ASSOCIAZIONE ITALIANA DI COLTURE CELLULARI

ITALIAN ASSOCIATION OF CELL CULTURES (ONLUS-AICC)

nam et ipsa scientia potestas est

DEPARTMENT OF BIOTECHNOLOGICAL AND APPLIED CLINICAL SCIENCES
UNIVERSITY OF L'AQUILA

29TH ANNUAL CONFERENCE OF ITALIAN ASSOCIATION OF CELL CULTURES (ONLUS-AICC)

CROSSROADS IN CELLULAR AND MOLECULAR BIOTECHNOLOGY

NOVEL DIAGNOSTIC AND THERAPEUTIC STRATEGIES FOR CANCER, IMMUNOLOGICAL DISORDERS AND RARE DISEASES

L'AQUILA
23 - 25 NOVEMBER 2016
AUDITORIUM
DEPT. OF HUMAN STUDIES
UNIVERSITY OF L'AQUILA

PROGRAMME & ABSTRACT BOOK

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ACKNOWLEDGEMENTS

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Istituto Superiore di Sanità
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Davide Vecchiotti, Barbara Di Francesco
University of L'Aquila
Phone: 0862 433538
e-mails: davide.vecchiotti@univaq.it;
barbara.difrancesco1@graduate.univaq.it

PRESENTATION OF THE CONFERENCE

The 29° Conference of Italian Association of Cell Culture will take place in L'Aquila from 23 to 25 November 2016 at the Auditorium of Department of Human Studies, University of L'Aquila.

The Conference will be focused on the intersection between cellular and molecular biotechnology and bio-medicine, in particular in the fields of cancer, immunological disorders and rare diseases. In the recent past years, some of the key contributions of red biotechnology to the human health regarded both the diagnostic and therapeutic approaches to human diseases. Some relevant examples are monoclonal antibodies, DNA probes, recombinant vaccine, valuable drugs like insulin, gene therapy, next generation sequencing and non coding RNA. The leitmotif of the Conference will be the translation of recent biotechnological achievements into novel strategies to treat human diseases.

The meeting is organized in three days involving six thematic sessions with national and international speakers leaders in their scientific fields, an opening lecture and a poster session. New insights into basic and translational research, as well as in clinical research, will be discussed, in order to provide an opportunity of interactions and collaborations between participants.

During the second day of the meeting, a prize to the memory of Prof. Alberto Gulino, a great man and excellent scientist, former Director of the Department of Experimental Medicine and Dean of the Faculty of Medicine at the University of L'Aquila, will be awarded to the best Junior Researcher with a Ph.D. in the field of Medical Biotechnology or Experimental Medicine. This prize, funded by the Department of Biotechnological and Applied Clinical Sciences of L'Aquila University, is intended to promote and support the research work of a young scientist who has recently completed the Ph.D. course.

We hope the Conference will be of your interest and look forward to meet you in L'Aquila.

Francesca Zazzeroni
Edoardo Alesse

SCIENTIFIC PROGRAMME

23 November 2016

13.00 REGISTRATION AND POSTER POSITIONING

14.00 **OPENING CERIMONY**
GREETINGS FROM THE AUTHORITIES
GREETINGS FROM AICC PRESIDENT

14:30 **OPENING LECTURE**
The p53 Family in Cancer Biology
Gerry Melino (Leicester University, UK and University of Rome Tor Vergata, Rome)

SESSION 1
TARGETING MOLECULAR PATHWAYS IN CANCER: TRANSLATING BASIC RESEARCH INTO FUTURE THERAPY
Chairpersons: Katia Scotlandi, Massimo Donadelli

15:15 **Insights into the Oncogenic Activity of TrkAIII and its Potential Therapeutic "Achilles Heel"**
Andrew Reay Mackay (University of L'Aquila)

15:40 **Targeting Notch Signaling in T-cell Acute Lymphoblastic Leukemia: Mouse Models and More**
Isabella Screpanti (University La Sapienza, Rome)

16:05 **Mutant p53 Surfs into Non-Coding RNAs Network**
Giovanni Blandino (Regina Elena National Cancer Institute, Rome)

16:30 COFFEE BREAK

17:00 **Targeting GLI Factors to Inhibit the Hedgehog-Dependent Tumorigenesis**
Lucia Di Marcotullio (University La Sapienza, Rome)

17:25 **DTP3: First-in-Class GADD45β/MKK7 Inhibitor Selectively Targeting the NF-κB Survival Pathway in Multiple Myeloma**
Laura Tornatore (Imperial College London, UK)

Selected communications:

17:50 **PARP Inhibition Enhances Replication Stress and Causes Mitotic Catastrophe in MYCN-Dependent Neuroblastoma** (P14)
Valeria Colicchia (University La Sapienza, Rome)

18:00 **GPER Couples IL1β Expression in Cancer-Associated Fibroblasts to IL1R1 Function in Breast Cancer Cells** (P41)
Rosamaria Lappano (University of Calabria)

18:10 General Discussion

24 November 2016

SESSION 2
CUTTING EDGE STRATEGIES IN CANCER DIAGNOSTICS
Chairpersons: Mauro Di Ianni, Maria Teresa Di Martino

9:00 **New Mini-Invasive Therapies in Interventional Radiology**
Carlo Masciocchi (University of L'Aquila)

9:25 **Exploring the Skin at Histologic Resolution: Confocal Microscopy for Pathologist and Clinicians**
Elvira Moscarella (Arcispedale S. Maria Nuova-IRCCS, Reggio Emilia)

9:50 **Unravelling Secrets of the Secretome: Approaches to Identifying Secreted Mesothelioma Cancer Proteins**
Elia Ranzato (University of Piemonte Orientale)

10:15 COFFEE BREAK

10:45 **NGS Applications to Breast and Colorectal Cancer Molecular Genetics**
Giuseppe Giannini (University La Sapienza, Rome)

11:10 **Integration of Genomic Technologies as a Successful Diagnostic Approach in Onco-Hematology**
Cristina Mecucci (University of Perugia)

Selected communications:

11:35 **Definition of microRNAs Signatures of Nodal Involvement in Laryngeal Cancer Patients** (P40)
Hiromichi Kawasaki (Second University of Naples)

11:45 **Heterogeneous Mutational Status of Melanomas in Multiple Primary Melanoma Patients** (P53)
Pellegrini Cristina (University of L'Aquila)

11:55 General Discussion

12:05 **Measuring Mitochondrial Function and Glycolysis using the Seahorse XF Analyzers**
Emma Dicapua (Agilent technologies)

12:20 **"PROF. ALBERTO GULINO" AWARD PRIZE CEREMONY**

13:00 LUNCH and POSTER SESSION

SESSION 3
NOVEL DIRECTIONS IN CLINICAL ONCOLOGY
Chairpersons: Michele Caraglia, Claudia Chiodoni

14:30 **Biological and Clinical Aspects of CAR-T Cells**
Attilio Bondanza (San Raffaele, MI)

14:55 **T-regulatory Cells and Stem Cell Transplantation**
Mauro Di Ianni (University of L'Aquila)

15:20 **New Evidences in the Pathogenesis and Treatment of Basal Cell Carcinoma**
Ketty Peris (Catholic University of the Sacred Heart, Rome)

15:45 **Immune check-point inhibitors: revolution for cure**
Pierpaolo Correale (University of Siena)

16:10 **Applying the Cancer Stem Cell Paradigm to Cancer Treatment**
Pier Adelchi Ruffini (Dompè, Milano)

16:35 COFFEE BREAK

Selected communications:

17:00 **Notch Signaling Deregulation In T Cell Acute Lymphoblastic Leukemia Promotes The Generation of Myeloid Derived Suppressor Cells** **(P38)**
Paola Grazioli (University La Sapienza, Rome)

17:10 **Diet-related Inflammation and Immune Dysfunction in Obesity: Potential Risk Factors for Colorectal Cancer** **(P30)**
Gessani Sandra (National Health Institute, Rome)

17:20 **Targeting CXCR1 on Breast Cancer Stem Cells: Signaling Pathways and Clinical Application Modelling** **(P04)**
Annamaria Cimini (University of L'Aquila)

17:30 **Structure Based Lead Optimization Approach in Discovery of Novel 5-Lipoxygenase Inhibitors and Cytotoxic Activity as New Anticancer Drugs on Human Glioblastoma Cancer Cell Lines** **(P72)**
Rosanna Filosa (Second University of Naples)

17:40 General Discussion

17.50 **M&M Services**: **Easily Improving Data with Flow Cytometry at CLNS/IIT**
Giovanna Peruzzi (Istituto Italiano di Tecnologia, Rome)

18:05 Meeting AICC Members

SOCIAL DINNER

25 November 2016

SESSION 4
NOVEL ADVANCES IN CELLULAR AND BIOMOLECULAR RHEUMATOLOGY
Chairpersons: Roberto Giacomelli, Edoardo Alesse

9:00 **Subspecificities of Anticentromeric Protein A Antibodies and Pulmonary Vascular Disease in Systemic Sclerosis**
Federico Perosa (University of Bari)

9:25 **Microbiome in the Pathogenesis of Spondyloarthropathies**
Giuliana Guggino (University of Palermo)

9:50 **Systemic Fibrosis as Human Experimental Model of Fibrosis: Role of the Endothelial and Mesenchymal Transition**
Roberto Giacomelli (University of L'Aquila)

Selected communications:

10:15 **Perivascular Cells of Diffuse Systemic Sclerosis Patients Overexpress Activated ADAM12, which Modulates the Profibrotic TGFb Activity, and Play an Active Role in Myofibroblasts Trans-Differentiation and Development of Fibrosis** (P24)
Paola Di Benedetto (University of L'Aquila)

10:25 **CARMA2sh and its Psoriasis-Linked Variants Regulate Inflammatory Pathways in Human Keratinocytes** (P65)
Ivan Scudiero (Biogem, Ariano Irpino)

10:35 General Discussion

10:45 COFFEE BREAK

SESSION 5

RARE DISEASES: FROM MOLECULAR CHARACTERIZATION TO THERAPY

Chairpersons: Nadia Rucci, Stefania Meschini

11:10 **Revisiting Fibrous Dysplasia of Bone Through Mouse Models**
Mara Riminucci (University La Sapienza, Rome)

11:35 **Autosomal Dominant Osteopetrosis Type 2: Molecular Features and Experimental Therapy**
Anna Maria Teti (University of L'Aquila)

12:00 **Modulation of CD99 in Ewing Sarcoma: therapeutic prospective**
Katia Scotlandi (Rizzoli Orthopaedic Institute, Bologna)

12:25 **The Non-Oncologic Bone Diseases, Paget Disease, Fibrous Dysplasia, Hyperparathyroidism: Clinical Approach and Differential Diagnosis**
Carmine Zoccali (Regina Elena National Cancer Institute, Rome)

Selected communications:

12:50 **Single Nucleotide Polymorphisms Associated with Gastrointestinal Symptoms in Fabry Disease** (P64)
Francesca Scionti (Magna Graecia University, Catanzaro)

13:00 **The Loss of ATP2C1 Impairs the DNA Damage Response and Induces Altered Skin Hmeostasis: Consequences for Epidermal Biology in Hailey-Hailey Disease** (P74)
Azzurra *Zonfrilli (University La Sapienza, Rome)*

13:10 **AICC POSTER PRIZE CEREMONY AND CLOSING REMARKS**

POSTERS INDEX

P01 **SCD5 enforced expression moves the balance toward a more epithelial phenotype in cancer cells**
Maria Bellenghi, Rossella Puglisi, Sabina Sangaletti, Giada Pontecorvi, Lisabianca Bottero, Marina Petrini, Luca Pasquini, Mario Paolo Colombo, Gianfranco Mattia and Alessandra Carè

P02 **Itch/βarrestin2-dependent non-proteolytic ubiquitylation of SuFu controls Hedgehog signalling and medulloblastoma tumourigenesis.**
F. Bernardi, P. Infante, R. Faedda, F. Bufalieri, R. Alfonsi, S. Pfister, D. Guardavaccaro, A. Gulino, L. Di Marcotullio.

P03 **Targeting oncogenic miR-17-92 primary transcripts by LNA gapmeRs in multiple myeloma: Molecular findings and therapeutic potential**
Lavinia Biamonte, Eugenio Morelli, Cinzia Federico, Maria Teresa Di Martino, Nicola Amodio, Maria Eugenia Gallo Cantafio, Niels M. Frandsen, Francesca Scionti, Maria Rita Pitari, Daniele Caracciolo, Annamaria Gullà, Maria Angelica Stamato, Marco Rossi, Pierosandro Tagliaferri, Pierfrancesco Tassone

P04 **Targeting CXCR1 on breast cancer stem cells: signaling pathways and clinical application modelling.**
Laura Brandolini, Loredana Cristiano, Maria De Pizzol M, Tiziana Marilena Florio, Giuseppina Confalone, Angelo Galante, Benedetta Cinque, Elisabetta Benedetti, Pieradelchi Ruffini, Maria Grazia Cifone, Antonio Giordano, Marcello Alecci, Marcello Allegretti, Annamaria Cimini

P05 **Parallel sequencing of a 50 genes panel in metastatic colorectal cancer (MCRC) patients (pts) treated with intensive first line FIr-B/FOx triplet chemotherapy plus bevacizumab (BEV): preliminary data and clinical outcome.**
Gemma Bruera, Umberto Malapelle, Francesco Pepe, Pasquale Pisapia, Antonella Dal Mas, Giuseppe Calvisi, Giancarlo Troncone, Enrico Ricevuto

P06 **Mutant p53 proteins influence secretome of pancreatic cancer cells**
Giovanna Butera, Marcello Manfredi, Jessica Brandi, Raffaella Pacchiana, Marco Cordani, Buzza Adriana, Conte Eleonora, Daniela Cecconi, Emilio Marengo, Massimo Donadell

P07 ***Mutant p53 proteins induce chemoresistance through stabilization of GAPDH protein in the cytoplasm of PDAC cells.***
Giovanna Butera, Raffaella Pacchiana, Marco Cordani, Massimo Donadelli

P08 ***Leptin is a key factor in the angiogenic process induced by adipose-derived stem cells***
Calgani Alessia, Bruni Angelo, Amicucci G, Gentile Warschauer Emilio, Delle Monache Simona

POSTERS INDEX

POSTERS INDEX

P26 **Essential ammino acids have a powerful apoptotic effect in *in vitro* colon cancer cells.**
Jacopo di Gregorio, Francesco Dioguardi, Vincenzo Flati

P27 **Amniotic epithelial stem cells phenotype and orientation can be influenced using electrospun poly(lactide-co-glycolide) scaffolds with high grade of fibers alignment mimicking tendon extracellular matrix.**
L. Di Marcantonio, V. Russo, R. Wyrwa, A. Mauro, P. Berardinelli, T. Walter, M. Schnabelrauch, B. Barboni

P28 **ErbB and BER pathways in human gastric carcinoma cell line AGS: implication for a molecular cross-talk.**
Di Marcantonio Maria Carmela, Mincione Gabriella, Moscatello Carmelo, Savino Luca, Lepore Stefania, Grande Rossella, Muraro Raffaella, Aceto Gitana Maria

P29 **Notch3 - EGFR crosstalk in triple negative breast cancers (TNBC): new therapeutic possibilities.**
G Diluvio, F Del Gaudio, MV Giuli, E Giuliani, D Bellavia, I Screpanti, S Checquolo

P30 **Diet-related inflammation and immune dysfunction in obesity: potential risk factors for colorectal cancer.**
Donninelli G., D'Archivio M., Del Cornò M., Conti L., Scazzocchio B., Varì R., Varano B., Stefania G., Pierdominici M., Pacella I., Piconese S., Masella R., Gessani S.

P31 **Perinatal progenitors from human cord blood (Cord Blood-Borne Fibroblasts, CB-BFs) generate complete ossicles in vivo.**
Samantha Donsante, Alice Pievani, Domenico Raimondo, Cristina Remoli, Alessandro Corsi, Marta Serafini, Mara Riminucci

P32 ***Wnt*/b-catenin *gene* expression in colon adenomas and adjacent colonic mucosa.**
G.Fabietti, A. Ricci, F. Fantini, C. Moscatello, C. Efthymatis, M. Neri, R. Valanzano, G. Aceto, A. Cama, M.C. Curia.

P33 **Mutational analysis of Tp53 gene using NGS to drive an alternative therapy for patients with chronic lymphocytic leukemia.**
Carmela Ferri, Margherita Russo, Mayra Rachele Zarone, Alessia Maria Cossu, Anna Grimaldi, Angela Lombardi and Michele Caraglia.

P34 **Enhancement of radiosensitivity by the novel anticancer quinolone derivative vosaroxin in preclinical glioblastoma models.**
Claudio FESTUCCIA, Andrea MANCINI, Assunta Leda BIORDI, Alessandro COLAPIETRO, Flora VITALE, Francesco MARAMPON, Simona POMPILI Antonella VETUSCHI, Judith A. FOX, Giovanni Luca GRAVINA.

POSTERS INDEX

P35 **Cytotoxic activity of Juglone against Notch3-overexpressing T-cell acute lymphoblastic leukemia: targeting the ER/UPR signaling.**
MV Giuli, G Diluvio, E Giuliani, D Bellavia, R Palermo, I Screpanti, S Checquolo

P36 **TRAIL induces pro-apoptotic crosstalk between the TRAIL-receptor signaling pathway and TrkAIII in SH-SY5Y cells.**
Luciana Gneo, Pierdomenico Ruggeri, Lucia Cappabianca, Antonietta Rosella Farina and Andrew Reay Mackay

P37 **The brain penetrating pan EPH receptor antagonist, UNIPR1331, shows potent antiangiogenic and anti-invasive effects in glioblastoma preclinical models.**
Giovanni Luca Gravina, Claudio Festuccia, Carmine Giorgio, Andrea Mancini, Alessandro Colapietro, Simona Delle Monache, Cristina Pellegrini, Vincenzo Mattei, Stefano Martelluci, Paola Chiodelli, Marco Rusnati, Riccardo Castelli, Federica Vacondio, Alessio Lodola, Massimiliano Tognolini

P38 **NOTCH signaling deregulation in T cell acute lymphoblastic leukemia promotes the generation of myeloid derived suppressor cells**
Paola Grazioli, Claudia Noce, Gaia Scafetta, Andrea Orlando, Elena Delfino, Isabella Screpanti, Antonio Francesco Campese

P39 **The KCASH2-KO mice: a mouse model with mild hedgehog-dependent phenotype.**
Moretti M, Izzo M*, Laricchiuta D, Fabretti F, De Feudis G, Spiombi E, Angrisani A, Gelfo F, Petrosini L, De Smaele E.*

P40 **Definition of microRNAs signatures of nodal involvement in laryngeal cancer patients.**
Hiromichi Kawasaki, Angela Lombardi, Rosanna Capasso, Gabriella Misso, Filippo Ricciardiello, Teresa Abate, Maurizio Iengo, Domenico Testa, Domenico Napolitano, Giovanni Motta, Marco Fornili, Elia Mario Biganzoli, Diego Ingrosso, Michele Caraglia

P41 **GPER couples IL1β expression in cancer-associated fibroblasts to IL1R1 function in breast cancer cells.**
Rosamaria Lappano, Francesca Cirillo, Sergio Abonante, Marcello Maggiolini, Paola De Marco

P42 **Extracellular vesicles mediate crosstalk between stromal and tumour cells in the bone microenvironment.**
Alexander Loftus, Chris George, Riccardo Paone, Marco Ponzetti, Alfredo Cappariello, Anna Teti, Nadia Rucci

P51 **A signature of long-non coding RNAs (lncRNAs) is associated with response to chemotherapy in Ewing Sarcoma.**
Alessandro Parra, Andrea Grilli, Cristina Baricordi, Stefano Ferrari, Piero Picci, Katia Scotlandi.

P52 **Effectiveness of Vemurafenib, PIK-75 and miR-126 triple combined therapy on B-RAF^{V600E} metastatic melanoma through MAPK and/or PI3K/AKT inhibition.**
F. Pedini, G. De Luca, A. Boe, A. De Feo, M. Spada, S. D'Atri, A. Giuliani, A. Carè, N. Felli

P53 **Heterogeneous mutational status of melanomas in multiple primary melanoma patients**
Cristina Pellegrini, Claudia Martorelli, Gianluca Cipolloni, Lucia Di Nardo, Maria Giovanna Maturo, Ambra Antonini, Maria Concetta Fargnoli

P54 **New 5-fluorouracil amphiphilic derivatives in liposome formulation to overcome drug resistance in colon cancer.**
Pellegrini E., Condello M., Giansanti L., Petaccia M., Mancini G., Meschini S.

P55 **The emerging role of Jagged1 in sustaining colorectal cancer aggressiveness.**
M. Pelullo, S. Zema, R. Quaranta, I. Screpanti and D. Bellavia.

P56 **Hemoglobin beta (HBB) increases breast cancer aggressiveness via "oxygen positive hypoxia".**
Marco Ponzetti, Mattia Capulli, Adriano Angelucci, Luca Ventura, Simona Delle Monache, Cinzia Mercurio, Alessia Calgani, Patrizia Sanità, Anna Teti, Nadia Rucci.

P57 **The G-quadruplex ligand EMICORON potentiates the antitumor efficacy of chemotherapy on colon cancer experimental models.**
Manuela Porru, Simona Artuso, Luca Pompili, Carla Caruso, Armandodoriano Bianco, Marcella Mottolese, Carla Azzurra Amoreo, Annamaria Biroccio, Carlo Leonetti

P58 **Maml1 acts cooperatively with Gli proteins to regulate Sonic hedgheog signaling pathway.**
Quaranta R., Pelullo M., Nardozza F., Zema S., Di Marcotullio L., Screpanti I., Bellavia D.

P59 **Targeting PDE4 cAMP phosphodiesterases to control hepatocellular carcinoma growth.**
Federica Ragusa, Silvia Cardarelli, Francesca Galli, Maria Federica Giardi, Mariacarla Carusi, Benedetta Cinque, Mauro Giorgi, Mara Massimi

P67 **Two simultaneous and very uncommon *PI3KCA* mutations in a liver metastasis from a colorectal cancer patient with aggressive and resistant disease.**
Tessitore, Alessandra, Bruera Gemma, Mastroiaco Valentina, Cannita Katia, Cortellini Alessio, Dalmas Antonella, Zazzeroni Francesca, Ficorella Corrado, Ricevuto Enrico, Alesse Edoardo.

P68 **Co-development of DTP3 and its biomarkers to therapeutically target the NF-kB survival pathway in multiple myeloma.**
Laura Tornatore, Daria Capece, Verzella Daniela, Gary Acton, Elizabeth A. Campbell, James Kelly, Michael Tarbit, Nigel Adams, Selina Bannoo, Federica Begalli, Jason Bennet, Daniel D'Andrea, Annamaria Sandomenico, Antonio Leonardi, Menotti Ruvo, Magda J. Al-Obaidi, Reuben Benjamin, Richard S. Kaczmarski, Holger Auner, Jane Apperley, Guido Franzoso.

P69 **Notch3 gene expression in T-ALL is mediated by a mutually exclusive recruitment of Notch1 and EZH2 on its intron1.**
Luca Tottone, Rocco Palermo, Michele Zampieri, Fabrizio Simeoni, Claudio Talora, Isabella Screpanti

P70 **NF-κB regulates Gli1 expression in Human PCa.**
Davide Vecchiotti, Daniela Verzella, Daria Capece, Mariafausta Fischietti, Barbara Di Francesco, Mauro Di Vito Nolfi, Stefania Meschini, Alessandra Tessitore, Edoardo Alesse, Francesca Zazzeroni

P71 **Coupling of angiogenesis and bone remodeling under mechanical unloading: Mechanistic insights.**
Vimal Veeriah, Mattia Capulli, Angelo Zanniti, Nadia Rucci, Anna Teti

P72 **Structure Based Lead Optimization Approach in Discovery of Novel 5-Lipoxygenase Inhibitors and Cytotoxic Activity as New Anticancer Drugs on Human Glioblastoma Cancer Cell Lines.**
Silvia Zappavigna, Antonella Peduto, Alessia Cossu, Chiara Schiraldi, Michele Caraglia, Rosanna Filosa

P73 **MiR-125b interferes with proliferation and induces cellular senescence in *in vitro* models of Multiple Myeloma: an integrative analysis of signaling pathways and molecular mediators.**
MR Zarone; G Misso; A Grimaldi; M Russo; A Galeone; M Caraglia

P74 **The loss of ATP2C1 impairs the DNA damage response and induces altered skin homeostasis: Consequences for epidermal biology in Hailey-Hailey disease.**
Zonfrilli A, Cialfi S, Le Pera L, De Blasio C, Mariano G, Palermo R, Uccelletti D, Palleschi C, Biolcati G, Barbieri L, Screpanti I, Talora C.

ABSTRACTS

Mutant p53 Surfs into Non-Coding RNAs Network

Federica Ganci and Giovanni Blandino

Oncogenomic and Epigenetic Unit, Italian National Cancer Institute Regina Elena, Rome, Italy

Head and neck squamous cell carcinoma (HNSCC) is typically characterized by a high incidence of local recurrences, which is directly related to an intrinsic tumor radio/chemo-resistance. This may be due to the existence of pre-neoplastic processes at multiple sites in the mucosa or small clusters of histopathologically undetectable tumor cells that survive to the treatment and proliferate leading to local recurrence. *TP53* mutation is one of the main players involved in chemo-radio resistance and it is associated with local recurrence development in HNSCC patients. Among the best promising biomarkers, miRNAs, are emerging as an appealing tool for screening, diagnosis and prognosis of cancer. By direct sequencing of exons 2 through 11 we analyzed *TP53* status of a prospective series of 121 HNSCC samples and assessed its association with clinical outcome. We performed microRNA expression profiling by array on 121 HNSCC samples, 66 peritumor and 66 normal counterparts and, combining this information with *TP53* status data, we identified 49 microRNAs associated with*TP53* mutation. Among them, we found a group of 4 miRNAs that correlates with cancer-specific survival and a group of 12 miRNAs whose expression correlates with recurrence-free survival. Additionally, the deregulation of a subgroup of these miRNAs is predictive of recurrence also when their expression is analyzed in tumor-surrounding (peritumoral) mucosa independently from other clinical variables, either when considered individually or as a group. Interestingly, this capability was lost when we analyzed normal samples collected behind surgical free of disease resection margins. Of note, the combination of the information on microRNAs signature expression in peritumoral in addition to tumor tissues significantly increases its capability to predict local recurrence in HNSCC.
Because the peritumoral tissue may represent a not-yet-cancer- transformed tissue, the identification of a specific signature may represent both a clinical model for early detection and for local recurrence development.

Biological and clinical aspects of CAR-T cells

Attilio Bondanza

Vita-Salute San Raffaele University and San Raffaele Hospital IRCCS

Chimeric antigen receptors (CARs) are synthetic biology molecules generated by fusing antigen-binding moieties from a monoclonal antibody to signal transduction components of the TCR. T cells genetically modified with CARs have demonstrated unprecedented antitumor efficacy when targeting the CD19 antigen in B-cell tumors. Despite these successes, there a still a number of problematic issues to be solved before the much anticipated promise of the CAR-T cell therapeutic revolution is fulfilled. These issues include, but are not restricted to, identifying sufficiently tumor-restricted target antigens in solid tumors, overcoming secondary resistance, decreasing associated toxicities and lowering the costs of personalized CAR-T cell production for every patient in need. In this presentation, I will update on the latest clinical results and on the different strategies my and other research group are investigating in order to tackle these barriers and finally establish CAR-T cell immunotherapy as an effective, safe and widely available anti-tumor approach.

Immune check-point inhibitors: revolution for cure

Pierpaolo Correale

Oncoimmunology specialist, Unit of Radiotherapy, Department of Medicine, surgery and Neuroscience, Siena University Hospital, Italy.

Systemic treatments, including chemotherapy, and molecular-target specific drugs, may provide initial disease control and benefit in cancer patients, however, the majority of them suffers tumor recurrence and/or progression due to the occurrence of drug resistance and adaptive response of the residual tumor cells. Cancer immunotherapy is a further anticancer treatment modality, based on the concept that tumor cells express molecular structures recognized by immune-surveillance system. Cytotoxic-T-cells (CTLs), represent the effectors responsible of tumor cells destruction. The general lack of therapeutic efficacy of this kind of strategies has been explained by a poor immunogenicity and low expression of the target antigens; immunosuppressive inflammation/hypoxia; immunological safeway/inhibitory feedbacks including regulatory-T-cells (T_{reg}s), myeloid-derivative-suppressive cells (MDSCs), and multiple inhibitory immune-checkpoints. In this context, the recent rise and therapeutic success of immune checkpoint blockade with newest mAbs, has renewed the scientific interest in the investigation of immunotherapy in patients with common solid malignancies including non-small cell-lung-cancer and colorectal carcinoma. In this context, PD-1/PDL1 immune-check point blockade seem to be a promising therapeutic tool for these patients. The main limitation of this kind of treatment is represented by an inefficient tumor specific CTL response and by a low tumor infiltration rate of these CTLs preceding the immune-checkpoint blockade. Thus a rationale combination of PD-1/PDL1 blockade with treatments, like chemotherapy, RT or cancer vaccines, able to elicit an efficient CTL response could produce more than additive therapeutic results in these patients.

T regulatory cells and stem cell transplantation

Mauro Di Ianni

Department of Life, Health and Environmental Sciences, University of L'Aquila

Post-transplant relapse is still the major cause of treatment failure in high-risk acute leukemia. Attempts to manipulate alloreactive T cells to spare normal cells while killing leukemic cells have been unsuccessful. In HLA-haploidentical transplantation we reported that donor-derived T regulatory cells (Tregs), co-infused with conventional T cells (Tcons), protected recipients against graft versus host disease (GvHD). The present phase II study investigated whether Treg-Tcon adoptive immunotherapy prevents post-transplant leukemia relapse.
43 adults with high-risk acute leukemia (AML 33; ALL 10) were conditioned with a TBI-based regimen. Grafts included $CD34^+$ cells (mean $9.7x10^6$/kg), Tregs (mean $2.5x10^6$/kg) and Tcons (mean $1.1x10^6$/kg). No post-transplant immunosuppression was given. 95% patients achieved full-donor type engraftment. 15% developed ≥ grade II acute GvHD. The probability of disease-free survival was 0.56 at a median follow-up of 46 months. The very low cumulative incidence of relapse (0.05) was significantly better than in historical controls. These results demonstrate the immunosuppressive potential of Tregs can be used to suppress GvHD without loss of the benefits of GvL activity. Humanized murine models provided insights into the mechanisms underlying separation of GvL from GvHD, suggesting the GvL effect is due to largely unopposed Tcon alloantigen recognition in bone marrow.

Targeting GLI Factors to Inhibit the Hedgehog-Dependent Tumorigenesis

Lucia Di Marcotullio

Department of Molecular Medicine, University "La Sapienza" Rome

The Hedgehog (Hh) pathway has emerged as an attractive target for anticancer therapy because its aberrant activation is implicated in several cancers. A critical goal in Hh-dependent tumor biology is the discovery of novel antagonists blocking the pathway both at upstream (Smo antagonists) and downstream (Gli inhibitors) level. The current development of new Smo antagonists associated with Hh pathway activation represents a major challenge for cancer therapy because most clinical data have been discouraging. Moreover, transcription factors are generally considered interesting targets in drug discovery and Glis factors, the final effectors of the Hh pathway, are among the most promising for the development of new anticancer drugs.
By a structure-based approach and multidisciplinary efforts, we recently identified novel compounds targeting Smo and Gli and investigated their pharmacological effects on Hh-dependent tumors. In particular, we discovered the first small molecule, GlaB, able to impair Hh oncogenic activity by inhibiting Gli/DNA interaction. This small molecule turned out to be an efficient inhibitor of the growth of Hh/Gli-dependent tumors *in vitro* and *in vivo*, indicating that Gli/DNA interference is an appealing therapeutic strategy to control the heterogeneous molecular changes leading to Hh/Gli pathway activation in cancer.

Systemic Fibrosis as Human Experimental Model of Fibrosis: Role of the Endothelial and Mesenchymal Transition

Roberto Giacomelli

Department of Biotechnological and Applied Clinical Sciences, Rheumatology Unit, School of Medicine, University of L'Aquila

Fibrosis is defined by the overgrowth, hardening, and/or scarring of various tissues and is attributed to excessive deposition of extracellular matrix components, including collagen. The key cellular mediator of fibrosis is the myofibroblast, which, after activation, produces large amount of collagen. Myofibroblasts are generated from a variety of sources including resident mesenchymal cells, epithelial and endothelial cells (ECs) by a process termed epithelial/endothelial-mesenchymal (EMT/EndMT) transition, as well as from circulating fibroblast-like cells.
Fibrosis in multiple organs is the distinguishing hallmark of Systemic Sclerosis (SSc). In this pathology, fibrosis and myofibroblast activation represents the irreversible end stage, maybe triggered by microvascular alteration, responsible of ischemia/reperfusion damage, impaired compensatory angiogenesis and capillary rarefaction. Although the complex link between vessels disappearance and fibroblasts activation is largely unknown, several papers focused on the interplay between ECs and perivascular cells, in SSc pathogenesis. In fact, the maintenance of the existing vasculature and the stabilization of the newly formed vessels depend by a strict interplay between ECs and pericytes, and an altered ECs-pericytes crosstalk, in SSc, may elicit an adverse phenotypic switch of multipotent perivascular cells toward a myofibroblastic phenotype. Different molecules such as Transorming Growth Factor- β (TGFβ), Endothelin-1 (ET-1), Caveolin-1 (Cav1), Vascular Growth Factor (VEGF), Angiopoietin-1/2 (Ang-1/2) and their receptors are involved in this altered crosstalk, modulating both ECs damage and fibrosis. We recently showed that SSc-endothelial cells (SSc-ECs), under the synergistic effect of TGFβ and ET-1, may further trans-differentiate toward myofibroblasts. During the trans differentiation, resident ECs loss cell-cell junctions and EC markers, such as Von Willebrand factor (vWF), CD31, and Vascular Endothelial-cadherin (VE-Cadherin), acquire invasive properties, associated with the gain of mesenchymal markers, such as alpha-Smooth Muscle Actin (αSMA), Smooth muscle 22 (Sm22) and Collagen1 (Col1A1), delaminate from the polarized cell layer thus invading the underlying tissue. Elucidating the myofibroblasts origin and EndoMT mechanisms, in *in vitro* experimental models of SSc pathogenesis, is essential in the quest to develop effective strategies to treat fibrotic disorders, a condition still needing an improved understanding of the roles of individual cell types and their products, in the development of fibrosis.

NGS Applications to Breast and Colorectal Cancer Molecular Genetics

Giuseppe Giannini

Department of Molecular Medicine, University La Sapienza, Rome

The advent of Next Generation Sequence technologies has had a terrific impact in genetics, and, more in general, in biology. Whole Genome Sequencing (WGS), Whole Exome Sequencing (WES) and Targeted Resequencing (TR) can provide detailed characterization of the genomic, exomic, or selected genes variations, at the level of point mutations, chromosomal structural aberrations and copy number alterations. Prior combination with specific steps allows NGS to be applied to qualitative and quantitative characterization of the transcriptome, miRNAome and epigenome.
Integrating NGS with other platforms, national or international consortiums have studied the genome, epigenome and transcriptome of relevant numbers of specific tumors. Highly sophisticated bioinformatic analysis translated the huge amount of data into a better understanding of cancer-associated biological pathways, or in the construction of molecular subtyping/classification profiles.
However, the clinical consequences of this revolution have not been as astonishing, yet. In example, while the TR of gene panels allows the identification of families exposed to a genetic risk for breast/ovarian cancer due to mutations in many non BRCA!/BRCA2 genes, guidelines for the clinical managements of these individuals are not yet available. Moreover, despite the dramatic drop in the costs of “sequencing”, the entire “omic” characterization of a tumor at the single patient level is far from being attainable, yet.
Nonetheless, a “clinical” use of NGS technologies is already ongoing. In example, targeted resequencing of predefined gene panels containing, but not limited to, clinically relevant targets (i.e. RAS genes and BRAF, in colorectal cancer), while absolving to the immediate clinical demand, may also provide useful information for a better subclassification of the patients and for more informed therapy decisions, with no significant extra costs. Our experience suggests that a clinically-oriented mutation screening may provide not only an increased awareness in “predictive oncology” but also interesting hints for a better biological understanding of cancer.

Microbiome in the Pathogenesis of Spondyloarthropathies

Giuliana Guggino, Francesco Ciccia, GIovanni Triolo

DIBIMIS, Rheumatology section, University of Palermo

Inflammatory innate and adaptive immune cell responses to commensal bacteria underlie the pathogenesis of human chronic inflammatory diseases. Intestinal dysbiosis has been described in patients with spondyloarthritis (SpA) and seems to be correlated with histologic and immunologic alterations. We will discuss the relationship occurring between intestinal dysbiosis and innate immune responses in patients with axial SpA.
Intestinal dysbiosis and differential activation of intestinal immune responses in patients with SpA have been demonstrated. Furthermore, innate cells that appear to be involved in the pathogenesis of SpA may control intestinal homeostasis through induction of apoptotic cell death and deletion of activated commensal bacteria-specific T cells.
Although the evidence shows that dysbiosis occurs in SpA, it is not clear the role of dysbiosis in regulating innate immune responses in SpA. Relationships between cause and effect remain to be answered.

Insights into the Oncogenic Activity of TrkAIII and its Potential Therapeutic "Achilles Heel".

Luciana Gneo, Pierdomenico Ruggeri, Lucia Cappabianca, Antonietta Rosella Farina and Andrew Reay Mackay.

Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila

The developmental and stress-regulated alternative TrkAIII splice variant of the TrkA NGF receptor is expressed by advanced stage primary neuroblastomas (NB), associates with worse prognosis, post therapeutic relapse and metastatic disease, and in NB models exhibits oncogenic activity and promotes chemotherapeutic-resistance. TrkAIII exhibits exon 6 and 7 skipping and is devoid of the extracellular D4 IG-C1 domain and several N-glycosylation sites that regulate cell surface expression and prevent spontaneous activation of fully spliced TrkA. As a consequence, TrkAIII is not expressed at the cell surface but accumulates within intracellular membranes, within which it exhibits spontaneous ligand-independent activation. Subsequent oncogenic activity from this alternative location results from IP3K/Akt/NF-kB survival signaling, induction of pro-angiogenic and stem cell-like gene expression, and recruitment of TrkAIII to the centrosome, inducing centrosome amplification and increasing chromosomal instability. Recently, we have gained further insight into the mechanism responsible for TrkAIII re-localisation, leading to oncogenic rather than the NB tumor suppressing activity exhibited by ligand-activated cell surface TrkA. In contrast to fully spliced TrkA, nascent immature TrkAIII accumulates above the spontaneous activation threshold within the ERGIC/COPI compartment, facilitated by omission of the spontaneous activation prevention D4 IG-C1 domain. Activation within this compartment not only results in oncogenic IP3K/Akt signaling but also in MT minus end-directed retrograde transport back to the ER, where TrkAIII is inactivated, and transport to the centrosome. TrkAIII, therefore, conserves the retrograde transport behaviour exhibited by fully spliced ligand-activated cell surface TrkA but within the wrong compartment. This, sets up self-perpetuating TrkAIII re-cycling between the ER and ERGIC/COPI compartments, blocking anterograde TrkAIII transport and maturation at the Golgi and ensuring continual accumulation and activation within ERGIC/COP1 membranes, unveiling a novel mechanism for RTK oncoprotein re-localisation, as a basis for oncogenic activity. We have also stumbled upon a potential therapeutic "Achilles heel" for the TrkAIII oncoprotein in human NB cells, with the observation that TrkAIII sensitizes TRAIL-resistant SH-SY5Y NB cells to TRAIL-induced apoptosis. Evidence will be presented that this results from one-way SHP/c-Src-mediated pro-apoptotic crosstalk between the TRAIL receptor pathway and TrkAIII, and supports a potential therapeutic use for TRAIL in TrkAIII expressing NB.

New mini-invasive therapies in Interventional Radiology

Carlo Masciocchi

Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila

Interventional Radiology (IR), thanks to technological developments, is achieving a first-line palliative as well as curative role in the management of different pathologies.
Apart from the well know application in the vascular and biliary fields, the oncological branch is experiencing quite an interesting growth with remarkable results. Thanks in particular to the techniques of ablation, IR can be proposed as "mininvasive surgery" allowing treatment of tumoral lesions with minimal injury to the surrounding structures. These techniques of ablation are today extremely powerful and precise in delivering energy and allow tretament of both benign and malignant lesions. In particular, in the last years, research has been moving to explore the possibilities of IR for the management of particular types or stages of tumors (i.e. when the diffusion of the disease is circumscribed). Usually a needle (or probe) is needed to deliver energy in the target point, but in the last years, an even less invasive technique has been developed: Focused Ultrasound Surgery by which it is possible to reach a lesion deeply located in the body without even cutting the skin of the patients. The ablation is obtained through thermal injury: both heat (Radiofrequency, Microwaves, etc.) and ice (cryoablation) can be used to the purpose.
All these procedures require an imaging technique (usually Ultrasound, Computer Tomography, Fluoroscopy or Magnetic Resonance) to guide the needle (or the focused ultrasound beam) to the target lesions. This accounts for the growing role of the interventional radiologist.
The choice of the most appropriate technique must be done in relation with the position and the characteristics of the lesions.
The main advantages of IR techniques are represented by the low grade of invasiveness, low incidence of complications and high rates of effectiveness, also in patients with poor health conditions.

Integration of Genomic Technologies as a Succesful Diagnostic Approach in Onco-Hematology

Cristina Mecucci and Roberta La Starza

Ematologia, Università di Perugia

Investigations on DNA structure and function in malignant cells provided us with exciting results to precisely diagnose and cure malignant hemopathies. Over last twenty years the technological revolution changed our diagnostic approach in hematology. Advances have been recognized by the WHO classification which incorporated recurrent genomic changes as hallmarks of myeloid and lymphoid malignancies. Available genomic technologies in advanced diagnostic laboratories include conventional and molecular cytogenetics, SNPs and CGH arrays, quantitative PCR, Sanger and next generation sequencing. Despite of the recent emphasis on the last technique, integration of more than one technology may be much rewarding in diagnostic algorithms. Multi-FISH and/or SNPs are helpful to properly classify complex karyotypes and unbalanced rearrangements. Normal cytogenetics in AML is the first step to address mutational analysis for NPM1, FLT3 and CEBPA, identifying distinct prognostic subgroups. FISH is a powerful approach to pick up rearrangements of promiscuous genes such as PDGFRB, PDGFRA and FGFR1 in Ph-negative myeloid neoplasms in which tyrosine kinase inhibitors are a successful targeted therapy. RNA-seq is also promising in all leukemia cases with founding lesions affecting promiscuous genes with rearrangements involving multiple genomic partners. Next generation sequencing is very interesting to interrogate a number of genes at once, as needed for definition of clonality in MDS. Genomic karyotyping by SNPs, in addition to unravel cryptic cytogenetic changes under the resolution power of conventional cytogenetics, is also a unique tool to identify copy neutral loss of heterozygosity as well as amplifications and chromotrypsis.

The p53 family in cancer biology

Ivano Amelio[1], Francesca Bernassola[2], Giovanni Chillemi[3], Tak Wah Mak[4], and Gerry Melino[1,2]

[1] MRC Toxicology Unit, LeicesterLE1 9HN, United Kingdom
[2] University of Rome Tor Vergata, Rome, Italy
[3] SCAI-SuperComputing Applications & Innovation Department, CINECA, Rome, Italy.
[4] The Campbell Family Cancer Research Institute, Toronto, Ontario M5G 2M9, Canada

The p53 family members p73 and p63 are involved in female infertility maternal reproduction (Nature Rev MCB 2011;12,4:259) and as well as in cancer formation (TiBS 2014;39,4:191). We identified their activation during DNA damage, several transcriptional targets, the mechanisms of regulation of cell death, and the protein degradation pathway.
To understand the p53 structure-function relationship, we performed a molecular dynamics study, showing an induced-fit interaction of the C-terminal domain with the DNA-binding domain. Direct intra- and intermonomeric long-range communications between the tetramerization and DNA-binding domains are noted, providing a biophysical rationale for the reported functional regulation of the p53 C-terminal region. We also detect 'dynamic' deformations switched on and off by particular p53 tetrameric conformations and measured by the roll and twist parameters in the same base pairs. These different conformations can indeed modulate the electrostatic potential isosurfaces of the whole p53-DNA complex (Oncogene, in press PMID: 26477317).
While TAp73$^{-/-}$ mice show high tumor incidence with hippocampal dysegensis, they show an elevated cancer incidence. Accordingly, TAp73 opposes HIF-1 activity, affecting tumour angiogenesis. TAp73 interacts with HIF-1a, promoting HIF-1a polyubiquitination and consequent proteasomal degradation. These findings demonstrate a novel mechanism for HIF-1 regulation and provide an additional explanation for the molecular basis of the growth, progression, and invasiveness of human cancers. (PNAS-USA 2015;112,1:226) (TiBS 2015;40,8:425).
P63 is a determinant of skin development. Using a MMTV-ErbB2 murine model, we found that ΔNp63 regulates mammary Cancer Stem Cells self-renewal and breast tumorigenesis via the direct transactivation of Sonic Hedgehog (Shh), GLI family zinc finger 2 (Gli2), and Patched1 (Ptch1) genes. (PNAS-USA 2015. 112,11: 3499-504. PMID: 25739959). At least in part, this seems to be exerted by regulation of the metabolism via Hexokinase II (PNAS-USA 2015. 112,37: 11577-82. PMID: 26324887).

Exploring the Skin at Histologic Resolution: Confocal Microscopy for Pathologist and Clinicians

Elvira Moscarella

Arcispedale S. Maria Nuova-IRCCS, Reggio Emilia

Reflectance confocal microscopy (RCM) is a non-invasive imaging technique that enables in vivo visualisation of the epidermis down to the papillary dermis in real time. Resolution is almost comparable to conventional histology. It has the advantage of allowing the clinician to do a “virtual biopsy” of the skin and obtain diagnostic clues while minimising unnecessary skin biopsies.
RCM uses the diode laser as a source of monochromatic and coherent light.
The light passes through a beam splitter, a scanning and focussing optical lens and a skin contact device. It penetrates the skin and illuminates a small tissue spot.
Light reflected from the focal point reflects back through the lens, which focusses it into a small pinhole and forms an image on a photodetector.
The pinhole only allows light from a focal point to pass through (i.e. it is confocal), and prevents light from another tissue point or out-of-focus plane from getting through.
RCM relies on reflectance (back-scattering) of light from structures with endogenous contrast, such as melanin, haemoglobin and some organelles. Reflectance occurs at the boundaries of two structures with different refractive indices, such as membranes, keratohyaline granules and melanosomes.
The commercially available device uses a laser of 830 nm, as this wavelength does not cause tissue injury for the patient or injury to the eyes of the operator. The depth of penetration is 200–300 µm, which generally corresponds to the papillary dermis or upper reticular dermis.
The main field of application is oncologic dermatology, as it allows the recognition of early skin tumors. However, numerous new applications are currently under examination, including inflammatory and infectious skin diseases.

New evidences in the pathogenesis and treatment of basal cell carcinoma

Ketty Peris

Institute of Dermatology, Catholic University, Rome, Italy

Basal cell carcinoma (BCC) is mainly an indolent and slowly progressive skin tumor that can be cured by surgical excision, however a small proportion of patients develop more invasive forms of BCC. Advanced BCC comprises locally advanced BCC (laBCC) where lesions are usually large, histologically invasive, unresectable or life threatening lesions, and metastatic BCC (mBCC), where the lesion spreads to lymph nodes and distant organs (bone, lung, and/or liver). Once metastasised, BCC is associated with a poor prognosis; these patients have a median survival of 8 months, and a 5-year survival of 10%. In patients with advanced BCC, radiation therapy and/or surgery are often inappropriate or the lesions are considered inoperable. Treatment of advanced BCC and multiple BCCs in Gorlin syndrome patients is currently problematic because there is no standard therapy and non-surgical treatment options are limited.
Abnormal activation of Hedgehog pathway signaling is a key driver in the pathogenesis of BCC. In BCC, the Hedgehog signaling pathway is typically activated by mutations leading to dysfunction of PTCH1, as observed in almost all patients with BCNS and in most patients with sporadic BCC tumors. An estimated 80%–90% of sporadic BCC tumors have PTCH mutations, whereas 10% harbor SMO mutations. Both types of mutations lead to constitutive SMO signaling and BCC development.
Vismodegib, a first-in-class small molecule inhibitor of Hedgehog pathway signaling represents an important treatment option for patients with advanced BCC, and is approved by regulatory authorities for the treatment of adults who have metastatic BCC or locally advanced BCC— a population for whom options were previously limited.

Subspecificities of anticentromeric protein A antibodies and pulmonary vascular disease in systemic sclerosis.

Federico PEROSA[a)], Elvira FAVOINO[a)], Isabella Eleonora FAVIA[a)], Serena VETTORI[b)], Marcella PRETE [a)], Ada CORRADO[c)], Francesco Paolo CANTATORE[c)], Gabriele VALENTINI[b)]

[a)] *Department of Biomedical Sciences and Human Oncology (DIMO), Rheumatologic and Systemic Autoimmune Diseases Unit, University of Bari Medical School, I-70124 Bari, Italy.*
[b)] *Department of Clinical and Experimental Internal Medicine "F. Magrassi, A.Lanzara"- Rheumatology Section, Second University of Naples, Naples, Italy.*
[c)] *Department of Medical and Surgery Sciences, Rheumatology Unit, University of Foggia, Foggia, Italy.*

Patients affected by systemic sclerosis (SSc) with anti-centromeric proteins (CENPs) autoantibodies are at risk of developing pulmonary vascular disease and pulmonary arterial hypertension (PAH) without fibrosis. At present no biomarkers are available to predict these complications. We previously characterized the fine specificity of anti-CENP-A antibodies in SSc by the use of a phage peptide display library, and identified phage clones whose peptides were differentially recognized by patients' autoantibodies. Here, we examined if subsets of SSc patients that differ in anti-CENP-A antibody subspecificities, also differ in terms of clinical features, and if serum antibody positivity for phage-displayed peptides can predict pulmonary vascular disease.
Blood samples and clinical data were collected from 84 anti-CENP-A-positive SSc patients. Serum reactivity to phage-displayed peptides was assessed by ELISA. Pulmonary vascular disease was defined as high systolic pulmonary arterial pressure (sPAP) and low diffusing lung capacity for carbon monoxide (DLCO; percent of predicted values).
Screening patient for reactivity to peptides expressed by phage clones pc4.2 and pc14.1, confirmed our earlier observation of differential specificities. Levels of antibodies specific for the 2 phage clones were associated with clinical features of pulmonary vascular disease, but in opposite ways. Specifically, anti-pc4.2 antibodies were positively associated with sPAP and inversely associated with DLCO, whereas anti-pc14.1 antibodies were inversely associated with sPAP and positively associated with DLCO. Levels of anti-pc4.2 and anti-pc14.1 antibodies predicted sPAP independently of DLCO. These correlations were confirmed by logistic regression using antibodies as predictors and dichotomized sPAP (cutoff, 45 mm Hg) as outcome. The ratio of the 2 antibody levels was a suitable biomarker in predicting high sPAP.
This study demonstrates that some SSc clinical condition associate with anti-CENP-A antibody subspecificities. Moreover, it shows a novel phage-based ELISA assay that can be used to predict SSc patients with high sPAP and low DLCO, hence those who are at greater risk of developing PAH. The ability to identify patients at risk for PAH may contribute to clinical efficiency and effectiveness. Further research into the peptides expressed by the phage clones may reveal the molecular mechanisms underlying increased risk of pulmonary vascular disease in anti-CENP-A-positive patients.

Unravelling secrets of the secretome: approaches to identifying secreted mesothelioma cancer proteins

Elia Ranzato

Università of Piemonte Orientale, DISIT - Dipartimento di Scienze e Innovazione Tecnologica, viale Teresa Michel, 11 - 15121 Alessandria, Italy

Despite major improvements on the knowledge and clinical management, cancer is still a deadly disease. Novel biomarkers for better cancer detection, diagnosis and treatment prediction are urgently needed. Proteins secreted, shed or leaking from the cancer cell, collectively termed the “cancer secretome”, are promising biomarkers since they might be detectable in blood or other bio-fluids.
Furthermore, the cancer secretome in part represents the tumor microenvironment that plays a key role in cancer promoting processes.
Malignant Mesothelioma (MMe) is characterized by a long latency period (20–30 years), a poor prognosis, and limited effective therapies. Finding novel diagnostic and therapeutic strategies is thus extremely important. MMe has a highly secretory cell type, and the factors released by cells may act in an autocrine or paracrine fashion on tumour and stroma, where they may modulate the extracellular environment.
The main goal is the characterization of the secretome of two MMe cell lines in comparison with a mesothelial cell line, and the evaluation of differences and similarities, of these two different MMe cancer model systems, to identify potential biomarkers.
We obtained the relative quantitation of secreted proteins by using the SWATH-MS (Sequential Window Acquisition of all Theoretical fragment ion spectra) analysis, which is a high throughput label-free method for protein quantitation that combines the traditional shotgun proteomics with the quantitative accuracy and reproducibility of selected reaction monitoring (SRM).
Our data outline the importance of current cancer secretome research and describe the innovative mass spectrometry-based analysis of the secretome of the two phenotypes of Mme.
The consistent and reproducible quantification of proteins by SWATH-MS provides insight in MMe progression, targeting the secretory pathway as a promising strategy for the management of this cancer.

Revisiting Fibrous Dysplasia of bone through mouse models

Mara Riminucci[1], Cristina Remoli[1], Rossella Labella[1], Biagio Palmisano[1], Alessandro Corsi[1], Isabella Saggio[1], Kenn Holmbeck[2], Pamela Gehron Robey[2] and Paolo Bianco[1]

[1]Sapienza University of Rome, Italy; [2]CSDB, NIDCR, NIH, Department of Health and Human Services, USA.

Fibrous Dysplasia (FD/MAS, OMIM174800) is a crippling skeletal disease caused by gain-of-function mutations of the Gsα gene (R201C, R201H). Gsα mutations associated with FD arise in early phases of embryo development. However, FD is a post-natal disease in which normally developed skeletal tissues are replaced by fibrous marrow and newly formed, mechanically unsound bone. We previously demonstrated that FD results from the dysfunction of mutated cells in the post-natal skeletal lineage and can be modeled by heterotopic transplantation of skeletal progenitors isolated from FD bones. However, the cellular and molecular pathogenetic mechanisms underlying the disease are as yet unknown and no effective therapies are currently available for FD patients. Recently, we generated transgenic mice expressing the mutated $Gs\alpha^{R201C}$ sequence either constitutively (EF1α-Gsa^{R201C} and PGK- $Gs\alpha^{R201C}$ mice) or as targeted to mature osteogenic cells (Col1A1- $Gs\alpha^{R201C}$ mice). Transgenic lines with constitutive expression of $Gs\alpha^{R201C}$ develop an exact replica of human skeletal lesions and provide the first-in-class mouse models of FD. In contrast, mice with osteoblast-targeted $Gs\alpha^{R201C}$ mutation develop a skeletal phenotype that is different from FD and reproduces human high bone mass disorders caused by dysregulated Wnt signaling. These transgenic lines demonstrate that the essential, morbidity-causing features of FD are not dependent on the direct impact of Gsα mutations on differentiated bone forming cells, and must emanate from the aberrant differentiation/function of cell types other than mature osteoblasts. To better understand the cellular determinants of FD, we have now established additional transgenic lines in which the expression of $Gs\alpha^{R201C}$ is targeted to different skeletal phenotypes in the bone microenvironment. Altogether, our transgenic mice are expected to provide conclusive evidence as to the cellular origin of FD lesions in the bone/bone marrow organ.

Applying the CSC paradigm to cancer treatment

Pier Adelchi Ruffini

Dompé farmaceutici s.p.a., Milano, Italy

Cancer Stem Cells (CSC) are a rare cell population within a tumor characterized by the ability to form tumors following injection into an immunocompromised host. While the role of CSC has been clearly established in animal models, retrospective clinical observations point to CSC as responsible for tumor recurrence and metastasis. Thus, it is theorized that the elimination of CSC would reduce the risk of cancer progression due to tumor regrowth after treatment. Hence, demonstration of the therapeutic elimination of CSC in the clinical setting is desired. A number of markers, or combination thereof, have been used to detect and measure CSC in almost all human tumors. Several pathways have been identified as crucial for, but not necessarily unique to, CSC survival and proliferation, and novel agents have been designed to target such pathways. A number of such agents have entered early phase development. Further, drugs that have long been marketed for non-oncological indications have been redirected to oncology as they appear to affect one or more of such pathways. However, the goal of CSC elimination following treatment thus far has remained elusive, and definitive demonstration of their clinical relevance as therapeutic target has not been achieved yet.
This presentation aims to review the available evidence on the clinical relevance of CSC from a drug development standpoint and the results of clinical trials of CSC targeting agents. It also discusses limitations of current clinical trial design and endpoints to demonstrate anti-CSC activity as well as possible strategies to overcome these limitations.

Modulation of CD99 in Ewing Sarcoma: therapeutic prospective

Scotlandi Katia

CRS Development of Biomolecular Therapies, Rizzoli Orthopaedic Institute, Bologna

CD99 is a trans-membrane protein of 32 Kda that regulates several important biological processes, such as cell migration, adhesion, apoptosis and cell differentiation. In physiology CD99 has been found to play a crucial role in the regulation of T- and B- cell differentiation together with in the trans-endothelial migration of immune cells during inflammation. In cancer the molecule shows a dual role playing as oncogene or oncosuppressor in dependence of the cellular context. In particular, in Ewing sarcoma and in acute lymphoblastic leukaemia CD99 acts as a potent oncogene that maintains cell stemness and favours cell migration and metastasis. In contrast in other tumours, such as osteosarcoma and gastric tumours, CD99 is expressed in the benign lesions and is lost during malignancy. Molecule replacement shifts malignant cells toward a less aggressive phenotype. In addition, recent papers have also shown a role of CD99 in the cross-talk between malignant and stromal or immune normal cells, thus modifying interactions in tumour microenvironment. Strategies to exploit the therapeutic potentialities of CD99 must be therefore different in the different cellular context, ranging from the use of antibodies to epigenetic drugs that may favours CD99 re-expression. Efficacy of different treatments in Ewing sarcoma and osteosarcoma, as paradigm of different situations, will be presented. Academia jointed the industry in the effort to take novel compounds at clinical level.

Targeting Notch signaling in T-cell Acute Lymphoblastic leukemia: mouse models and more

Rocco Palermo, Saula Checquolo, Diana Bellavia and Isabella Screpanti

Sapienza University of Rome and IIT - CLS@Sapienza, Rome, Italy

Deregulation of Notch signaling is known to play a key role in T-cell transformation, since about 60% of T-ALL harbor activating *NOTCH1* mutations and the vast majority of T-cell Acute Lymphoblastic Leukemia (T-ALL)-bearing patients displays increased expression and function of Notch3. T-ALL is an aggressive hematological cancer that accounts for 10-15% of pediatric and 25% of adult ALL cases. Although efficient chemotherapy protocols rendered T-ALL a curable cancer, 25-30% of children and up to 60% of adults still relapse, undergoing a poor prognosis.
Our previous studies, involving the generation of a mouse model of T-ALL, driven by the enforced expression of constitutively active Notch3 intracellular domain (N3-IC) in immature thymocytes, and of several double mutant mice, suggested that the initial dysregulation of T cell development in N3-IC tg mice takes place at the intrathymic preT/T cell transition phase of thymocyte differentiation and results in the disruption of proliferation and maturation process checkpoints, recapitulated in the pre-TCR- and NF-kB-dependent steps. In keeping with our results, more recently it has been shown that 50% of a T-ALL patient cohort display activation of Notch3 and 2 out of 12 of them exhibit activation of NOTCH3 without activation of NOTCH1, thus supporting a non redundant role of either Notch paralogue in T-ALL.
Several Notch-blocking agents have been developed up to preclinical research and a number of them have been moved to clinical trials for the therapy of Notch-driven tumors, including T-ALL. To this regard, it is worth noting that the most promising pharmacologic approach to block Notch signaling relies in the suppression of the last proteolytic cleavage operated by γ-secretase, which leads to the generation of the functional Notch-IC. Unfortunately, as revealed by clinical trials, the potential clinical applications of γ-secretase inhibitors is limited by primary resistance and/or by severe off-target side effects, especially those occurring within the gastrointestinal tract.
Through the use of *in vivo*, *ex vivo* and *in vitro* experimental models we approached the specific role of Notch3 in development and progression of T-ALL, as well as the possible pharmacological targeting of Notch signaling, including the identification of new specific Notch inhibitors.

Autosomal Dominant Osteopetrosis Type 2: Molecular Features and Experimental Therapy

Anna Teti

Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila

Autosomal Dominant Osteopetrosis Type 2 (ADO2) is a heritable osteosclerotic disorder dependent on osteoclast impairment. In most patients, it results from heterozygous missense mutations in the chloride channel 7 (*CLCN7*) gene, encoding for the CLC7 $2Cl^-/1H^+$ antiporter. By a knock-in strategy we generated a mouse model of ADO2 carrying the *Clcn7* mouse homologue (p.G213R) of the most frequent heterozygous mutation found in humans (p.G215R). The ADO2 model holds true, presenting with higher bone mass and increased numbers of poorly resorbing osteoclasts than wildtype mice, and a lethal phenotype in the homozygous state. The ClC7 protein is expressed in many organs and ADO2 mice presented with lung perivascular fibrosis and increased anxiety and depression, with no changes in memory and motor activity. Increased β-amyloid accumulation and increased Glo1 and Gad1 enzyme mRNA expression were observed in ADO2 brains, indicating a neural phenotype. Furthermore, the mutant ClC7 was accumulated in cultured neuron Golgi apparati, which appeared enlarged in brain cryosections. Similar Golgi changes were observed in monocytes and osteoclasts, suggesting a common pathogenic mechanism in cells involved in the ADO2 multiorgan phenotype. ADO2 cells presented no abnormal ClC7 accumulation in the upstream ER organelle and no Bip1 increase, while the downstream lysosomes showed less ClC7 and exhibited reduced acidification. Furthermore, increased LC3 expression suggested altered autophagy. ADO2 is incurable and we found this disease to be suitable for siRNA therapy. ADO2-specific siRNAs, identified in vitro through a systematic mutation-driven strategy, reduced the ADO2 mRNA without affecting the normal mRNA, mimicking a condition of haplosufficiency. In vivo, this siRNA rescued a normal bone phenotype returning bone mass to baseline. In the rescued ADO2 mice, serum biomarkers of bone resorption, osteoclast number and erosion surface were also normalized. Treatment was well tolerated, with no overt adverse events, suggesting that our strategy could be translated to the clinic. To advance our discovery, we patented the ADO2 siRNAs and are negotiating industrial partnerships. We believe that our strategy could have high chances of bringing a benefit to ADO2 patients, as it would be the first actual treatment available for this therapeutically neglected form of osteopetrosis.

DTP3: first-in-class GADD45β/MKK7 inhibitor selectively targeting the NF-κB survival pathway in multiple myeloma

Laura Tornatore, Gary Acton, Elizabeth A. Campbell, James Kelly, Michael Tarbit, Nigel Adams, Selina Bannoo, Daria Capece, Federica Begalli, Verzella Daniela, Jason Bennet, Daniel D'Andrea, Annamaria Sandomenico, Antonio Leonardi, Menotti Ruvo, Magda J. Al-Obaidi, Reuben Benjamin, Richard S. Kaczmarski, Holger Auner, Jane Apperley, and Guido Franzoso

Imperial College, London

Pathologic NF-κB signaling promotes survival in multiple myeloma (MM) and other cancers, yet current NF-κB-targeting strategies lack cancer-cell specificity. Recently, we identified the interaction between the NF-κB-regulated anti-apoptotic factor, Gadd45β, and the JNK kinase, MKK7, as a therapeutic target in MM. Gadd45β is upregulated in MM cells by NF-κB, associated with poor outcome in patients, and promotes myeloma cell survival by suppressing MKK7/JNK signaling. Through a drug discovery approach, we developed DTP3, a D-tripeptide, which disrupts the Gadd45β/MKK7 interaction, kills MM cells effectively and, importantly, lacks toxicity to normal cells. DTP3 also displayed potent and cancer-selective activity against MM in preclinical animal models, with no apparent side-effects.

The regulatory 28-day intravenous (i.v.) repeat dose toxicology studies in rat and dog demonstrated that DTP3 is well tolerated, causing only mild and transient clinical signs, no significant target organs of toxicity and no histopathology nor laboratory signals upon daily dosing, at up to 17 times the effective exposure. DTP3 did not accumulate on repeat dosing. No potential for drug-drug interaction *via* cytochrome P450 and no significant off-target activity were identified. Safety pharmacology studies reported no adverse effect on the central nervous, cardiovascular or respiratory systems. In the rat, i.v. DTP3 rapidly and extensively distributed to tissues, did not pass the blood-brain barrier, and was readily eliminated in urine and faeces. The pharmacokinetic (PK) and toxicokinetic (TK) evaluation of DTP3 in rat and dog identified long plasma half-lives, modelled into a half-life of 20-24 hr in man.

Collectively, the preclinical package demonstrated that DTP3 combines therapeutic efficacy with selective pharmacology and favorable PK/TK profile, thus supporting the progression of this first-in-class Gadd45β/MKK7 inhibitor into clinical development.

A companion biomarker programme has also been developed in order to inform patient stratification, and also demonstrate pathway-specific pharmacodynamic response and proof of mechanism.

Currently, we are conducting the first-in-human phase I/IIa trial of DTP3 in patients with relapsed/refractory multiple myeloma to deliver clinical proof of concept for a cancer-selective NF-κB-targeting strategy as a safe and effective therapy, which promises profound benefit to myeloma patients, and potentially, other further areas of unmet need.

The non oncologic bone diseases, Paget Disease, Fibrous Dysplasia, Hyperparathyroidism: Clinical Approach and differential diagnosis

Carmine Zoccali*, Alessandra Scotto di Uccio**

**Oncological Orthopedics Department, Regina Elena National Cancer Institute, Rome*
*** School of Medicine, Tor Vergata University, Rome*

Bone lesions are a very heterogeneous group of pathologies and often it is very difficult to diagnose so biopsy and histology become mandatory to differentiate one from another.
The correct approach has to take several aspects into account such as clinical symptoms and imaging, but often biopsy and relative histology become mandatory.

Paget disease is very common, its aspect is quite characteristic and its diagnosis is based on bone metabolic enzymes dosage and imaging but sometimes biopsy is mandatory to differentiate it from metastatic lesions or to exclude the onset of osteosarcoma. Therapy is based on the assumption of bisphosphonates and surgeries for the complications.

Fibrous dysplasia is rarer dysplastic lesion and its recognition is often an occasional observation; biopsy is important but sometimes it is not so easy to differentiate it from low-grade osteosarcoma;
even high grade osteosarcoma can onset in a previous fibrous dysplasia. Therapy is just observation but sometimes intralesional surgery and bone graft is necessary.

Hyperparathyroidism causes bone lesions similar to those of a giant cell tumor so differential diagnosis is based on histology and on dosage of parathormone. Therapy consists in removing the cause of hyperparathyroidism such as parathyroid adenoma that determines calcification of the bone lesions.

SCD5 enforced expression moves the balance toward a more epithelial phenotype in cancer cells

Maria Bellenghi[1], Rossella Puglisi[1], Sabina Sangaletti[2], Giada Pontecorvi[1], Lisabianca Bottero[1], Marina Petrini[1], Luca Pasquini[1], Mario Paolo Colombo[2], Gianfranco Mattia[1] and Alessandra Carè[1]

[1]Dept. of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy.
[2]Dept. of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy.

OBJECTIVE: The Epithelial-Mesenchymal Transition (EMT) is a critical step associated with cancer progression toward metastatic dissemination. Although non canonical, an EMT-like program, characterized by the aberrant expression of EMT driven Transcription Factors (EMT-TFs) has been reported in melanoma. Considering our previous results demonstrating the reduction of malignancy associated with Stearoyl-CoA desaturase 5 (SCD5) expression, we have further characterized SCD5 looking for its possible role in reverting the EMT program in melanoma and carcinoma cells.
MATERIALS AND METHODS: SCD5 was enforcedly expressed in human melanoma and 4T1 murine mammary carcinoma cell lines. All the expression studies and biological assays were performed according to standard procedures.
RESULTS: We previously demonstrated *in vivo* that in human melanoma the antimetastatic role of SCD5 was associated with decreased stromal deposition consequent to SPARC reduced secretion. As in this tumor the EMT-like program is controlled by SPARC, we looked for the possible combined effects played by SCD5 overexpression and intracellular SPARC. Results showed increased expression of Microphthalmia-associated Transcription Factor (MITF), the master-gene of melanoma phenotype switch, underlying the balance between epithelial and mesenchymal cell behaviors. Accordingly, we observed the reduction of ZEB1, SLUG and SNAI1 expression paralleled by ZEB2 upregulation. In line with MITF differentiation-driver role, an increment of Tyrosinase expression eventually associated with induced melanin synthesis was also observed.
Finally, SCD5 capabilities in promoting an EMT reversion were confirmed in the murine 4T1 mammary carcinoma cell line. According to the substantial reduction of spontaneous metastases observed in the syngeneic Balb/c *in vivo* model, the enforced expression of SCD5 evidenced a typical switch from EMT to MET phenotype, chiefly characterized by E-cadherin reexpression at membrane surfaces besides the canonical modulation of the EMT transcription factors.
CONCLUSION: Overall, the molecular and functional changes induced by SCD5 in human melanoma as well as in murine mammary carcinoma cell
lines indicate that at least part of the antimetastatic potential of this desaturating enzyme goes through an EMT reversion toward a more differentiated phenotype.

Itch/βarrestin2-dependent non-proteolytic ubiquitylation of SuFu controls Hedgehog signalling and medulloblastoma tumourigenesis.

F. Bernardi, P. Infante, R. Faedda, F. Bufalieri, R. Alfonsi, S. Pfister, D. Guardavaccaro, A. Gulino, L. Di Marcotullio.

Università "La Sapienza", Dipartimento di Medicina Molecolare
ADD Guardavaccaro e Pfister institution

OBJECTIVE: Suppressor of Fused (SuFu), a tumour suppressor mutated in medulloblastoma (MB), is a central player of Hh signalling, a pathway crucial for development and deregulated in tumors. Although the control of Gli transcription factors by SuFu is critical in Hh signalling, our understanding of the mechanism regulating this key event remains limited. To this end, the main objective of this study is to investigate the role of the ubiquitylation processes in the regulation of SuFu/Gli interaction**.**
MATERIALS AND METHODS: We evaluated the effect of several E3 ubiquitin ligases and adaptor proteins on SuFu ubiquitylation by in vivo ubiquitylation assay. Subsequently, we investigated the ability of the HECT E3 ligase Itch and the adaptor β-arrestin2 to bind and ubiquitylate SuFu in MEF cells and during cerebellar development in order to gain insight into the biological role of this post-translational modification. We also analysed the mechanisms by which Itch-dependent SuFu ubiquitylation regulates SuFu/Gli interaction through nanobit technology and co-immunoprecipitation assays. Finally, proof-of-concept in vivo studies (xenograft/ortotopic models and PET/SPECT/CT analysis) were performed in human MB Daoy cells infected with lentiviral vectors expressing SuFu WT or SuFu mutant insensitive to Itch E3 ligase activity.
RESULTS: In the present study, we demonstrate that Itch, in complex with β-arrestin2, ubiquitylates SuFu through K63-mediated linkages, without affecting SuFu stability, characterizing a regulatory rather than a degradative pathway. In this regard, we observed that this process increases the association of SuFu with Gli3, promoting its conversion into Gli3R and keeping the Hh pathway off. Notably, SuFu mutant, missing lysines interested in Itch-dependent ubiquitination, enhances MB cell proliferation in vivo, highlighting the relevance of our new mechanism in tumor growth.
CONCLUSION: Our findings identify a novel molecular mechanism in the negative control of Hh signaling and unveil, for the first time, that Itch-dependent K63-polyubiquitylation of SuFu may play an important role in the MB onset.

Targeting oncogenic miR-17-92 primary transcripts by LNA gapmeRs in multiple myeloma: Molecular findings and therapeutic potential

Lavinia Biamonte[1], Eugenio Morelli[1], Cinzia Federico[1], Maria Teresa Di Martino[1], Nicola Amodio[1], Maria Eugenia Gallo Cantafio[1], Niels M. Frandsen[2], Francesca Scionti[1], Maria Rita Pitari[1], Daniele Caracciolo[1], Annamaria Gullà[1], Maria Angelica Stamato[1], Marco Rossi[1], Pierosandro Tagliaferri[1], Pierfrancesco Tassone[1]

[1]UMG of Catanzaro, Catanzaro, Italy; [2]Exiqon A/S, Vedbaek, Denmark

There is emerging evidence that miR-17-92 plays a crucial role in c-Myc driven tumorigenesis of multiple myeloma (MM). We attempted to antagonize its full-oncogenic activity by targeting primary transcripts (pri-miR-17-92) with RNase H-triggering antisense oligonucleotides (LNA gapmeRs). Specifically, 7 different molecules were generated and screened for their ability to inhibit pri-miR-17-92 expression. The most effective molecule, henceforth named miR17-92-i-PT, was selected for further investigation. As assessed by qRT-PCR, transfection of MM cells with miR17-92-i-PT resulted in downregulation of both pri-miR-17-92 and all 6 mature miRNA transcripts. Importantly, miR17-92-i-PT inhibited MM cell proliferation more effectively than inhibitors targeting each cluster's miRNA. Lack of relevant off-target effects was demonstrated by specifically-designed negative (LNA mixmeRs) and positive (not-phosphorothioated LNA gapmeRs) control oligos. Importantly, treatment of MM cells with naked miR17-92-i-PT resulted in miR-17-92 downregulation. Low micromolar concentrations of miR17-92-i-PT significantly affected survival of MM patient plasma cells (n=14) and MM cell lines (n=15), while the viability of CD138+ cells from MGUS patients (n=3) or PBMCs from healthy donors (n=3) was not impaired. Molecular perturbations in MM cells exposed to miR17-92-i-PT were firstly investigated by gene expression profiling (HTA 2.0, Affimetrix). Ingenuity pathway analysis (IPA) of differentially expressed genes highlighted alteration of relevant molecular functions, including "cell cycle", "DNA replication, recombination, and repair", and "cell death and survival", which were validated by functional assays. Moreover, impairment of BRD4 activity was indicated by upstream regulator analysis (Zscore<2). Indeed, Hexim1 -the endogenous antagonist of BRD4- was upregulated upon treatment with miR17-92-i-PT and is currently being validated as a direct target of miR-18a, miR-19a and miR-19b cluster members. Consistent with a BET bromodomain inhibitor-like activity, miR17-92-i-PT downregulated c-Myc, thus indicating a novel feedback loop between miR-17-92 and c-Myc. Finally, we found a significant in vivo anti-tumor activity of systemically delivered naked miR17-92-i-PT against human MM xenografts in SCID/NOD mice. Different animal models were utilized, including SCID-hu and mouse orthotopic models, besides classic subcutaneous xenografts. Lack of systemic toxicity was demonstrated both in mice (balb/c) and in monkeys (cynomolgus monkeys). Overall, our results provide the rational framework for development of miR17-92-i-PT-based therapies in MM.

Targeting CXCR1 on breast cancer stem cells: signaling pathways and clinical application modelling

Laura Brandolini[1], Loredana Cristiano[2], Maria De Pizzol M[3], Tiziana Marilena Florio[2,4], Giuseppina Confalone[2], Angelo Galante[2,4], Benedetta Cinque[2], Elisabetta Benedetti[2], Pieradelchi Ruffini[3], Maria Grazia Cifone[2], Antonio Giordano[5,6], Marcello Alecci[2,4], Marcello Allegretti[1], Annamaria Cimini[2,4,6]

[1]Dompé Farmaceutici SpA, Via Campo di Pile, L'Aquila, Italy
[2]Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy
[3] Dompé Farmaceutici SpA, Via Santa Lucia, Milano, Italy
[4]National Institute for Nuclear Physics (INFN), Gran Sasso National Laboratory (LNGS), Assergi, Italy
[5]Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy
[6]Sbarro Institute for Cancer Research and Molecular Medicine and Center for Biotechnology, Temple University, Philadelphia, USA

OBJECTIVE: In breast cancer, has been proposed that the presence of cancer stem cells may drive tumor initiation, progression and recurrences. IL-8, up-regulated in breast cancer, and associated with poor prognosis, increases CSC self-renewal. It signals via two cell surface receptors, CXCR1 and CXCR2. In this work we have studied the effects of reparixin, a CXCR1/2 inhibitor, alone or in combination with paclitaxel, on mammospheres derived from a highly aggressive triple-negative breast cancer cell line MDA-MB-231 and also in a murine model of breast cancer metastasis into the brain.
MATERIALS AND METHODS BCSCs were isolated from MDA-MB 231 cells. BCSCs were firstly purified by clonal selection and then characterized by cytofluorimetry and western blotting for stemness markers, such as ABGC2, ALDH1, CD44, CXCR1, CXCR2. On BCSCs, cell cycle, cell proliferation, adhesion, mammosphere size were analyzed, upon paclitaxel, reparixin, rep+Pac treatments,. For the in vivo study, Nude Balb/c mice were used. The animals were divide in 4 groups, anaesthetized and injected in the internal carotid artery with 250.000 MDA-MB-231 cells. The tumor size and distribution in brain parenchyma, under the different treatment conditions, was followed by MRI.
For the *in vitro* experiments samples were processed by SPSS software. Statistical analysis of two population means was performed by the unpaired Student's *t* test, while statistical differences comparing multiple means were analyzed by the analysis of variance (ANOVA) followed by Scheffe's post hoc test analysis. *P < 0.05; **P < 0.005, **P < 0.0005.
RESULTS: The obtained data indicate a beneficial use of the drug combination reparixin and paclitaxel to counteract brain tumour metastasis due to CSC, probably due to the combined effects of the two drugs, the pro-apoptotic action of paclitaxel and the cytostatic and anti-migratory effects of reparixin.
CONCLUSIONS: CSC represents a potential target as they are responsible for disease relapse and metastasis. Administration of a CSC-targeting agent in patients at high risk for developing brain metastases (i.e., HER2+ and TNBC patients) could readily test this hypothesis. The data presented here suggest that the combination strategy (paclitaxel/reparixin) can be translated into future clinical trials in dedicated patient populations.

Parallel sequencing of a 50 genes panel in metastatic colorectal cancer (MCRC) patients (pts) treated with intensive first line FIr-B/FOx triplet chemotherapy plus bevacizumab (BEV): preliminary data and clinical outcome.

Gemma Bruera[1,2], Umberto Malapelle[3], Francesco Pepe[3], Pasquale Pisapia[3], Antonella Dal Mas[4], Giuseppe Calvisi[4], Giancarlo Troncone[3], Enrico Ricevuto[1,2]

[1]Oncology Network ASL1 Abruzzo, Oncology Territorial Care, S. Salvatore Hospital, ASL1 Abruzzo, University of L'Aquila, L'Aquila, Italy; [2]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy; [3]Department of Public Health, University Federico II, Napoli, Italy; [4]Pathology, S. Salvatore Hospital, ASL1 Abruzzo, L'Aquila, Italy.

BACKGROUND: *RAS/BRAF* genotype guide MCRC treatment. First line triplet chemotherapy/BEV significantly improved PFS and OS. OS was significantly worse in *KRAS* c.35G>A and *BRAF* mutant (mt). Most CRC (86%) harbored mt genes, prevalently *TP53*, *RAS, BRAF*, *PIK3CA*.
METHODS: MCRC samples of 67 pts treated with FIr-B/FOx (77% overall) were analyzed through a 50 genes panel (PGM/Colon Lung Cancer) by ION Torrent: 57 (85%) primary, 10 (15%) metastatic samples; 59 (88%) pre-, 8 (12%) post-treatment. Molecular diagnostic criteria: ≥50% coverage; >1% mutant allelic fraction. Clinical outcomes were evaluated and compared by log-rank.
RESULTS: All wild-type (wt) and mt MCRC were 6 (8.9%) and 61 (91.1%), respectively; median mt genes 3 (1-12). Mt genes, 35 (%): *KRAS* 44 (65.6%), *TP53* 38 (56.7%), *APC* 26 (38.8%), *KIT* 23 (34.3%), *PDGFRA* 19 (28.3%), *PIK3CA* 18 (26.8%), *EGFR* and *NRAS* 15 (22.3%), *SMAD4* 10 (14.9%), *FBXW7* and *MET* 7 (10.4%), *GNAS* and *PTEN* 6 (8.9%), *BRAF* and *NOTCH1* 5 (7.4%), *ATM, PTPN11* and *SMARCB1* 4 (5.9%), *HRAS, KDR*, *JAK3* and *VHL* 3 (4.4%), *ERBB2*, *FGFR2*, *FGFR3*, *IDH1* and *STK11* 2 (2.9%), *ABL1, AKT1, CDKN2A, FGFR1, FLT3, HNF1A, RB1, RET* 1 (1.4%). *BRAF* mutations: c.1756G>A, 1796C>T, 1405G>A, 1406G>C. Median follow-up 21 months (m), overall PFS 13, OS 27m: *KRAS* exon 2 ($KRAS_2$) wt/mt, PFS 14/12m, OS 28/21m, respectively, not significantly different; c.35G>A *KRAS* mt showed trendy worse OS 14m; *BRAF* mt trendy worse PFS (8 vs 14m) and OS (11 vs 28m); RAS_{2-4}/$BRAF_{15}$ wt/mt, PFS 18/12m (p .866), OS 28/22m (p .956). *TP53* wt/mt, PFS 14/12m, OS 28/23m. All wt, PFS 24, OS 44m, not significantly different vs ≥1 mt gene.
CONCLUSIONS: Multigenic analysis of MCRC patients treated with FIr-B/FOx shows trendy worse clinical outcome conferred by uncommon *BRAF* mutations.

Mutant p53 proteins influence secretome of pancreatic cancer cells

Giovanna Butera[1], Marcello Manfredi[2,3], Jessica Brandi[4], Raffaella Pacchiana[1], Marco Cordani[1], Buzza Adriana[3], Conte Eleonora[2], Daniela Cecconi[4], Emilio Marengo[3], Massimo Donadelli[1]

[1] Department of Neuroscience, Biomedicine and Movement, Biochemistry Section, University of Verona, Verona, Italy.
[2] ISALIT, Spin-off of Department of Sciences and Technological Innovation, University of Piemonte Orientale, Alessandria, Italy.
[3] Department of Sciences and Technological Innovation, University of Piemonte Orientale, Alessandria, Italy.
[4] Department of Biotechnology, Proteomics and Mass Spectrometry Laboratory, University of Verona, Verona, Itlay.

The cancer secretome is a rich repository in which to mine useful information for both cancer biology and clinical oncology. A better understanding of biological features that are common or peculiar to different tumors could help devise new targeted therapeutic approaches and allow the identification of specific and sensitive prognostic/predictive biomarkers for early diagnosis and tumor progression monitoring. This would be particularly relevant for pancreatic adenocarcinoma (PDAC), in which its extremely high mortality rate is mainly due to early metastasis, resistance to conventional treatments, and lack of recognizable symptoms and tests for early detection. Indeed, the main issue against successful therapy for PDAC is represented by the absence of early diagnostic and prognostic markers. Among the various important genes altered in pancreatic malignancy, the tumor suppressor p53 is considered crucial. p53 gene is a master transcriptional regulator controlling several key cellular pathways and is found to be mutated in more than 50% of human cancers, including PDAC (~70%). These mutations generally causing conformational changes and not only block the p53 tumor suppressor functions but also determine the acquisition of new oncogenic activities, such as the stimulation of tumor cell proliferation, chemo-resistance, local diffusion and metastasis.
This project aims to recognize and validate a specific signature of biomarkers secreted by PDAC cells carrying GOF mutant p53, using SWATH-MS technology. This signature would permit to easy discriminate PDAC patients into two main groups selecting those having oncogenic mutant p53 from those bearing wtp53. In this way, we compare the secretome of p53-null AsPC1 PDAC cells before and after ectopic overexpression of R273H-mutp53 and the secretome of Panc1 PDAC cells (bearing R273H mutant *TP53* gene) before and after knock-down of mutp53.Thus, we discover a number of common proteins having opposite regulation after overexpression and knock-down of R273H-mutp53. These proteins might constitute a sort of secreted signature driven by the hot-spot mutant R273H-p53 in PDAC and will be validated in serum samples of PDAC patients having WT or mutant *TP53* gene. These data might also suggest the identification of targeted therapies specifically addressed to inhibit growth of PDACs carrying oncogenic mutant p53, which are strongly resistant to traditional chemotherapies.

Mutant p53 proteins induce chemoresistance through stabilization of GAPDH protein in the cytoplasm of PDAC cells.

Giovanna Butera, Raffaella Pacchiana, Marco Cordani, and Massimo Donadelli

Department of Neuroscience, Biomedicine and Movement, Biochemistry Section, University of Verona, Verona, Italy

Pancreatic adenocarcinoma (PDAC) is a dreadful disease and is the fourth leading cause of cancer-related deaths worldwide. Surgery is possible in only 10-15% of cases and treatment with the drug gemcitabine (2',2'-difluoro-2'-deoxycytidine; GEM) has a response rate of less than 20%. Therefore, during the last years the identification of evaluable targets and novel therapeutic strategies to improve GEM effects in pancreatic adenocarcinoma have been extensively investigated. *TP53* is one of the most frequently mutated genes in the landscape of the human PDAC (~70%). Its missense mutations generally causing conformational changes and can add novel functions (gain-of-function, GOF) that promote chemoresistance, invasion, metastasis and the counteraction of apoptosis and cellular senescence.

Mutant p53 was reported to promote tumor metabolic change as a novel gain-of-function in promoting tumor development. Here, we show novel mechanisms by which mutp53 promotes pancreas cancer cell proliferation and chemoresistance, in particular demonstrating a functional link between mutp53 and the cellular localization of the multifunctional and pleiotropic glycolytic enzyme GAPDH. By knocking-down the endogenous mutp53 proteins or by ectopically expressing mutp53 variants in PDAC cells, we functionally demonstrated that mutp53 proteins inhibit the nuclear translocation of GAPDH stabilizing its cytoplasmic localization. This event contributes to both the establishment of the Warburg effect of cancer cells and the prevention of cell death mechanisms mediated by nuclear GAPDH. Mechanistically, we showed that the cytoplasmic stabilization of GAPDH by mutp53 is dependent on both the stimulation of the Akt signaling and the repression of the AMPK pathway, which are both involved in the phosphorylation of GAPDH required to translocate into the nuclei. By using a specific siRNA-GAPDH or conformational inhibitors of the enzyme, we functionally demonstrated that the maintenance of GAPDH in the cytosol has a critical role on the anti-apoptotic effect driven by mutp53. Furthermore, the blockage of its mutp53-dependent cytoplasmic stabilization is able to restore the sensitivity PDAC cells to GEM treatment. These results reveal a novel mechanism by which mutp53 promotes pancreas cancer cell proliferation and chemoresistance with the concomitant inhibition of apoptosis. Finally, these data suggest the triggering of nuclear GAPDH as a potential personalized therapeutic approach in human cancers carrying mutant TP53 gene.

Leptin is a key factor in the angiogenic process induced by adipose-derived stem cells

Calgani Alessia, Bruni Angelo, Amicucci G, Gentile Warschauer Emilio, Delle Monache Simona

University of L'Aquila

OBJECTIVE: Although the in vivo potential of adipose-derived stem cells (ASCs) is largely unclear, there is a growing evidence supporting the hypothesis that ASCs can indirectly modulate the surrounding environment by releasing several growth factors, cytokines and adipokines. Adipose tissue is highly vascularized and, in adults, it is constantly remodelled requiring angiogenesis and vascular regression. The reduction in oxygen tension may represent an important angiogenic switch for adipose resident cells and it could produce a specific niche for stem cells towards a pro-angiogenic phenotype. Leptin, is a pleiotropic adipokine involved in several processes such as proliferation, inflammation, angiogenesis and reproduction and a correlation between angiogenesis and the secretion of leptin from adipose tissue is well known. The aim of this study has been to investigate the role of hypoxia in modulating the angiogenic potential of ASCs and the molecular proangiogenic mechanism underlyng leptin effects on endothelial cells

MATERIALS AND METHODS: For this purpose we compared the secreted cytokine profile of hypoxia-conditioned ASCs (hASCs) with normoxic ASCs (nASCs) and we analyzed the effect of ASCs conditioned medium (CM) on endothelial cells. Human ASCs were obtained from male obese (35±10years) donors during abdominal surgery after written consent. After isolation cells were expanded for several passages showing "stemness" characteristics.

RESULTS: We found that hypoxia induced a transient upregulation of VEGF in ASCs and a notable and enduring upregulation of leptin mRNA expression 30-fold greater than control after 24 h and up to 60-fold greater than control at day 7. CM from hASC stimulated EC tube formation to a significantly greater extent than CM from nASC. This might be due to leptin-secreted factor. Indeed, exogenous leptin stimulated the expression of HIF2-α, but not HIF1-α, and upregulated the expression of Flt-1 and Tie-1 proangiogenic receptors.

CONCLUSION: In conclusion, hASCs may be particularly efficient in sustaining angiogenesis through the release of leptin.

Leptin modulates mitochondrial homeostasis and sustains hypoxic phenotype in prostate cancer cells.

Calgani Alessia[1], Delle Monache Simona[1], Bologna Mauro[2], Vicentini Carlo[2], Angelucci Adriano[1].

[1] Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, 67100 L'Aquila, Italy.[2] Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy

OBJECTIVE: Leptin is a cytokine produced by the adipose tissue in response to food intake; its serum levels are chronically high in obese subjects. In different carcinomas, obesity and high serum leptin are risk factors for advanced tumor stage and poor prognosis. It has been proposed that adipose tissue, through the release of leptin, can regulate whole-body energy homeostasis.
Physiological action of leptin in modulating the metabolic adaptation of different peripheral tissues supports the hypothesis that it could also exert a direct effect on cancer cells. Prostate cancer cell lines with different energetic metabolisms were evaluated for their responsiveness to leptin treatment in hypoxic conditions.
MATERIALS AND METHODS: We investigate leptin signaling in prostate cancer cell lines PC3 and LNCaP, in normoxic and hypoxic conditions.
RESULTS: Leptin treatment stimulated proliferation of prostate cancer cells in hypoxia and anoxia. Leptin effects were OXPHOS-independent and were associated with mitochondrial homeostasis, stabilization of mitochondrial membrane potential and of ROS production. In normoxia, leptin determined a reduction in mitochondrial respiration with a concomitant upregulation of UCP2 and of uncoupled respiration. In hypoxia leptin treatment sustained an additional stabilization of HIF-1α and the upregulation of the lactate exporter MCT4, determining the maintenance of a high lactate production rate. Furthermore, leptin counteracted the down modulation of SIRT1 induced by hypoxia, and persistent high levels of SIRT1 were involved in HIF-1α stabilization.
CONCLUSION: Leptin action, through the modulation of cancer metabolism, may explain, at least partially, the association between obesity and prostate cancer. Leptin can sustain cancer progression through the direct modulation of cancer cell energetics. This effect could be particularly important in hypoxic environment and when mitochondrial respiration is impaired. The proposed mechanism, involving new targetable intermediates, such as MCTs and SIRT1, offers new opportunities in diagnosis stratification and treatment for prostate carcinoma patients with high serum leptin levels.

Molecular characterization of the positive feedback between glioblastoma and stromal cells

Alessia Calgani (1), Francesca Tucci (1), Claudio Festuccia (1), Giovanni Luca Gravina (1), Silvia Schenone (2), Maurizio Botta (3)(4), Adriano Angelucci (1)

1) Dipartimento di Scienze Cliniche Applicate e Biotecnologiche, Università dell'Aquila, 67100, L'Aquila (IT)
2) Dipartimento di Farmacia, Università di Genova, Viale Benedetto VX 3, 16132, Genova (IT)
3) Dipartimento Biotecnologie, Chimica e Farmacia, Università degli Studi di Siena, via Aldo Moro 2, 53100, Siena (IT)
4) Lead Discovery Siena S.r.l., via Vittorio Alfieri 31, 53019, Castelnuovo Berardenga, Siena (IT)

OBJECTIVE: Natural progression of glioblastoma (GB) involves the development of a dynamic molecular interaction between cancer cells and the microenvironment. In fact, GB cells efficiently interact and infiltrate the surrounding normal tissue, determining the curative failure of surgical resection and adjuvant chemo/radiotherapy. A new therapeutic approach, able to interfere with GB capacity to synergize with normal brain tissue could be effective in an adjuvant protocol.
MATERIALS AND METHODS: Molecular interaction of GB cells with stromal cells was investigated in vitro and in vivo, by modulating signalling pathway associated with SRC and TGF-β.
RESULTS: U-87 glioblastoma cells induced in vitro myofibroblastic differerentiation of normal fibroblasts and this effect was particularly effective in hypoxia. Myofibroblasts had a positive feedback on GB growth by releasing PDGF, sustaining survival of cancer cells in hypoxia. Blocking Src kinase or TGFbR, in presence of conditioned medium from U-87 cells, rendered fibroblasts ineffective in up-regulating myofibroblastic markers, α-SMA and β-PDGFR. The in vivo combination treatment with Src inhibitor and radiotherapy was strongly active in reducing U-87 xenograft growth respect to control and single treatments. The histology revealed a significant difference in stromal compartment of tumoral tissue from control or RT-treated samples respect to Src-inhibitor-treated samples, showing a reduced presence of fibrosis and a-SMA positive cells.
CONCLUSION: Drugs that target SRC and TGF-β axis could represent an effective therapeutic strategy in GB able to block positive paracrine loop with stromal cells and the development of unfavourable tumor microenvironment. This approach could result important in combination with conventional treatments in the effort to reduce tumor resistance to therapy.

Extracellular vesicles (EVs) as new mean in intercellular bone crosstalk.

Cappariello A., Paone R, Ucci A., Rucci N., Muraca M., Teti A.

University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences

Bone is the site of crowded cell-to-cell crosstalk, and various molecules are exchanged to ensure tissue homeostasis. To investigate the EV-shuttled communication between bone cells, we isolated EV pellets from osteoblast conditioned media (3.03±0.79mg), increasing their yield with 10-8M hrPTH(1-34) (4.05±1.19mg,p=0.0405). By FACS we sorted 16.67±1.93% events showing, by transmission electron microscopy, membrane integrity, size and structure typical of EVs. EVs shuttled fluorochromes into osteoblasts, monocytes and endothelial cells. 97.1+0.26% EVs contained RNAs transferred to target osteoblasts. 53.95±3.48% EVs were RANKL-positive, which increased up to 63.6±4.20% after PTH treatment (p=0.037). EVs targeted the bone ex-vivo, because murine calvaria, incubated with fluorochrome-loaded EVs, showed fluorochrome integration in bone cells with a vesicular pattern. We injected i.p. 30.000 FACS-sorted RANKL-positive EVs in 5-days-old CD1 pups and observed a fast uptake of EV-shuttled fluorochrome in bone, peaking at 1.5 hours from injection and declining thereafter to a lower plateau within 24 hours. To investigate the in-vivo impact of RANKL-positive EVs on osteoclastogenesis, we injected i.p. 4-days-old RANKL-/- mice with 30,000, 60,000, and 120,000 RANKL-positive EVs/mouse, every other day for 5 times. Tibia sections revealed Tartrate-Resistant Acid Phosphatase (TRAcP) positive cells in treated mice, which were instead totally absent in vehicle-treated RANKL-/- mice. TRAcP-positive cell area steadily increased with increasing EV densities (PBS: ND; 30,000 EVs: 398.92+54.97µm2; 60,000 EVs: 810.17+169µm2; 120,000 EVs: 2403.91+932.30µm2, p<0.05), indicating dose-dependent osteoclastogenic potential. Our data demonstrate that EVs are physiologically involved in intercellular communication in bone and contribute to RANKL-induced osteoclastogenesis, representing a potential means of bone virtuous cycle.

The Notch2 pathway mediates the dormancy of breast cancer cells in the bone marrow

Mattia Capulli, Dayana Hristova, Zoe Valbret, Isabella Baldini, Ronak Arjai, Antonio Maurizi, Argia Ucci, Alfredo Cappariello, Nadia Rucci, Anna Teti.

Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy

Breast Cancer Cells (BCC) transit through the bone marrow where they can remain dormant before spreading to other organs. We hypothesized that dormant BCC interact with Spindle-shaped N-cadherin^{high} endosteal niche Osteoblasts (SNO) and share mechanisms of quiescence with bone marrow Hematopoietic Stem Cells (HSC). N-Cadherin^{high} SNO were sorted and cultured in monolayers on which EGFP^{+}-human MDA-MB231 or PKH26-stained mouse 4T1 BCC were attached for 1 hour, showing an adhesion rate similar to BCC cultured on SNO-depleted osteoblasts (NON-SNO). In contrast, while the number of BCC on SNO was not changed after 24 hours of co-culture, their number on NON-SNO doubled ($p=0.02$), forming +3.3fold clones ($p<0.05$). Similar to HSC, SNO-attached BCC showed increased expression of Notch2 (2.8fold, $p=0.013$), whose ligand, Jag1, was expressed by SNO. Notch2-specific siRNA increased BCC proliferation on SNO (1.83fold, $p=0.04$), achieving the proliferation rate of BCC attached to NON-SNO. BCC injected in tibias of immunocompromised mice progressively reached the trabecular endosteal surface ($p=0.02$) and remained non-proliferating for the timeframe of 1 month. They expressed Notch2 and localized near N-Cadherin^{high} endosteal cells, whereas cells located far from the endosteal surface (>2 cell distance) were Notch2^{low}. Only very rare asymmetric divisions were observed in BCC localized at the endosteal surface, suggesting their stem phenotype, with the farthest daughter cells being Notch2^{low}. In vivo competition assay performed injecting 10^5 HSC and increasing (10^5-10^6) BCC in busulfan/cyclophosphamide conditioned immunocompromised mice, showed the engraftment of HSC declining with the increase of administered BCC ($p=0.037$, $R=0.28$), suggesting that BCC and HSC share commonalities for the occupancy of the HSC bone marrow niche. Consistently, Notch2^{high}-sorted BCC showed +5.37fold Sca1 ($p=0.002$), +2.6fold Tie2 ($p=0.0001$) and +1.34fold CXCR4 mRNAs than Notch2^{low} BCC. Interestingly, Sca-1 is a biomarker of HSC stemness, and Tie-2 and CXCR4 are implicated in the quiescence of HSC attached to SNO. Finally, a single injection of 4.8mg/kg dibenzazepine, a γ-secretase inhibitor dampening the Notch pathway, in mice intratibially injected with BCC after 1 month of presumed dormancy (no overt tumour evident), showed 2 months later an increase of liver metastases (+2.5fold, $p=0.04$), suggesting Notch impairment to be implicated in dormant BBC reactivation.

EPHA2 inhibition reverts epithelial-mesenchymal transition (EMT) phenotype and reduces proliferation of colorectal cancer cells.

Alessandro Colapietro[1], Francesco Marampon[1], Loredana Cristiano[2], Vincenzo Mattei[3], Stefano Martellucci[3], Giovanni Luca Gavina [1] and Claudio Festuccia[1].

1 Department of Biotechnological and Applied Clinical Sciences, Laboratory of Radiobiology, University of L'Aquila, Italy; 2 Department of Life, Health and environmental Sciences, University of L'Aquila, Italy 3 Laboratory of Experimental Medicine and Environmental Pathology, "Sabina Universitas", Rieti, Italy

OBJECTIVE: The Eph-Ephrin system is involved on different biological cellular processes, but at the same time, reveals to be an important player on tumor progression including colorectal cancer cells. High levels of EphA2 are observed on colorectal cancer cells compared to normal counterpart. This is associated with a worse prognosis and a more invasive and metastatic behaviour. Furthermore, EphA2 promotes and supports the EMT, making tumor cells more aggressive. The aim of the study is the evaluation of the anti-tumor effects of a tyrosine kinase EphA2 inhibitor on a cohort of five colorectal cancer cells in vitro.
MATERIALS AND METHODS: We analyzed antitumor effects of this compound performing growth analysis by direct cell count, MTT assay and FACS analysis. Western blot assay were performed for the evaluation of EMT phenotype by using antibody against E-cadherin (negative EMT marker), survivin, β-catenin, SNAIL, c-myc and PCNA.
RESULTS: EphA2 inhibition reduces significantly the growth of colorectal cancer cells. We selects two models of tumor cells: HCT115 (Kras wild type) and HCT116 (Kras mutated) to perform western blot. Analyses of cell cycle reveals an accumulation of cells in G0/G1 phase and a reduction of G2/M phase. Few apoptosis was observed. Major effects were, indeed, observed in the EMT reversion and growth inhibition with strong enhance in E-cadherin expression, and reduction of PCNA, survivin, β-catenin, c-myc and SNAIL.
CONCLUSIONS: In our knowledge, this work, for the first time, evaluates the effects of tyrosine kinase EphA2 inhibition on colorectal cancer cell models.
Our data are in agreement with previous studies stating that EphA2 signaling correlates with tumor aggressiveness and progression. Further in vitro and in vivo investigations are, however, necessary to better elucidate the overall involved molecular arrangements.

PARP inhibition enhances replication stress and causes mitotic catastrophe in MYCN-dependent neuroblastoma

[1]Valeria Colicchia, [1]Marialaura Petroni, [2]Giulia Guarguaglini, [1]Biancamaria Ricci, [1]Francesca Sardina, [1]Maria Sahun Roncero, [2]Patrizia Lavia, [1]Alberto Gulino, [1]Giuseppe Giannini

[1]Dept. of Molecular Medicine, University La Sapienza, Rome, Italy; [2]Institute of Molecular Biology and Pathology, National Research Council, Rome, Italy

OBJECTIVE: High-risk neuroblastomas with MYCN amplification (MNA) have a very poor outcome, making the search for new therapeutic approaches an absolute priority. PARP inhibitors-based combination schemes have been tested in neuroblastoma preclinical models with encouraging results. However, the expression of PARPs and the biochemical consequences of their inhibition on the DNA damage response (DDR) were not characterized.
MATERIALS AND METHODS: Taking advantage of the R2-Genomics analysis and visualization platform, we performed in silico analysis of the expression of PARP family members in primary human neuroblastoma datasets. In vitro, we have used neuroblastoma cell lines to characterize the effects of PARP inhibitors on cell proliferation, death, replication stress and DNA damage, by using multiple assays.
RESULTS: Analysis of R2-datasets indicates that among the seventeen PARP family members, PARP1 and PARP2, as well as PAR-degrading enzyme PARG are highly expressed in high-risk and MNA primary neuroblastomas. In addition, their expression is significantly associated with poor survival, suggesting a potential and new prognostic value. In this context, inhibition of PARP activity via olaparib induces cell death by mitotic catastrophe, anticipated by the accumulation of DNA damage. Indeed, olaparib treated MYCN-overexpressing cells show DDR signs including H2AX and p53 phoshorylation, 53BP1 foci, micronuclei and anaphase bridges. Furthermore, at earlier time, olaparib treatment yields activation of a typical replication stress-checkpoint via CHK1-CDC25A pathway causing a transient delay in the S-phase of cell cycle. The mechanism by which olaparib induces such effects is mainly related to its ability to trap PARP onto DNA rather than to its catalytic inhibition. In fact, PARP silencing and treatment with others PARP inhibitors, with different trapping potency, showed that PARP trapping is essential for cytotoxicity, DNA damage and replication stress checkpoint in MYCN-overexpressing cells. CHK1 inhibition abrogates the S-phase checkpoint anticipating and increasing the occurrence of mitotic catastrophe induced by olaparib.
CONCLUSION: Due to the known role of MYCN and PARP in replication stress, we propose the introduction of PARP inhibitors, in combination with CHK1 inhibitors, in therapeutic approaches for neuroblastomas with high MYCN activity, to exacerbate the MYC-driven replication stress and to induce cell death.

Gain-of-function mutant p53 enhances mitochondrial ROS through the inhibition of PGC-1α/UCP2 axis in cancer cells

Marco Cordani, Giovanna Butera, Elena Butturini, Raffaella Pacchiana, Elisa Oppici, Sofia Mariotto, Barbara Cellini, Massimo Donadelli.

Department of Neurosciences, Biomedicine and Movement, Biochemistry Section, University of Verona, Verona, Italy.

Mutations in the *TP53* gene occur in over 50% of the human cancers and most of them are missense mutations that result in the expression of mutant forms of p53. In addition, p53 mutated proteins acquire new biological properties referred as gain-of-function (GOF) that contribute to the induction and maintenance of cancer. Reactive oxygen species (ROS) are radicals, ions or molecules highly reactive that are produced as an inevitable byproduct of mitochondrial oxidative phosphorylation. ROS can act as second messengers in cellular signaling in human cancer and are implicated in a plethora of biological events addressed to sustain each aspect of its progression. Uncoupling protein 2 (UCP2) is located in the mitochondrial inner membrane and plays an essential role is critical in energy regulation and in the maintenance of cellular ROS homeostasis by limiting the production of mitochondrial superoxide.
We have investigated the molecular mechanisms by which mutant p53 regulates the redox status in cancer cells and its role in sustaining cancer progression and chemoresistance. We found that mutant p53 proteins, contrarily to wild type p53, enhance mitochondrial ROS in cancer cells which are crucial mediators of their oncogenic activity leading: i) cancer cell proliferation, ii) inhibition of apoptosis, and iii) chemoresistance. Importantly, we unveiled that mutant p53 inhibits SESN/AMPK-α interaction leading an inhibition of AMPK phosphorylation. Consequently to the deregulation of AMPK signaling by mutant p53, but not by wild type p53, the expression of its effector PGC-1α was also affected, driving a reduction of UCP2 expression and an increase of mitochondrial superoxide. These data reveal a novel mechanism by which mutant p53 sustains tumor progression and lightened on the importance that plays the redox cellular status in the tumors carrying oncogenic mutant p53 proteins.

The 1,4 benzoquinone-featured 5-Lipoxygenase Inhibitor RF-Id Induces Apoptotic Death Through Downregulation of IAPs in Human Glioblastoma Cells

Cossu A. M.[a], Zappavigna S.[a], Scuotto M.[b], Ingrosso D.[a], De Rosa M.[b], Schiraldi C. [b], Filosa R.[b] , Caraglia M.[a]

[a]*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, via Costantinopoli 16, Naples, 80138, Italy.*
[b]*Department of Experimental Medicine, Second University of Naples, Via Costantinopoli, 16, Naples, 80138 Italy.*

BACKGROUND: Embelin is a potent dual inhibitor of 5-lipoxigenase (5-LOX) and microsomal prostaglandin E2 synthase (mPGES)-1 that suppresses proliferation of human glioma cells and induces apoptosis by inhibiting XIAP and NF-κB signaling pathway. Synthetic structural modification yielded the derivative 3-((decahydronaphthalen-6-yl)methyl)-2,5-dihydroxycyclohexa-2,5-diene-1,4-dione (**RF-Id**), an embelin constrained analogue, with improved efficiency against 5-LOX in human neutrophils and anti-inflammatory activity in vivo. Taking into account that lipoxygenase (LOX) metabolites, from arachidonic acid and linoleic acid, have been implicated in tumor progression, here, we determined whether **RF-Id** was able to hinder glioblastoma (GBM) cancer cell growth and the related mechanisms.
METHODS: U87MG and LN229 cells were plated in 96-wells and treated with increasing concentrations of **RF-Id**. Cell viability was evaluated by MTT assay. The effects of the compounds on cell cycle, apoptosis, oxidative stress and autophagy were assessed by flow cytometry (FACS). The mode of action was confirmed by Taqman apoptosis array and evaluating caspase cascade and NFκB pathway by western blotting technique.
RESULTS: Here, we found that **RF-Id** induced a stronger inhibition of GBM cell growth than treatment with embelin. Flow cytometry analysis showed that **RF-Id** induced about 30% apoptosis and a slight increase of autophagy after 72h on U87-MG cells. Moreover, the compound induced an increase in the percentage of cells in G2 and S phase that was paralleled by an increase of p21 and p27 expression but no significant changes of the mitochondrial membrane potential; array analysis showed a significant upregulation of *CASP8* and a downregulation of *IAP* family and *NFκB* genes in cells treated with **RF-Id**. **RF-Id** induced a significant cleavage of caspases 8, 9, 3 and 7, blocked c-IAP2/XIAP interaction by inducing XIAP degradation and inhibited NFκB pathway.
CONCLUSIONS: **RF-Id** induced a caspase-dependent apoptosis in GBM cells by inhibiting IAP family proteins and NFκB pathway and represented a promising lead compound for designing a new class of anti-cancer drugs with multiple targets.

Effects of UVr exposure and Cetuximab treatment in HaCaT cells.

[1]Costantini E, [1]D'Angelo C,[2] Amerio P, [1]Reale M, [1]De Tursi M, [2]Auriemma M, [1]Muraro M.

[1]Dep. of Medical and Oral Sciences and Biotechnologies, University "G. D'Annunzio" Chieti-Pescara
[2]Dep. of Neurosciences, Imaging and Clinical Sciences, University "G. D'Annunzio" Chieti-Pescara

BACKGROUND: Ultraviolet (UVr) exposure seems responsible to cause various biological events on the skin, with different implications. For metastatic colon-cancer patients, treated with an anti-EGFR monoclonal antibody, Cetuximab, therapeutic guidelines suggest to avoid solar exposure. Since the main negative effect, for patients, is the development of skin rash leading to therapy suspension and considering the successfully use of UVr in different cutaneous conditions, we evaluated the effects of UVr and Cetuximab in an in vitro model, using human keratinocytes, HaCaT cells.
METHODS*:* HaCaT cells were grown until confluence in complete medium and then stimulated with Cetuximab and UVr, alone or in combination, to evaluate EGFR phosporilation and cells viability. Cytokines/chemokines expression and release were assessed using Real-Time PCR and ELISA assay respectively. In addition, pro-inflammatory cytokines pattern was assessed in Cetuximab-treated patient's serum and it's combined effect with UVr-exposure was evaluated in our cell model. Considering the high frequencies of fissuration, in Cetuximab-treated patients, HaCaT cells were cultured with their serum and exposed to UVr to observe wound repair.
RESULTS*:* UVr-exposure did not affect HaCaT cells viability and EGFR phosphorylation. In Cetuximab-treated HaCaT a decrease of IL-1β, IL-8, CCL-5 and CCL-2 was observed while UVr-exposure induce their increase. Therefore combined effects seem restore IL-1β and IL-8 basal levels. Pro-inflammatory cytokines production was increased in Cetuximab-treated patients serum. The combined treatments with Cetuximab-treated patients serum and UVr-exposure, of HaCaT cells reduce the pro-inflammatory cytokines expression, compared to un-exposed cells. Wound assay show that UVr-exposure quicken wound closure in presence of patients serum, compared to controls.
CONCLUSIONS*:* UVr exposure decreasing IL-1β and IL-8 pro-inflammatory cytokines and promote wound healing in HaCaT cells cultured in presence of Cetuximab-treated patients serum. Our results suggest that is possible to use the UVr-exposure to treat the adverse cutaneous reactions in order to avoid therapy suspension and that solar exposure should not be avoided by Cetuximab-treated patients.

Anti-myeloma effects of Trabectedin are induced through DNA-damage and cell stress in tumor cells and through microenvironment NK activation

Maria Cucè, Cirino Botta, Daniele Caracciolo, Francesca Scionti, Nicoletta Staropoli, Marco Rossi, Pierosandro Tagliaferri, Pierfrancesco Tassone and Maria Teresa Di Martino

Magna Graecia University of Catanzaro

OBJECTIVE: Trabectedin is a marine-derived anti-cancer drug, originally isolated from the sea squirt Ecteinascidia turbinata, currently approved for advanced soft tissue sarcomas and relapsed platinum-sensitive ovarian cancer treatment. Trabectedin binds to DNA, interacts with transcription factors and interferes with DNA-repair machinery, thus exerting effects on both cancer cells and tumor-microenvironment. Here we investigated, for the first time, the activity of trabectedin in multiple myeloma (MM) against MM cell lines and patient derived cultures.
MATERIALS AND METHODS: MM cells and patients-derived primary MM cells were exposed to different trabectedin concentrations (0.1-2.5 nM). Apoptosis and cell cycle analysis were performed by flow-cytometry. Anti-myeloma activity was also studied by a 3D matrigel-spheroid model that recapitulates the microenvironment of MM disease and reduce the need for in vivo study. Gene-expression profiling (GEP) was carried out with HTA 2.0 Affymetrix array. DNA-damage response was evaluated through western-blot, immunofluorescence (ɣ-h2ax foci) and DNA fragmentation assay (COMET assay). Cellular stress was evaluated by analyzing reactive oxygen species (ROS) production and alteration of mitochondrial membrane potential (MMP) in MM cells. NK activator ligands modulation was evaluated by GEP and flow-cytometry while NK activation was assessed with the CD107a degranulation assay.
RESULTS: Trabectedin induces apoptosis in primary MM cells and in all 9 MM cell lines, with IC50 values ranging from picomolar to nanomolar concentrations. GEP analysis revealed DNA damage response, cellular stress and cell cycle as the main affected pathways. Accordingly, we observed an early S-phase arrest. Furthermore, we found a reduction of BCL-2 and MCL-1 and a strong increase in c-CASP3, c-PARP and p21. DNA-damage was confirmed by a marked increase of ɣ-h2ax, pBRCA1, pATR, p-chk1 and p-chk2 together with an increase in ɣ-h2ax nuclear foci as well as COMET DNA fragments in cell lines treated with trabectedin. Moreover, trabectedin induces ROS production and MMP depolarization in MM-cells. GEP analysis showed that trabectedin induces an up-regulation of MICA/MICB genes together with the upregulation of their transcription factor E2F1 and the downregulation of their main repressors IRF4, IKZF1 and IKZF3. We confirmed these results by western-blot and flow-cytometry and functionally, demonstrating an increase in the percentage of degranulating/activated NK cells after trabectedin treatment.
CONCLUSION: Trabectedin exerts a potent anti-MM activity inducing cell stress and DNA damage in the malignant plasma cells and NK activation. Our findings support the investigation of trabectedin in MM translational therapeutics.

Oxidative Stress and 5-HT turnover in human neuroblastoma cells: ELF-EMF effects.

D'Angelo C[1], Costantini E[1], Hellmann-Regen J[2], Regen F[2], Reale M[1]

[1]University "G. d'Annunzio" - Chieti, Italy; [2]University "Charité" - Berlin, Germany

OBJECTIVE: Nowadays, an increasing exposure to environmental extremely low-frequency electromagnetic fields (ELF-EMF) occurs, causing a great debate on harmful effects of ELF-EMF for human health. Oxidative stress and 5-HT turnover play an important role during neurological disorders.
In the present study, using neuronal-like SH-SY5Y neuroblastoma cells, we investigated if ELF-EMF exposure might influence oxidative stress and 5-HT turnover, representing a possible risk factor for neuroderenerative disorders.
MATERIALS AND METHODS: SH-SY5Y cells were grown in DMEM and 10% FBS, at 37°C and 5%CO2. Reached 60-70% confluency, cells were exposed to a 50-Hz ELF-EMF, 1 mT (r.m.s.) produced by an electromagnetic generator for 1, 3 and 24h. Non-exposed cell cultures were grown simultaneously. After ELF-EMF exposure a MTT cell viability assay was performed and intracellular ROS were measured with H2DCF-DA fluorescent probe. Exposed and unexposed homogenate cells, were spectrophotometrically analysed to monitoring NOS activity, measuring conversion of L-[2,3-3H]arginine in L- [2,3-3H]citrulline, and CAT activity through H2O2 decomposition. Thereafter 5-HT and 5-HIAA levels were determined by RP-HPLC in cellular homogenates. Data were expressed as means ± SD of at least three independent experiments performed in triplicate.
RESULTS: ELF-EMF-exposure did not affect cell viability. However our experimental conditions impacted significantly ROS production, which is resulted increased after 1-3h, and have caused an early stimulation in NOS activity. Intracellular 5HT and 5HIAA were significant affected by ELF-EMF exposure; the ratio 5HIAA/5HT was strikingly increased. CAT activity remained unaltered at early time-point but a higher activity was observed in 24h-exposed cells, compared to unexposed.
CONCLUSION: Although ELF-EMF exposure induces an early increase of ROS and NOS, sustained by the increased 5-HIAA/5-HT ratio, we demonstrated that there is a possible modulatory effect of our ELF-EMF. In fact, after 24h of exposure, the antioxidant CAT activity was increased reverting the ROS production.
Thus CAT activity may represent a mechanism of neuroprotection against early rise, ELF-EMF induced, of NOS and ROS.
We emphasize the need of additional *in vitro* and *in vivo* studies to better understand the link between ELF-EMF exposure and neurological disorders.

The dichotomous role of Notch signaling in cancer.

De Blasio C, Mariano G, Cialfi S, Zonfrilli A, Le Pera L, Palermo R, Screpanti I, Talora C.

Sapienza University of Rome. Dept. Molecular Medicine

OBJECTIVE: The oncosuppressor role of Notch1 in epithelial cells is generally accepted. Nevertheless, the amplifications of Notch gene locus and Notch-pathway genes, have been described in epithelial cell-derived tumors, underlying the difficulty of manage an univocal *context-dependent pathway* and switching to the hypothesis of a *cell signaling-dependent pathway* under which Notch1 could promotes tumorigenesis rather than be protective. Which are the reasons of those divergent functions of Notch1 are not fully understood. In our work we attempt to understand this double nature of Notch signaling.
MATERIALS AND METHODS: Gamma-secretase treated and control squamous cell carcinoma cell lines were used to identify Notch1 targets by whole-genome expression profiling using RNA-seq technology. The expression of differentially expressed genes was validated by RT-PCR. The effect of Notch1 on the expression of GSI-regulated genes was examined by Western blot, RT-PCR and Chromatin immunoprecipitation (ChIP) assays.
RESULTS: We found that although the components of the Notch-transcriptional machinery are expressed in SCC, the canonical Notch/Hes-1 axis is defective, as compared to other keratinocyte-derived cell lines. By RNA-Seq analysis in GSI-treated SCC-cells, we have identified hundreds of genes differentially modulated. In our work we have revealed a subset of Notch1 regulated genes which function is required for SCC tumor phenotype. Although the canonical Notch-signaling is defective we identified novel targets, e.g. TP53INP1/INP2. These genes are stress-induced p53-target genes, however a p53-independent TP53INP1/INP2 expression has been described. In *silico* analysis of the TP53INP1/INP2 promoters revealed binding sites for Notch1/RBP-Jk. TP53INP genes are tumor-suppressors often down-regulated in tumors.
CONCLUSION: We have found that Notch1 represses TP53INP1/INP2 gene expression. Although the underlying mechanism remains to be elucidated, we provide evidence that regardless of the cellular context the switch between the pro or anti-oncogenic function of the Notch signaling depends on recruitment of different signaling pathways.

KCASH2 expression in mouse testis: a role in sperm differentiation and maturation?

De Feudis G., Moretti M., Basciani S., Izzo M., Spiombi E., Angrisani A., Cucchi D., Gnessi L., De Smaele E.

SAPIENZA Università di Roma

The KCASH family, consisting of KCASH1, 2 and 3, negatively regulates the Hedgehog (Hh) pathway. The Hh family of genes codifies for specific secreted proteins, which act as crucial morfogens during embryonic development. Perturbations of the Hh signalling pathway are linked to tumorigenesis and many developmental defects. Indeed, it appears to be involved in the testis and epididymis development and function. In particular Desert Hh is secreted from the Sertoli cells and modulates development and differentiation of Leydig cells, while Sonic Hh is expressed in the epididymus and is involved to the sperm maturation. We used the KCASH2-KO mouse model (generated in our lab), which expresses the β-gal reporter in lieu of KCASH2, to monitor KCASH2 expression in testis and to investigate its role in sperm maturation and fertility.
MATERIALS AND METHODS: We generated the KCASH2-KO mouse model using the "gene targeting" technology by homologous recombination in mESCs of a reporter gene LacZ cassette.
Standard immunohistochemistry techniques have been used on paraffin embedded or criopreserved tissues, and standard β-galactosidase assays were also performed. We also collected sperm samples from mouse epididymis and conduced motility and morphology sperm analysis by microscopic observation of sperm suspensions.
RESULTS: We used the reporter gene expression in KCASH2-KO mice to reveal cellular localization in Leydig cells and in the epididymal epithelium (with a pattern caput-cauda). We confirmed these data analyzing the KCASH2 mRNA levels and protein expression in mouse testis and epididymis. Comparative analysis between ko and wt animals shows more frequent spermatozoa anomalies in KCASH2-KO mice and alteration in sperm motility, suggesting a role for KCASH2-dependent modulation of the Hh pathway in this context.
CONCLUSION: The KCASH2-KO mouse previous analysis does not show apparent morphological alteration of the testis and epididymis. Interestingly, though, we are able to observe anomalies in the spermatozoa morfology (increased atipicity) and altered sperm mobility (dyscinesia). Although, the mice are still able to breed, we are setting experiments to evaluate the fertility rate. We want to verify the modulation of the Hh pathway in KCASH-KO tissues compared with WT samples, and identify the developmental and differentiation steps (Hh dependent or independent) that are altered by KCASH2 loss.

New bioinformatics procedure to identify target genes for dysregulated microRNAs in a chemically-induced hepatocellular carcinoma mouse model.

Filippo Del Vecchio[1], Francesco Gallo[2], Antinisca Di Marco[2], Valentina Mastroiaco[1], Pasquale Caianiello[2], Francesca Zazzeroni[1], Edoardo Alesse[1], Alessandra Tessitore[1].

[1]*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Via Vetoio, Coppito 2, 67100 L'Aquila*
[2]*Department of Computer Engineering and Science, and Mathematics, University of L'Aquila, Via Vetoio, Coppito 1, 67100 L'Aquila*

INTRODUCTION: Hepatocellular carcinoma (HCC) is an aggressive tumor. MiRNAs are non-coding RNAs acting in post-transcriptional gene regulation, whose dysregulated activity plays a role in cancer. A single miRNA can exert its action on hundreds of putative target genes. We used a chemically-induced HCC mouse model to identify miRNAs differential expression during the progression of hepatic damage up to HCC. In this context, we generated an original bioinformatic approach to predict putative target genes and protein networks involved in hepatocarcinogenesis.
MATERIAL AND METHODS: C57BL/6J mice were treated with DEN and sacrificed after 3, 6 and 11 months. MiRNA expression related to controls was evaluated. Dysregulated miRNAs were analyzed by bioinformatics prediction tools (miRanda, TargetScan, PITA and RNA-22) to identify target genes. Genemania software was then exploited for enrichment annotation analysis and protein construction network. Immunoblotting was used to validate target genes' expression.
RESULTS: Bioinformatics tools globally identified 15 putative target genes of four upregulated miRNA (miR-125a-5p, miR-27a, miR-182, miR-193b) subjected to analysis. The protein product of one among them (Ankyrin-G) was further validated by immunoblotting to assess the strength of the approach. Enrichment annotation analysis highlighted 26 significant functional clusters putatively involved in DEN-induced HCC, and a network including links between selected miRs, targets, and possible interactions among them and other proteins was built.
CONCLUSIONS: We combined the results of microRNAs expression analysis, from an *in vivo* HCC mouse model, with new bioinformatics approach. Interactions between miRs, target genes and related proteins putatively involved in HCC initiation and progression were identified.

Targeted therapy of human glioblastoma via delivery of a toxin protein through cell surface directed peptides

AC. Dhez [2,3], E. Benedetti[1], F. Giansanti[1], A. Antonosante[1], L. Cristiano[1], J. Courty[2,3], A. Cimini [1], R. Ippoliti[1]

[1]*Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy,*
[2]*Department of Cell Biology, Université Paris-Est, UPEC, Créteil, France,*
[3]*Laboratoire de Recherche sur la Croissance Cellulaire, la Réparation et la Régénération Tissulaires (CRRET) CNRS, Créteil, France*

Targeted therapy of cancer demands the discovery of new cellular targets to be exploited for delivery of toxic molecules and drugs. In this perspective, one interesting surface marker have been identified to be used for the therapy of glioblastoma: nucleolin.
The multifunctional protein nucleolin (NCL) is overexpressed on the surface of activated endothelial and tumor cells. Previous studies reported that the NCL binding multivalent pseudopeptide NucAnt-6L (Nucleolin Antagonist, N6L) that suppressed both tumor growth and angiogenesis in several carcinoma cell lines and the proliferation of endothelial cells. The effects of N6L on human glioblastoma cells in primary culture was previously investigated by our group. The results obtained indicated an anti-proliferative effect of N6L and point towards its possible use as adjuvant agent to the standard therapeutic protocols presently utilized for glioblastoma. To obtain greater activity of N6L we further explored the possibility to target glioblastoma cells linking N6L to a toxin (saporin) to obtain an active conjugate exploiting the toxicity in a two steps process: the binding via N6L and the toxicity via saporin.
We observed that toxicity of SAP-N6L is 1000 fold more efficient than saporin alone in glioblastoma primary cells and 100 fold more in the model cell line (U87-MG). SAP-N6L induces cell death with concentrations in the nanomolar range as determined by cell viability assay. At these nanomolar concentrations, N6L acts only as a targeting agent because it has no biological effect, so we hypothesize that toxicity comes only from saporin. The mechanisms of intoxication and cell death induced by saporin are not so clear but apoptosis seems to be the main detectable effect. In vivo studies were performed on BALB/c-nu/nu athymic mice injected intracranially with U87-LUC cells. The bioluminescence data, analyzed weekly, showed a significant decrease of tumor growth in SAP-N6L treated group. Glioblastoma is the most invasive and aggressive brain tumor in humans, and despite the latest chemical and radiation approaches, it is still poorly sensitive to these treatments. Therefore, targeting of cell-surface NCL with an antagonist such as N6L and enabling toxin internalization may represent a suggested therapy against this kind of tumor.

Perivascular cells of diffuse Systemic Sclerosis patients overexpress activated ADAM12, which modulates the profibrotic TGFβ activity, and play an active role in myofibroblasts trans-differentiation and development of fibrosis.

Paola Cipriani[1], Paola Di Benedetto[1], Piero Ruscitti[1], Vasiliki Liakouli[1], Onorina Berardicurti[1], Francesco Carubbi[1], Francesco Ciccia[2], Giuliana Guggino[2], Francesca Zazzeroni[3], Edoardo Alesse[3], Giovanni Triolo[2], Roberto Giacomelli[1].

[1]Department of Applied Clinical Sciences and Biotechnology, Rheumatology Unit, School of Medicine, University of L'Aquila, Delta 6 Building, Via dell'Ospedale, 67100 L'Aquila, Italy
[2]Dipartimento Biomedico di Medicina Interna e Specialistica, Sezione di Reumatologia, Università degli Studi di Palermo, Palermo, Italy
[3]Department of Applied Clinical Sciences and Biotechnology, General Pathology Unit, University of L'Aquila, Coppito 2, 67100 L'Aquila, Italy

OBJECTIVE: The microvascular damage is a pivotal event in the pathogenesis of Systemic Sclerosis (SSc), preceding fibrosis, whose trigger is not still fully understood. Perivascular progenitor cells, with profibrotic fate and function, are identified by the expression of the isoform 12 of A Disintegrin And Metalloprotease (ADAM12) and this molecule may be up-regulated by TGFβ. The goal of this work was to evaluate if pericytes in the skin of diffuse SSc patients expressed ADAM12, suggesting their potential contribution to the fibrotic process and if TGFβ might modulate this molecule.
MATERIALS AND METHODS: After ethical approval, mesenchymal stem cells (MSCs) and fibroblast (FBs) were isolate from bone marrow and skin samples collected from 20 patients, affected by diffuse SSc. ADAM12 expression was investigated in the skin, and in isolated MSCs and FBs treated with TGFβ, by immunofluorescence and qRT-PCR. Furthermore, we silenced ADAM12 expression in both dSSc-MSCs and -FBs, to confirm the TGFβ modulation
RESULTS: Pericytes and FBs of SSc skin showed an increased expression of ADAM12 when compared with HC-skin. TGFβ in vitro treatment induced a significantly increase of ADAM12 in both SSc-MSCs and FBs, the higher levels observed in SSc cells. After ADAM12 silencing, the TGFβ ability to upregulate α-smooth muscle actin (αSMA) in both SSc-MSCs and SSc-FBs, was inhibited.
CONCLUSION: Our results suggest that, during SSc, already committed pericytes to trans-differentiate toward activated fibroblast are present in the vascular tree and TGFβ, increasing ADAM12 expression, may modulate this trans-differentiation.

Identification and characterization of c.4117G>T pathogenic variant of *BRCA1* gene recurrent in the Center Italy population in the territory of Lazio and Abruzzo regions.

Daniela Di Giacomo[1,2,3], Martina Calicchia[4], Elisabetta Buccieri[4], Stefania Candria[2,3], Tina Sidoni[5], Gemma Bruera[2,3], Emanuela Lucci Cordisco[4], Mario Tosi[6], Maurizio Genuardi[4], Enrico Ricevuto[2,3].

[1]Fellow, Umberto Veronesi Foundation
[2]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy [3]Oncology Network ASL1 Abruzzo, Oncology Territorial Care, S. Salvatore Hospital, ASL1 Abruzzo, University of L'Aquila, L'Aquila, Italy
[4] Institute of Genomic Medicine, "A. Gemelli ", Catholic University, Rome, Italy
[5] Oncology, S. Salvatore Hospital, L'Aquila, Italy
[6]Inserm U1079-IRIB, University of Rouen, Normandy Centre for Genomic and Personalized Medicine, Rouen, France

Constitutional alterations in the *BRCA1* and *BRCA2* tumor-suppressor genes predispose to early-onset breast and ovarian cancer; mutational status is heterogeneous; some pathogenic variants occur in specific populations and ethnic groups with a founder effect. *BRCA1* nonsense variant, c.4117G> T - p. (Glu1373 *) occurs in families of Central Italy. In the program of identification and surveillance of genetic predisposition to breast and ovarian cancer in the Oncology Network ASL1 Abruzzo, University of L'Aquila, this variant was identified in 9 un- related families with familial origin in the region between Tagliacozzo (L'Aquila) and Sora (Frosinone), along the Liri river.
In these families, the c.4117G>T variant was always associated with *BRCA1* polymorphism, c. .3119G> A - p.Ser1040Asn, to A allele (allelic frequency 1.3% according to data ExAC), in 17/17 carriers tested, 8 affected and 9 unaffected. In collaboration with Institute of Genomic Medicine, "A. Gemelli ", Catholic University of Rome, additional 8 families with c.4117G> T variant were identified, all coming from the same regional area. Among 16 out of the 17 overall families carrying this nonsense variant, the co-segregation with the variant allele c.3119G>A was confirmed in all 34 (14 affected and 20 unaffected) identified carriers ($p < .05$). The analysis is in progress in the remaining family members. The analysis with microsatellite markers in the 17q21 region (D17S846, D17S1328, D17S855 intragenic, D17S902, D17S806), completed in 15 families, confirms the presence of a common haplotype. This haplotype is associated with the deleterious variant comprising the region between D17S1328 and D17S902 markers.
The results show that *BRCA1* c.4117G> T is a new founder variation, common in the territory of Central Italy between Abruzzo (L'Aquila) and southern Lazio (Frosinone) regions. Furthermore, this variation is associated with allelic variant *BRCA1* c. 3119G>A.
The project is ongoing to investigate the relative frequency of the variant in the high-risk population and in this territory, the associated phenotypes, and the clinical and preventive implications.

Essential ammino acids have a powerful apoptotic effect in *in vitro* colon cancer cells

Jacopo di Gregorio[1], Francesco Dioguardi[2], Vincenzo Flati[1]

[1]Department of Biotechnological and Applied Clinical Sciences, University of l'Aquila; [2]Dipartimento di Scienze Cliniche e di Comunità, Università degli studi di Milano

OBJECTIVE Nutrients such as ammino acids are able to modulate cell metabolism and control cell survival, activating metabolic regulatory pathways such as mTOR, in the form of the two complexes mTORC1 and MTORC2. Moreover, it has been proposed that ammino acids can mantain protein synthesis and anabolism, activating also pathways upstream of mTOR. This is relevant in normal, healty cells; however, ammino acids activity may also affect the metabolism of cancer cells. We performed our study to determine ammino acids effects *per se* in tumor cells.
MATERIALS AND METHODS We treated HCT-116 (colon cancer cells) with a mixture of essential ammino acids (EAA), with untreated HCT-116 cells used as control. Then, we collected the whole protein from the cells and performed Western Blot analysis for Caspase 3, Bax and Bcl2.
RESULTS We observed a cleavage of Caspase 3, and an enhancement of the Bax/Bcl2 ratio, in the EAA-treated cells compared to the controls.
CONCLUSION These data suggest an EAA mediated apoptotic activation, in the form of the intrinsic apoptotic pathway that involves mitochondrial activation. This finding could open possibilities about use of ammino acids as an additive anti-cancer therapy.

Amniotic epithelial stem cells phenotype and orientation can be influenced using electrospun poly(lactide-co-glycolide) scaffolds with high grade of fibers alignment mimicking tendon extracellular matrix

L. Di Marcantonio[*], V. Russo[*], R. Wyrwa[§], A. Mauro[*], P. Berardinelli[*], T. Walter[§], M. Schnabelrauch[§], B. Barboni[*]

**University of Teramo, Faculty of Veterinary Medicine, Teramo Italy*
[§]Innovent e.V., Biomaterials Department, Jena Germany

OBJECTIVE: Electrospun poly(lactide-co-glycolide) (PLGA) is a biocompatible copolymer, and can be engineered with amniotic derived stem cells (AECs) (Russo et al, Mater Sci Eng C Mater Biol Appl 2016 69:321). AECs are known for their easy retrieval, non-ethical concerns, non-tumorigenic and immunomodulatory properties, thus ideal in allo and xenotransplantation settings. They are able to differentiate toward the tenogenic linage when co-cultivated *in vitro* with tendon explants or when transplanted *in vivo* in a tendon injury model. Indeed, when transplanted, AECs contribute to tissue remodeling also directly by producing Collagen Type 1 (COL1), which is the major protein expressed in a tendon.
In this study, PLGA electrospun scaffolds were fabricated with a high degree of aligned fibers, in order to mimic tendon extracellular matrix (ECM), and with random fibers (control). Then, these scaffolds were cultured with ovine AECs verifying their biocompatibility and if fiber alignment, mimicking a tendon structure, could influence cell phenotype and orientation.
MATERIALS AND METHODS: To this aim, oAECs were seeded on scaffolds and cultivated for 48h. Then, scaffold ultrastructure (SEM), Calcein AM, PKH26 vital dyes and Ki67, a cell proliferation marker, and COL1 mRNA and protein expression were analyzed.
RESULTS: Immunostaining showed that nearly all cells were alive and able to proliferate (20% cells of highly aligned fibers vs. 15% random fibers, $p<0.05$). Additionally, oAECs spatial distribution and orientation was influenced by scaffold fibers' alignment. In fact, when oAECs were cultivated on highly aligned electrospun PLGA fibers they changed their morphology acquiring a spindle tenocyte-like shape, and were able to align along the longitudinal axis of the fibers, whereas in random electrospun PLGA scaffolds oAECs maintained their cuboidal morphology. Moreover, several oAECs were able to express in their cytoplasm COL1 only on aligned fibers scaffolds and not on the random oriented fibers ones. These findings indicate that when oAECs are seeded on electrospun PLGA scaffolds with highly aligned fibers, their phenotype and orientation are influenced by this artificial tendon ECM structure acquiring an early tenogenic-like phenotype.
CONCLUSION: Electrospun PLGA scaffolds engineered with oAECs could be used for future clinical application in the treatment of tendon disorders.

ErbB and BER pathways in human gastric carcinoma cell line AGS: implication for a molecular cross-talk

Di Marcantonio Maria Carmela[1], Mincione Gabriella[1], Moscatello Carmelo[1], Savino Luca[1], Lepore Stefania[1], Grande Rossella[2], Muraro Raffaella[1], and Aceto Gitana Maria[1]

[1]Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy
[2]Department of Pharmacy, University G. d'Annunzio Chieti-Pescara, Chieti, Italy

OBJECTIVE: In many human cancers deregulation of the intracellular signals are the driving forces of the disease. We hypothesize the presence of a cross-talk between ErbB and BER pathways in pathological gastric tissue and its potential role as a molecular signature for progression of the disease, altering the tumor microenvironment. For this purpose, we evaluated the role of EGF/ErbB and BER systems in AGS human gastric cancer cell line.
MATERIALS AND METHODS: ErbB receptors, BER and Nrf2/ARE gene and protein expressions were evaluated by qRT-PCR and Western Blot. Effects of treatment on cell cycle was analyzed by flow cytometry, while the viability was tested by MTT assay. In particular, the AGS cells, cultured in serum-free basal medium, were treated with H_2O_2, EGF, LY294002 and PD98059 alone and combined with each other.
RESULTS: In AGS cells H_2O_2 induced changes in cell morphology associated with an elongation phenotype and formation of microparticles. The combined treatments with H_2O_2 significantly reduced cell viability, and in particular the co-treatment with LY294002. In the presence of H_2O_2 a reduction of the population in S-phase after 48 hours compared to 24 hours of treatment was observed. An up-regulation of *ErbB2* gene expression was observed after EGF treatment, while *ErbB3* and *ErbB4* increased in the single treatment with LY294002 and H_2O_2. The expression level of the BER system molecules, specifically MUTYH, OGG1 and APE1, increased in the combined treatment of H_2O_2 with EGF and LY294002 (H_2O_2+EGF; H_2O_2+LY294002) compared to single treatment (EGF; LY294002); while NRF2 gene expression was reduced after H_2O_2 plus LY294002 treatment.
Although no significant change of EGFR expression was shown, an increase of phospho-EGFR after treatments with H_2O_2 alone or combined with EGF, LY294002 or PD98059 was demonstrated. Interestingly, an increased activation of MAPK was detected with H_2O_2 (alone and in co-treatment with EGF and LY294002), while in the same conditions, phospho-Akt was downregulated.
CONCLUSION: These preliminary data suggest that a pronounced stress with H_2O_2 may induce a mutual regulation of BER system and ErbB receptors. Further studies are necessary to know whether the expression of BER system molecules is MAPK-dependent.

Notch3 - EGFR crosstalk in triple negative breast cancers (TNBC): new therapeutic possibilities.

G Diluvio[1], F Del Gaudio[2], MV Giuli[1], E Giuliani[1], D Bellavia[1], I Screpanti[1,3,4], S Checquolo[5]

[1]Laboratory of Molecular Pathology, Department of Molecular Medicine, Sapienza University, Rome, Italy;
[2]Department of Cellular and Molecular Biology, Karolinska Insitutet, Stockolm, Sweden;
[3]Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy;
[4]Institute Pasteur-Foundation Cenci Bolognetti, Sapienza University, Rome, Italy;
[5]Department of Medico-Surgical Sciences and Biotechnology, Sapienza University, Latina, Italy.

OBJECTIVES. Triple negative Breast Cancers (TNBC) are heterogeneous and aggressive tumors lacking the expression of the therapeutically targetable Estrogen, Progesteron and ErbB2 receptors, making this subtype difficult to treat. EGFR receptor is overexpressed in about 60% of TNBC, thus several therapies are being developed that target this specific biomarker, such as a tyrosin kinase inhibitor (TKI) gefitinib. Unfortunately the majority of data with EGFR inhibitors revealed a limited clinical activity against breast cancers, often due to the activation of compensatory pathways that confer intrinsic resistance to EGFR inhibition, such as Notch receptor signaling. Our main aim is to investigate the Notch-EGFR axis in TNBC, focusing on the cellular response to TKI-treatment, in order to gain new insights into the pathobiology and to develop therapies to this form of breast cancer, which is currently lacking.
METHODS. TNBC MDA-MB 468 TKI-resistant cells; RNA intereference; Gefitinib treatment; PLA-IF; CRISPR/Cas9 genome editing; Membrane lipid rafts isolation.
RESULTS. Here we demonstrated that: 1. Notch3-silencing (rather than Notch1) induces growth arrest; 2. Notch3-silencing (rather than Notch1) leads to a strong re-sensitization to TKI-gefitinib treatment. Based on these data we can suppose a different role of Notch3 and Notch1 in the same cellular context, thus suggesting a possible different crosstalk with EGFR, as we demonstrated that both receptors are able to directly interact with EGFR itself. Thus, we focused our studies on the role of Notch3 upon EGFR. MDA-MB 468 Notch3-silenced cells, with or without gefitinib, show: 1. a decreased EGFR phosphorylation at the Tyr1173; 2. a significative EGFR internalization. These data suggest that Notch3 is important to maintain EGFR at the plasma membrane and in an active "status", ready to be "targetable" when Notch3 is removed. *Mechanistically*, our preliminary results suggest a different localization of Notch3 and Notch1 at the membrane lipid raft compartment, where EGFR mainly localize in TNBC TKI-resistant cells. This could be a starting point to explain the different molecular mechanism underlying Notch3-EGFR *vs* Notch1-EGFR crosstalk in this context.
CONCLUSIONS. Notch3 (rather than Notch1) could be a new molecular marker associated with EGFR TKI-resistance in TNBC, enabling patients to optimize their personal treatment options.

Diet-related inflammation and immune dysfunction in obesity: potential risk factors for colorectal cancer

Donninelli G. (a), D'Archivio M. (b), Del Cornò M. (a), Conti L. (a), Scazzocchio B. (b), Varì R. (b), Varano B. (a), Stefania G. (b), Pierdominici M. (c), Pacella I. (d), Piconese S. (d), Masella R. (b), Gessani Sandra (a)

(a) Dept Hematology, Oncology and Molecular Medicine, ISS - Rome.
(b) Dept Veterinary Public Health and Food Safety, ISS - Rome.
(c) Dept of Cell Biology and Neurosciences, ISS – Rome
(d) Dipartimento di Medicina Interna e Specialità Mediche, Sapienza Università di Roma- Rome.

PURPOSE: The risk of developing colorectal cancer (CRC) is elevated in the obese (Ob) population, and diet plays a key role (1). Our hypothesis is that fatty acids (FA), major components of white adipose tissue (WAT), are key determinants in inflammation (2), and WAT may represent the initial place where dietary influences are transmitted to the immune system.
METHODS: WAT biopsies and blood samples were collected from 4 groups of subjects: normal weight [Nw, Body Mass Index (BMI) 22.0-24.9 Kg/m^2] and Ob (BMI > 30.0 Kg/m^2) with or without CRC. AT stromovascular fraction (SVF) was recovered after adipocyte isolation. WAT FA composition was determined by gas chromatography.
The frequencies of blood T_{reg} and γδ T lymphocytes as well as AT innate immunity cells were analyzed by flow cytometry.
To evaluate the effects of WAT microenvironment on DC-γδ T cell cross-talk, DC, generated in the presence of adipocyte conditioned medium (ACM), were co-cultured with autologous γδ T cells and lymphocyte activation was measured by assessing IFN-γ secretion.
RESULTS: We observed a reduced number of circulating activated T_{reg} ($CD4^+CD45RA^-Foxp3^{high}$) and a reduced $Vγ9Vδ2^+/γδ^+$ T cell ratio in Ob and CRC subjects as compared to healthy donors. Comparative analysis of T_{reg} frequency in SVF versus blood pointed to AT as a T_{reg}-hostile microenvironment, as suggested by their lower accumulation and increased OX40 expression. Preliminary results also indicated a preferential accumulation of NKT cells in SVF versus blood. The altered lymphocyte profile observed in Ob and CRC subjects was associated to a pro-inflammatory WAT microenvironment, as indicated by distinct individual ω6 PUFA profiles.
Additionally, adipocyte microenvironment from Ob and CRC subjects altered the capacity of ACM-DC to induce γδ T cell activation, as assessed by reduced IFN-γ production.
CONCLUSION: All together these results suggest the existence of complex interactions among WAT microenvironment, FA composition and immune dysfunction, at systemic and local level, that may contribute to the risk of developing CRC.

Perinatal progenitors from human cord blood (Cord Blood-Borne Fibroblasts, CB-BFs) generate complete ossicles *in vivo*

Samantha Donsante[1], Alice Pievani[2], Domenico Raimondo[1], Cristina Remoli[1], Alessandro Corsi[1], Marta Serafini[2] and Mara Riminucci[1]

[1] *Department of Molecular Medicine, Sapienza University of Rome, Italy;*
[2] *Dulbecco Telethon Institute, University of Milano-Bicocca, Italy.*

OBJECTIVE: Human Cord Blood-Borne Fibroblasts (hCB-BFs) are perinatal progenitor cells that may be isolated from cord blood according to plastic adherence. We recently demonstrated that, like post-natal stem/progenitor cells isolated from bone marrow (Human Bone Marrow Stromal Cells, hBMSCs), hCB-BFs form heterotopic bone when transplanted in vivo in the presence of osteoconductive carriers. Unlike marrow-derived progenitors, hCB-BFs generate cartilage in addition to bone and do not establish a hematopoietic microenvironment in standard, scaffold-based transplantation systems.
In this study, we further investigated the skeletogenic activity of hCB-BFs by using an in vivo experimental approach, free of exogenous scaffolds and based on transplantation of chondroid pellets generated in vitro.
MATERIALS AND METHODS: Cells isolation and cartilage pellet generation BMSCs were isolated from washouts of discarded bone marrow collection bags. CB-BFs were isolated from umbilical cord blood samples. BMSC and CB-BF strains were expanded for two/three passages and then cultured using a pellet culture system. Cultures were maintained in Chondrogenic Differentiation Medium. Chondrogenic differentiation was evaluated by histological and molecular analysis. In vivo transplantation Pellets were transplanted into 8-15-week old female SCID/beige mice and harvested after 8 weeks. Untransplanted cartilage pellets and heterotopic ossicles harvested at 8 weeks were fixed in formaldehyde in phosphate buffer, decalcified in EDTA, processed for paraffin embedding and used for qualitative and quantitative analysis.
RESULTS: we report that in vitro, hCB-BFs formed chondroid pellets that were larger in size than those generated by hBMSCs. Following in vivo transplantation, hCB-BF pellets were entirely converted into ossicles composed of bone and functional bone marrow cavities. In agreement with in vitro results, ossicles derived from hCB-BFs were larger in size than those generated by hBMSCs. The hematopoietic microenvironment of ossicles produced by hCB-BFs contained murine short-term and long-term HSCs as well as committed hematopoietic cells. In addition, as hBMSC samples, hCB-BF ossicles supported the engraftment and multi-lineage differentiation of human $CD34^+$ cells.
CONCLUSION: these data demonstrate that hCB-BFs display differentiation activity and functional properties similar to those of skeletal stem/progenitor cells residing in the bone marrow. For this reason, hCB-BFs warrant further exploration as tool for skeletal tissues and HSC niche regeneration.

*Wnt/*β-catenin *gene* expression in colon adenomas and adjacent colonic mucosa

[1]G.Fabietti, [1]A. Ricci, [1]F.Fantini, [1]C. Moscatello, [2]C. Efthymatis, [2]M.Neri, [3]R.Valanzano, [1]G.Aceto, [4]A. Cama, [1]M.C.Curia.

[1]*Department of Medical, Oral and Biotechnological Sciences,'G. d'Annunzio' University, Chieti, Italy;* [2]*Unit of General and Laparoscopic Surgery, SS. Annunziata Hospital, Chieti, Italy;* [3]*Unit of Surgery, Department of Surgery and Translational Medicine, Careggi University Hospital, Firenze, Italy*; [4]*Department of Pharmacy,'G. d'Annunzio' University, Chieti, Italy*.

BACKGROUND. The importance of canonical Wnt/β-catenin signalling for colon development and cancer progression has long been recognised. Adenomatous polyposis coli (APC) gene, a Wnt component, contributes to adenoma formation but some its roles remain to elucidate. Transcript dosage may influence transcriptome. To elucidate the role of altered gene expression in colon adenomas we analyzed *APC* and other components of Wnt pathways.
MATERIALS AND METHODS. Patients with familial and sporadic polyps were enrolled following inclusion (age≥ 18 years) and exclusion (inflammatory bowel diseases) criteria. Donors with no family history of cancer were recruited as controls. mRNA expression levels were investigated by quantitative Real-Time PCR (qRT-PCR). The correlation among clinical and molecular features has been evaluated.
RESULTS. We analyzed expression in colon tumour tissues (n. 26), adjacent mucosa (n.19) from 11 patients with FAP (familial adenomatous polyposis) and 25 with sporadic adenomas , and in normal colonic mucosa from 12 healthy controls. qRT-PCR results showed a reduced *APC* expression in colon tumour tissues (0.15 $2^{-}\Delta CT$) as compared to the adjacent mucosa (0.32 $2^{-}\Delta CT$). Intriguingly the *APC* expression in adjacent colonic mucosa was higher also compared to healthy controls colonic mucosa. The differences in *APC* expression between colon tumour tissues and adiacent mucosa in familial and sporadic cases, were statistically significant in familial group (p=0.0054). We also correlated *APC* expression with age. In patients the expression levels tend to decrease more rapidly with age. Instead in control group there is a constant *APC* expression trend in life. Correlation with sex showed that the *APC* reduced expression is more evident in men than female. Downstream Wnt/β-catenin components *BCL9* and *LEF1* showed a reduced expression in adjacent colonic mucosa vs adenomas in cases analyzed. Expression analysis of Wnt ligands is in progress.
CONCLUSIONS. This study showed that the *APC* gene is lower expressed in colon tumor tissue compared to the adjacent mucosa but more expressed in adjacent mucosa compared to mucosa of healthy controls. The increased APC expression in adjacent mucosa could be due to a cross talk between tumor and surrounding colonic epithelium.

Mutational analysis of Tp53 gene using NGS to drive an alternative therapy for patients with chronic lymphocytic leukemia

Carmela Ferri, Margherita Russo, Mayra Rachele Zarone, Alessia Maria Cossu, Anna Grimaldi, Angela Lombardi and Michele Caraglia

Second University of Naples, Department of Biochemistry, Biophysics and General Pathology

OBJECTIVE: Chronic lymphocytic leukemia (CLL) is a malignant neoplasm characterized by accumulation of monoclonal B lymphocytes that infiltrates the hematopoietic tissues. Recently, more and more evidences support that there is a *TP53* network which could involve most prognostic factors. It plays an important role in the pathogenesis of CLL and may be an effective tool to guide the treatment in this disease. Defects of the *TP53* gene in CLL are more strongly associated with aggressive phenotypes, poor clinical outcomes, and resistance to therapy with purine analogues. Alemtuzumab is a humanized anti-CD52 monoclonal antibody that recently was approved for clinical use in fludarabine-refractory CLL.
The frequency of TP53 defects at diagnosis or before first therapy is only between 5 and 15%, but the proportion of affected patients is significantly higher (44%) after conventional treatment in a fludarabine-refractory cohort. This led to the suggestion that TP53 mutations should be investigated before each therapy in CLL patients. On these grounds, the assessment of TP53 status is essential for clinical decision-making. Treatment with alemtuzumab should ideally be initiated earlier in the disease, before multiple therapies and/or advanced stage.
Next-generation sequencing (NGS) technologies currently enable mutation analyses in cancer patients with previously unattainable sensitivity, reaching as far as fractions of percentages. Our project involves the use of NGS technology to assess the presence of Tp 53 gene mutations in patients with CLL.
MATERIALS AND METHODS: Mutations of the p53 gene were assessed by extracting DNA by mononuclear cells which are isolated from peripheral blood. We used the Ion Torrent PGM sequencing platform in combination with the Ion AmpliSeq Panel to sequence frequently mutated regions, included *TP53* gene. For variant calling, Ion Torrent analysis software was supplemented with additional variant annotation and filtering.
RESULTS: In all patients were found SNP mutations (point mutations with substitution of a single nucleotide). In particular we have found the presence of a novel *TP53* gene mutation in many patients. This mutation, present on chromosome 17, is located at position 7,579,472, exon 4 and was discovered several time with very high frequency (100%).
This polymorphism arises from a single-base-pair substitution at codon 72, where either CCC encodes proline or CGC encodes arginine (16). Clearly this is a non conservative amino acid change, and furthermore, it results in a structural change in the protein. Both proteins are structurally wild type but are not functionally equivalent, and this may have important implications for the management of patients with wild-type p53-containing tumors, depending on their p53 genotype.
We also found in some patients other two hotspot mutations: the mutation c.916C> T (p.Arg306Ter) and mutation c.742C> T (p.Arg248Trp). The first type of substitution gives rise to a non-sense mutation (change of a codon encoding an amino acid to a stop codon). Several articles associate these mutations to Hereditary cancer-predisponding syndrome (Li-Fraumeni syndrome).

Enhancement of radiosensitivity by the novel anticancer Quinolone derivative Vosaroxin in preclinical glioblastoma models.

Claudio Festuccia[1], Andrea Mancini[1], Assunta Leda Biordi[1], Alessandro Colapietro[1], Flora Vitale[1], Francesco Marampon[1], Simona Pompili[1] Antonella Vetuschi[1], Judith A. Fox[2] and Giovanni Luca Gravina[1]

[1]Department of Biotechnological and Applied Clinical Sciences, Laboratory of Radiobiology, University of L'Aquila, L'Aquila, Italy; [2]Sunesis Pharmaceuticals Inc, South San Francisco, CA, USA.

PURPOSE: Glioblastoma multiforme (GBM) is the most aggressive brain tumor. The activity of vosaroxin, a first-in-class anticancer quinolone derivative that intercalates DNA and inhibits topoisomerase II, was investigated in GBM preclinical models as a single agent and combined with radiotherapy (RT).
MATERIALS AND METHODS: Cellular, molecular and anti-proliferative effects of vosaroxin alone or combined with RT were evaluated in 12 GBM cell lines. Tumor growth delay was determined in U87MG, U251 and T98G xenograft mouse models. Disease free survival (DFS) and Overall Survival (OS) were assessed in orthotopic intra-brain models using luciferase-transfected U251 cells by bioluminescence and magnetic resonance imaging.
RESULTS: Vosaroxin had antitumor activity in clonogenic survival assays with IC20 of 10-100 nM and caused radiosensitization. Combined treatments exhibited significantly higher g-H2AX levels compared to controls. Vosaroxin reduced tumor growth and showed enhanced activity with RT; vosaroxin/RT combined was more effective than temozolomide/RT. Vosaroxin/RT triggered rapid and massive cell death with characteristics of necrosis. Only a minor proportion of treated cells underwent caspase-dependent apoptosis in agreement with in vitro results. Vosaroxin/RT inhibited RT-induced autophagy increasing necrosis. This was associated with increased recruitment of granulocytes, monocytes and undifferentiated bone marrow-derived lymphoid cells. Pharmacokinetic analyses revealed adequate blood-brain penetration of vosaroxin. Vosaroxin/RT increased DFS and OS significantly compared to RT, vosaroxin alone, temozolomide and temoxolomide/RT in the U251-luciferase orthotopic model.
CONCLUSIONS: Vosaroxin demonstrated significant activity in vitro and in vivo in GBM models, and showed additive/synergistic activity when combined with RT in O6-methylguanine methyltransferase (MGMT) negative and positive cell lines.

Cytotoxic activity of Juglone against Notch3-overexpressing T-cell acute lymphoblastic leukemia: targeting the ER/UPR signaling.

MV Giuli[1], G Diluvio[1], E Giuliani[1], D Bellavia[1], R Palermo[2], I Screpanti[1,2,3], S Checquolo[4]

[1]Laboratory of Molecular Pathology, Department of Molecular Medicine, Sapienza University, Rome, Italy; [2]Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy; [3]Institute Pasteur-Foundation Cenci Bolognetti, Sapienza University, Rome, Italy; [4]Department of Medico-Surgical Sciences and Biotechnology, Sapienza University, Latina, Italy.

OBJECTIVES. Aberrant Notch signaling has been implicated in the development of several diseases, including T-cell acute lymphoblastic leukemia (T-ALL), a malignant disorder that originates from hematopoietic precursors committed to T-cell lineage. Survival rates in T-ALL patients have greatly improved in the last decades but still a substantial number of patients will relapse and die. An increased understanding of T-ALL biology has already translated into new prognostic biomarkers and has opened opportunities for the development of targeted therapies for the treatment of this disease. Recently, several studies suggest the role of the unfolded protein response (UPR) in acute leukemias. UPR is a conserved adaptive signaling pathway which tries to restore protein homeostasis mainly after Endoplasmatic Reticulum (ER) stress. It has been demonstrated that cancer cells are able to maintain malignancy by acquiring therapy resistance through its UPR signaling. Our main aim is to demonstrate that Juglone, a naturally-occurring naphtoquinone, is able to induce ER stress/UPR perturbation, thus having therapeutic effects on T-ALL cells.
METHODS. Notch3-overexpressing murine 232T and human TALL-1 leukemia cells; pharmacological treatments; PI/Annexin staining and cytofluorimetric analysis; RNA interference; RT-qPCR; Western blot.
RESULTS. Juglone is considered a promising anticancer agent for its strong activity against cancer cells *in vitro* and *in vivo* models. Here we demonstrate that this molecule induces ER stress by inhibiting proteasome system, leading to the accumulation of ubiquitinated proteins and the subsequent up-regulation of Bip, an ER chaperone known to be involved in ER stress signalling starting. The underlying molecular mechanism might be Notch3-dependent, as we observed a strong decrease in the Notch3 expression after Juglone treatment, in keeping with what observed after Thapsigargin treatment, a known ER stress inducer. Moreover, TALL-1 Notch3-silenced cells show up-regulation of Bip. Interestingly, we further demonstrate that Juglone is also able to alter cellular proteins homeostasis by disrupting the cytoprotective network of the UPR through the down-regulation of the UPR proteins (IRE1α and PERK), finally inducing pro-apoptotic events.
CONCLUSIONS. Juglone induces apoptotisis in Notch3-overexpressing T-ALL cells, via perturbation of ER/UPR signaling.

TRAIL induces pro-apoptotic crosstalk between the TRAIL-receptor signaling pathway and TrkAIII in SH-SY5Y cells

Luciana Gneo, Pierdomenico Ruggeri, Lucia Cappabianca, Antonietta Rosella Farina and Andrew Reay Mackay

Department of Applied Clinical and Biotechnological Sciences, University of L'Aquila, Via Vetoio, Coppito 2, L'Aquila 67100, Italy

TrkAIII expression in neuroblastoma associates with advanced stage disease, worse prognosis, post therapeutic relapse, promotes chemotherapeutic-resistance and exhibits oncogenic activity in NB models. The objectives of this study were to identify novel ways to kill neuroblastoma cells that express TrkAIII. Using methodologies including transient transfection, siRNA knockdown, RT-PCR, Immunoprecipitation, Western blotting, cell death assays, tumourigenic growth *in vitro* and ligand precipitation of TRAIL-activated death receptors, we report a potential therapeutic "Achilles heel" for the TrkAIII oncoprotein in a SH-SY5Y neuroblastoma model. This is characterised by TRAIL-induced one-way, pro-apoptotic crosstalk between the TRAIL receptor signaling pathway and TrkAIII, resulting in delayed induction of apoptosis via the extrinsic pathway and complete abrogation of tumourigenic activity *in vitro*. This effect initiates with TRAIL-induced SHP-dependent c-Src activation and induction of TrkAIII/SHP-1/c-Src complexing, leads to SHP-mediated TrkAIII de-phosphorylation and induction of complexing between de-phosphorylated TrkAIII and cFLIP, resulting in a time-dependent increase the caspase-8 to cFLIP ratio at activated death receptors, explaining delayed caspase cleavage and caspase-dependent apoptosis. We also confirm central roles for cFLIP and Mcl-1 in regulating the sensitivity of TrkAIII expressing SH-SY5Y cells to TRAIL-induced apoptosis, and show that inhibition of cFLIP and Mcl-1 expression both accelerates and augments TrkAIII SH-SY5Y cell-sensitivity to TRAIL. Our study unveils a novel mechanism for killing TrkAIII expressing NB cells that depends upon TRAIL-induced SHP/Src-mediated crosstalk between the TRAIL-receptor signaling pathway and TrkAIII, supporting a potential pro-apoptotic therapeutic use for TRAIL in TrkAIII expressing NB.

The brain penetrating pan EPH receptor antagonist, UNIPR1331, shows potent antiangiogenic and anti-invasive effects in glioblastoma preclinical models.

Giovanni Luca Gravina[1], Claudio Festuccia[1], Carmine Giorgio[2], Andrea Mancini[1], Alessandro Colapietro[1], Simona Delle Monache[3], Cristina Pellegrini[1], Vincenzo Mattei[4], Stefano Martelluci[4], Paola Chiodelli[5], Marco Rusnati[5], Riccardo Castelli[2], Federica Vacondio[2], Alessio Lodola[2] and Massimiliano Tognolini[2]

(1) Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, 67100 L'Aquila, Italy; (2) Dipartimento di Farmacia, Università degli Studi di Parma, Viale delle Scienze 27/A, 43124, Parma, Italy. (3) Department of Biotechnological and Applied Clinical Sciences, Neurobiology Laboratory, University of L'Aquila; (4) Laboratory of Experimental Medicine and Environmental Pathology, University Consortium "Sabina Universitas", University of Rome "Sapienza" in Rieti, Italy. (5) Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy.

BACKGROUND. Angiogenesis is often deregulated and exploited in tumor growth. After initial responses to anti-VEGF/VEGFR compounds, glioblastomas (GBM) tend to grow back in a more invasive manner and relapse. Here we tested UniPr1331, a novel brain penetrating pan Eph receptor antagonist, in combination with bevacizumab or sunitinib.
METHODS: The effects of bevacizumab, sunitinib and UniPR1331, were tested alone or in combination in subcutaneous xenografts of U87MG, U251 and T98G cells as well as on intra-cranial xenografts of luciferase tagged U87MG cells injected in CD1-nu/nu mice.
RESULTS: UniPr1331 reduces the in vitro migratory and invasive capacities of GBM and endothelial cells as well as vasculomimicry in U87MG cells at doses which were not able to modify cell proliferation. Reduction of the tube formation of endothelial cells was observed in vitro and in chick embryo chorioallantoic membrane model. UniPr1331 was tested in orthotopic intra-brain tumors by using luciferase-taggeted U87MG and TPC8 cells. We observed that UniPr1331 inhibited the tumor growth of GBM cell models and was additive/synergistic with s bevacizumab or sunitinib increasing significantly Disease Free Survival and Overall Survival. Our data indicate that UniPR13l may represent a novel therapeutic strategy to tackle GBM tumors increasing and bypassing resistance to VEGF- based anti angiogenetic treatments.

NOTCH signaling deregulation in T cell acute lymphoblastic leukemia promotes the generation of myeloid derived suppressor cells

Paola Grazioli[1], Claudia Noce[1], Gaia Scafetta[2], Andrea Orlando[1], Elena Delfino[1], Isabella Screpanti[1] and Antonio Francesco Campese[1]

[1]Dept. of Molecular Medicine, 'Sapienza' University, Rome, Italy [2]Dept. of Medical-Surgical Sciences and Biotechnologies, 'Sapienza' University, Rome, Italy

OBJECTIVE: Notch receptors deeply influence both T cell differentiation and T-cell leukemia development. Notch3 constitutive activation inside T cell compartment of transgenic (*N3tg*) mice induces an aggressive form of 'T-cell acute lymphoblastic leukemia' (T-ALL), characterized by the 'aberrant' presence of extrathymic immature $CD4^+CD8^+$ T cells.
'Myeloid Derived Suppressor Cells' (MDSCs) include a heterogeneous group of immature/progenitor myeloid cells, that in mice are broadly identified as $CD11b^+Gr\text{-}1^+$ cells. MDSCs are expanded and activated in tumor microenvironment, where they suppress host immune responses, thus facilitating disease progression. MDSCs have been extensively described in solid tumors and, recently in hematological malignancies.
Thus, we would explore the possibility that Notch3-deregulation in T cells may induce *in trans* the appearence of MDSCs in the context of T-ALL.
MATERIALS AND METHODS: We used cells from *N3-tg* mice, and *wt* controls, at different age, to describe by FACS analysis, the distribution of both $CD4^+CD8^+$ (DP) T and $CD11b^+Gr\text{-}1^+$ cells. These subsets were also purified by 'FACS-assisted cell sorting', and processed for real-time qRT-PCR experiments and for *in vitro* 'suppression' assay on activated *wt* T cells and co-colture experiments. ELISA test was performed to check cytokines concentration in mouse serum.
RESULTS: We observed alterations inside myeloid compartment of *N3-tg* mice, with a systemic expansion of $CD11b^+Gr1^+$ cells, that display features of MDSCs, such as the overexpression of functional molecules (i.e. Arginase-1, ROS), and the ability to suppress *in vitro* proliferating *wt* T cells. Moreover, we suggested that in Notch3-dependent T-ALL, the massive presence of aberrant DP T cells in BM microenvironment may influence differentiation of myeloid compartment, through non-cell autonomous mechanisms. Indeed, BM DP T cells from *N3-tg* mice promote the generation of MDSCs, at least *in vitro*, through a mechanism that is partially dependent on the IL-6 produced.
CONCLUSION: Collectively, our data represent the first demonstration of the presence of MDSCs in T-ALL and shed new light on the cross-talk between T cell and myeloid cell compartment, that may influence the disease outcome and thus would have an important impact on the development of innovative therapies.

The KCASH2-KO mice: a mouse model with mild hedgehog-dependent phenotype

Moretti M*, Izzo M*, Laricchiuta D, Fabretti F, De Feudis G, Spiombi E, Angrisani A, Gelfo F, Petrosini L, De Smaele E.

SAPIENZA Università di Roma

The identification of the KCASH family has provided new informations on the regulation of the hedgehog pathway during cerebellar development and differentiation. The KCASH family is composed by three members KCASH1, KCASH2, KCASH3, which share a high sequence homology, and modulate HDAC1 levels by inducing its ubiquitin-dependent degradation. In turn, HDAC1 modulates Hedgehog (Hh) signaling, deacetylating its effectors Gli1/2 and enhancing their transcriptional activity.
KCASH2 expression is observed in adult cerebellum, whereas epigenetic silencing and allelic deletion are observed in human Medulloblastoma, the most aggressive pediatric brain tumor. Our aim was to generate and characterize a new mouse model knocked-out for KCASH2 and obtain a greater understanding of KCASH2 role as a negative regulator of Hh pathway *in vivo* during cerebellar development, differentiation, function and tumorigenesis.
MATERIALS AND METHODS: We generated the KCASH2-KO mouse model using the "gene targeting" technology by homologous recombination in mESCs of a reporter gene LacZ cassette, Immunohistochemistry and immunofluorescence techniques have been used on paraffin embedded or criopreserved tissues. Standard β-galactosidase assays were also performed. To study the KCASH2-KO mouse behaviours we performed Morris water maze test and Rotarod test.
RESULTS: The KCASH2-KO mouse is a Knock-out first and it is vital, fertile and so far does not develop Mb. The KCASH2 protein is expressed during cerebellum development, its expression is greater in the adult, confirming its role in the silencing of Hh pathway and cellular differentiation. The reporter gene expression shows KCASH2 cellular localization in cerebellar granules in the IGL, as expected, and in the Purkinje cells, confirmed by specific immunostaing. Furthermore, accurate morphological analysis shows an overgrowth in the cerebellum corresponding to a thicker IGL in IV and V lobes compared with the WT controls. Behavioral experiments exhibit a delay in learning ability of the KCASH2-KO mice.
CONCLUSION: The KCASH2-KO mouse analysis shows mild cerebellar morphology alteration during development. Such alteration is compensated in adulthood, probably due to a partial redundant effect of the other KCASH members. Preliminary data suggest that KCASH-KO mice also present behavioural abnormalities, which may be linked to a cerebellar phenotype. Further analysis will elucidate the relevance of the KCASH2 expression in Purkinje cells.

Definition of microRNAs signatures of nodal involvement in laryngeal cancer patients

Hiromichi Kawasaki[1,2], Angela Lombardi[1], Rosanna Capasso[1], Gabriella Misso[1], Filippo Ricciardiello[3,5], Teresa Abate[3], Maurizio Iengo[3], Domenico Testa[4], Domenico Napolitano[5], Giovanni Motta[4], Marco Fornili[6], Elia Mario Biganzoli[6], Diego Ingrosso[1], and Michele Caraglia[1]

[1] *Department of Biochemistry, Biophysics, and General Pathology, Second University of Naples*
[2] *Drug Discovery Laboratory, Wakunaga Pharmaceutical Co., Ltd.*
[3] *Department of Ear Nose and Throat Unit, University of Naples Federico II*
[4] *Department of Ear Nose and Throat Unit, Second University of Naples*
[5] *Department of Ear Nose and Throat Unit, Cardarelli Hospital, Naples*
[6] *Department of Clinical Sciences and Community Health, University of Milan*

OBJECTIVE: This study aims to investigate deregulated miRNA expression patterns to use as a powerful indicator of prediction, diagnosis, and prognosis of laryngeal cancer (LCa) with malignant metastases.
MATERIALS AND METHODS: In this current study, we investigated aberrant miRNA expressions in LCa with nodal metastases using a comprehensive microarray screening and quantitative real-time PCR (qRT-PCR) analysis. Both tissue and serum specimens were collected from patients suffering from laryngeal carcinoma either with lymph node metastasis (N+) or no metastasis (N-), and non-pathological serum samples were obtained from healthy individuals. Total RNA, including miRNA, was extracted from the tissues. Additionally, circulating miRNA was isolated from the sera with a miRNA specific bead capture system. The RNA was then converted into cDNA by a reverse transcription step for the profiling and validation.
RESULTS: We enrolled 46 laryngeal carcinoma tissues, containing 23 N+ and 23 N-, and 30 their adjacent normal tissues in this study. We carried out a high-throughput screening assay in order to explore deregulated miRNA candidates using the microarray approach. The analysis showed that miR-449 and miR-652 were the most deregulated in N+ when comparing in N- ($p < 0.05$). Additionally, the expression levels of miR-133b ($p = 0.073$) and miR-223 ($p = 0.10$) were relatively highly altered, respectively.
For further validation study, we chose 4 miRNAs, miR-133b, miR-223, miR-449, and miR-652, which were much differentially deregulated in between N+ and N-. Of the 4 miRNAs, only miR-449 was significantly deregulated ($p < 0.05$) in N+ compared to in N-.
We used 13 sera (6 N+ and 7 N-) and 7 non-pathological sera for the serum miRNA screening. In the microarray study, the expression of miR-454 was the highest up-regulated in N+, whereas miR-202 was the most down-regulated.
CONCLUSION: Concluding, we have performed the microarray screening and qRT-PCR validation for the investigation of deregulated miRNA expression in LCa with both nodal involvement and no metastasis. This study indicates that miR-449 may contribute to develop potential biomarkers in order to predict and prognosticate laryngeal cancer with malignant metastases.

GPER couples IL1β expression in cancer-associated fibroblasts to IL1R1 function in breast cancer cells

Rosamaria Lappano[1], Francesca Cirillo[1], Sergio Abonante[2], Marcello Maggiolini[1], Paola De Marco[1]

[1]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy; [2]Breast Cancer Unit, Regional Hospital, Cosenza, Italy.

OBJECTIVE: IL1β/IL1R1 axis plays a main role in the functional crosstalk between cancer-associated fibroblasts (CAFs) and cancer cells, therefore triggering a pro-tumorigenic inflammatory phenotype. The G protein estrogen receptor (GPER) has been recently involved in estrogenic signaling in diverse types of tumor cells as well as in CAFs, which strongly contribute to tumor progression. We here evaluated the potential of GPER to regulate IL1β/IL1R1 expression and function in both CAFs and breast cancer cells.
MATERIALS AND METHODS: In order to provide novel insights into the mechanisms through which GPER facilitates the functional liaison between CAFs and tumor cells, we performed gene expression studies, immunoblotting analysis and gene silencing experiments, F-actin staining, enzyme-linked immunosorbent (ELISA), migration and polarization assays in MCF-7 and SkBr3 breast cancer cells and CAFs that we obtained from breast tumor patients.
RESULTS: We found that GPER triggers the EGFR/ERK/PKC transduction pathway in mediating the estrogen-induced up-regulation of IL1β and IL1R1 respectively in CAFs and breast cancer cells. We also determined that GPER contributes to the functional interplay between tumor cells and the surrounding stroma by up-regulating IL1β/IL1R1 target genes like PTGES, COX2, RAGE and ABCG2. Then, we ascertained that conditioned medium from CAFs exposed to GPER agonists stimulates breast cancer cells toward biological responses that characterize cancer progression like the acquisition of a fibroblastoid cytoarchitecture, the reorganization of F-actin and cell migration.
CONCLUSION: Collectively, our data show that ligand activated-GPER couples IL1β induction in CAFs to IL1R1 expression and function in breast cancer cells, leading to relevant features involved in breast tumor progression. Hence, GPER-mediated IL1β/IL1R1 regulation may be considered in further comprehensive therapeutic strategies targeting breast cancer.

Extracellular vesicles mediate crosstalk between stromal and tumour cells in the bone microenvironment

Alexander Loftus, Chris George, Riccardo Paone, Marco Ponzetti, Alfredo Cappariello, Anna Teti, Nadia Rucci

Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy

The bone microenvironment is a fertile soil for cancer dissemination. Malignant cells that colonise the skeleton are capable of disrupting bone cell biology, either by interaction with stromal cells of the bone microenvironment or by expressing bone-modulating factors themselves. Regulatory molecules can be exocytosed by cells to passively reach their targets, or can be released within extracellular vesicles (EVs). EVs are membrane-bound containers of biologically active molecules shed by cells, which are emerging as mediators of a range of pathological processes, including cancer. However, their role in primary and metastatic cancers of the bone has been poorly explored. We set out to investigate EV-mediated communication between tumour cells (i.e. breast cancer and osteosarcoma cells), and the bone residing cells (osteoblasts and osteoclasts). We found that osteoblasts and tumour cells participate in reciprocal communication via EVs. In osteoblasts, tumour-derived EVs significantly increased mRNA expression of the pro-osteoclastogenic factors, *IL-1β* (12-fold,$p<0.001$) and *IL-6* (10fold,$p=0.05$), while inhibiting expression of osteoblast differentiation and activity-related transcripts, including *Runx2* (-80%,$p<0.001$), *Osx* (-85%,$p<0.001$), *Col1α2 (*-70%,$p=0.001$*) and Cyclin D1* (-75%,$p=0.02$). Tumour-derived EVs also induced *Nos2* mRNA expression (15-fold,$p=0.015$). Furthermore, tumour-secreted factors promoted RANK-L protein expression in osteoblasts and enriched its presence in their EVs, as demonstrated by FACS analysis, which in turn led to an increased osteoclastogenic potential. As EV-mediated communication between osteoblasts and tumour cells is reciprocal, we investigated osteoblast-derived EVs as a means of delivery for therapeutic agents. Delivery of the chemotherapeutic drug doxorubicin (DXR) encapsulated within osteoblast-derived EVs caused death in osteoclasts, in breast cancer and in osteosarcoma cell lines comparable to that caused by 4μM free DXR, while osteoblast-derived EVs alone had no effect. Moreover, a dramatically decreased mass of DXR was required to mediate this effect when encapsulated in EVs. Together, these data implicate EVs in cancer-associated disruption of bone biology and point EVs as a potential novel and highly efficient vector for targeted delivery of chemotherapeutics.

Development of urotensin II-targeted liposomes: a new selective drug delivery system for prostate and colon cancer cells

Luce Amalia[1], Zappavigna Silvia[1], Lusa Sara[1], Cossu Maria Alessia[1], Stiuso Paola[1], Di Lorenzo Raffaella[2], Yousif Ali Munaim[2], De Rosa Giuseppe[2], Grieco Paolo[2] and Caraglia Michele[1]

[1] *Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, Italy;*
[2] *Department of Pharmacy, University of Naples "Federico II", Italy.*

OBJECTIVE: Urotensin II (UT-II) is an 11-amino acid peptide known for its potent vasoconstrictive actions in mammalian tissues. In addition, UT-II and its receptor (UTR) play an important role in the regulation of cell proliferation, invasion and motility in human prostate, colorectal and bladder cancer cells. Liposomes are used as a drug delivery system to improve the pharmacokinetic and pharmacodynamic profiles of chemotherapeutic agents and reduce systemic toxicity compared with free drug. Ligand-mediated targeting of liposomal anticancer drugs allow a selective and specific binding to cell surface receptors expressed on cancer cells. On these bases, the purpose of this study was to develop liposomes conjugated with UT-II (LipoUT) in order to target cancer cells overexpressing UTR.
MATERIALS AND METHODS: UTR expression on prostate (DU145, PC3 and LNCaP) and colorectal (WIDR and LoVo) cancer cell lines were evaluated by FACS and western blotting analysis.
We developed stealth liposomes encapsulating doxorubicin (Lipo-DOX). Cys-hUT-II(4-11) was conjugated to liposomes modified with maleimide-polyethylene glycol (DSPE-PEG-Mal) to obtain LipoUT-DOX. The resulting liposomes were then purified.
The effects of different formulations on cell viability were quantified by FACS analysis. Liposome uptake by cells were investigated by confocal microscopy and FACS analysis using Bodipy-labelled liposomes.
RESULTS: UTR protein was expressed in all cell lines tested and the expression level was higher in DU145, PC3 and LNCaP cells than in LoVo and DU145. The evaluation of cell viability, after 72 hours of treatment, showed that LipoUT-DOX were more active than Lipo-DOX on the growth inhibition of cells that overexpressed UTR (PC3, LNCaP and WIDR) while in LoVo and DU145 cell lines the activity was similar or lower than that one of Lipo-DOX. Moreover, fluorescent-labelled liposome uptake in PC3 and DU145 was higher for LipoUT than the not armed counterparts in both cell lines, mostly in UTR overexpressing PC3 cells (about 2-fold higher) as evaluated by both confocal and FACS. Preliminary results obtained by confocal microscopy, using LipoUT-DOX and labelled UTR confirmed the uptake of targeted liposomes.
CONCLUSION: These results open a new perspective on the use of urotensin II-targeted liposomes for selective drug delivery in prostate and colorectal cancer.

The brain penetrating CXCR4 antagonist PRX177561 combines synergistically with anti-angiogenic agents to inhibit progression of glioblastoma.

Andrea Mancini[1], Giovanni Luca Gravina[1], Francesco Marampon[1], Alessandro Colapietro[1], Simona Delle Monache[1], Roberta SFERRA, Flora Vitale[2], Peter J Richardson[3], Lee Patient[3], Stephen Burbidge[3] and Claudio Festuccia[1]

(1) Department of Biotechnological and Applied Clinical Sciences, Radiobiology Laboratory, University of L'Aquila; (2) Department of Biotechnological and Applied Clinical Sciences, Neurobiology Laboratory, University of L'Aquila; (3) Proximagen Ltd, Babraham Research Campus, Cambridge CB22 3AT, UK.

BACKGROUND: Glioblastoma recurrence after treatment with the anti-vascular endothelial growth factor (VEGF) antibody bevacizumab is characterized by a highly infiltrative and malignant behavior that renders surgical excision and chemotherapy ineffective. It has been demonstrated that anti-VEGF/VEGFR therapies control the invasive phenotype and that relapse occurs through the increased activity of CXCR4. We therefore hypothesized that combining bevacizumab or sunitinib with the novel CXCR4 antagonist, PRX177561, would have superior anti-tumor activity.
METHODS: The effects of bevacizumab, sunitinib and PRX177561, were tested alone or in combination in subcutaneous xenografts of U87MG, U251 and T98G cells as well as on intra-cranial xenografts of luciferase tagged U87MG cells injected in CD1-nu/nu mice. Animals were randomized to receive vehicle, bevacizumab (4 mg/kg iv every 4 days), sunitinib (40 mg/kg po qd) or PRX177561 (50mg/kg po qd).
RESULTS: these in vivo experiments demonstrated that bevacizumab and sunitinib increase the in vivo expression of SDF-1, CXCR4, IL-1β, TNFα, MCP-1 and TGFβ and that the co-administration of PRX177561 with bevacizumab or sunitinib inhibited the increase in these mediators, reduced the infiltration of leukocytes and slowed tumor progression to a greater extent than seen with either agent alone. The combination of PRX177561 with bevacizumab or sunitinib resulted in synergistic effects on the reduction of tumor growth, the increment of both disease free survival (DSF) and overall survival (OS) in these preclinical murine models of glioblastoma.
CONCLUSIONS: The CXC4 antagonist PRX177561 may be a valid therapeutic complement to anti- angiogenic therapy, particularly when used in combination with VEGF/VEGFR inhibitors. Therefore this compound deserves to be considered for future clinical evaluation.

Ephrin receptor kinase inhibition reverts oncophenotype, induces myogenic differentiation and radiosensitizes embryonal rhabdomyosarcoma cell lines.

Francesco Marampon[1,6,§,*], Francesca Megiorni[2,§,*], Simona Camero[2], Alessandro Colapietro[1], Cinzia Marchese[3], Simona Ceccarelli[3], Cristina Antinozzi[5], Heather P. McDowell[2,4], Roberto Maggio[7], Vincenzo Tombolini[8], Clara Crescioli[5], Carlo Dominici[2], Claudio Festuccia[1,#],Giovanni Luca Gravina[1,#]

[1]Department of Biotechnological and Applied Clinical Sciences, Division of Radiation Oncology, University of L'Aquila, 67100 L'Aquila, Italy.
[2]Department of Paediatrics and Infantile Neuropsychiatry, Sapienza University, Viale Regina Elena 324, 00161, Rome, Italy.
[3]Department of Experimental Medicine, Sapienza, University of Rome, Viale Regina Elena 326, 00161, Rome, Italy;
[4]Department of Oncology, Alder Hey Children's NHS Foundation Trust, Eaton Road, L12 2AP Liverpool, United Kingdom.
[5]Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Rome, Italy

Ephirin receptor tyrosine kinases (RTK EPH), the largest of the RTK families, are a clinically relevant class of targets in cancer. GLPG1790 is a new potent pan-EPH inhibitor. This report describes the effects of GLPG1790 on human embryonal rhabdomyosarcoma (ERMS) RD and TE671 cell lines in which the role of EPH signalling is unknown. GLPG1790 induced a dual effect promoting a caspase-3-mediated cell apoptosis and a G1 growth arrest as demonstrated by the reduction ofRB phosphorylation, Cyclin A and B1protein expression, and increase of p21 and p27 protein expression. GLPG1790 decreased migratory capacity and clonogenic potential both in adherent and non-adherent conditions, counteracting the expression of integrin-b1, b3 and b5.Interestingly, GLPG1790 prevented rhabdosphere formation, down-regulated stem cell markers CD133, CXCR4 and Nanog expression, and committed ERMS cells towards myogenic terminal differentiation by inducing a myogenic-like phenotype, decreasing DNMT3B and increasing MYOD1, Myogenin and MyHC myogenic marker expression. Furthermore, GLPG1790 significantly radiosensitized ERMS cells by increasing the radiation-therapy-induced DNA damages and affecting the NHEJ and HR pathways responsible of the DNA double-strand break repair. Finally, our study showed, for the first time, a significant up-regulation of EPHA2 and related ligand Ephrin A1 in 7ERMS tumour samples and ERMS cell lines in comparison to normal skeletal muscle. Taken together, our data indicate that altered EPH signalling plays a key role in ERMS development and that its pharmacological inhibition can represent a potential therapeutic strategy able to impair steaminess and reverse ERMS phenotype by rescuing myogenic program.

SWATH-MS analysis of mitochondrial proteome impaired by eiF6 shRNA

Simona Martinotti[1], Marcello Manfredi[2], Elisa Robotti[1], Emilio Marengo[1], Stefano Biffo[3,4], Elia Ranzato[1]

[1]University of Piemonte Orientale, DISIT - Dipartimento di Scienze e Innovazione Tecnologica, viale Teresa Michel, 11 - 15121 Alessandria, Italy
[2]Isalit srl, via Bovio 6, 28100, Novara – Politecnico di Torino, viale T. Michel 5, 15121, Alessandria, Italy.
[3]Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi", Via Sforza 28, 20122, Milano, Italy.
[4]University of Milan, Department of Biosciences, Via Celoria, 26, 20133, Milano, Italy.

OBJECTIVE: The aim of this work was to analyse, by using the SWATH-MS approach, the expression of mitochondrial proteome of AML-12 (non-tumourigenic murine liver hepatocytes) cell line where eiF6 was down-regulated by shRNA.
Eukaryotic Initiation Factor 6 (eIF6) is an initiation factor that binds 60S ribosomal subunits and has an anti-association property, by impeding 60S premature joining to 40S. In general, eIF6 is rate limiting for tumour onset and progression.
Mitochondria are the main compartments of energy production, and some lines of evidence have shown that mitochondrial alterations contribute to the development of metabolic syndrome.
MATERIALS AND METHODS: Liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) is the best analytical method for the identification and quantification of proteins in biological samples. Sequential window acquisition of all theoretical fragment-ion spectra mass spectrometry (SWATH-MS) is a data independent analysis (DIA) workflow that uses a first quadrupole isolation window to step across a mass range, collecting high resolution full scan composite MS/MS spectra at each step, and generating an ion map of the fragments from all the detectable precursor masses.
RESULTS: Mitochondria are the main compartments of energy production, and some lines of evidence have shown that mitochondrial alterations contribute to the development of metabolic syndrome.
CONCLUSION: We found that depletion of eIF6 by shRNA induce profound and varied impact on mitochondrial proteome, triggering mitochondrial hyperpolarization, impairing the energy production, steering the metabolism toward the up-regulation of aerobic glycolysis and the inhibition of oxidative phosphorylation.

MIR-182 dysregulation in a diet-induced NAFLD-NASH-Hepatocellular carcinoma mouse model.

Mastroiaco Valentina, Tessitore Alessandra, Cicciarelli Germana, Sferra Roberta, Vetuschi Antonella, Del Vecchio Filippo, Verzella Daniela, Fischietti Mariafausta, Vecchiotti Davide, Di Francesco Barbara, Zazzeroni Francesca, Alesse Edoardo.

Department of Biotechnological and Applied Clinical Sciences (DISCAB), University of L'Aquila, L'Aquila, Italy.

INTRODUCTION: Nonalcoholic fatty liver disease (NAFLD) is a frequent liver disorder. It can progress through the more severe nonalcoholic steatohepatitis (NASH), fibrosis and, lastly, HCC. MiRNAs are small non-coding RNAs acting as regulators of gene expression at the post-transcriptional level. In this study, a mouse model was used to investigate the effects of high-fat (HF) and low-fat/high-carbohydrate (LF-HC) diets on miRNA expression during liver damage progression.
MATERIALS AND METHOD: C57BL/6J mice were HF or LF-HC diet fed for 3, 6, 12, and 18 months. Control mice were standard diet fed. Hepatic tissues were collected. Histological analyses were performed. TaqMan qRT-PCR was used to analyze miRNAs in liver tissues. Target gene protein products were examined by immunoblotting.
RESULTS: Progressive liver damage was observed in HF and LF-HC mice. Tumors were detected in HF after 12 and 18 months and in LF-HC after 18 months. Molecular analysis showed several miRNAs differentially expressed during the disease progression and in tumors. Among them, miR-182 showed early dysregulation, being overexpressed in HF vs LF-HC fed mice after 3 months. The trend was maintained in HF after 6, 12 and 18 months and, in particular, in tumors compared to peritumoral tissues. The transcription factor FOXO1, a miR-182 target, was analyzed by immunoblotting and found ipo-expressed.
CONCLUSIONS: MiRNA expression was evaluated in livers from mice HF or LF-HC diet fed during the progression of liver damage up to HCC. MiR-182 was up-regulated in HF tissues and HF/LF-HC tumors. The expression of its target FOXO1 decreased and the role of this will be discussed.

Transcriptomic analysis in mouse osteoclasts carrying the ClC7^{G213R} mutation causing Autosomal Dominant Osteopetrosis type2 (ADO2)

Antonio Maurizi, Rajvi Patel, Nadia Rucci, Anna Teti, Mattia Capulli

Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, Italy

OBJECTIVE: We expect this research to pave the way for the identification of druggable targets that could improve the osteoclast function in ADO2.
MATERIALS AND METHODS: Total RNA was extracted from osteoclasts isolated from WT and ADO2 mice and subjected to RNA deep sequencing using Illumina Hi Seq 2500 technologies.
RESULTS: Preliminary cellular studies in primary osteoclasts isolated from WT and ADO2 mice carrying the ClC7^{G213R} mutant demonstrated that the ClC7 protein was significantly accumulated in the Golgi (+8.5fold,p<0.01), which appeared enlarged (+4fold,p=0.01). In contrast, it was reduced in lysosomes (-0.52%,p=0.02), harming lysosome acidification (+1.64 pH units,p<0.001) and increasing LC3-mediated autophagy. These data suggest that ADO2 osteoclasts can be affected at various levels. The RNA deep sequencing analysis showed 387 over- and 63 under-expressed transcripts in ADO2 vs WT osteoclasts (p<0.05). The highest expressed genes were grouped by the Gene Ontology (GO) terms. Statistically significant enrichment was observed for genes involved in the biological processes of cell adhesion (p<0.0001), integrin signaling (p<0.001) and regulation of apoptosis (p=0.05). Grouping these genes for their molecular functions, revealed an enrichment of the metal and ion binding function (p<0.05), peptidase activity (p=0.008) and integrin binding function (p=0.01). The analysis of the cellular components revealed an enrichment of transcripts for proteins localized in endoplasmic reticulum (p<0.001) and basement membrane (p<0.001). Conversely, biological processes such as immune response (p=0.04), chemotaxis (p<0.0001), cell proliferation (p<0.001) and regulation of endocytosis (p=0.05) were enriched in the group of under-expressed transcripts of ADO2 vs WT osteoclasts. Interestingly, further investigations using the KEEG pathway analysis revealed higher expression in ADO2 of genes involved in osteoclast differentiation (p<0.05). Accordingly, in vitro osteoclastogenesis was faster and more prominent in ADO2 bone marrow cultures vs WT (1.7fold,p<0.01). Interestingly, we found other pathways more active in ADO2 osteoclasts such as cytokines-cytokine receptors (p<0.001), Jak/Stat signal (p=0.001), extracellular matrix receptor (p<0.001) and focal adhesion (p<0.001).
CONCLUSION: All together, these results suggest that the impairment of ClC7 function in ADO2 osteoclasts does not cause only a defective resorption lacuna acidification, but also an impairment of pathways essential for other osteoclast functions.

Wnt/β-catenin and EGFR pathways evaluation in primary pleural cancers.

Carmelo Moscatello[1], Simone Di Russo[1,2], Maria Carmela Di Marcantonio[1], Fabiana Fantini[1], Maria Cristina Curia[1], Pasquale Batista[1], Raffaella Muraro, Gabriella Mincione[1], Felice Mucilli[2], Gitana Maria Aceto[1].

[1]Department of Medical, Oral and Biotechnological Sciences, University 'G. d'Annunzio'-Chieti-Pescara
[2]Department of Surgery, University of Chieti

OBJECTIVE: Malignant Pleural Mesothelioma (MPM) and Pleural Synovial Sarcoma (PSS) are rare and aggressive pleural cancers. Compared to MPM, PSS occur more often in youth, although both forms are unified by a poor prognosis. Wnt/β-catenin and EGFR signaling play key roles in embryonic development, in the determination of cell fate, tissues stem cell renewal and homeostasis. It is well documented that dysregulation of these pathways leads to tumorigenesis with poor prognosis but the possible crosstalk between them is largely unknown.
MATERIALS AND METHODS: This study investigates the possible convergence between Wnt/β-catenin and EGFR signaling by gene and protein expression in eight pleural cancers, six epitelial MPM (eMPM), one sarcomatous MPM (sMPM) and one PSS tissues from patients treated at the Department of Thoracic Surgery, SS. Annunziata Hospital in Chieti, from 2013 to 2014. Fresh tumour tissues were evaluated for protein and gene expression by Western blotting and qRT-PCR analysis.
RESULTS: Western blotting for total β-catenin reveals a lower expression in sMPM compared to eMPMs and a strong EGFR activation. The PSS and 1/5 eMPM display higher amount of activated β-catenin compared to the remaing samples. Gene expression of Wnt/β-catenin signalling components highlights a very strong expression of *LEF1* transcription factor in all cancers. *APC* gene increases about three times in 3/5 eMPMs whereas the PSS, sMPM and 1/5 eMPM showed a lower expression compared to pleural non-cancerous tissues. *WNT3a* a prototype of the canonical signaling showed an opposite trend respect to *APC*. *WNT5a,* a prototype of the noncanonical pathway, decreased although the β-catenin was expressed al low levels. *Axin2*, *LRP6* and *BCL9* decreased. ErbB reseptors were shown to be overexpressed in all tumors analyzed, except *ErbB4* overexpressed only in PSS.
Moreover, in one eMPM from a patient treated with neoadjuvant chemotherapy we observed a reduction of Wnt/β-catenin and EGFR signalling markers, although the reduction was less marked in LEF1 and ErbB3 genes.
CONCLUSION: These preliminary data confirm the involvement of Wnt/β-catenin and ErbB pathways in pleural cancers and also underlined the noncanonical Wnt signalling implication. To highlight the solutions of unanswered questions, will be imperative the rational exploration of these pathways in future molecular treatment strategies.

A signature of long-non coding RNAs (lncRNAs) is associated with response to chemotherapy in Ewing Sarcoma

Alessandro Parra[1,2], Andrea Grilli[1,3], Cristina Baricordi[1,2], Stefano Ferrari[4,2], Piero Picci[2], Katia Scotlandi[1,2]

1 CRS Development of Biomolecular Therapies, Istituto Ortopedico Rizzoli, Bologna, Italy;
2 Experimental Oncology Laboratory, Istituto Ortopedico Rizzoli, Bologna, Italy;
3 Center for Genome Research Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy.
4 Struttura Semplice Dipartimentale di Chemioterapia dei Tumori dell'Apparato Locomotore, Istituto Ortopedico Rizzoli, Bologna, Italy.

OBJECTIVE: Treatments of Ewing sarcoma (EWS), the second most common bone tumor in pediatric age, are still firmly confined to conventional chemotherapy and few validated biomarkers have been selected so far. Around 30% of patients do not respond to the therapy and undergo disease progression even during the treatments. In this study, we evaluated the genetic landscape of 15 patients with a very different outcome.
MATERIALS AND METHODS: 250 ng of TRIZOL extracted RNA from EWS untreated samples were used for the synthesis of cDNA libraries with TruSeq RNA Sample Prep Kit v2 (Illumina), and sequenced by synthesis at 75bp in paired-end mode on HiScanSQ sequencer (Illumina). Reads were aligned with TopHat2/BowTie2 to the reference human genome hg19/GRCh37. Defuse and Chimerascan packages were used to detect chimeric transcripts from RNA-seq data. Raw reads were aligned using TopHat (version 2.1.0; to build version hg19 of human genome from UCSC. Counts for UCSC annotated genes were calculated from the aligned reads using HTSeq (version 0.6.0). Normalization and differential analysis were carried out using edgeR package (version 2.12.0) and R (version 3.2.2).
RESULTS: Fusion transcript analysis identified no other translocations than the canonical EWS/ETS, while SNV analysis detected a total of 809 SNVs (87 in at least 2 patients). Analysis of SNV differential expression in good- vs poor-responders identified: 1. missense or non-sense p53 mutations in 4/7 (57%) poor responders vs 0/8 (0%, p=0.02 Fisher's test) 2. Mutations in 5 genes characterize good responders vs poor responders. In particular, we observed mutations in 3/8 (38%) good responders vs 0/7 (0%) poor responders for: NOMO1, NUMP153, OGG1, PIEZO1 and MICB. In addition, we focused our attention to lncRNA expression. We found a signature that clearly distinguished good vs poor responders. Differential expression of selected lncRNAs was confirmed by qPCR and functional studies validated their impact on cell chemosensitivity
CONCLUSION: Besides confirming the role of p53 mutations as indicator of bad prognosis, our studies actually identifies novel SNVs that appeared to define good responders to chemotherapy. In addition, we found a signature of lncRNAs that define different patient response to chemotherapy.

Effectiveness of Vemurafenib, PIK-75 and miR-126 triple combined therapy on B-RAF^{V600E} metastatic melanoma through MAPK and/or PI3K/AKT inhibition.

F.Pedini[1],G.De Luca[1], A.Boe[1], A.De Feo[1], M. Spada[1], S.D'Atri[2]., A.Giuliani[1], A.Carè[1] and N. Felli[1]

[1]Dept. of Hematology, Oncology and Molecular Medicine, Istituto Superiore Sanità, 00161 Rome, Italy,[2]Laboratory of Molecular Oncology, "Istituto Dermopatico dell'Immacolata"-IRCCS, Rome, Italy

OBJECTIVE: One of the most important challenges facing the oncologists today is to prevent the escape of cancer cells from the action of chemotherapeutic agents. BRAF-mutated (V600E) is the most prevalent alteration in human melanoma, representing one of the key elements underlying the constitutive activation of the Ras/Raf/MEK/ERK (MAPK) signaling. The inhibition of MAPK and/or PI3K/AKT represents one of the more promising new strategies for melanoma therapy. The major trouble in these approaches is the existence of multiple cross-talks between those pathways, as the inhibition of AKT phosphorylation appears to induce MAPK activation and *vice versa.* Accordingly, the majority of patients relapse quite rapidly after treatment due to acquired resistance. The focus of our work was to find a new combined therapy including selective MAPK and PI3K/AKT pathways inhibitors together with microRNA-mediated wider action.
METHODS: We investigated the effect of anticancer compounds (Selleck-Library) through cell viability assay, pathways inhibition by western blot analysis and *in vivo* experiments.
RESULTS: Looking for new drug combinations, we tested 349 anticancer-compounds against B-RAF^{V600E} metastatic melanoma cell lines. Among several others, we focused on PIK-75, an inhibitor of PI3K/p110α, which showed a strong effect on metastatic melanoma cell viability and appeared to synergistically act with vemurafenib in keeping off both PI3K/AKT and MAPK pathways. In addition, this anticancer activity was further enhanced by overexpressing the oncosuppressor miR-126, already known to target the p85β subunit of PI3K that, forming a complex with p110α, regulates the PI3K/AKT pathway. We found that the triple combination of Vemurafenib, PIK-75 and miR-126 significantly induced apoptosis in turn reducing cell growth. Results were confirmed on early passage melanoma cells obtained from bioptic metastatic samples as well as in *in vivo* preliminary preclinical models. Interestingly a strong effect was also obtained when the Vemurafenib resistant B-RAF^{V600E} metastatic melanoma cell line (A375M-VR) was co-treated with PIK-75.
Conclusion: Our study confirms the close interplay between Ras/Raf/MEK/ERK and AKT pathways in human melanomas offering direct evidences for the effectiveness of combining inhibitory drugs and oncosuppressor microRNAs.

Heterogeneous mutational status of melanomas in multiple primary melanoma patients

Cristina Pellegrini1, Claudia Martorelli1, Gianluca Cipolloni2, Lucia Di Nardo1, Maria Giovanna Maturo1, Ambra Antonini1, Maria Concetta Fargnoli1

1. Dept. of Dermatology, University of L'Aquila, L'Aquila, Italy, Italy.
2. Dept. of Pathology, University of L'Aquila, L'Aquila, Italy, Italy.

OBJECTIVE: Multiple primary melanomas (MPM) develop in about 5% of sporadic melanoma patients and in up to 19% of melanoma patients with a positive family history. Many genetic alterations, including predisposing and/or somatic mutations, may contribute to the development of MPM and data on the genetic diversity of MPMs are limited. We aimed to assess the frequency and distribution of *BRAF*, *NRAS* and *TERT* promoter somatic mutations in subsequent melanomas of the same patient and evaluate the association of somatic alterations with germline mutational profile of MPM patients.
MATERIALS AND METHODS: Sixty-six synchronous/asynchronous FFPE melanoma tissues from 31 patients were analyzed by Real-Time PCR, Sanger Sequencing and SNaPshot molecular methods for BRAF, NRAS and TERT promoter mutations. Germline DNA was screened by Sanger sequencing for mutations in CDKN2A, CDK4, POT1, MITF, MC1R genes and TERT promoter.
RESULTS: BRAF V600 mutations were found in 46.2% of melanomas, NRAS mutations in 10.6% and TERT promoter rs2853669 polymorphism in 64.2%. Mutational somatic profile (BRAF, NRAS) was concordant between first and subsequent primary tumors in 65.4% of patients. Somatic mutational status was not associated with any of the clinical characteristics of patients or melanomas, including age, melanoma thickness and body site. At the germline level, MPM patients were wild-type for mutations in CDKN2A, CDK4, POT1 and MITF genes. TERT-promoter polymorphisms (rs2853669 and rs35226131) were identified in 46.1% of patients and MC1R allelic variants in 73.1%. Carriers of MC1R changes were diagnosed more frequently with melanoma on the trunk compared to wild-type patients (p=0.02), but no association between MC1R status and somatic mutations was identified in our patients.
CONCLUSION: In conclusion, our results support the heterogeneity of molecular profiles in MPM of the same patient with implication in clinical practice due to the difficulties in molecularly classifying patients with discrepant primary melanomas.

New 5-Fluorouracil amphiphilic derivatives in liposome formulation to overcome drug resistance in colon cancer

Pellegrini E.(1), Condello M. (1), Giansanti L. (2), Petaccia M. (2), Mancini G. (3), Meschini S.(1)

(1) Dipartimento Tecnologie e Salute, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 (RM), Italy.
(2) Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila, Via Vetoio 67100 Coppito (Aq), Italy
(3) CNR – Istituto di Metodologie Chimiche, Via Salaria km 29.300, 00016 Monterotondo Scalo (RM), Italy.

OBJECTIVE: 5-Fluorouracil (5-FU), one of the first-line chemotherapeutic agents for the treatment of gastrointestinal malignancies has shown limited efficacy. 5-FU exhibits a high toxicity, the occurrence of acquired resistance, low tumor affinity and risk of severe side effects for the patients. Numerous derivatives have been synthesized to improve the physicochemical, biopharmaceutical and pharmacokinetic properties of 5-FU diminishing or circumventing some of its disadvantages. Three 4-substituted 5-fluoropyrimidines with characterized by the presence of a polyoxyethylene chain of different length and a hydrophobic alkyl chain were synthesized and characterized.
MATERIALS AND METHODS: Physicochemical characterization of liposomes formulation and biological evaluation by MTT colorimetric test for evaluation of cell viability after 48 h of treatment with liposomes formulated without the 5-FU derivatives or with free 5-FU.
RESULTS: The new derivatives were obtaining by alkylatingthe N3 position of 5-FU with a chain consisting of six, seven or eight units of ethylene glycol linked to an alkyl chain of 12 carbon atoms (amphiphile 1, 2 or 3, respectively. The new 5-FU derivatives were included in liposomes composed of a natural phospholipid, dioleoyl-snglycero-phosphocholine (DOPC), in the presence of a gemini cationic surfactant (2S, 3S)-2,3-dimethoxy-1,4-bis(Nhexadecyl- N,N-dimethylammonium) butane bromide,that had been shown to attribute to phospholipid formulations high efficacy in drug delivery and to be characterized by low toxicity. The cytotoxicity of these formulations was evaluated by MTT assay on colorectal carcinoma HCT116 cell line 48 h after the treatment. These formulations were found active against HCT116 tumor cells; it was shown that the efficacy of the treatment increases as a function of the length of the polyoxyethilenic segment of the 5-FU derivatives, and that the polyoxyethilenic chain itself has no influence on their biological activity.
CONCLUSION: The investigated liposome formulations showed a higher cytotoxic compared to the free drug, thus showing a great potential in cancer therapy. Ongoing studies by flow cytometry, confocal and electron microscopy on the mechanism of interaction and internalisation of liposomes with colon cancer cells will clarify the mechanism of action of these new formulations.

The emerging role of Jagged1 in sustaining colorectal cancer aggressiveness

M. Pelullo, S. Zema, R. Quaranta, I. Screpanti and D. Bellavia.

Dept. of Molecular Medicine, Sapienza University, Rome, Italy

OBJECTIVE: Colorectal cancer (CRC) is the third and second most common malignancy in terms of incidence and mortality worldwide in male and female respectively. The Notch signalling has an important role in regulating the intestinal development and homoeostasis. In fact, alterations of Notch pathway contribute to the CRC onset and malignancy. It is widely demonstrated that the Notch ligand Jagged1 is aberrantly expressed in 50% of CRC and some studies correlate the levels of Jagged1 expression with tumour differentiation parameters and CRC clinical stages. Recently, it has been proposed that the Jagged1 ligand protein undergoes to sequential proteolytic cleavages that involve two distinct enzymes: a A-Disintegrin and Metalloprotease ADAM-17 and PS/γ-secretase complex, ending in the release of Jagged1 intracellular domain (Jag1-ICD), able to move into the nucleus and activate genes expression. We speculate that Jagged1 is able to trigger an own signal transduction via release of Jag1-ICD intracellular fragment, in a Notch-indipendent way, with an important impact on CRC development. We aim to better understand the molecular mechanism responsible of Jagged1-ICD-dependent signaling in CRC in order to be able to define a new potential target therapy.
MATERIALS AND METHODS: Western Blot analysis; qRT-PCR; Pharmacological treatment; Cell Cycle analysis; Invasion assay; Stable transfection; Xenograft tumorigenesis in nude mice.
RESULTS: We observed that the ligand Jagged1 is not only abundantly over-expressed, but also constitutively cleaved, releasing the soluble intracellular fragment Jag1-ICD, able to move into the nucleus, in CRC cell lines. TAPI-2 pharmacological treatment significantly inhibits the Jagged1 intracellular processing, with an important effect on CRC cell growth and cell migration ability. Intriguingly, colon cancer cells, stably expressing Jag1-ICD, showed increasing cell growth in xenograft mouse model with a considerable up-regulation of cell metastasis markers (MMP-9, Snail1 and Snail2).
CONCLUSIONS: Accordingly to our *in vitro* and *in vivo* data, we demonstrate the existence of a Jag1-ICD signalling that regulates transcriptional events able to empower the aggressiveness of CRC cells, in a Notch-independent manner. A better understanding of this signalling could be useful to provide new CRC therapeutic treatment directly targeting Jagged1.

Hemoglobin beta (HBB) increases breast cancer aggressiveness via "oxygen positive hypoxia"

Marco Ponzetti, Mattia Capulli, Adriano Angelucci, Luca Ventura, Simona Delle Monache, Cinzia Mercurio, Alessia Calgani, Patrizia Sanità, Anna Teti, Nadia Rucci

University of l'Aquila; San Salvatore hospital (L'Aquila)

Abstract

Besides its role as O_2 and CO_2 transporter, recent findings suggest that hemoglobin beta (HBB) may have roles in other contexts. In our previous work, we found HBB to be upregulated in bone metastases of breast cancer patients that then developed also visceral metastases compared to bone metastasis-only patients. In this work, we wanted to investigate whether HBB could be important also in the progression of the primary breast cancer by *in vitro* and *in vivo* studies. We observed a significantly higher expression of HBB in invasive carcinoma histotypes versus *in situ*, and a positive correlation between HBB and the Ki67 proliferation marker in breast cancer patients. More aggressive breast cancer cells (MDA-MB-231) expressed more HBB compared to less aggressive (MCF7, HCC1954) cells. We generated stable HBB-overexpressing breast cancer cells, and found them to migrate and invade more, to have HIF-1α upregulation (a biological situation that we called "Oxygen-positive hypoxia") and their conditioned media enhanced angiogenesis. Blocking the oxygen-binding site of HBB with the carbon monoxide releasing molecule (CORM)-2, reverted the increase of migration and the oxygen-positive hypoxia observed in HBB-overexpressing breast cancer cells. Orthotopically implanted MDA-MB-231 overexpressing HBB (MDA-HBB) generated tumors with a faster growth rate and increased vascularization. Moreover, local recurrence and visceral metastases were observed only in MDA-HBB cell-implanted mice. Similar results were observed using 4T1 mouse breast cancer cells. Intriguingly, MCF7, a less aggressive breast cancer cell line, showed no increase of invasion when engineered to overexpress HBB. Finally, bioinformatics analyses of public data sets correlated high HBB expression with lower overall survival, especially in more aggressive subtypes of breast cancer. These data demonstrate that HBB expression increases breast cancer cells aggressiveness and associates with poor prognosis. Therefore, HBB could be a novel prognostic biomarker of breast cancer progression, which increases aggressiveness by causing "oxygen-positive hypoxia".

The G-quadruplex ligand EMICORON potentiates the antitumor efficacy of chemotherapy on colon cancer experimental models

Manuela Porru[1], Simona Artuso[1], Luca Pompili[1,2], Carla Caruso[2], Armandodoriano Bianco[3], Marcella Mottolese[1], Carla Azzurra Amoreo[1], Annamaria Biroccio[1], Carlo Leonetti[1]

[1]Regina Elena National Cancer Institute, Rome; [2]University of Tuscia, Viterbo; [3]University "La Sapienza", Rome, Italy.

G-quadruplex structures, present at the telomeric ends of chromosomes and in the promoters of a wide range of genes important in cell signaling, gained interest as therapeutic targets and several small molecules able to bind and stabilize G4 structures have been developed. However, due to the poor drug-like properties and/or selectivity profile none of the G4 ligands has progressed through the drug discovery pipeline. Our group recently showed that the novel G4 ligand EMICORON exhibited a favourable pharmacological profile and elicited antitumor efficacy against advanced experimental models of colon cancer.

OBJECTIVE. Our aim was to study the ability of EMICORON to increase the efficacy of the standard chemotherapy for human colon cancer.

MATHERIALS AND METHODS*.* We assessed the *in vitro* cytotoxic activity of EMICORON in combination with SN-38, 5-Fluorouracil and Oxaliplatin by clonogenic and 3D tumor-spheroid formation assay. *In vivo* experiments were performed in mice bearing HT29 tumors treated with Irinotecan followed by EMICORON in one or two cycles of administration. Moreover, efficacy of the standard FOLFIRI or FOLFOX was evaluated in two different colon cancer patient-derived xenografts.

RESULTS AND CONCLUSIONS. The exposure of HT29 cells to the combination of EMICORON with chemotherapeutics resulted strongly synergistic when the drugs were administered following the sequence SN-38→EMICORON, EMICORON→5-Fluoruracil or EMICORON→Oxaliplatin. The high activity of SN-38 followed by EMICORON was confirmed in the HT29-formed spheroids. When mice bearing HT29 xenografts were treated with the combination Irinotecan→EMICORON, a tumor weight inhibition of 80% was observed and an increase in overall survival of mice of 85%. Interestingly, the administration of a second cycle of treatment produced an impressive increase of survival of mice to 114%. Then, we tested the efficacy of FOLFOX or FOLFIRI therapeutic regimens on two colon cancer PDXs and as expected these treatments were very effective in reducing the tumor mass, but the relapse of the disease was observed in all the mice. In conclusion, these results provide a compelling argument to suggest that the integration of EMICORON in standard chemotherapeutic regimens could be a highly valuable strategy for the treatment of human colon cancer.

Maml1 acts cooperatively with Gli proteins to regulate Sonic hedgheog signaling pathway

Quaranta R., Pelullo M., Nardozza F., Zema S., Di Marcotullio L., Screpanti I., Bellavia D.

Dept. of Molecular Medicine, Sapienza – University of Rome

OBJECTIVE. Sonic hedgehog (Shh) signaling plays a critical role in development, embryogenesis and tumorigenesis. The effects of this pathway are mediated by Gli transcription factors, which control the expression of many target genes, including Gli1 itself. Shh is essential for proliferation of cerebellar granule cell progenitors (GCPs) and its misregulation has been linked to various disorders, including cerebellar cancer medulloblastoma.
Transcriptional coactivators are proteins that are associated with transcription factors in regulating the expression of specific target genes, therefore they are now recognized as important elements in the mechanisms of signal transduction. An example of transcriptional coactivators is represented by Mastermind-like (MAML) proteins family. MAML1 was originally identified as a foundamental element in Notch signaling and is now emerging as coactivator in other pathways, such as p53 and β-catenin ones. Based on these observations and that Maml1 expression is higher in cerebellum than other human tissues, we sought to examine Maml1 role in the activation of Shh pathway.
MATERIALS AND METHODS. MEFs (Mouse Embryonic Fibroblasts) and GCPs were isolated from wild-type and mutant mice. *In vitro* cell lines treatments and expression of recombinant proteins as well as preparation of whole cell lysates and proteins coimmunoprecipitation were realized. Analysis of gene expression with qPCR, Chromatin Immunoprecipitation and immunofluorescence staining were also essential to achieve our results.
RESULTS. We found that MAML1 physically interacts with Gli proteins, working as a potent transcriptional coactivator. Notably, Maml1 silencing in NIH3T3 and *Patched1*$^{-/-}$ MEFs results in a significant reduction of Gli-target genes expression, also downstream SAG activation, with a negative impact on cell growth. Significantly, Shh pathway activity results severely compromised both in MEFs and GCPs deriving from *Maml1*$^{-/-}$ mice with an effect on GCPs proliferation and cerebellum foliation, suggesting an intrinsic requirement for Maml1 in cerebellum development.
CONCLUSION. Our observations suggest that MAML1 might be the new coactivator of Gli transcription factors and reveal an important role of MAML1 in Shh signaling. This foundings highlight the relevance of understanding the molecular network in cerebellum development in order to identify new potential therapeutic targets for diseases associated to dis-regulation in Shh pathway, such as medulloblastoma.

Targeting PDE4 cAMP phosphodiesterases to control hepatocellular carcinoma growth

[1]Federica Ragusa, [2]Silvia Cardarelli, [1]Francesca Galli, [1]Maria Federica Giardi, [1]Mariacarla Carusi,[1]Benedetta Cinque, [2]Mauro Giorgi and [1]Mara Massimi

[1]Dept. Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila. [2]Dept. Biology and Biotechnology Charles Darwin, Sapienza University of Roma, Rome.

OBJECTIVES: - To examine the potential of specific PDE4 inhibitors (rolipram and GEBR-7b) to selectively inhibit proliferation, survival and invasion of hepatocarcinoma cells, also focusing on the molecular mechanisms involved.
- To uncover PDE4 isoforms specifically modulated in hepatocellular carcinomas of different origin, with the goal of finding new targets for treatment.
MATERIALS AND METHODS: Total PDE activity was measured in three different hepatocarcinoma cell lines (HepG2, Huh7 and Hep3B) and in terminally differentiated HepaRG cells. Cells were then treated with PDE4 specific inhibitors: rolipram (inhibitor of all PDE4 isoforms) and GEBR-7b (inhibitor of the PDE4D isoform, with effect also on PDE4A). Following treatments, inhibition of cell proliferation was evaluated by neutral red and Trypan blue assays, cell cycle and apoptosis by flow cytometry, and cell migration and invasion by wound healing and transwell invasion assays. In addition, the expression of key molecules of cell cycle progression and apoptosis was analysed by Western blotting.
RESULTS: Cyclic AMP PDE activity increased in the more tumorigenic cells (Hep3B and Huh7), with the major portion of the activity from the PDE4 family. PDE4A and PDE4D, in particular, represented the major isoforms in all cell lines examined, with PDE4D4 highly expressed in highly proliferative cells.
Treatment with rolipram or GEBR-7b produced a significant reduction in cell growth, especially in the more tumorigenic cells. Flow cytometry assay showed that HepG2 accumulated in S phase with Hep3B and Huh7 manly in the G2/M phase. Changes in cell cycle progression and cell survival were paralleled by modulation of expression of p53, p21 and p27, as well as of cyclin D1, A and B1.
CONCLUSION: The results indicate that inhibitors of PDE4 are able to slow the proliferative activity of human hepatocellular carcinoma cells, with no effects on terminally differentiated hepatocytes. These inhibitors showed the highest efficacy towards cells characterized by a higher degree of tumour aggressiveness (Huh7 and Hep3B cells), which also expressed higher levels of PDE4A and PDE4D isoforms. PDE4 inhibitors are thus able to selectively inhibit actively growing hepatocarcinoma cells with low levels of toxicity and may be potential candidates as adjuvant chemotherapeutic drugs.

The role of BK channels in the hypoxia-induced aggressiveness of human glioblastoma cells

P. Rosa[a], G. Mangino[a], D. Bastianelli[a], S. Carlomagno[a], F. Franciolini[b], L. Catacuzzeno[b] and A. Calogero[a]

[a]Dept. of Medical-Surgical Sciences and Biotechnologies, University of Rome "Sapienza" -Polo Pontino-, Italy. [b]Dept. of Chemistry, Biology and Biotechnology, University of Perugia, Italy

OBJECTIVE. Glioblastomas (GBMs) are brain tumors of glial origin characterized by a heavy hypoxic microenvironment, which correlates with tumor aggressiveness. GBM cells abundantly express large-conductance, calcium-activated potassium (BK) channels and, since hypoxia modulates their activity in many tissues, the aim of the present work was to explore their role in some aspects of the hypoxia-induced aggressiveness of GBM cells, such as migration, chemoresistance and clonogenic ability.

MATERIALS AND METHODS. U87-MG cells were grown under hypoxia and normoxia and tested for their expression of BK channels currents, mRNA, protein and intracellular Ca^{2+} and ROS concentrations levels. Hypoxia-induced migration was evaluated by wound healing and transwell assays in presence or not of paxilline. Chemoresistance and clonogenic capacity were evaluated by MTS, trypan blue and colony formation assays by treating cells with cisplatin and adding or not paxilline in conditions of normal and low oxygen concentrations. Immunoistochemical analysis for BK channels and HIF-1α was performed on paraffin embedded sections of two GBM patients, compared to normal brain.

RESULTS. Hypoxia induces up-regulation of BK channels activity in U87-MG, without interfering with their expression, likely related with an increase in intracellular Ca^{2+} and ROS concentrations. Wound healing and transwell assays showed that hypoxia increased U87-MG cells migratory ability and that this effect could be prevented by BK channel inhibition. These results were also confirmed in a primary culture derived from a GBM patient. Furthermore, toxicological experiments showed how hypoxia was able to induce chemoresistance to cisplatin in U87-MG cells and that the concomitant inhibition of BK channels had opposite effects, depending if the cells were grown under normal or low oxygen concentrations, with an increase or decrease, respectively, of cell viability and clonogenic capacity. Finally, using immunoistochemical analysis, we highlighted the presence of BK channels in hypoxic areas of human GBM tissues, suggesting that our findings may have physiopathological relevance *in vivo*.

CONCLUSION. BK channels are able to limit several aspects of the aggressive potential of GBM cells induced by hypoxia, such as migration and chemoresistance to cisplatin. All these results point to BK channels as a potential therapeutic target in the treatment of GBM.

ZNF216 and EGF/EGFR mutual regulation in human carcinoma cells.

Savino Luca[1], Di Marcantonio Maria Carmela[1], Tarantelli Chiara[1,4], Sancilio Silvia[2], Ponti Donatella[3], Muraro Raffaella[1], and Mincione Gabriella[1]

[1]Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio Chieti-Pescara, Chieti, Italy
[2]Department of Pharmacy, University G. d'Annunzio Chieti-Pescara, Chieti, Italy
[3]Department of Medico-Surgical Sciences and Biotechnologies, University of Rome Sapienza, Latina, Italy
[4]Current Address: Lymphoma and Genomics Research Program, IOR Institute of Oncology Research, Bellinzona, Switzerland.

OBJECTIVE: Epidermal Growth Factor Receptor (EGFR), a member of the ErbB family of receptor tyrosine kinase (RTK) proteins, is mutated or overexpressed in tumors and plays pivotal roles in cancer onset and metastatic progression. ZNF216 gene has been identified as one of Immediate Early Genes (IEGs) induced by RTKs. Overexpression of ZNF216 protein sensitizes 293 cell line to TNF-α induced apoptosis but on the other hand ZNF216 overexpression has been reported in medulloblastomas and metastatic nasopharyngeal carcinomas. Thus, at present, only few studies addressed the physiopathological role of ZNF216 in tumorigenesis.
MATERIALS AND METHODS: Thus, we first investigated the correlation between ZNF216 and EGFR expression in various cancer cell lines by Real-Time quantitative PCR and by RT-PCR and then we evaluated their functional relationship in a model of NIH3T3 transfected with the ZNF216 and EGFR genes (NIH3T3-EGFR/ZNF216) compared to NIH3T3 transfected with EGFR alone (NIH3T3-EGFR).
RESULTS: Interestingly, our results evidenced an inverse correlation between ZNF216 and EGFR mRNA expressions and proteins in human prostate carcinoma cell lines and in human cancer cell lines characterized and selected for different EGFR expressions. In addition, we demonstrated that: a) ZNF216 significantly increased the levels of phosphorylated EGFR in the cytosolic and nuclear fractions in NIH3T3-EGFR/ZNF216, compared to NIH3T3-EGFR, as determined by Western blot and immunofluorescence; b) EGFR activation increased the expression and nuclear translocation of ZNF216 in a time dependent manner; c) interestingly, EGF treatments induced apoptotic cell death in NIH3T3-EGFR/ZNF216, compared to NIH3T3-EGFR, as determined by intense positivity in TUNEL assay and enhanced PARP cleavage. In addition, EGF treatment induced an overall increased cell size, consistent with a G1 phase block in NIH3T3-EGFR/ZNF216, compared to NIH3T3-EGFR, as detected by flow cytometry.
CONCLUSION: Our results provide some initial clues on the ZNF216 biological function, signalling and interaction with EGFR oncogenic properties. Since detection of nuclear EGFR in tumors was strongly correlated with resistance to therapy and poor prognosis, we suggest an important role for ZNF216 in modulating the oncogenic EGFR biological outcomes.

Cladribine and Clofarabine as novel small molecule inhibitors targeting CD99 in the treatment of Ewing sarcoma

Sciandra M[1,2], Manara MC[1,2], Çelik H[3], Uren A[3], and Scotlandi K[1,2]

[1]CRS Development of Biomolecular Therapies, Experimental Oncology Laboratory, Rizzoli Orthopaedic Institute, 40136 Bologna, Italy
[2] PROMETEO Laboratory, STB, RIT Department, Rizzoli Orthopaedic Institute, 40136 Bologna, Italy
[3] Department of Oncology, Georgetown University Medical Center, Washington, D.C. 20007, USA

Ewing sarcoma (EWS) is a rare and aggressive tumour which affects children and adolescents. The outcome has been improved to 60-70% in localized disease thanks to multimodality therapy. Instead, for patients with metastasis at the onset or which relapse following initial therapeutic regimen the prognosis still remains dismal.
EWS cells express high levels of CD99, a membrane antigen currently used as diagnostic marker for the disease. This molecule has been proposed as an effective target to treat EWS, being easily accessible on cell surface and having a role in the pathogenesis of this tumour.
In the present study, we took advantage of an innovative screening approach based on Surface Plasmon Resonance (BIAcore™) technology. From libraries of chemical compounds we identified small molecules that can directly bind to CD99, isolating two drugs already FDA-approved (Food and Drug Administration) for the management of some hematological malignancies: cladribine and clofarabine.
After drug treatment, EWS cell lines exhibited lower IC_{50} values when compared with non-EWS cells from different histotypes such as osteosarcoma (OS) and rhabdomyosarcoma.
As nucleoside analogues, cladribine and clofarabine induced a cell cycle arrest in S phase both in OS and EWS cells but they selectively triggered cell death in EWS. These findings suggest that the pharmacological effect (cytotoxic or cytostatic) is related to the presence of CD99 as supported by the correlation between protein expression and cladribine and clofarabine sensitivity in cancer cells.
The knockdown of CD99 in EWS models was able to rescue partially drug sensitivity and specifically the phenotype of cell death after drug treatment.
Signalling analysis indicated an effect against ROCK2 which was modulated by cladribine/clofarabine, thus inhibiting migration of EWS cells.
Drug effectiveness in EWS was confirmed also in anchorage-independent conditions and in EWS xenograft mouse model.
These observations shed new light on two small molecule inhibitors, cladribine and clofarabine, already used in other paediatric tumors. Our results demonstrated the specific action of both drugs against CD99 protein, supporting their potential application in EWS treatment and strengthening the therapeutic interest towards CD99 as an appealing target in the management of this cancer.

ZNF521 potentiates the Hedgehog pathway activity by interacting with Gli factors and promoting transactivation of responsive genes.

Scicchitano S*, Lucchino V*, Giordano M°, Montalcini Y*, Zoppoli P*, Spoleti CB*, Chiarella E*, Codispoti B*, Nappo G@, Aloisio A*, Marafioti MG*, Mesuraca M*, De Smaele E^, Bond HM*, Morrone G.*

** Dept. of Experimental and Clinical Medicine, University Magna Græcia, Catanzaro, Italy.*
° Molecular Medicine Program, European Institute of Oncology, Milano, Italy.
^ Dept. of Experimental Medicine, University La Sapienza, Rome, Italy
@ YCR Cancer Research Unit - Department of Biology, University of York, United Kingdom.

BACKGROUND AND OBJECTIVE:
ZNF521 is a large multifunctional transcription co-factor implicated in the control of the stem cell compartment in the haematopoietic, osteo-adipogenic and neural system. Our previous studies have shown that: ZNF521 expression is abundant in the cerebellum, and particularly in the granule neuron progenitors, considered candidate cells-of-origin of medulloblastomas (MBs); particularly high levels of ZNF521 are detected in an MB subgroup characterised by constitutive activation of the Hedgehog (HH) pathway; ZNF521 strongly enhances the growth, clonogenicity, motility and tumourigenicity of human and mouse medulloblastoma cells. Based on these data, we have sought to gain further insight into the mechanism of action for ZNF521 with particular regard to its potential co-operation with the HH pathway.
MATERIALS AND METHODS: The cross-talk between ZNF521 and HH signalling was investigated by coimmunoprecipitation experiments, by Chromatin immunoprecipitation assays, by assessing the co-operation between ZNF521 and HH pathway effectors in the transcriptional activation of HH-dependent promoters, as well as in the induction of the expression of cellular HH target genes in response to enforced expression of ZNF521.
RESULTS:- ZNF521 co-immunoprecipitates specifically with both GLI1 and GLI2 transcription factors, the main mediators of HH signalling;
- in the presence of GLI factors, ZNF521 localises to GLI-binding sites in the promoters of HH-responsive genes;
- ZNF521 strongly potentiates the of GLI1 and GLI2-induced transcription of reporter constructs containing GLI-binding sequences derived from HH responsive promoters;
- this effect requires the integrity of an N-terminal motif of ZNF521 that recruits the nucleosome remodelling and histone deacetylase (NuRD) complex; it is abrogated by histone deacetylase inhibitors and HH inhibitors;
- enforced expression of ZNF521 in NIH3T3 cells and DAOY medulloblastoma cells enhances the expression of endogenous HH target genes – including GLI1 and PTCH1
- both in basal conditions and in response to the HH pathway agonist, SAG.
CONCLUSIONS: These results identify ZNF521 as a potent HH agonist, whose mechanism of action depends on its interaction with the NuRD complex. This cross-talk may be of considerable relevance in several tumours where HH contributes to control the homeostasis of the stem cell compartment and ZNF521 is highly expressed.

Single nucleotide polymorphisms associated with gastrointestinal symptoms in Fabry disease

Francesca Scionti[1], Simona Sestito[2], Angela Nicoletti[2], Mariamena Arbitrio[3], Pietro Hiram Guzzi[4], Valentina Talarico[2], Federica Altomare[2], Maria Teresa Sanseviero[2], Antonio Pisani[5], Eleonora Riccio[5], Daniela Concolino[2], Licia Pensabene[2] and Maria Teresa Di Martino[1]

[1]Department of Experimental and Clinical Medicine, Magna Graecia University, Salvatore Venuta University Campus, Catanzaro, 88100 Italy;[2]Department of Medical and Surgical Sciences, Pediatric Unit, Magna Graecia University, Catanzaro, 88100 Italy;[3]ISN-CNR, Roccelletta di Borgia, Catanzaro, Italy;[4]Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, 88100 Italy;[5]Departement of Nephrology University Federico II, Naples, 80138 Italy

OBJECTIVE: Fabry disease (FD) is a rare X-linked lipid storage disorder caused by mutations in the *GLA* gene, encoding the lysosomal enzyme α-galactosidase A (α-GAL A). Deficient activity of α-GAL A leads to a progressive accumulation of neutral glycosphingolipids, predominantly globotriaosylceramide (Gb3), in the vascular endothelium of skin, kidney, nervous system, heart and other tissue with consequent multiorgan dysfunction. Gastrointestinal symptoms (GIS) are reported in approximately 60% of FD patients and show a high degree of inter-individual and intra-familial variability in patients carrying the same mutation. The aim of this study was to explore the association between genetic polymorphisms in drug absorbtion, distribution, metabolism and excretion (ADME) related genes and susceptibility to GIS in FD.
MATERIALS AND METHODS: Genomic DNA of 49 FD patients was extracted from peripheral blood and genotyped for 1936 genetic variants across 231 genes that encode for drug-metabolizing enzymes and drug transport proteins using the DMET Plus platform. Genotypes were calculated by DMET Console software version 1.1 using the Dynamic Genotype Boundaries algorithm. Patients with a call rate less than 95% were excluded from further analysis. Genotype frequencies were analyzed by DMET-Analyzer software using two-tailed Fisher exact test. Results of potential interest were limited to those in which the p-value was ≤0.05. Results were validated using pre-designed TaqMan Genotyping assays.
RESULTS: Nine single nucleotide polymorphisms (SNPs), mapped within four genes (*ABCB11*, *SLCO1B1*, *NR1I3* and *ABCC5*), involved in the enterohepatic circulation of bile acids, showed statistically significant differences in genotype frequencies between FD patients who experienced GIS (N=12) and patients without GIS (N=37).
CONCLUSION: In this study we examined for the first time the relationships between genetic heterogeneity in ADME-related genes and GIS in FD. Our findings provide a potential novel genetic variant framework which warrants further investigation for precision medicine in FD.

CARMA2*sh* and its psoriasis-linked variants regulate inflammatory pathways in human keratinocytes

Ivan Scudiero, Pellegrino Mazzone, Gianluca Telesio, Maddalena Pizzulo, Pasquale Vito

Biogem, Ariano Irpino (AV) Italy

OBJECTIVE: The molecular complexes formed by specific members of the family of CARMA proteins, the CARD domain-containing adapter molecule BCL10, and MALT1 represent a central hub in regulating activation of the pleiotropic transcription factors NF-κB. Recently, dominant germline mutations in CARMA2*sh* have been shown to cause with high penetrancy psoriasis and pityriasis rubra pilaris, two related skin inflammatory disorders. Aim of our work was to dissect the molecular mechanisms by which CARMA2*sh* and its variants associated with psoriasis control NF-κB activity in the skin.
MATERIALS AND METHODS: Molecular partners of CARMA2*sh* were searched by a yeast two-hybrid screening and tested by co-immunoprecipitation in HEK293T cells. Immunoblot analysis, in vitro kinase and phosphatase assays were used to monitor the phosphorylation status of CARMA2*sh*. Deletion mutants of CARMA2*sh* served to map the phosporylation region and its functional significance. Murine strains expressing psoriasis-linked mutants of CARMA2*sh* in their keratinocytes were generated via homologous recombination in the Rosa26 locus. NF-κB activation signaling and the expression of inflammatory genes were investigated by biochemical and genetic assays in human and murine keratinocytes. A BCL10 inhibitory peptide was delivered by liposome in NHEK cells exposed to heat-killed bacteria or fungi and NF-κB target genes expression levels were assessed by Real Time RT-PCR.
RESULTS: 1) We identified a serine/threonine kinase, termed CIK (CARMA2*sh* Inhibitory Kinase), which binds to and phosphorylates CARMA2*sh*. Phosphorylation of CARMA2*sh* is required to promote lysosomal degradation of BCL10 and to turn off CARMA2*sh*-mediated NF-κB signaling. 2) We demonstrate that CARMA2*sh* mutants associated with psoriasis escape CIK inhibition. 3) We show that in human keratinocytes CARMA2*sh* plays an essential role in the signal transduction pathway connecting Pathogen Associated Molecular Patterns recognition to NF-κB activation. 4) A peptide blocking CARD-mediated BCL10 interactions reduces the capacity of psoriasis-linked CARMA2*sh* mutants to activate NF-κB.
CONCLUSIONS: Our work elucidates a basic signaling mechanism operating in human keratinocytes and opens to novel potential tools for the therapeutical treatment of human skin disorders.

Non-canonical Hedgehog/AMPK-mediated control of polyamine metabolism is necessary for medulloblastoma growth

D'Amico D., Sdruscia G., Antonucci L., Di Magno L., Coni S., Serrao S.M. and Canettieri G.

IIIT@Sapienza - Department of Molecular Medicine, La Sapienza University of Rome

INTRODUCTION: Developmental Hedgehog signaling controls proliferation of cerebellar granule cell precursors (GCPs) and its aberrant activation is a leading cause of Medulloblastoma (SHH-MB, Hedgehog molecular subgroup). Treatment of SHH-MB patients with the FDA-approved Hh inhibitor Vismodegib (which targets the transducer Smoothened) has been disappointing because of the occurrence of resistance, attributed to Smo inactivating mutations or to activation of downstream effectors. Thus, it is now believed that the identification and targeting of novel downstream components represents a preferable option for this disease.
MATERIAL AND METHODS: We used biochemical, cell biology and molecular biology approaches to unmask and dissect the molecular features of a novel mechanism involved in polyamine production upon Hh activation. The biological relevance of our findings has been evaluated in mice and patients with SHH MB and other subgroups.
RESULTS: We show here that Hedgehog promotes polyamine biosynthesis in GCPs by engaging a non-canonical axis leading to ODC translation. This process is regulated by AMPK, which phosphorylates threonine 173 of CNBP in response to Hedgehog activation. Phosphorylated CNBP increases its association with Sufu, followed by CNBP stabilization, ODC translation and polyamine biosynthesis. Notably, CNBP, ODC and polyamines are hallmarks Hedgehog-dependent Medulloblastoma (SHH-MB) and genetic or pharmacological inhibition of this axis efficiently blocks Hedgehog-dependent proliferation of Medulloblastoma cells in preclinical settings.
CONCLUSIONS: Together, these data illustrate a novel auxiliary mechanism of metabolic control by a morphogenic pathway with relevant implications in cancer.

Two simultaneous and very uncommon *PI3KCA* mutations in a liver metastasis from a colorectal cancer patient with aggressive and resistant disease.

Tessitore, Alessandra[1]; Bruera, Gemma[1,2]; Mastroiaco, Valentina[1]; Cannita, Katia[2]; Cortellini, Alessio[1,2]; Dalmas, Antonella[3]; Zazzeroni, Francesca[1]; Ficorella, Corrado[1,2]; Ricevuto, Enrico[1,2]; Alesse, Edoardo[1].

[1]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, [2]Medical Oncology, S. Salvatore Hospital, L'Aquila, [3] Pathology Unit, S. Salvatore Hospital, L'Aquila.

INTRODUCTION. Colorectal cancer (CRC) is a widespread tumor. *KRAS*/*NRAS* genotype drives metastatic CRC treatment with targeted therapies. The prognostic and predictive significance of other genes (*PIK3CA*, *BRAF)* is under investigation. Here, we describe a CRC patient carrying a *KRAS* and two very uncommon *PIK3CA* mutations in liver metastasis.
MATERIAL AND METHOD. Primary colorectal and metastatic liver tumor samples were collected from an early-onset patient with synchronous metastases, treated with first-line intensive triplet chemotherapy plus bevacizumab (FIr-B/Fox). *KRAS*/*NRAS* (exons 2, 3, 4), *BRAF* (exon 15), *PI3KCA* (exons 9, 20) were analyzed by direct sequencing. PTEN, Akt and ^{S473}p-AKT were examined by immunohistochemistry.
RESULTS. The *KRAS* exon 2 c.34G>A, G12S mutation was detected in primary tumor and liver metastasis. Two *PIK3CA* exon 9 mutations onto the same allele (c.1633G>C, E545Q; c.1645G>C, D549H) were detected only in the latter sample. Interestingly, the c.1633G>C mutation was reported with very low frequency in databases; on the contrary, the c.1645G>C mutation was never described in CRC, although it was reported in just two samples from hepatocellular and cervical carcinoma. Immunohistochemistry analysis revealed differences in Akt and p-AKT expression between primary and metastatic samples. Patient showed aggressive and resistant disease (7 months PFS, 15 months OS).
CONCLUSIONS. A *KRAS* and two rare *PI3KCA* mutations, whose one never reported in CRC, are described in liver metastasis from a patient with very aggressive disease. Differences were detected in Akt and p-Akt expression by comparing primary tumor/metastasis, leaving hypothesize a functional role of these rare PI3KCA mutations in cancer aggressiveness.

Co-development of DTP3 and its biomarkers to therapeutically target the NF-kB survival pathway in multiple myeloma

Laura Tornatore, Daria Capece, Verzella Daniela, Gary Acton, Elizabeth A. Campbell, James Kelly, Michael Tarbit, Nigel Adams, Selina Bannoo, Federica Begalli, Jason Bennet, Daniel D'Andrea, Annamaria Sandomenico, Antonio Leonardi, Menotti Ruvo, Magda J. Al-Obaidi, Reuben Benjamin, Richard S. Kaczmarski, Holger Auner, Jane Apperley, and Guido Franzoso

Imperial College London, UK

OBJECTIVE: Pathologic NF-κB signaling promotes survival in multiple myeloma (MM) and other cancers, yet current NF-κB-targeting strategies lack cancer-cell specificity. Consequently, there is a need for more effective therapeutic approaches.
MATERIALS AND METHODS: Through a drug discovery approach, we developed DTP3, a D-tripeptide, which disrupts the Gadd45β/MKK7 interaction, which is an essential survival module downstream NF-κB pathway, and a novel attractive therapeutic target in MM. Pharmacodynamics (PD), safety pharmacology, pharmacokinetic (PK), and toxicology studies have been performed. A companion biomarker programme has also been developed in order to inform patient stratification, demonstrate pathway-specific PD activity and proof of mechanism.
RESULTS: DTP3 selectvely kills myeloma cells and totally lacks toxicity in heathy cells. DTP3 ablates myeloma xenografts in mice. The preclinical package demonstrates that DTP3 combines therapeutic efficacy with selective pharmacology and favourable PK/TK profile, thus supporting progression into clinical development. The companion biomarker programme demonstrated cancer-selective pharmacodynamic response.
CONCLUSION: Collectively, our preclinical and clinical data strongly suggest that cancer-selective targeting of the NF-κB pathway is possible and, at least for myeloma patients, promises to provide profound benefit.

Notch3 gene expression in T-ALL is mediated by a mutually exclusive recruitment of Notch1 and EZH2 on its intron1

Luca Tottone, Rocco Palermo, Michele Zampieri, Fabrizio Simeoni, Claudio Talora, Isabella Screpanti

Molecular Medicine Department, Sapienza - University of Rome, Italy

Aberrant Notch activation plays a prominent role in T-cell acute lymphoblastic leukaemia (T-ALL) oncogenesis. Somatic Notch1 activating mutations have been identified in more than 50% of all T-ALL cases analyzed, and Notch3 is among the highest expressed genes in T-ALL. Although evidences indicate Notch3 as a transcriptional Notch1 target gene, the mechanism of action is not fully elucidated. In this regard, ChIPon-chip assays against Notch1 failed when addressing Notch3 proximal promoter, but revealed Notch1 bound to the intron1, thus suggesting a potential regulatory function for this region. Recently, it has been shown a transcriptional regulatory antagonism between Notch1 and the DNA methyl-transferase Enhancer of Zeste Homolog 2 (EZH2) in the occupancy of the regulatory regions of several Notch1 target genes.
In our hand, by gene expression and ChIP analysis on human T-ALL cell lines, we unveiled a strict correlation between Notch1 ectopic iper-activation, increased transcription of Notch3, and Notch1 recruitment on Notch3 intron1. Conversely, Notch blockage by specific Notch1-blocking antibodies, or by gamma secreatase inhibitors (GSI), resulted in the opposite effects. Consistently, GSI-washout assays allowed Notch3 gene expression recovery and Notch1 occupancy on Notch3 intron1. In addition, we observed a mutual exclusive binding activity between Notch1 and EZH2 on the Notch3 regulating regions, combined with lysine-27 tri-methylation of histone 3 (H3K27me3) status changing.
Overall, our findings demonstrate that Notch1 regulates Notch3 gene transcription by modulating the intron1 mark H3K27me3 of Notch3, via antagonizing EZH2 binding on this regulatory region. These results not only unveil a novel non-canonic and intra-genic system of gene transcription regulation, but candidate epigenetic modulation as an effective strategy in Notch pathway inhibition.

NF-κB regulates Gli1 expression in Human PCa

Davide Vecchiotti[1], Daniela Verzella[1], Daria Capece[1], Mariafausta Fischietti[1], Barbara Di Francesco[1], Mauro Di Vito Nolfi[1], Stefania Meschini[2], Alessandra Tessitore[1], Edoardo Alesse[1], Francesca Zazzeroni[1]

[1]*Dipartimento di Scienze Cliniche Applicate e Biotecnologiche, Università degli Studi di L'Aquila*
[2]*Dipartimento Tecnologie e Salute, Istituto Superiore di Sanità, Roma*

OBJECTIVE. Prostate cancer (PCa) is the most commonly diagnosed cancer in men. Common genomic alterations in PCa involve the androgen receptor and PI3K pathways, rearrangements of the ETS transcription factors, and loss of function of the prostate tumor suppressor NKX3.1. In addition, constitutive activation of both NF-κB transcription factor and Hedgehog (Hh) pathway have been suggested to play a role during the development and progression of PCa. This project aimed at investigate if a cross-talk between NF-κB and Hh pathways exists in PCa.
MATERIALS AND METHODS. PCa cell lines (PC-3, Du145, LnCaP, 22Rv1) and BPH-1 were cultured in standard conditions. RelA silencing was performed by lentiviral infection of pLentiLox3.7-human *RelA shRNA.* NF-κB-p65 (NeoMarker), Shh and Gli1 (Santa Cruz Biotech) IHC was performed on PCa-Normal Tissue Array (CA3; SuperBioChips Tissue). Immunofluorescence, Western blots, Q-PCRs (Thermo), MTS assay (Promega), NF-κB DNA-binding assay (TransAM™ActiveMotif) were performed following standard protocols. GraphPad Prism software was used for statistical analysis.
RESULTS. Strong correlation between NF-κB activation (high expression levels; nuclear localization), Shh and Gli1 expression was observed.
Hyperactivation of both NF-κB and SHh-Gli1 pathways were shown in androgen-independent PC-3 and Du145 cell lines, which correlate with the higher proliferative rate capacity of these cell lines respect to androgen-dependent cell lines. Knockdown of RelA determined a significant decrease in Gli1 level but had no effects on SHh expression. As expected, a significant reduction of RelA knockdown cell lines proliferative capacity was observed.
CONCLUSIONS. We demonstrated a close relationship between Gli1 and NF-κB in human PCa. Knockdown of NF-κB subunits resulted in decreased GLI1 expression, indicating that NF-κB is a regulator of Gli1 expression. These data could be relevant both in diagnosis and prognosis of PCa, because the combination of IHC for p65 and Gli1 expression may predict the progression through advanced stage of PCa and could help clinicians to evaluate the prognosis of the disease in view of the fact that higher levels of p65 and Gli1 matches with higher gleason score and stage. Further experiments are needed to demonstrate whether NF-κB regulates Gli1 at transcriptional or post-transcriptional level and if Gli1 could be the target for new drugs useful for treatment of PCa patients with constitutive activation of NF-κB.

Coupling of angiogenesis and bone remodeling under mechanical unloading: Mechanistic insights

Vimal Veeriah, Mattia Capulli, Angelo Zanniti, Nadia Rucci, Anna Teti.

Bone Biopathology Lab, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy.

Molecular mechanisms underlying coupling of angiogenesis and bone remodeling in the skeletal biomechanical response is uncertain. To identify a novel molecular cascade involved in the mechanoresponsive crosstalk between angiogenesis and osteogenesis, we subjected mouse and human endothelial cells (EC) to simulated microgravity in the NASA-developed RWV bioreactor, which minimizes shear stress and mimics unloading. We observed a microgravity intensity-dependent increase of IL1β release in EC conditioned medium (0.08*g* 12pg/ml; 0.008*g* 36pg/ml,p<0.001), which stimulated LCN2 expression in mouse and human osteogenic cells (12.7fold,p<0.001). This induction impaired osteoblast differentiation (-52% ALP p=0.024,-66% mineralization, p=0.010) in wildtype but not in LCN2-deleted osteogenic cells, and was definitely IL1β-dependent as suggested by the blunted effected of IL1β-depleted microgravity EC conditioned medium. Simulated microgravity activated the IL1β upstream transcription factor NF-κB in EC, and IL1β prompted NF-κB activation and the NOS2/NO/COX2 pathway in osteogenic cells, in which they stimulated proliferation (1.7fold,p=0.003) and cyclin d1 expression (5.3fold,p=0.002) in a manner blunted by IL1β depletion. LCN2 was also released in the conditioned medium of osteogenic cells directly subjected to microgravity (6fold,p<0.001) and contributed to the inverse mechanoresponsive EC-osteoblast crosstalk, stimulating EC proliferation (1.5fold,p=0.002) migration (2fold,p=0.027), tube formation (2.3fold,p<0.008) *in-vitro*, sprouting from mouse aortic rings (4fold,p<0.001) *ex-vivo* and new blood vessels formation (3.5fold,p=0.009) in CAM (chick chorioallantoic membrane) assay *in-vivo.* These events were mediated in EC by LCN2-induced VEGF signaling (mRNA fold increase: VEGF, 6.2, p=0.001; Hif1α 5.4, p=0.02), and were dampened by incubating EC in conditioned medium from LCN2-deleted osteogenic cells subjected to microgravity, or in the presence of recombinant LCN2 along with the VEGF receptor antagonist, Avastin. Importantly, the IL1β/NOS2/LCN2 cascade was induced *ex-vivo*, in calvarias cultured in the presence of microgravity EC conditioned medium, and *in vivo*, in calvarias and tibias injected with microgravity EC conditioned medium, or in tibias from mice subjected to tail suspension or treated with botulin toxin to cause hind limb disuse, in which the VEGF mRNA was also upregulated (+10.6fold, p<0.001). Our results suggest a pathogenic relevance of the IL1β/NOS2/LCN2/VEGF cascade in bone biomechanical failures and disuse osteoporosis.

Structure Based Lead Optimization Approach in Discovery of Novel 5-Lipoxygenase Inhibitors and Cytotoxic Activity as New Anticancer Drugs on Human Glioblastoma Cancer Cell Lines

Silvia Zappavigna[a], Antonella Peduto[b], Alessia Cossu[a], Chiara Schiraldi[b], Michele Caraglia[a], Rosanna Filosa[b]

[a]Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, via Costantinopoli 16, Naples, 80138, Italy.
[b]Department of Experimental Medicine, University of Naples, Via Costantinopoli, 16, 80138 Naples, Italy.

OBJECTIVE: 5-lipoxygenase (5-LO) is a key enzyme in the synthesis of leukotrienes (LTs) that are involved in the carcinogenesis. We investigated 5-LO expression and examined whether the 5-LO pathway is associated with the proliferation of human glioma tumors.
MATERIALS AND METHODS: Several benzoquinones have been found effective in treating some forms of cancer; in particular it has been shown that these compounds act on cells by regulating numerous mechanisms, such as apoptosis, cell cycle, production of reactive oxygen species (ROS).[1-2]
We perform a sophisticated medicinal chemistry lead generation and optimization to improve efficacy of our lead compounds. We therefore evaluated the effects of the new compounds on apoptosis, cell cycle and autophagy in order to define the molecular mechanism underlying growth inhibition. Moreover, human telomere G-quadruplex DNA was investigated as a molecular target for EA 100c using UV–vis, CD, fluorescence, NMR, melting temperature.
RESULTS: We demonstrated that EA100c Red induced apoptosis mediated by the reticulum stress associated with autophagy and modulation of the cell cycle on LN-229 but not on the U87-MG. The reticle stress led to higher levels of CHOP, via activation of NF-kB and JNK that ended with the induction of the caspase cascade.
CONCLUSION: Taken together, our results indicate that the treatment of LN-229 cancer cells with **EA100c Red** can be useful in inhibiting in vitro cancer cell growth, representing a promising lead compound for designing a new class of anti-cancer treatment.

MiR-125b interferes with proliferation and induces cellular senescence in *in vitro* models of Multiple Myeloma: an integrative analysis of signaling pathways and molecular mediators.

MR Zarone[1]; G Misso[1]; A Grimaldi[1]; M Russo[1]; A Galeone[2]; M Caraglia[1]

[1]Department of Biochemistry, Biophysics and General Pathology, Second University of Naples, via Costantinopoli 16, 80138, Naples, Italy.
[2]Department of Pharmacy, University of Naples Federico II, via Montesano 49, 80131, Naples, Italy.

OBJECTIVE: MicroRNAs (miRNAs) are short non-coding RNAs that regulate gene expression at post-transcriptional level. Several miRNAs have been found deregulated in Multiple Myeloma (MM) cells; among these not much is known about the function of miR-125b. Here, we have evaluated the effect of miR-125b re-expression in MM cell lines in order to study the biochemical effects, as well as to attribute a possible oncogenic or tumor suppressor role. Furthermore we have developed a series of chemical modifications aimed at both improve the resistance to nucleases and at increase the stability and binding specificity of the mRNA/miRNA duplex.
MATERIALS AND METHODS: U266, RPMI-8226, KMS-12, OPM2 and SKMM-1 MM cell lines were grown in RPMI-1640 medium. MiR-125b levels were quantified by Real-time PCR Viia7. The 2'-OMet,LNA and 2'-F oligonucleotides were synthesized by a Millipore Cyclone Plus DNA synthesizer. The MM cell lines were electroporated with miR-125b and its modified analogs using Neon Trasfection System. Cell viability assay was performed using Cellometer® Auto 1000 after Trypan blue staining. The evaluation of microRNAs targeting sites by Western Blotting analysis was performed in U266 cells and the signal was detected using ChemiDoc XRS$^+$ imaging system. Cellular senescence was evaluated by Colorimetric Detection of Senescence-associated β-Galactosidase activity at pH 6.0 and by the analysis of cell cycle negative regulators' expression levels.
RESULTS: We have determined the anti-proliferative *in vitro* effects of synthetic miR-125b and its modified analogs, correlating it with the modulation of several targets responsible for the regulation of multiple intracellular signaling involved in proliferative processes. Our data have shown that the anti-proliferative effects detected following miR-125b transfection may be correlated with baseline miRNA levels in MM cells. Furthermore, we found that miR-125b replacement, more largely using its O-met analog, can induce senescence in U266 cells.
CONCLUSION: Ectopic expression of miR-125b and its modified analogs induces an anti-proliferative effect and increases the expression of the main cell cycle negative regulators correlated with the triggering of senescence. This study provide the rationale for the development of a new therapeutic strategy based on miRNA-therapy aimed at improve MM patients outcome.

The loss of ATP2C1 impairs the DNA damage response and induces altered skin homeostasis: Consequences for epidermal biology in Hailey-Hailey disease.

Zonfrilli A, Cialfi S, Le Pera L, De Blasio C, Mariano G, Palermo R, Uccelletti D, Palleschi C, Biolcati G, Barbieri L, Screpanti I, Talora C.

Sapienza University of Rome. Dept. Molecular Medicine

OBJECTIVE: Hailey-Hailey disease is a rare genetic disease caused by deregulation in ATP2C1 genes. However, how ATP2C1 loss of function impact on the disease manifestation is completely unknown. In this context, the overall objective of our work was to understand the molecular mechanisms underlying HHD in order to identify therapeutic targets and exploring novel therapeutic intervention strategies.
MATERIALS AND METHODS: We performed whole-genome expression profiling using RNA-seq technology from lesioned and unaffected skin of three individuals with HHD to identify the differentially expressed genes. Additionally, we performed whole-exome sequencing of two lesion-derived keratinocytes using human all-exon targeted capture (Agilent) followed by massively parallel sequencing (Illumina) to address the mutation burden in HHD-lesions.
RESULTS: Here, we identified ATP2C1 as a crucial regulator of epidermal homeostasis through the regulation of oxidative stress. Upon ATP2C1 inactivation, oxidative stress and Notch1 activation were increased in cultured human keratinocytes. Using RNA-seq experiments, we found that the DNA damage response (DDR) was consistently down-regulated in keratinocytes derived from the lesions of patients with Hailey-Hailey disease. Although oxidative stress activates the DDR, ATP2C1 inactivation down-regulates DDR gene expression. We showed that the DDR response was a major target of oxidative stress-induced Notch1 activation. Here, we show that this activation is functionally important because early Notch1 activation in keratinocytes induces keratinocyte differentiation and represses the DDR.
CONCLUSION: Our results indicate that an ATP2C1/NOTCH1 axis might be critical for keratinocyte function and cutaneous homeostasis, suggesting a plausible model for the pathological features of Hailey-Hailey disease.

Measuring mitochondrial function and glycolysis using the Seahorse XF analyzers

Emma Dicapua (Agilent technologies)

Mitochondrial function and glycolysis play critical roles in a variety of vital cellular processes, including cellular activation, proliferation, differentiation, cell death, and disease progression. Seahorse Bioscience has developed a technology that enables the measurement of various metabolic parameters and functions using live cells, in real-time, in a microplate. Seahorse Analyzers profile cellular metabolic functions, using label-free, solid-state disposable optical sensors. The Seahorse Analyzers simultaneously measure mitochondrial respiration (oxidative phosphorylation; OXPHOS) via the oxygen consumption rate (OCR), and glycolysis via the extracellular acidification rate (ECAR). Integrated drug injection ports allow for up to 4 reagent additions (e.g. drug or substrate) that can be programmed for automated delivery into the independent cell culture wells. Assay kits and reagents provide standard methods for quantifying mitochondrial respiration, glycolytic activity, endogenous and exogenous fatty acid oxidation, substrate oxidation, and metabolic phenotype. Seahorse XF technology has been applied to multiple research areas, including cancer, obesity, diabetes, metabolic disorders, immunology, cardiovascular function, neurodegeneration, virology, and aging.

M&M Services: **Easily improving data with flow cytometry at CLNS/IIT**

Giovanna Peruzzi (Istituto Italiano di Tecnologia, Rome)

The Flow Cytometry laboratory at CLNS-IIT@Sapienza is equipped with laser-based instruments whose intended use is to characterize and isolate a variety of viable cell populations from different speciemen mammalian (human and mouse) or other origin. The instrument technology allows simultaneous multiparametric analysis of thousands of cells per second for rapid analysis of complex cell populations and is able to describe in detail a complex sample or to identify low frequency subset of cells. This is achieved by the use of different fluorochrome labeled antibodies or dyes to target specific molecules expressed at the surface or in the cytoplasm of the cell.
Through these instruments, a variety of fluorochromes conjugated to different antibodies can identify the molecular features of cells (flow cytometer) and permit to specifically select cells for isolation (cell sorter).
In details, the instruments in the laboratory are the BD LSRFortessa cell analyzer, a 20 parameter flow cytometer, and the the BD FACSAriaIII, a cell sorter able to measure 10 fluorescence and sort up to 4 different populations, both running a BD FACSDiva software on XP.
The convention recently started with M&M Services is aimed at offering a complete FACS Facility service to all those labs where cytometry is not commonly used.

LIST OF AUTHORS

LIST OF AUTHORS

Abate T	P40	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Abiusi E	P43	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Abonante S	P41	*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria*
Aceto GM	P28, P32, P50	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Acton G	P68	*Imperial College London*
Adams N	P68	*Imperial College London*
Alecci M	P4	*Department of Life, Health and Environmental Sciences, University of L'Aquila,*
Alesse E	P22, P24, P48, P67	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Alfonsi R	P2	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Allegretti M	P4	*Dompé Farmaceutici SpA*
Al-Obaidi MJ	P68	*Imperial College London*
Aloisio A	P63	*Department of Experimental and Clinical Medicine, University Magna Græcia*
Altomare F	P64	*Department of Medical and Surgical Sciences, Pediatric Unit, Magna Graecia University*
Amerio P	P17	*Department of Neurosciences, Imaging and Clinical Sciences, University "G. d'Annunzio"*
Amicucci G	P8	*University of L'Aquila*
Amodio N	P3	*UMG of Catanzaro*
Amoreo CA	P57	*Regina Elena National Cancer Institute*
Angelucci A	P9, P10, P56	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Angrisani A	P21, P39	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Antinozzi C	P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Antonini A	P53	*Department of Dermatology, University of L'Aquila, L'Aquila*
Antonosante A	P23	*Department of Life, Health and Environmental Sciences, University of L'Aquila,*
Antonucci L	P66	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Apperley J	P68	*Imperial College London*
Arbitrio M	P64	*ISN-CNR, Roccelletta di Borgia*
Arjai R	P12	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Artuso S	P57	*Regina Elena National Cancer Institute*
Auner H	P68	*Imperial College London*
Auriemma M	P17	*Department of Neurosciences, Imaging and Clinical Sciences, University "G. d'Annunzio"*
Baldini I	P12	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Bannoo S	P68	*Imperial College London*
Barbieri L	P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Barboni B	P27	*University of Teramo, Faculty of Veterinary Medicine*
Baricordi C	P51	*CRS Development of Biomolecular Therapies, Istituto Ortopedico Rizzoli; Experimental Oncology Laboratory, Istituto Ortopedico Rizzoli*
Basciani S	P21	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Bastianelli D	P60	*Department of Medical-Surgical Sciences and Biotechnologies, University of Rome Sapienza*
Batista P	P50	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Begalli F	P68	*Imperial College London*
Bellavia D	P29, P35, P55, P58	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Bellenghi M	P1	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Benedetti E	P4, P23	*Department of Life, Health and Environmental Sciences, University of L'Aquila,*
Benjamin R	P68	*Imperial College London*
Bennet J	P68	*Imperial College London*
Berardicurti O	P24	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Berardinelli P	P27	*University of Teramo, Faculty of Veterinary Medicine*

LIST OF AUTHORS

Bernardi F	**P2**	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Bertini E	P43	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Biamonte L	P3	*UMG of Catanzaro*
Bianco A	P57	*University "La Sapienza"*
Biffo S	P47	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Biganzoli EM	P40	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Biolcati G	P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Biordi LA	P34	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Biroccio A	P57	*Regina Elena National Cancer Institute*
Boe A	P52	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Bologna M	P9	*Department of Life, Health and Environmental Sciences, University of L'Aquila,*
Bond HM	P63	*Department of Experimental and Clinical Medicine, University Magna Græcia*
Botta C	P18	*UMG of Catanzaro*
Botta M	P10	*Dipartimento Biotecnologie, Chimica e Farmacia, University of Siena*
Bottero L	P1	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Brandi J	P6	*Department of Biotechnology, Proteomics and Mass Spectrometry Laboratory, University of Verona*
Brandolini L	P4	*Dompé Farmaceutici SpA*
Bruera G	P5, P25, P67	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Bruni A	P8	*University of L'Aquila*
Buccieri E	P25	*Institute of Genomic Medicine, "A. Gemelli ", Catholic University*
Bufalieri F	P2	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Burbidge S	P45	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Butera G	P6, P7, P15	*Department of Neuroscience, Biomedicine and Movement, Biochemistry Section, University of Verona*
Butturini E	P15	*Department of Neuroscience, Biomedicine and Movement, Biochemistry Section, University of Verona*
Buzza A	P6	*Department of Sciences and Technological Innovation, University of Piemonte Orientale*
Caianiello P	P22	*Department of Computer Engineering and Science, and Mathematics, University of L'Aquila,*
Calgani A	P8, P9, P10, P56	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Calicchia M	P25	*Institute of Genomic Medicine, "A. Gemelli ", Catholic University*
Calogero A	P60	*Department of Medical-Surgical Sciences and Biotechnologies, University of Rome,La Sapienza*
Calvisi G	P5	*Pathology, S. Salvatore Hospital, ASL1 Abruzzo*
Cama A	P32	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Camero S	P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Campbell EA	P68	*Imperial College London*
Campese AF	P38	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Candria S	P25	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Canettieri G	P66	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Cannita K	P67	*Medical Oncology, S. Salvatore Hospital*
Capasso R	P40	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Capece D	P68	*Imperial College London*
Cappabianca L	P36	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Cappariello A	P11, P12, P42	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Capulli M	P12, P71, P49, P56	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Caracciolo D	P3, P18	*UMG of Catanzaro*

LIST OF AUTHORS

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LIST OF AUTHORS

Lospinoso Severini L	**P43**	***Department Of Molecular Medicine, University of Roma "La Sapienza"***
Lucchino V	P63	*Department of Experimental and Clinical Medicine, University Magna Græcia*
Luce A	P44	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Lusa S	P44	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Mackay AR	P36	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Maggio R	P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Maggiolini M	P41	*Department of Pharmacy, Health and Nutritional Sciences, University of Calabria*
Malapelle U	P5	*Department of Public Health, University Federico II*
Manara MC	P62	*PROMETEO Laboratory, STB, RIT Department, Rizzoli Orthopaedic Institute*
Mancini A	P34, P37, P45	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Mancini G	P54	*CNR – Istituto di Metodologie Chimiche*
Manfredi M	P6, P47	*Department of Sciences and Technological Innovation, University of Piemonte Orientale*
Mangino G	P60	*Department of Medical-Surgical Sciences and Biotechnologies, University of Rome Sapienza*
Marafioti MG	P63	*Department of Experimental and Clinical Medicine, University Magna Græcia*
Marampon F	P13, P34, P45, P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Marchese C	P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Marengo E	P6, P47	*Department of Sciences and Technological Innovation, University of Piemonte Orientale*
Mariano G	P20, P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Mariotto S	P15	*Department of Neuroscience, Biomedicine and Movement, Biochemistry Section, University of Verona*
Martellucci S	P13, P37	*Laboratory of Experimental Medicine and Environmental Pathology, "Sabina Universitas"*
Martinotti S	P47	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Martorelli C	P53	*Department of Dermatology, University of L'Aquila, L'Aquila*
Masella R	P30	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Massimi M	P59	*Department of Life, Health and Environmental Sciences, University of L'Aquila,*
Mastroiaco V	P22, P67, P48	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Mattei V	P13	*Laboratory of Experimental Medicine and Environmental Pathology, "Sabina Universitas"*
Mattia G	P1	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Maturo MG	P53	*Department of Dermatology, University of L'Aquila, L'Aquila*
Maurizi A	P12, P49	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Mauro A	P27	*University of Teramo, Faculty of Veterinary Medicine*
Mazzone P	P65	*Institution Biogem*
McDowell HP	P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Megiorni F	P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Menotti R	P68	*Imperial College London*
Mercuri E	P43	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Mercurio C	P56	*University of l'Aquila; San Salvatore hospital*
Meschini S	P54	*Dipartimento Tecnologie e Salute, Istituto Superiore di Sanità*
Mesuraca M	P63	*Department of Experimental and Clinical Medicine, University Magna Græcia*
Mincione G	P28, P50, P61	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Misso G	P40, P73	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Montalcini Y	P63	*Dept. of Experimental and Clinical Medicine, University Magna Græcia*
Morandi L	P43	*Department Of Molecular Medicine, University of Roma "La Sapienza"*

LIST OF AUTHORS

Morelli E	**P3**	***UMG of Catanzaro***
Moretti M	P21, P39	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Morrone G	P63	*Department of Experimental and Clinical Medicine, University Magna Græcia*
Moscatello C	P28, P32, P50	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Motta G	P40	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Mottolese M	P57	*Regina Elena National Cancer Institute*
Mucilli F	P50	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Muraca M	P11	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Muraro R	P17, P28, P50, P61	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Napolitano D	P40	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Nappo G	P63	*YCR Cancer Research Unit - Department of Biology, University of York,United Kingdom.*
Nardozza F	P58	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Neri M	P32	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Nicoletti A	P64	*Department of Medical and Surgical Sciences, Pediatric Unit, Magna Graecia University*
Noce C	P38	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Oppici E	P15	*Department of Neuroscience, Biomedicine and Movement, Biochemistry Section, University of Verona*
Orlando A	P38	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Pacchiana R	P6, P7, P15	*Department of Neuroscience, Biomedicine and Movement, Biochemistry Section, University of Verona*
Pacella I	P30	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Palermo R	P20, P35, P69, P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Palleschi C	P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Paone R	P11, P42	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Parra A	P51	*CRS Development of Biomolecular Therapies, Istituto Ortopedico Rizzoli; Experimental Oncology Laboratory, Istituto Ortopedico Rizzoli*
Pasquale V	P65	*Institution Biogem*
Pasquini L	P1	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Patel R	P49	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Patient L	P45	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Pedini F	P52	*Dept. of Hematology, Oncology and Molecular Medicine, ISS*
Peduto A	P72	*Department of Experimental Medicine, University of Naples*
Pellegrini C	P37, P53	*Department of Dermatology, University of L'Aquila, L'Aquila*
Pellegrini E	P54	*Dipartimento Tecnologie e Salute, Istituto Superiore di Sanità*
Pelullo M	P55, P58	*Departmentof Molecular Medicine, Sapienza University*
Pensabene L	P64	*Department of Medical and Surgical Sciences, Pediatric Unit, Magna Graecia University*
Pepe F	P5	*Department of Public Health, University Federico II*
Petaccia M	P54	*Dipartimento di Scienze Fisiche e Chimiche, Università degli Studi dell'Aquila*
Petrini M	P1	*Dept. of Hematology, Oncology and Molecular Medicine, ISS*
Petroni M	P14	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Petrosini L	P39	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Pfister S	P2	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Picci P	P51	*Experimental Oncology Laboratory, Istituto Ortopedico Rizzoli*
Piconese S	P30	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Pierdominici M	P30	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Pievani A	P31	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Pisani A	P64	*Departement of Nephrology University Federico II*
Pisapia P	P5	*Department of Public Health, University Federico II*

LIST OF AUTHORS

LIST OF AUTHORS

Schiraldi C	**P16, P72**	***Department of Experimental Medicine, Second University of Naples***
Schnabelrauch M	P27	*Innovent e.V., Biomaterials Department*
Sciandra M	P62	*PROMETEO Laboratory, STB, RIT Department, Rizzoli Orthopaedic Institute*
Scicchitano S	P63	*Dept. of Experimental and Clinical Medicine, University Magna Græcia*
Scionti F	P3, P18, P64	*Department of Experimental and Clinical Medicine, University Magna Græcia*
Scotlandi K	P51, P62	*CRS Development of Biomolecular Therapies, Istituto Ortopedico Rizzoli; Experimental Oncology Laboratory, Istituto Ortopedico Rizzoli*
Screpanti I	P20, P29, P35, P38, P55, P58, P69, P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Scudiero I	P65	*Institution Biogem*
Scuotto M	P16	*Department of Experimental Medicine, Second University of Naples*
Sdruscia G	P66	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Serafini M	P31	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Serrao SM	P66	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Sestito S	P64	*Department of Medical and Surgical Sciences, Pediatric Unit, Magna Graecia University*
Sferra R	P45, P48	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Sidoni T	P25	*Oncology, S. Salvatore Hospital*
Simeoni F	P69	*Molecular Medicine Department, Sapienza - University of Rome*
Spada S	P52	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Spiombi E	P21, P39	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Spoleti CB	P63	*Dept. of Experimental and Clinical Medicine, University Magna Græcia*
Stamato MA	P3	*UMG of Catanzaro*
Staropoli N	P18	*UMG of Catanzaro*
Stefania G	P30	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Stiuso P	P44	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Tagliaferri P	P3, P18	*UMG of Catanzaro*
Talarico V	P64	*Department of Medical and Surgical Sciences, Pediatric Unit, Magna Graecia University*
Talora C	P20, P69, P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Tarantelli C	P61	*Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio Chieti-Pescara, Chieti, Italy. Lymphoma and Genomics Research Program, IOR Institute of Oncology Research, Bellinzona, Switzerland.*
Tarbit M	P68	*Imperial College London*
Tassone P	P3, P18	*UMG of Catanzaro*
Telesio G	P65	*Institution Biogem*
Tessitore A	P22, P47, P48	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Testa D	P40	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Teti A	P11, P12, P49, P56, P71	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Tiziano FD	P43	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Tognolini M	P37	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Tombolini V	P46	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Tornatore L	P68	*Imperial College London*
Tosi M	P25	*Inserm U1079-IRIB, University of Rouen, Normandy Centre for Genomic and Personalized Medicine*
Tottone L	P69	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Tramontano A	P43	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Triolo G	P24	*Dipartimento Biomedico di Medicina Interna e Specialistica, Sezione di Reumatologia, Università degli Studi di Palermo*
Troncone G	P5	*Department of Public Health, University Federico II*
Tucci F	P10	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Uccelletti D	P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*

LIST OF AUTHORS

Ucci A	**P11, P12**	***Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila***
Uren A	P62	*Department of Oncology, Georgetown University Medical Center*
Vacondio F	P37	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Valanzano R	P32	*Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio"*
Valbret Z	P12	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Varano B	P30	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Varì R	P30	*Department of Hematology, Oncology and Molecular Medicine, ISS*
Vecchiotti D	P48, P70	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Veeriah V	P71	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Ventura L	P56	*University of l'Aquila; San Salvatore hospital*
Verzella D	P48, P68	*Imperial College London*
Vetuschi A	P34, P48	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Vicentini C	P9	*Department of Life, Health and Environmental Sciences, University of L'Aquila,*
Vitale F	P34, P45	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Walter T	P27	*Innovent e.V., Biomaterials Department*
Wyrwa R	P27	*Innovent e.V., Biomaterials Department*
Yousif AM	P44	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Zampieri M	P69	*Molecular Medicine Department, Sapienza - University of Rome*
Zanniti A	P71	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Zappavigna S	P16, P44, P72	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Zarone MR	P33, P73	*Department of Biochemistry, Biophysics and General Pathology, Second University of Naples*
Zazzeroni F	P22, P24, P67, P48	*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila*
Zema S	P55, P58	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Zonfrilli A	P20, P74	*Department Of Molecular Medicine, University of Roma "La Sapienza"*
Zoppoli P	P63	*Department of Experimental and Clinical Medicine, University Magna Græcia*

GENERAL INFORMATION

CONFERENCE VENUE

Auditorium
Department of Human Studies
University of L'Aquila
Viale Nizza 14, 67100 L'Aquila
Web: www.univaq.it/section.php?id=1525

LANGUAGE

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

REGISTRATION FEE (VAT INCLUDED)

AICC MEMBER € 200,00
GISM MEMBER € 200,00
NON-MEMBER € 250,00 (€ 200,00 + € 50,00 mandatory membership fee)
Ph.D. € 120,00 (€ 70,00 + € 50,00 mandatory membership fee)
STUDENT € 50,00
ONE-DAY FEE (AICC and GISM member) € 70,00
ONE-DAY FEE (non-member) € 120,00 (€ 70,00 + € 50,00 mandatory membership fee)

The registration fee includes the following:
Attendance at the 29° Annual Conference of Italian Association of Cell Cultures (ONLUS AICC)
Attendance Certificate
Conference Kit, Coffee Breaks, Lunches

SOCIAL EVENT

Thursday, November 24th 2016
Social dinner at "Delfina Restaurant"
Social dinner contribution fee € 40,00
Reservation for social dinner will be accepted until 8th November 2016.
After this date and on site the participants will be subjected to availability.

HOW TO REGISTER

The registration and payment forms are available on line at the website: **www.onlus-aicc.org**.

ABSTRACT SUBMISSION

Deadline for abstract submission is **October 30th, 2016**. Abstract form is available at the website: **www.onlus-aicc.org**.
The Scientific Committee will select the abstracts received into accepted as oral presentation or accepted as a poster presentation. E-mail notification of acceptance will be sent by the Organizing Secretariat.
Please note: for each accepted abstract, the registration fee of at least one of the Authors will be required.

GENERAL INFORMATION

"Prof. ALBERTO GULINO" PRIZE

During the Conference, a prize to the memory of Prof. Alberto Gulino will be awarded to the best Junior Researcher. Announcement and application form are available at the website: **www.univaq.it**; **http://discab.univaq.it**; **www.onlus-aicc.org**. Deadline for submission is **October 15th, 2016**.

POSTER AWARD

During the Conference, AICC will assign 2 "Poster Award" of € 500 each to the first author of the 2 best posters who is under 35 years of age at the time of the Conference. As prescribed by law, a tax deduction as income tax will be applied. The awards will be delivered during the Poster Awards Ceremony directly to the winners (delegation for collection is not allowed – the possible absence will cause disqualification from the contest). If you wish to take part in the contest, please fill in the appropriate box in the abstract form.

GENERAL INFORMATION

HOW TO REACH THE CONFERENCE VENUE

L'Aquila is about 100 Km (62 miles) East of Rome and 100 km West of Pescara. The nearest airports are The international Airport L. da Vinci - Fiumicino (FCO) and “GB Pastine – Ciampino Airport (CIA) in Rome, and The Abruzzo International Airport (PSR) in Pescara. L'Aquila can be easily reached by bus or car.

BY BUS FROM ROME

From “L. da Vinci” (Fiumicino) Airport and “GB Pastine (Ciampino) Airport:
Direct Bus: A coach bus service connects directly Rome airports (Fiumicino/Ciampino) to L'Aquila. Time table and other information are available at www.gasparitours.it.

From “Termini” Railway Station: The “B” line subway connects Termini Station to Tiburtina Station. Outside the Tiburtina Railway Station there is Tiburtina bus terminal. Coach buses to downtown L'Aquila are provided by the regional transportation agency (ARPA; ticket costs and timetable at www.arpaonline.it). The trip takes about 1h 40', stopping in front of Hotel Amiternum and then ending at Bus Terminal-Collemaggio (downtown L'Aquila).

BY BUS FROM PESCARA

From The Abruzzo International Airport - Pescara: catch the local bus 38 (every 10 minutes, from 5:30 to 23:30; the journey takes about 15 minutes) from Pescara Airport Terminal to downtown Pescara (Piazza della Repubblica).

From “Pescara” Railway Station: Outside of the railway station of Pescara, In Piazza della Repubblica, there is the bus terminal. The regional transportation agency (ARPA) coaches connect Pescara with L'Aquila (ticket costs and timetable at www.arpaonline.it). The trip takes about 1 hour and 50 minutes, ending at Bus Terminal-Collemaggio (downtown L'Aquila).

BY CAR

From Rome: take L'AQUILA-TERAMO A24 Highway. Take exit L’AQUILA OVEST.

From Adriatic cost: coming from north along Highway A14 (Direction Bari), exit to TERAMO-GIULIANOVA-MOSCIANO S. ANGELO, take freeway SS80racc (direction Teramo), and then Highway A24 to L’Aquila. Coming from south along Highway A14 (direction Bologna), exit to 'AUTOSTRADA A25 Torano Pescara' to take Highway A25 (direction Rome). Exit at BUSSI-L'AQUILA, then follow the Freeway 'SS17' to L'Aquila.

GENERAL INFORMATION

HOTEL ACCOMODATION

HOTEL FEDERICO II
Via Strinella, 6 - 67100 L'Aquila
T: +39 0862 21191
E: info@hotelfedericosecondo.it
W: www.hotelfedericosecondo.it

HOTEL SAN MICHELE
Via dei Giardini, 6
67100 L'Aquila (AQ)
Tel +39 0862.420260
Fax +39 0862.27060
E: info@stmichelehotel.it
W: www.stmichelehotel.it

HOTEL CASTELLO
P.zza Battaglione Alpini
67100 L'Aquila
tel. +39 0862 419147
fax +39 0862 419140
E: info@hotelcastelloaq.com
W: www.hotelcastelloaq.com

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ITALIAN ASSOCIATION
OF CELL CULTURES
(ONLUS-AICC)

nam et ipsa scientia potestas est

DEPARTMENT OF BIOTECHNOLOGICAL AND
APPLIED CLINICAL SCIENCES
UNIVERSITY OF L'AQUILA

www.onlus-aicc.org