERIVED TUMOR XENOGRAFTS

Paola Ostano[1], Alessia Anastasia[2], Maria Rosa Bani[2], Eugenio Morani[3], Raffaella Giavazzi[2] and Giovanna Chiorino[1].

[1] Genomics Lab, Fondazione Edo ed Elvo Tempia, Biella, Italy; [2] Department of Oncology, Mario Negri Institute for Pharmacological Research, Milano, Italy; [3] Ospedale di Rho, ASST-Rhodense, Rho (MI), Italy

Pancreatic ductal adenocarcinoma (PDA) is a lethal disease with an overall survival rate of 5% and a median survival time of less than 6 months. Current systemic treatments offer only a modest benefit in symptom control and survival, also due to the biological heterogeneity of this type of cancer. The aim of this study is to exploit our patient-derived primary PDA xenografts (PDA-PDX) growing into the pancreas of recipient mice, in order to characterize tumor parenchyma and surrounding stromal tissue, at the molecular level. Gene expression profiling of 5 PDA-PDXs at passage 2 and/or 4 was performed on both human and mouse whole genome microarrays. Raw data were elaborated and integrated in order to understand the weight of the tumor or stroma counterpart in the characterization of each PDA-PDX. Starting from gene expression profiling of the tumor parenchyma (human), Bailey and Collisson classifiers [1,2] revealed that our tumors all belong to the same molecular subtype (Classical/Pancreatic Progenitor). Applying Moffitt activated stroma signature [3] to the profiles of the stromal counterpart, two distinct clusters emerged that are also associated with different response rates to therapy. Direct class comparison applied to the two clusters revealed other peculiar molecular features. Stroma contribution is determinant to predict response to therapy in homogeneous PDA subtypes.

REFERENCES

1. Bailey P et al. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 2016 Mar 3;531(7592):47-52. PMID: 26909576;
2. Collisson EA et al. Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy. Nat Med. 2011 Apr;17(4):500-3
3. Moffitt RA et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat Genet. 2015 Oct;47(10)

Name MARIA TERESA PALANO

Email maria.palano@unimi.it

Position PhD student

Affiliation Università degli Studi di Milano, Department of Health Science; Via Antonio di Rudinì, 8 - 20100 Milano

Education:

2012- BS in Biotecnologie Mediche - Università degli Studi di Milano; Milano, Italy

2015- MS in Biotecnologie mediche, cellulari e molecolari – Libera Università 'Vita Salute San Raffaele'; Milano, Italy

Representative Careers:

05/2011-07/2011: Bachelor trainee at Istituto Auxologico Italiano, Cusano Milanino (MI), Italy.

03/2012-09/2012: Volunteer at Istituto Auxologico Italiano, Cusano Milanino (MI), Italy.

09/2013-03/2015: Master trainee, Experimental Oncology Dep., Lymphoid Organ Development Laboratory, Libera Universita' 'Vita Salute S. Raffaele', Milan.

04/2015-09/2015: Scholarship at San Raffaele Institute, Experimental Oncology Dep., Lymphoid Organ Development Laboratory, Milan.

10/2015- present: PhD student at the Molecular and Translational Medicine PhD Program, Department of Health Sciences, University of Milano, Milano, Italy.

Representative Awards:

Silver poster Awards BTM-Novara 2016

Interesting Research Areas:

Cancer; Notch signaling pathway; Tumoral microenvironment end extracellular matrix role in cancer development and maintenance; Endothelial compartment in cancer; 3D organoid development;

Selected Publications:

1. Platonova N., Lesma E., Basile A., Bignotto M,. Garavelli S., **Palano MT**., Moschini A.,Neri A., Colombo M., Chiaramonte R. "Targeting Notch as a therapeutic approach for human malignancies". Curr Pharm Des. 2016 Oct 6. PMID:27719637

ABSTRACT

NOTCH-JAG AXIS IN THE INTERPLAY BETWEEN MULTIPLE MYELOMA AND ENDOTHELIUM

Palano MT.[1], Saltarella I.[2], Garavelli S.[1], Ria R.[2], Chiaramonte R.[1].

[1]Department of Health Sciences, University of Milano, Milano, Italy. [2]Department of Internal medicine and Clinical Oncology, University of Bari Medical School, Bari, Italy.

Multiple myeloma(MM) is an incurable plasma cells malignancy. MM is characterized by aberrant activation of Notch signaling pathway due to Jag1 and 2 ligands overexpression. Notch activation occurs through homotypic interaction among MM cells and through heterotypic interaction of MM with surrounding bone marrow(BM) cells. In BM microenvironment, stromal cells support MM through Jag-Notch interaction and endothelial cells (ECs) are involved in MM progression and dissemination, indeed MM patients display high level of angiogenesis. The aim of this project is to study if MM cells influences BM-ECs behavior through Jag-mediated activation of Notch signaling.

Jag1 and 2 were silenced in MM cell line using lentiviral vector (RPMI8226$^{shJAG1/2}$) were cultured with human pulmonary artery endothelial cells (HPAECs) used as model of ECs. Angiogenesis was assessed by Matrigel assay at 24 h. To assess the exclusive effect of Jag ligands on angiogenesis, HPAEC were directly stimulated with soluble Jag1. Wound healing assay was set up to assess variation in the migration ability of HPAEC using conditioned media from RPMI8226^{SCR} and RPMI8226$^{shJAG1/2}$ cells. Moreover, a 3D organoid was set up to mimic BM niche to study the interplay between MM cells and ECs.

Matrigel assays indicate that an active Jag-Notch axis directed by MM cells stimulates angiogenesis. Indeed, the absence of Jag ligands in MM cells reduces their angiogenic potential. Moreover, Jag1 alone is sufficient to promote EC angiogenic capability. Also ECs motility is modulated through Notch signaling, more specifically CM from silenced MM cells have a reduced ability to activate cell motility suggesting that MM cells release Notch dependent soluble factors that stimulate migration. Assays with 3D organoids show that MM cells support ECs survival. These data suggest that the Jag-Notch axis is necessary for MM-associated angiogenesis.

NATALIA PLATONOVA

natalia.platonova@unimi.it

Temporary Research Associate

Department of Health Sciences, Università degli Studi di Milano, via Rudinì,8 Milano, Italy.

Education:

1997: Specialist degree in process engineering of Biotechnology, Laurea magna cum laude. Department of Molecular Biotechnology, Faculty of Fine Organic and Microbiological Synthesis, St.-Petersburg State Technological Institute, Russia

2000: Ph.D. in Biochemistry, Research Institute of Experimental Medicine, Russian Academy of Medical Sciences, St.-Petersburg, Russia

2013: Ph.D. in Molecular Medicine, Università degli Studi di Milano, Milan, Italy Representative

Careers:

2000 – 2004 Junior investigator, Department of Molecular Genetics, Research Institute of Experimental Medicine, St.-Petersburg, Russia

2004 - 2007 Post doctoral fellow, Laboratory of Development Genetics, Dulbecco Telethon Institute at CNR-ITB, Milan, Italy

2007- 2009 Post doctoral fellow, Laboratory of Molecular Mechanisms of Angiogenesis, INSERM U920, University of Bordeaux I, Bordeaux, France

2009-2013 Ph.D. in Molecular Medicine, Università degli Studi di Milano, Milan, Italy

2014-2015 Post doctoral fellow, Laboratory of General Pathology, Department of Health Sciences, San Paolo Hospital, Università degli Studi di Milano, Milan, Italy

2015-2016 Post doctoral fellowship of Fondazione Umberto Veronesi 2015, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy

2016-2016 2016- up to date Post doctoral fellowship of Fondazione Umberto Veronesi 2016, Department of Oncology and Hemato-oncology, Università degli Studi di Milano, Milan, Italy Temporary Research Associate Department of Health Sciences, San Paolo Hospital, Università degli Studi di Milano, Milan, Italy

HONORS and AWARDS:

1995, 1998 Personal student grants appropriated by St.-Petersburg government (Russia)

2000 Honorary title of Soros Graduate Student

2002, 2003 Personal Grants from Russian Foundation for Basic Research for young scientists

2005 NATO-CNR Advanced fellowship

2015 Immunotools Special Award

2015,2016 Post-doctoral fellowships from Fondazione Umberto Veronesi

Interesting Research Areas:

Notch signaling, bone microenvironment, multiple myeloma. Drug discovery and design.

Selected Publications:

1. Platonova N, Parravicini C, Sensi C, Paoli A, Colombo M, Neri A, Eberini I, Chiaramonte R. Identification of small molecules uncoupling the Notch::Jagged interaction through an integrated high-throughput screening. Plos One. 2017, in print.
2. Platonova N, Lesma E, Basile A, Bignotto M, Garavelli S, Palano MT, Moschini A, Neri A, Colombo M, Chiaramonte R. Targeting Notch as a Therapeutic Approach for Human Malignancies. 2017. Curr Pharm Des 23: 108-134.
3. Chiaramonte R, Colombo M, Bulfamante G, Falleni M, Tosi D, Garavelli S, De Simone D, Vigolo E, Todoerti K, Neri A, Platonova N. Notch pathway promotes ovarian cancer growth and migration via CXCR4/SDF1α chemokine system. Int J Biochem Cell Biol. 2015 Sep; 66:134-40.
4. Platonova N, Manzo T, Mirandola L, Colombo M, Calzavara E, Vigolo E, Cermisoni GC, De Simone D, Garavelli S, Cecchinato V, Lazzari E, Neri A, Chiaramonte R. PI3K/AKT signaling inhibits NOTCH1 lysosome-mediated degradation. Genes Chromosomes Cancer. 2015 Jun 6. doi: 10.1002/gcc.22264.
5. Platonova N, Miquel G, Regenfuss B, Taouji S, Cursiefen C, Chevet E, Bikfalvi A. Evidence for the interaction of fibroblast growth factor-2 with the lymphatic endothelial cell marker LYVE-1. Blood. 2013 Feb 14;121(7):1229-37.

ABSTRACT

A NEW STRATEGY FOR SELECTIVE NOTCH2 TARGETING IN MULTIPLE MYELOMA BASED ON SMALL MOLECULES HAMPERING RECEPTOR-LIGAND INTERACTION

Platonova N[1], Parravicini C[2], Palazzolo L[2], Saporiti S[2], Colombo M[1], Vallelonga V[1], Colella R[1], Baccianti F[1], Neri A[3], Eberini I[2] and Chiaramonte R[1].

[1]Department of Health Sciences, H. S. Paolo, University of Milano; [2]Department of Pharmacological and Biomolecular Sciences, University of Milano. [3]Department of Oncology and Hemato-oncology, University of Milano; Hematology, Fondazione Cà Granda IRCCS Policlinico, Milano.

Multiple myeloma (MM) is an incurable hematological cancer characterized by MM cells accumulation in the bone marrow (BM) that promotes tumor survival and drug resistance. The oncogenic Notch signaling consists of 4 receptors (Notch1-4) and 5 ligands (Jag1,2 and Dll1,3,4) and plays a crucial role in MM. In particular, aberrant Notch2 activation and Jag1 and 2 overexpression stimulate MM cells to establish pathological interactions with BM that trigger MM progression (1). These effects can be interfered by knocking down of Jag1 and 2 expression (2). This evidence prompts us to develop a therapeutic tool to selectively inhibit Notch2 signaling triggered by Jag1/2.

We applied in silico protein-protein docking and virtual high-throughput screening (HTS) of a chemoteque to select druglike small molecules. The biological activity was validated by a Notch responsive reporter assay and co-culture assay that allows to measure Notch2 transcriptional activity induced either by Jag or Dll ligands. Based on previous setup integrated in silico pipeline (3), we applied a strategy to exclusively uncouple Notch2- Jag1/2, leaving unaltered the interaction with Dll that allows to overcome a gut toxicity causing by Notch pan- inhibitors. 100 top-scoring compounds directed exclusively to Notch2-Jag2 surface were selected by HTS of the chemoteque. 2 of 100 compounds validated in vitro significantly reduced Notch activity in the reporter assay on HEK293T cells. The co-culture assay of Hela cells overexpressing Notch2 with NIH3T3 overexpressing Jag1 or Dll4 ligands identified one promising compound that specifically inhibited Notch2-Jag1 and not Notch2-Dll4 interactions.

We have identified compounds that selectively antagonize Notch activation. This lays a basis for an effective and safe Notch-directed anti-tumor therapy in MM and other Notch-dependent tumors.

1.Houde C, et al. Blood. 2004;104:3697-704 2.Colombo M, et al. Oncotarget. 2014;5:10393-406 3. Platonova N, et al. PlosOne. 2017, in print.

ELTJONA RRAPAJ

eltjona.rrapaj@med.uniupo.it
PhD student

Università del Piemonte Orientale, Department of Health Sciences; 'Maggiore della Carità' Hospital of Novara, Laboratory of Molecular Biology, Anatomic Pathology Unit,

Via Solaroli, 17, Novara.

Education:

2010 –Laurea Bachelor degree (BSc) in Biotechnology- University of Perugia, Faculty of Mathematical, Physical and Natural Sciences (Italy).

2012 –Master of Science degree in Pharmaceutical Biotechnology (Class LM-9 – Medical, Veterinary and Pharmaceutical Biotechnology), University of Perugia (Italy). Final evaluation: 110/110 with honours.

Representative Careers:

05/2009 – 02/2010: Traineeship and bachelor thesis activity at the Department of Experimental Medicine and Biochemical Sciences, Section of Pharmacology; University of Perugia. Experimental thesis entitled "Cloning project of the TLR9 mouse receptor into the expression vector pEFBOS".

10/2010-10/2012: Master thesis activity and traineeship (2 years) in the Laboratory of Molecular and Cellular biology, by the Department of Clinical and Experimental Medicine, Section of Physiopathology, University of Perugia. Experimental thesis entitled " *In vivo* and *in vitro* analyses on the HOPS (Hepatocyte Odd Protein Shuttling) control in the proliferative processes".

03/2013- 07/2013: Volunteer and training activity by Universita' Vita'-Salute San Raffaele, Division of Genetics and Cellular Biology; Research Unit: Age Related diseases.

04/2014-10/2014: Post-graduate research activity and Fellowship at San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Chromatin Dynamics Unit.

10/2014-PRESENT : PhD student at Laboratory of Molecular Biology, Anatomic Pathology Unit, Universita' di Piemonte Orientale, Novara. PhD research activity: The role of HMGB1 in Malignant mesothelioma pathogenesis, prognosis and as predictive biomarker.

Interesting Research Areas:

- Toll-like receptors in innate immunity.
- In vivo (NOD/SCID mices) and in vitro (MEF cells) analysis on the HOPS (Hepatocyte Odd Protein Shuttling) control in the proliferative processes.

- Dissection of the mechanisms that ensure proteostasis (proteins homeostasis) and PI (Proteosome Inhibitors) resistance in multiple myeloma cells.
- Study of HMGB1 role into Malignant mesothelioma pathogenesis, prognosis and predictivity.
- In vitro and In vivo models generation for the study of the role of HMGB1 protein in mesothelioma.
- MicroRNAs prognostic impact and significance in lung cancer.

Selected Publications:

1. Mezzapelle, R.; **Rrapaj, E.**; Gatti E.; Ceriotti, C.; De Marchis, F.; Preti, A.; Spinelli, A.E.; Perani, L.; Venturini, M.; Valtorta, S.; Moresco, .R. M.; Pecciarini, L.; Doglioni, C.; Frenquelli, M.; Crippa, L.; Recordati, C.; Scanziani, E.; de Vries, H.; Berns, A.; Frapolli, R.; Boldorini, R.; D'Incalci M.; Bianchi, M.E. and Crippa, M.P. (2016) - Human malignant mesothelioma is recapitulated in immunocompetent BALB/c mice injected with murine AB cells – *Scientific Reports* **6**, 22850, doi:10.1038/srep22850.
2. Mancuso, G.; Bovio E.; Rena O.; **Rrapaj, E**.; Mercalli, F.; Veggiani, C.; Paganotti, A.; Andorno,S.; Boldorini, R. (2016)- Prognostic impact of a 3-Micro-RNA signature in cytological samples of small cell lung cancer.- Cancer Cytopathology.
3. **Rrapaj, E.;** Trisolini E.; Bertero L.; Salvo M.; Indellicato R.; Andorno S.; Garcia-Manteiga JM, Rena O, Boldorini R.(2017)- Expression analysis of High-mobility group box-1 (HMGB1) in histologic samples of malignant pleural mesothelioma.-Modern Pathology. (Submitted, under review).

ABSTRACT

MALIGNANT MESOTHELIOMA ORGANOIDS, MESOSPHERES AND PATIENT DERIVED XENOGRAFTS AS TOOLS TO STUDY DRUG SENSITIVITY AND TUMOR MICROENVIRONMENT.

Rrapaj E [1,2], Mezzapelle R [2], De Marchis F [2], Rena O [3], Carretta A [4], Boldorini R [2], Crippa MP[2] and Bianchi ME[2,5].

[1] Division of Pathology "Maggiore Della Carità" Hospital, Novara, Italy. [2] Chromatin Dynamics Unit, Division of Genetics and Cell Biology, San Raffaele Hospital, Milano, Italy. [3] Unit of Thoracic Surgery, Maggiore della Carità Hospital, Novara, Italy. [4] Department of Thoracic Surgery, Vita-Salute San Raffaele University, San Raffaele Scientific Institute, Milan, Italy. [5] San Raffaele Vita-Salute University, Milano, Italy.

Malignant mesothelioma is a very aggressive tumor with poor prognosis and no effective therapies available. Recent findings showed that malignant mesotheliomas can originate as polyclonal tumors. Moreover, tumor formation and outgrowth is not only determined by genetic alterations in tumor cells, but also by the inflammatory microenvironment. Evaluating mesothelioma as a complete organ becomes crucial to understand the biology of the tumor and to develop new therapeutic strategies. Recently, many types of in vitro 3D culture systems have been developed to recapitulate the in vivo growth conditions of cancer.[1] Our aim is to develop a 3D in vitro model of mesothelioma in order to create a collection of patient-derived human organoids,[2] to screen for drug sensitivity and to study the crosstalk between tumor and immune cells. Murine mesothelioma spheroids and patient derived xenograft (PDX) are alternative models that help us to pursue the same goal. Human mesothelioma samples obtained by extrapleural pneumonectomy and biopsies were used for in vitro organoids generation[3]. The same tumor fragments were transplanted in immune deficient mice (NOD *scid* gamma) to generate and expand patient derived tumor xenografts. Murine mesothelioma cell line AB1 was cultured in non-adherent conditions in order to generate spheroids (mesospheres). Organoid formation from human epithelioid mesothelioma was observed after 2 days post seeding. Organoids are small in size (starting at around 200 µm diameter and growing to 700 µm) and can be processed to form second-, third- and fourth- generation organoids. Hematoxylin&eosin staining showed that human mesothelioma organoids have inside-outside polarity, and an internal matrix. We obtained three PDX; immunophenotyping showed that the tumors propagated in mice are similar to the original tumor. Finally, preliminary experiments of co-culture of murine mesospheres and monocytes demonstrated interaction between the two cell types. We have the ability to generate organoids and PDX models of mesothelioma, but further characterization is needed. We expect these models will help us to better understand the mesothelioma pathogenesis.

REFERENCES:

1)John J. Tentle et al. (2012) Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 9: 338–350. 2)Mahé MM et al. (2013) Establishment of gastrointestinal epithelial organoids. Curr Protoc Mouse Biol. 3: 217–240.3)Drost J et al. (2016) Organoid culture systems for prostate epithelial tissue and prostate cancer tissue. Nat Protoc. 11: 347–358.

ALBERTO RUBIN PEDRAZZO

alberto.rubinpedrazzo@unito.it

PhD Student in Chemical and Materials Sciences

Università degli studi di Torino, Dipartimento di Chimica, Via P. Giuria, 7 - 10125 Torino

Education:

2014 – Laurea - Chemistry and Chemical Technology (B.D.) 2016 – Laurea *Summa cum Laude* – Industrial chemistry (M.D.)

Representative Careers:

October 2016-now

PhD Programme in CHEMICAL AND MATERIAL SCIENCE at Polymer Chemistry Group of Chemistry Department, under supervision of Prof. Francesco Trotta and Prof. Guido Viscardi

Interesting Research Areas:

Synthesis and characterization of biopolymers for drug delivery and for coating/absorption of active substances.

Photoreactive squaraines for photodynamic therapy.

Development of nanostructured oxides for ultrasensitive gas sensors (for detection of acetone and carbon monoxide) and for photocatalytic application.

Selected Publications:

1. Fioravanti, A.; Morandi, S.; **Rubin Pedrazzo**, A.; Bracco, P.; Zanetti, M.; Manzoli, M.; Mazzocchi, M.; Carotta, M.C. Ultrasensitive Gas Sensors Based on Electrospun TiO2 and ZnO. Proceedings 2017, 1, 485

ABSTRACT

DEXTRIN-BASED NANOSPONGES AS INNOVATIVE TOOL FOR ANTICANCER DRUG DELIVERY

Alberto Rubin Pedrazzo [1], Maria Tannous [1], Nilesh Kumar Dhakar [1], Fabrizio Caldera [1], Monica Argenziano [2], Roberta Cavalli [2], Francesco Trotta [1]

[a] Department of Chemistry, University of Torino, via P. Giuria 7, 10125, Torino, Italy; [b] Department of Drug Science and Technology, University of Torino, via P. Giuria 9, 10125, Torino, Italy;

The design of new nanocarriers as a strategy for anticancer drug delivery offers an interesting way to overcome some limitations of current clinical treatments, such as the inadequate targeting in tumor sites and the high toxicity of many anticancer drugs. Nanoformulations can improve the therapeutic efficacy of anticancer drugs by increasing their solubility and modifying the biodistribution of the active substances, reducing the exposure of not-target tissues. Cyclodextrin-based nanosponges (NS) are a novel nanosized delivery system composed of hyper-cross-linked cyclodextrins connected in a three-dimensional network. They show a good capacity for incorporating active molecules, ions and macromolecules as well, within their structure. Nanosponges can be used as nanocarriers to deliver and protect from light or from chemical degradation encapsulated molecules. A significant example was established by incorporating insoluble camptothecin (CPT), wide-range effective anticancer drug, into cyclodextrin nanosponges. The *in vitro* drug release studies indicated slow and prolonged CPT release over a period of 72 h and the NS formulations protected the lactone ring of CPT. Furthermore, *in vivo* experiments showed that CPT-loaded NS significantly inhibited the growth, the metastatization and the vascularization of orthotopic anaplastic thyroid carcinoma xenografts in SCID/beige mice without apparent toxic effects. Recently, also, glutathione (GSH)-responsive nanosponges have been designed for targeted intracellular drug release and *in vitro* experiments fully proved the ability of GSH-NS to release doxorubicin as a function of the GSH cell concentration. GSH-NS enhanced doxorubicin accumulation in tumor tissue and were more effective than free drug in reducing tumor growth in xenograft models. In conclusion, cyclodextrin-based nanosponges are a very promising tool for the improvement of cancer chemotherapy, for the challenging characteristics and versatility.

REFERENCES

1. Trotta F, Dianziani C, Caldera F, Mognetti B, Cavalli R. The application of nanosponges to cancer drug delivery. Expert Opin. Drug Deliv. (2014) 11(6):931-941. Trotta F, Caldera F, Dianziani C, Argenziano M, Barrera G, Cavalli R. Glutathione Bioresponsive Cyclodextrin Nanosponges. ChemPlusChem (2016), 81:439-443

RAJESHWARI REDDY MERUPATI

rajeshwarireddy26@gmail.com

Msc student in Medical Biotechnology

Laboratory of Molecular Pathology and Nanobioimaging, Università del Piemonte Orientale, Department of Health Sciences,
via Solaroli 17, 28100 Novara (Italy)

Education:

October 2016-present Master degree in Medical Biotechnology Università del Piemonte Orientale, Novara, Italy.

January 2015 Master degree in Pharmaceutical Science, University of Greenwich, Medway London, U.K.

July 2013 Bachelor of Pharmacy, Osmania University, GokarajuRangaraju College of Pharmacy, Hyderabad, India.

Representative Careers:

March 2017-present Research Internship, Laboratory of Molecular Pathology and Nanobioimaging, Università del Piemonte Orientale. (Mentor: Prof. Ciro Isidoro)

October 2016 Attendance of the International Conference "Basic to Translational Medicine 2016: focus on cancer", October 6th-7th 2016, Università del Piemonte Orientale, Novara, Italy.

March 2014-December2014 Internship, "Analysis of Fluroquinolones using Dart ionization by Mass Spectrometry", University of Greenwich, Medway London, U.K. (Mentor: Prof.Frank Pullen)

July 2013 Internship, "Dendrimers in Pharmacy", Osmania University, GokarajuRangaraju College of Pharmacy, Hyderabad, India (Mentor: Dr Sheela Modani).

Representative Awards:

2016 Scholarship from EDISU, Università del Piemonte Orientale Novara, Italy.

Interesting Research Areas:

Cancer Metabolism - autophagy – long non-coding RNAs - Nanoparticles.

ABSTRACT

RESVERATROL REVERTS LPA-MEDIATED LNC-RNAS PROFILING IN OVARIAN CANCER CELLS

Merupati Rajeshwari Reddy[1], Christian Seca[1], Maria Rizzo[1], Giovanna Chiorino[2], Danny N Dhanasekaran[3] Ciro Isidoro[1#]

1) Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", via Solaroli 17, 28100 - Novara (Italy). 2) Fondazione Edo&Elvo Tempia, Biella (Italy). 3) Stephenson Cancer Center and Department of Cell Biology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA. # E-mail: ciro.isidoro@med.uniupo.it

INTRODUCTION Long non-coding RNAs (lncRNA) are one subtype of non-coding RNAs (ncRNAs) that exceed 200 nucleotides in length. lncRNAs are a relatively abundant component of the mammalian transcriptome and are involved in several cellular functions, including cancer cell proliferation, drug resistance and the regulation of glycolysis [1]. Very recently, our group demonstrated that Resveratrol, a natural occurring polyphenol, is implicated in the epigenetic regulation of autophagy and cancer cell invasion [2].

RESULTS Our previous findings showed that UCA-1, a long non-coding RNA reported to be crucial in tumour progression, is up-regulated during amino acid starvation, thus implicating a role in cancer cell metabolism. Moreover, we found that Resveratrol could differentially modulate LPA-associated lnc-RNA profiling. Among the seven lnc-RNA identified, the LINCOO473 has been reported to be involved in chemo-resistance and metastasis.

CONCLUSION Further investigations are necessary to elucidate the mechanisms through which the lnc-RNA identified in our study may be potential targets for the treatment of ovarian cancer patients.

REFERENCES

1. Ma Z, Huang H, Xu Y, He X, Wang J, Hui B, Ji H, Zhou J, Wang K. Current advances of long non-coding RNA highly upregulated in liver cancer in human tumors. Onco Targets Ther. 2017 Sep 25;10:4711-4717. doi: 10.2147/OTT.S136915. eCollection 2017. Review.
2. Ferraresi A, Phadngam S, Morani F, Galetto A, Alabiso O, Chiorino G, Isidoro C. Resveratrol inhibits IL-6- induced ovarian cancer cell migration through epigentic up-regolation of autophagy. Mol. Carcinog. 2017 Mar; 56(3):1164-1181

MARINA RICCI

marina.ricci@unito.it

PhD Student

University of Torino, Department of Clinical and Biological Sciences, Corso Raffaello, 30 - 10125 Torino (Italy)

Education:

2015 - 2nd level degree – Master in Cellular and Molecular Biology (LM-6), University of Torino (Italy), School of Natural Sciences – Department of Life Sciences and Systems Biology.

2012 - 1st level degree – Bachelor in Biology (L-13), University of Torino (Italy), Faculty of Mathematical, Physical and Natural Sciences.

Representative Careers:

October 2014 – present: PhD student in Experimental Medicine and Therapy at the Department of Clinical and Biological Sciences (University of Torino, Italy).

January 2014 – August 2014: Student in Cellular and Molecular Biology at the Prof. Josep Maria Argilés' laboratory, Department of Biochemistry and Molecular Biology (University of Barcelona, Spain). Experimental thesis entitled: *"A rat immobilization model based on cage volume reduction: a new model for bed-rest?".*

January 2012 – July 2012: Student in Biology at the Prof. Mirella Giovarelli's laboratory, Research Center for Experimental Medicine (San Giovanni Battista Hospital, Turin, Italy). Thesis entitled: *"HER-2 as a target for cancer therapy*".

Interesting Research Areas:

Cancer; Nanomedicine; Biomaterials; PPARs; Polyunsaturated fatty acid

Selected Publications:

1) Muzio G, Miola M, Ferraris S, Maggiora M, Bertone E, Puccinelli MP, **Ricci M**, Borroni E, Canuto RA, Verné E, Follenzi A. Innovative superparamagnetic iron-oxide nanoparticles coated with silica and conjugated with linoleic acid: Effect on tumor cell growth and viability. Mater Sci Eng C Mater Biol Appl. 2017 Jul 1;76:439-447.

2) Marmonti E, Busquets S, Toledo M, **Ricci M**, Beltrà M, Gudiño V, Oliva F, López-Pedrosa JM, Manzano M, Rueda R, López-Soriano FJ, Argilés JM. A Rat Immobilization Model Based on Cage Volume Reduction: A Physiological Model for Bed Rest? Front Physiol. 2017 Mar 29;8:184.

3) Muzio G, **Ricci M**, Traverso N, Monacelli F, Oraldi M, Maggiora M, Canuto RA. 4-Hydroxyhexenal and 4-hydroxynonenal are mediators of the anti-cachectic effect of n-3 and n-6 polyunsaturated fatty acids on human lung cancer cells. Free Radic Biol Med. 2016 Oct;99:63-70.

ABSTRACT

SUPERPARAMAGNETIC IRON-OXIDE NANOPARTICLES FUNCTIONALIZED WITH CONJUGATED LINOLEIC ACID: ANTITUMOR ACTIVITY ON MOUSE BREST CANCER CELLS

Ricci M.[1], Muzio G.[1], Miola M.[2], Ferraris S.[2], Maggiora M.[1], Bertone E.[2], Puccinelli M.P.[3], Borroni E.[4], Vernè E.[2], Follenzi A.[4], Canuto R.A.[1]

[1] Department of Clinical and Biological Sciences, University of Turin, Turin; [2] Department of Applied Science and Technology, Politecnico di Torino, Turin; [3] Department of Laboratory Medicine, Azienda Ospedaliera Universitaria, Città della Salute e della Scienza, Turin; [4] Department of Health Sciences, University "Amedeo Avogadro" of East Piedmont, Novara

Cancer nanomedicine pays particular attention to superparamagnetic iron oxide nanoparticles (SPIONs) that can reach the tumor sites carrying chemotherapeutic drugs, nucleic acids, monoclonal antibodies or engineered viral vectors. In the past, also conjugated linoleic acid (CLA) antitumor properties were demonstrated. This study aimed to prepare SPIONs functionalized with CLA able to affect 4T1 mouse breast cancer cell viability.

EXPERIMENTAL MODEL. Cell number and type of death were evaluated by flow cytometry; proliferation modulation *(PPARγ)* by western blotting; inflammation markers by western blotting (PPARα) and ELISA (TNFα, IL-1β). In vivo biodistribution was evidenced by iron staining.

RESULTS. SPION uptake was confirmed by increased CLA content in 4T1 cells incubated with CLA-SPIONs. Flow cytometry analysis showed that both SPIONs, especially the CLA functionalized ones, decreased the alive cell number and induced necrosis, peaking at 48 hours. After 72 hours, the cells treated with CLA-SPIONs restarted growing. Decreased cell proliferation inversely correlated with *PPARγ, a negative modulator of cell proliferation.* The induction of cell death associated with the increase of pro-inflammatory TNFα and IL-1β in the medium and by the decrease of anti-inflammatory PPARα. In-vivo (NSG mice) biodistribution analysis evidenced SPION accumulation mainly in the spleen and, to a lesser extent, in the liver, especially the CLA functionalized ones.

CONCLUSION. The results show that CLA-SPIONs impair breast cancer cell growth triggering necrotic cell death more than SPIONs alone, and that the effect seems to be modulated by PPARs. This transient cytostatic/cytotoxic effect suggests that a repeated treatment might effectively abrogate cancer cells survival.

REFERENCES. 1. Kandasamy G. and Maity D. Int. J. Pharm. 496: 191-218, 2015; 2. Park J. et al. Nat Mater. 3, 891-895, 2004; 3. Belury M.A. J. Nutr. 132, 2995-2998, 2002.

SUYANEE THONGCHOT

suyanee.thongchot@gmail.com

Ph.D. student

Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand; Laboratory of Molecular Pathology, Department of Health Sciences Università del Piemonte Orientale Via Solaroli 17, 28100 Novara, Italy

Education:

2016 One year experience at laboratory of Molecular Pathology, Department of Health Sciences Università del Piemonte Orientale Novara, Italy

2014-Now Study for Doctor of Philosophy (Medical Biochemistry and Molecular biology), Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

2014 Master degree (Medical Biochemistry and Molecular biology), Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand.

2011 Bachelor degree of Science in Medical Technology (second class honor), Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand

Representative Awards:

2016: Silver award for outstanding Poster presentation at International Conference "Basic to Translational Medicine 2016: focus on Cancer", Novara October 7th-8th 2016.

2015: Outstanding Poster Presentation, "International Congress of Liver Flukes and Cholangiocarcinoma: Towards Control and Elimination" (Thailand)

Interesting Research Areas:

Autophagy in cholangiocarcinoma. Molecular, pathology and cell biology of CCA. Role of autophagy in Liver fluke-associated cholangiocarcinoma in term of progression, chemoprevention and treatment

Selected Publications:

1. **Thongchot S**, Yongvanit P, Loilome W, Seubwai W, Phunicom K, Tassaneeyakul W, Pairojkul C, Promkotra W, Techasen A, Namwat N. *. High expression of HIF-1α, BNIP3 and PI3KC3: hypoxia-induced autophagy predicts cholangiocarcinoma survival and metastasis. Asian Pac J Cancer Prev. 2014;15(14):5873-8.

2. **Thongchot S**, Loilome W, Yongvanit P, Dokduang H, Thanan R, Techasen A, Namwat N. * Chloroquine exerts anti-metastatic activities under hypoxic conditions in cholangiocarcinoma cells. Asian Pac J Cancer Prev. 2015;16(5):2031-5.

3. Silakit, R., Loilome, W., Yongvanit, P., **Thongchot, S**., Sithithaworn, P., Boonmars, T., Koonmee, S., Titapun, A., Khuntikeo, N., and Chamadol, N. (2015). Urinary microRNA-192 and microRNA-21 as potential indicators for liver fluke-associated cholangiocarcinoma risk group. Parasitology international.

4. Dai, X., **Thongchot, S.,** dokduang, H., loilome, W., khuntikeo, N., titapun, A., ungarreevittaya, P., yongvanit, P., techasen, A., and namwat, N. (2016). Potential of Selenium Compounds as New Anticancer Agents for Cholangiocarcinoma. Anticancer Research *36*, 5981-5988.

5. Phanthaphol N, Techasen A, Loilome W, **Thongchot S**, Thanan R, Sungkhamanon S, Namwat N*. Upregulation of TCTP is associated with cholangiocarcinoma progression and metastasis. Oncology Letters. 2017;14(5):5973-9.

6. Dai X, **Thongchot S**, Loilome W, Techasen A, Yongvanit P, Namwat N*. Expression Patterns of Selenoprotein P in Human Cholangiocarcinoma. Srinagarind Med J 2014;29 (Suppl).

7. Kittirat Y, **Thongchot S**, Loilome W, Techasen A, Yongvanit P, Namwat N*. Expression of 14-3-3 Sigma Protein in Cholangiocarcinoma Progression. Srinagarind Med J 2014;29 (Suppl).

ABSTRACT

CANCER ASSOCIATED FIBROBLASTS INFILTRATION AND DEREGULATION OF AUTOPHAGY PLAY A ROLE IN CHOLANGIOCARCINOGENESIS

Suyanee Thongchot[1,2,4], Watcharin Loilome[1,2], Puangrat Yongvanit[1,2], Narong Khuntikeo[2,3], Attapol Titapun[3], Ciro Isidoro[4]* and Nisana Namwat[1,2]*

[1]Department of Biochemistry, Faculty of Medicine, [2]Liver Fluke and Cholangiocarcinoma Research Center, Khon Kaen University, Khon Kaen (TH); [3]Department of Surgery, Faculty of Medicine, [4]Laboratory of Molecular Pathology, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Novara (I).

INTRODUCTION Cholangiocarcinoma (CCA) is a primary cancer that originates from the neoplastic transformation of the bile duct epithelium. In Thailand, the highest incidence rate is in the northeast region and is associated with chronic inflammation and infection with the human liver fluke Opisthorchis viverrini (Ov). Recently, our group demonstrated that the combination of OV and human carcinogen nitrosamine N-nitrodimethylamine (NDMA) intake greatly contribute to CCA development in endemic areas (1). It is now clear that cancer associated fibroblasts (CAFs) and epithelial cancer cells reciprocally influence their metabolism through cytokines and the exchange of autophagy metabolites (2). In this study, we focused on the role of fibroblast infiltration and autophagy in cholangicarcinogenesis and CCA progression.

EXPERIMENTAL MODEL The expression of LC3 and p62 (markers of autophagy) and of α-SMA (marker of cancer associated fibroblasts, CAF) was examined by immunohistochemistry, immunofluorescence and western blotting in paraffin liver sections of hamsters in which CCA was induced by OV plus NDMA combined treatment.

RESULTS α-SMA expression was significantly increased and correlated with the accumulation of fibrosis and carcinogenesis in hamster-CCA model. The expression of autophagy markers in epithelial cells showed a biphasic pattern: it increased during 1 to 4 months and it decreased at 6 month when cancer transformation was obvious and in concomitance with the highest level of CAF infiltration.

CONCLUSION The present data indicate that CAF infiltration accompanies and possibly promotes cholangiocarcinogenesis in association with deregulation of autophagy in the epithelial cells of bile ducts.

REFERENCES

1. Prakobwong S, Yongvanit P, Hiraku Y, Pairojkul C, Sithithaworn P, Pinlaor P, et al. Involvement of MMP-9 in peribiliary fibrosis and cholangiocarcinogenesis via Rac1-dependent DNA damage in a hamster model. Int J Cancer. 2010;127(11):2576-87.

2. Thuwajit C, Ferraresi A, Titone R, Thuwajit P, Isidoro C. The metabolic cross-talk between epithelial cancer cells and stromal fibroblasts in ovarian cancer progression: Autophagy plays a role. Med Res Rev. 2017 Sep 19. doi:10.1002/med.21473.

KONDURU SAI SANDEEP VARMA

sandeep.varma567@gmail.com

Post-graduate fellow

Università del Piemonte Orientale, Department of Translational Medicine; via Paolo Solaroli 17 –28100, Novara, **Italy**

Education:

2017: Master Degree in Medical Biotechnologies; Università del Piemonte Orientale, Novara, Italy

2014: Bachelor of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore, India

Representative Careers:

08/2017 – to date: Post-graduate fellow in the laboratory of Biochemistry, department of translational medicine, Università del Piemonte Orientale, Novara, Italy

11/2015 – 07/2017: Master trainee at laboratory of Biochemistry, Università del Piemonte Orientale, Novara, Italy

Interesting Research Areas:

Diacylglycerol kinase α, Osteopontin and T-cell malignancies.

ABSTRACT

ROLE OF DIACYLGLYCEROL KINASE α IN TRANSDUCING OSTEOPONTIN SIGNALLING AND TUNING CELL SENSITIVITY TO CELL DEATH

Konduru Sai Sandeep Varma[1], Suresh Velnati[1], Elisa Ruffo[2], Elena Boggio[3], Nausicaa Clemente[3], Umberto Dianzani[3], Annalisa Chiocchetti[3], Andrea Graziani[2], Gianluca Baldanzi[1].

[1]Department of Translational Medicine, "A. Avogadro" University of Piemonte Orientale, via Solaroli 17 28100, Novara, Italy; [2]Division of Experimental Oncology, School of Medicine, University Vita e Salute San Raffaele, Milan, Italy; [3]Department of Health Sciences and Interdisciplinary Research Center of Autoimmune Diseases (IRCAD), "A. Avogadro" University of Piemonte Orientale (UPO), 28100 Novara, Italy

INTRODUCTION: DGKs (diacylglycerol kinases) are members of a unique and conserved family of intracellular lipid kinases that phosphorylate DAG (diacylglycerol), catalysing its conversion into PA (phosphatidic acid). DGKα, DGKζ and DGKδ as the major isoforms expressed in T cells. DGKα and DGKζ regulates metabolism of DAG downstream to T cell receptor (TCR) activation. TCR signal strength and particularly DAG signaling directly correlates with sensitivity to re-stimulation induced cell death (RICD) a form of apoptosis counteracting lymphocyte expansion. Previous work clarified that excessive DGKα activity reduces DAG and promotes the RICD resistance typical of lymphocytes from XLP-1 patients [1]. In order to verify whether the role of DGKα in the control of lymphocyte apoptosis is peculiar of XLP-1 derived lymphocytes, we used osteopontin (OPN) treatment as an alternative way to promote RICD resistance. Our speculation is that if DGKα plays a general role in tuning RICD sensitivity, DGKα inhibitors should restore the RICD sensitivity also in OPN treated cells.

METHODS: RICD was induced with anti-CD3 (OKT3) antibody treatment of purified PBLs pre-activated with PHA and IL-2. Live cells were counted using the trypan blue exclusion test and results expressed as % cell loss with respect to untreated cells. In this setting, OPN treatment promotes a resistance to RICD as previously described [2]. To verify the involvement of DGK in this resistance, we used two specific DGKα inhibitors (R59949 and ritanserin). As both of them are also serotonin receptor antagonist [3], we also used ketanserin, which selectively antagonizes serotonin receptors without affecting DGKα.

RESULTS: CD3 treatment resulted in a consistent cell loss at 1µg/ml and, as expected, osteopontin (1 µg/ml) significantly decreased this cell loss. Interestingly, both R59949 and ritanserin (5 µM) have no effect on the % cell loss of untreated or CD3 treated cells, indicating that those inhibitors are not influencing directly the TCR signaling promoting RICD. However, the presence of R59949 or ritanserin completely blocks OPN effect, suggesting that DGKα is an essential mediator of OPN protective signaling. Surprisingly, ongoing experiments with ketanserin shows some effect also of this molecule, suggesting a possible role of serotonin signaling in the onset of RICD resistance.

CONCLUSION: Those results indicates that DGK activity plays a key role in the onset of RICD resistance of osteopontin treated cells and suggest that R59949 and ritanserin, by restoring RICD in OPN treated cells, may reduce the accumulation and proliferation of T cells not only in XLP-1 but also in OPN promoted autoimmune diseases.

REFERENCES: 1. Ruffo, E., et al., *Inhibition of diacylglycerol kinase alpha restores restimulation-induced cell death and reduces immunopathology in XLP-1.* Sci Transl Med, 2016. **8**(321): p. 321ra7. **2.**Boggio, E., et al., *Thrombin Cleavage of Osteopontin Modulates Its Activities in Human Cells In Vitro and Mouse Experimental Autoimmune Encephalomyelitis In Vivo.* J Immunol Res, 2016. **2016**: p. 9345495. **3.** Boroda, S., et al., *Dual activities of ritanserin and R59022 as DGKalpha inhibitors and serotonin receptor antagonists. Biochem Pharmacol, 2016.*

CHIARA VIDONI

chiara.vidoni@med.uniupo.it

Postdoctoral fellow

Laboratory of Molecular Pathology, Department of Health Sciences,
University Piemonte Orientale, Novara
Via Paolo Solaroli17 – 28100, Novara (Italy)

Education:

30th May 2017 Doctor of Philosophy (PhD) in Science and Medical Biotchnologies, Università del Piemonte Orientale, Novara, Italy

March 26th 2012 Master Degree in Medical Biotechnologies, Università del Piemonte Orientale "A. Avogadro"Novara, Italy. Graduation mark: 108/110.

October 28th 2009 Bachelor Degree in Biotechnology, Università del Piemonte Orientale "A. Avogadro"Novara Italy. Graduation mark: 98/110.

2006 Scientific High school Diploma "G.Ferrari", Vercelli Italy. Final mark: 76/100

Representative Careers:

March 2017 – present: Fellowship recipient for the project "REGOLAZIONE DELLA PROTEOLISI AUTOFAGICO-LISOSOMICA NELLA PROGRESSIONE DEL CARCINOMA OVARICO", Università del Piemonte Orientale, Department of Health Sciences, Laboratory of Molecular Pathology, Novara, Italy

February 2017 – present: Tutoring activities for laboratory techniques, Department of Health Sciences, University of Piemonte Orientale, Novara, Italy

6-7 October 2016: International Congress "Basic to Translational Medicine 2016: Focus on cancer"; Novara, Italy

June 2014/June 2015/June 2016: Contract of tutoring activities in laboratory of "fondamenti di patologia e immunologia" Prof. Isidoro, at Biological Science Faculty, University of Piemonte Orientale, Alessandria, Italy

November 2013-October 2016: PhD student in Sciences and Medical Biotechnologies, Università del Piemonte Orientale, Department of Health Sciences, Laboratory of Molecular Pathology, Novara, Italy.

January 2013-October 2013: Stage, Università del Piemonte Orientale, Department of Health Sciences, Laboratory of Molecular Pathology, Novara, Italy. Under the supervision of Prof. Isidoro.

2009-2012: MS stage, Department of Health Sciences, Università del Piemonte Orientale, Laboratory of Medical Clinic. Under the supervision of Prof. Pirisi

2008-2009: BS stage, Department of Department of Chemical, Food, Pharmaceutical and Pharmacological Sciences, Università del Piemonte Orientale, Laboratory of Microbiology. Under the supervision of Prof.ssa Martinotti.

Representative Awards:

July 2013 Award of research activity, Laboratory of Molecular Pathology, Prof. Isidoro, University of Piemonte Orientale, Novara

Interesting Research Areas:

Molecular mechanisms involved in neurodegenerative disease and cancer – autophagy – cancer – epigenetics- cell migration - programmed cell death/cell toxicity - biogenesis and function of lysosomes, lysosomal cathepsins and lysosome-related organelles.

Selected Publications:

1. **Chiara Vidoni,** Eleonora Secomandi, Andrea Castiglioni, Mariarosa A.B. Melone and Ciro Isidoro. Resveratrol protects neuronal-like cells expressing mutant Huntingtin from Dopamine toxicity by rescuing ATG4-mediated autophagosome formation. Neurochem Int. 2017 May 19. pii: S0197-0186(17)30243-7. doi: 10.1016/j.neuint.2017.05.013.
2. **Chiara Vidoni**, Andrea Castiglioni, Christian Seca, Eleonora Secomandi, Mariarosa A.B. Melone and Ciro Isidoro. Dopamine exacerbates mutant Huntingtin toxicity via oxidative-mediated inhibition of autophagy in SH-SY5Y neuroblastoma cells: Beneficial effects of anti-oxidant therapeutics. Neurochem Int. 2016 Dec;101:132-143. doi:10.1016/j.neuint.2016.11.003.
3. **Vidoni C,** Follo C, Savino M, Melone MA, Isidoro C. The Role of Cathepsin D in the Pathogenesis of Human Neurodegenerative Disorders. Med Res Rev. 2016 Sep;36(5):845-70. doi: 10.1002/med.21394. Review.
4. **Chiara Vidoni**, Rossella Titone, Federica Morani, Carlo Follo and Ciro Isidoro. Epigenetic control of Autophagy in cancer: the role of microRNAs. Minerva Biotecnologica 2014 June;26(2):87-92.
5. Rossella Titone, Federica Morani, Carlo Follo, **Chiara Vidoni**, Delia Mezzanzanica and Ciro Isidoro. Epigenetic control of autophagy by microRNAs in Ovarian Cancer. Biomed Res Int. 2014;2014:343542. doi: 10.1155/2014/343542.

ABSTRACT

RESVERATROL COUNTERACTS OVARIAN CANCER PROGRESSION STIMULATED BY INTERLEUKIN-6: ROLE OF GLUCOSE METABOLISM AND OF AUTOPHAGY

Chiara Vidoni[1], Alessandra Ferraresi[1], Suratchanee Phadngam[1], Andrea Castiglioni[1*], Alessandra Galetto[2], Ciro Isidoro[1]

[1]Laboratory of Molecular Pathology and Nanobioimaging, Department of Health Sciences, Università del Piemonte Orientale "A. Avogadro", Via Solaroli 17, 28100 - Novara (Italy). [2]Unit of Oncology, Department of Translational Medicine, Università del Piemonte Orientale "A. Avogadro", Novara (Italy). [*]Program of Molecular Medicine, European Institute of Oncology, Via Ripamonti 435, 20141, Milan, Italy. Department of Oncology and Hemato-Oncology, Università degli Studi di Milano, 20142 Milano, Italy.

INTRODUCTION: IL-6 is a pro-inflammatory cytokine produced by cancer-associated fibroblasts and known to increase the proliferative and invasive properties of ovarian cancer cells (1). Moreover, ovarian cancer cells display altered glucose metabolism, thus becoming addicted (2). Resveratrol (RV) is a naturally polyphenolic compound with cancer-preventing properties, including cancer cell migration and glucose uptake inhibition (3).

RESULTS: Here we show that RV contrasts IL-6-induced cell migration and invasion effects. Transcriptomic and microRNomic analyses reveal that *ARH-I* (*DIRAS3*), a Ras homolog GTPase known to interact with BECLIN 1, is differentially modulated by RV and IL-6. IL-6 down-regulates ARH-I expression, whereas RV enhances the ARH-I-BECLIN 1 interaction, thus promoting autophagy. Moreover, RV prevents the mitochondrial ROS production and the autophagy inhibition induced by IL-6 at the migration front in a glucose dependent manner.

CONCLUSION: Our data suggest that RV abrogates ovarian cancer cell migration stimulated by IL-6 through suppressing glucose uptake, mitochondrial ROS generation, and up-regulating autophagy.

REFERENCES:

1. Plante M, Rubin SC, Wong GY, Federici MG, Finstad CL, Gastl GA. Interleukin-6 level in serum and ascites as a prognostic factor in patients with epithelial ovarian cancer. Cancer. 1994 Apr 1;73(7):1882-8.
2. Phadngam S, Castiglioni A, Ferraresi A, Morani F, Follo C, Isidoro C. PTEN dephosphorylates AKT to prevent the expression of GLUT1 on plasmamembrane and to limit glucose consumption in cancer cells. Oncotarget. 2016 Dec20;7(51):84999-85020.
3. Ferraresi A, Phadngam S, Morani F, Galetto A, Alabiso O, Chiorino G, Isidoro C. Resveratrol inhibits IL-6-induced ovarian cancer cell migration through epigenetic up-regulation of autophagy. Mol Carcinog. 2017 Mar;56(3):1164-1181.

NOTES

RINGRAZIAMENTI PERSONALI

Sono grato a Tutti gli speakers invitati, per aver accettato con entusiasmo l'invito. Grazie a tutti coloro che con la partecipazione attiva alle sessioni scientifiche avranno dato un contribuito all'avanzamento delle nostre conoscenze sul cancro.

Ringrazio il Magnifico Rettore Prof. C. Emanuel, le Personalità politiche e tutti i colleghi e amici, insomma tutti i "V.I.P. Invited Guest", che hanno partecipato alla cerimonia inaugurale e ai lavori congressuali.

Anche in questa occasione, come già per il Congresso "Basic to Translational Medicine 2016: focus on Cancer" dello scorso anno, ho sentito la vicinanza del Città di Novara e percepito il clima di entusiasmo e collaborazione per la buona riuscita dell'evento.

Il mio ringraziamento alle Istituzioni, Fondazioni, Associazioni e Aziende che con i loro contributo hanno reso possibile far fronte a tutte le spese per l'organizzazione e l'accoglienza degli ospiti e dei partecipanti.

Ma sono le Persone che fanno muovere le Istituzioni. Il ringraziamento va dunque alle persone che hanno messo a disposizione il loro tempo e le loro energie.

Il Sindaco Alessandro Canelli, l'Assessore Valentina Graziosi, e Benedetta Baraggioli, Antonella Viviani e tutto lo staff del Comune di Novara hanno dato un supporto logistico fondamentale alla realizzazione dell'evento. Grazie alla Città di Novara per aver offerto ai nostri Ospiti momenti indimenticabili con l'unicità dell'arte architettonica e della storia e cultura rappresentati dalla Cupola Antonelliana, dal Broletto e dal Castello Sforzesco-Visconteo.

Grazie alla Dott.ssa Maria Rosa Fagnoni dell'Azienda Turistica Locale per aver curato nei dettagli l'accoglienza turistica dei nostri ospiti.

Ringrazio la Dott.ssa Laura Bianchi della Fondazione Castello per aver cortesemente messo a disposizione le sale del Castello per il Gala Dinner, una cornice storica che i nostri ospiti porteranno nella loro memoria.

Ringrazio la Prof.ssa Alessandra Aina, il Prof. Renato Politi e il Prof. Renato Meucci per aver organizzato il Concerto di Musica Classica come benvenuto agli ospiti. Un grazie speciale alla dott.ssa Anna Belfiore, Presidente del Conservatorio, che da subito ha promosso l'iniziativa mettendo a disposizione la sala Musica del Conservatorio.

Grazie alla dott.ssa Loreta Guacci e alla Prof.ssa Marina Lunghi, e alle allieve e allievi dell'I.P.S. G. Ravizza per aver collaborato nell'accoglienza dei partecipanti durante le giornate della Conferenza.

Al Signor Francesco Martelli di Italgrafica devo il mio Grazie per tutto il materiale tipografico a stampa (e grazie a Michele, per la sua professionalità e dedizione).

La Signora Giusi Ferrari dell'Agenzia Novarseti ha curato con professionalità ogni esigenza di viaggio e alloggiamento dei nostri ospiti. Alla professionalità impeccabile, Giusi unisce disponibilità e simpatia, ciò che rende il lavoro più leggero e piacevole.

Un grazie a parte lo meritano i tanti Amici di Novara, che con la loro presenza autorevole hanno dato la fiducia e la forza necessarie per affrontare l'impresa. Da subito, mi han fatto onore della loro Amicizia, e dunque mi permetto di menzionarli per nome, senza titoli. Tra questi, Franco, Paolo, Davide, Giuse, Maria Rosa, Laura, Piero, Francesco, e poi Alessandro, Benedetta, Valentina e Antonella (vi riconoscete?).

Grazie a Roberto Serra, per aver ideato e gestito la struttura dei weblink per la registrazione e up-load di CV e Abstract, e per l'invio automatico dei Certificati di Partecipazione, e grazie allo staff amministrativo della Scuola di Medicina (Francesco Cellerino, Simona Pasquino e Maurizio Pietroni). Grazie all'Ufficio Stampa e Comunicazioni dell'Ateneo (Paolo Pomati e Stefano Boda).

In chiusura, vorrei ringraziare con un affettuoso abbraccio tutti i miei allievi e collaboratori, passati e presenti, che hanno profuso tutte le loro energie per la buona riuscita del Congresso.

Grazie a Chiara, Alessandra, Andrea, Sara, Eleonora, Maria, Merupati (Rajeshwari), Angelica, Arianna, Giulia, Carlo, Claudia, Ilaria, Chiara, e Ada (Suyanee, Christian e Valentina erano lontani, ma vicinissimi con il pensiero) per la dedizione e professionalità con cui hanno curato nei minimi dettagli tutti gli aspetti pratici organizzativi. In questa occasione, non ho dovuto dir loro nulla… sapevano già cosa e come fare (hanno fatto esperienza, ormai!).

Ciro Isidoro