Summary

- Editorial June 2019
- 31st Pezcoller Symposium
  - Program
  - Abstracts of oral presentations
  - Abstracts of posters
- Call for nomination 2020 Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research
- Call for nomination Scholar-In-Training Awards
2019 Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research winner: Prof. Alberto Mantovani
First of all we are reporting with a great pleasure that the recipient of 2019 Pezcoller Foundation - AACR International Award for Extraordinary Achievement in Cancer research is Prof. Alberto Mantovani, scientific Director at the Istituto Clinico Humanitas and President of the Fondazione Humanitas for Research in Milan. Mantovani was chosen among 21 nominees, by the Selection Committee chaired by Michael B. Kastan MD, PhD, who met in Philadelphia on November 30 2018. The other members of the Committee were: Mariano Barbacid, PhD, FAACR; Nina Bhardwaj, MD, PhD; Carlos M. Caldas MD; Elisabetta Dejana PhD; Joe W. Gray PhD FAACR; Lorenzo Moretta MD; David A. Tuveson MD, PhD; Jennifer Wargo MD.

The Award to Mantovani was formally announced at the AACR annual Meeting in Atlanta, on Sunday, March 31, 2019, where he delivered his award lecture. The award prize was then officially presented to Mantovani by the President of the Pezcoller Foundation Prof. Enzo Galligioni, MD on May 11 in the Teatro Sociale of Trento, during the Award Ceremony at the presence of the of Elizabeth M. Jaffee, MD Past President of AACR, Margaret Foti PhD, MD, CEO of AACR, Gios Bernardi MD, President Emeritus of the Pezcoller Foundation and large part of the Trento Community.

Before the Award Ceremony Mantovani gave a Lectio Magistralis at the at the Department of Molecular Medicine of the University of Padova and a second lecture at the CIBIO (Centre for Integrative Biology) of the University of Trento. In the Award Ceremony, President Galligioni highlighted the main points of Mantovani’s researches and discoveries.

“Dr. Mantovani is an eminent physician-scientist, he said, a leader in the field of tumor immunology for decades. Presently Scientific Director at the Istituto Clinico Humanitas and Full Professor of General Pathology at the Humanitas University, Mantovani was for many years Head of the Department of Immunology and Cell Biology, Istituto di Ricerche Farmacologiche “Mario Negri”, Milan, Italy. Furthermore he holds the Chair of Inflammation and Therapeutic Innovation, at the Queen Mary University, London, UK.

By identifying macrophages in tumors as corrupted policemen promoting cancer progression, and by discovering relevant genes and functions, Alberto Mantovani highlighted the role of inflammation and immunity in the tumor microenvironment, a paradigm shift fundamental for the development of tumor immunology and immunotherapy. In addition, his researches included Chemokines (CCL2), IL-1/Toll-like receptors (TLR) and Humoral innate immunity (PTX3).”

“Alberto Mantovani is the most cited Italian scientist working in Italy (Scopus, Web of Science, Google scholar) and a bibliometric analysis indicates that he is one of the 10 most quoted immunologists in the world.

He currently serves as president of International Union of Immunological Societies (IUIS) and is past president of the Italian Society of Immuni-
Mantovani is actively involved in the fostering of science and scientific policies in Italy and cofounded the association “Gruppo2003” of Italian highly cited scientists (http://www.gruppo2003.it) and the website http://www.scienzainrete.it. “

During the ceremony as President Emeritus I made some remarks where I tried to point out the human and social personality of the winner, referring to Mantovani’s publications aimed to young scholars:

…”Alberto Mantovani want to convey the typical sense of adventure of science. He recommends a passion for the work, the modesty of learning from everyone, especially from collaborators and technicians..... Mantovani, although deeply engaged on scientific researches, never forget his being a physician and that the ultimate concern, not only for a physician but also for any scientific researcher, must be the patient, from the bench to the hospital bed...”

In addition to the Award, I would like to mention that on the next June 17-18, we will hold our 31st Pezcoller Symposium. In this year’s Symposium, the focus will be on the newest insights into the processes that give rise to cancer tissue and its persistence. Cancers are, in essence, products of organ and tissue development gone wrong. In fact, many critical cellular and organ-based operations from genome integrity, the behavior of one’s own microbiome, oxygen utilization and cell metabolism, orderly tissue and organ anatomy, disciplined cell behavior, and the immune response may become disordered in the interest of tumor development. Also included will be recent findings that have the potential to influence clinical cancer therapeutics in novel ways. In the next pages you will find the program and the faculty.

Finally I’m pleased to particularly remember, among all other activities of the Pezcoller Foundation, the Agreement between the Pezcoller Foundation and the Museum of Science of Trento (MUSE), aimed to mutually collaborate to the diffusion of the scientific awareness among general population.

Gios Bernardi
Editor
### 31th Pezcoller Symposium

**CANCER AS A CORRUPTED TISSUE**

Trento, Italy • June 17 - 18, 2019

---

### PROGRAM

#### MONDAY JUNE 17, 2019

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00</td>
<td>Registration</td>
</tr>
<tr>
<td>8.30</td>
<td>Enzo Galligioni Welcome</td>
</tr>
<tr>
<td>8.40</td>
<td>David Livingston Focus &amp; Goals</td>
</tr>
</tbody>
</table>

**The Enrico Mihich Lecture**

Chair: David Livingston

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.50</td>
<td>Sean Morrison</td>
</tr>
<tr>
<td></td>
<td>The metabolic regulation of cancer progression</td>
</tr>
<tr>
<td>9.35</td>
<td>Discussion</td>
</tr>
</tbody>
</table>

**Session 1 - The Microbiome and Cancer**

Chair: Maria Rescigno

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.50</td>
<td>Romina Goldszmid</td>
</tr>
<tr>
<td></td>
<td>Microbiota as a key modulator of the tumor microenvironment</td>
</tr>
<tr>
<td>10.15</td>
<td>Discussion</td>
</tr>
<tr>
<td>10.30</td>
<td>Jennifer Wargo</td>
</tr>
<tr>
<td></td>
<td>The role of the gut and tumor microbiome in response to cancer therapy</td>
</tr>
<tr>
<td>10.55</td>
<td>Discussion</td>
</tr>
<tr>
<td>11.10</td>
<td>Coffee Break</td>
</tr>
</tbody>
</table>

**Session 2 - Cancer Metastasis**

Chair: Stefano Piccolo

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.30</td>
<td>Christoph Klein</td>
</tr>
<tr>
<td></td>
<td>From early dissemination to manifest metastasis: Theoretical and practical challenges of an unsolved problem.</td>
</tr>
<tr>
<td>11.55</td>
<td>Discussion</td>
</tr>
<tr>
<td>12.10</td>
<td>Mikala Egeblad</td>
</tr>
<tr>
<td></td>
<td>Neutrophil extracellular traps generated during inflammation drive cancer cell proliferation and a pro-metastatic microenvironment</td>
</tr>
<tr>
<td>12.35</td>
<td>Discussion</td>
</tr>
<tr>
<td>12.50</td>
<td>Lunch</td>
</tr>
</tbody>
</table>

**Session 3 - Genome Order and Disorder**

Chair: Fabrizio D’Adda di Fagagna

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.00</td>
<td>Roger Greenberg</td>
</tr>
<tr>
<td></td>
<td>The necessity of noncanonical DNA damage responses for human cancer</td>
</tr>
<tr>
<td>14.25</td>
<td>Discussion</td>
</tr>
<tr>
<td>14.40</td>
<td>Daniel Durocher</td>
</tr>
<tr>
<td></td>
<td>Charting the genetic architecture of the DNA damage response</td>
</tr>
<tr>
<td>15.05</td>
<td>Discussion</td>
</tr>
<tr>
<td>15.20</td>
<td>Simon Boulton</td>
</tr>
<tr>
<td></td>
<td>Maintaining telomeres in ALT cancers</td>
</tr>
<tr>
<td>15.45</td>
<td>Discussion</td>
</tr>
<tr>
<td>16.00</td>
<td>Fabrizio D’Adda di Fagagna</td>
</tr>
<tr>
<td></td>
<td>The role of non coding RNA in genome integrity</td>
</tr>
<tr>
<td>16.25</td>
<td>Discussion</td>
</tr>
<tr>
<td>16.40</td>
<td>Poster View</td>
</tr>
<tr>
<td>17.30</td>
<td>Adjourn</td>
</tr>
<tr>
<td>19.30</td>
<td>Symposium Dinner</td>
</tr>
</tbody>
</table>

---

#### TUESDAY JUNE 18, 2019

**Session 4 - Tissue and Organ Formation**

Chair: Cathrin Briskens

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.30</td>
<td>Cedric Blampain</td>
</tr>
<tr>
<td></td>
<td>Cancer cell of origin, tumor heterogeneity and EMT transitional state</td>
</tr>
<tr>
<td>08.55</td>
<td>Discussion</td>
</tr>
<tr>
<td>09.10</td>
<td>Stefano Piccolo</td>
</tr>
<tr>
<td></td>
<td>Tumors as wounds that never heals: a YAP/TAZ perspective</td>
</tr>
<tr>
<td>09.35</td>
<td>Discussion</td>
</tr>
<tr>
<td>09.50</td>
<td>Nikolaus Rajewsky</td>
</tr>
<tr>
<td></td>
<td>Principles of Gene Regulation by Single-Cell RNA Sequencing</td>
</tr>
<tr>
<td>10.15</td>
<td>Discussion</td>
</tr>
<tr>
<td>10.30</td>
<td>Coffee Break</td>
</tr>
</tbody>
</table>

**Session 5 - Cancer and Metabolism**

Chair: Massimo Loda

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.50</td>
<td>William Sellers</td>
</tr>
<tr>
<td></td>
<td>The Next Generation Characterization of the Cancer Cell Line Encyclopedia - implications for targeting metabolic alterations in cancer</td>
</tr>
<tr>
<td>11.15</td>
<td>Discussion</td>
</tr>
<tr>
<td>11.30</td>
<td>Matt Vander Heiden</td>
</tr>
<tr>
<td></td>
<td>Metabolic limitations of cancer progression</td>
</tr>
<tr>
<td>11.55</td>
<td>Discussion</td>
</tr>
<tr>
<td>12.10</td>
<td>Lunch</td>
</tr>
</tbody>
</table>

**Session 6 - Cancer and Immunology**

Chair: Alberto Bardelli

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.20</td>
<td>Andrea Ablasser</td>
</tr>
<tr>
<td></td>
<td>Expanding roles of cGAS in immunity and inflammation</td>
</tr>
<tr>
<td>13.45</td>
<td>Discussion</td>
</tr>
<tr>
<td>14.00</td>
<td>Catherine Wu</td>
</tr>
<tr>
<td></td>
<td>Addressing cancer heterogeneity: personalized cancer vaccines</td>
</tr>
<tr>
<td>14.25</td>
<td>Discussion</td>
</tr>
<tr>
<td>14.40</td>
<td>Poster Discussion and Poster Presentation (led by Massimo Loda)</td>
</tr>
<tr>
<td>15.40</td>
<td>David Livingston</td>
</tr>
<tr>
<td></td>
<td>Concluding Remarks</td>
</tr>
</tbody>
</table>
FACULTY

• Ablasser Andrea  
  Swiss Federal Institute of Technology, Lausanne, CH
• Bardelli Alberto  
  Istituto di Ricerche di Cancro, University of Torino, IT
• Blanpain Cédric  
  Université Libre de Bruxelles, BE
• Boulton Simon  
  The Francis Crick Institute, London, UK
• Bruskin Cathrin  
  Swiss Federal Institute of Technology, Lausanne, CH
• D'Adda di Fagagna Fabrizio  
  IFOM – The FIRC Institute of Molecular Oncology, Milano, IT
• Durocher Daniel  
  Lunenfeld-Tanenbaum Research Institute Mount Sinai Hospital, Toronto, Canada
• Egeblad Mikala  
  Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
• Goldszmid Romina  
  National Cancer Institute, Bethesda, MD
• Greenberg Roger  
  Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
• Klein Christoph  
  University of Regensburg, Regensburg, D
• Livingston David  
  Dana Farber Cancer Institute, Boston, MA
• Loda Massimo  
  Weill Cornell Medicine, New York, NY
• Morrison Sean  
  Children's Research Institute at UT Southwestern, Dallas, TX
• Piccolo Stefano  
  Department of Molecular Medicine, University of Padova, Padova, IT
• Rajewsky Nikolaus  
  The Berlin Institute for Medical Systems Biology, Berlin, D
• Rescigno Maria  
  Humanitas University, Milano, IT
• Sellers William  
  Broad Institute of MIT, Cambridge, MA
• Vander Heiden Matthew  
  Koch Institute for Integrative Cancer Research at MIT, Cambridge, MA
• Wargo Jennifer  
  MD Anderson Cancer Center, Houston, TX
• Wu Catherine  
  Dana Farber Cancer Institute, Boston, MA

INVITED PARTICIPANTS
The metabolic regulation of melanoma metastasis

Sean J. Morrison

Investigator, Howard Hughes Medical Institute
Professor, Children’s Research Institute
University of Texas Southwestern Medical Center

To study the mechanisms that regulate distant metastasis by melanoma cells, we developed a xenograft assay in which small numbers of cells from primary human melanomas engraft in NOD/SCID IL2Rγnull (NSG) mice and spontaneously metastasize (Nature 456:593). We have now banked and characterized the metastatic potential of melanomas from more than 150 patients in this assay and observed a correlation between spontaneous metastasis in NSG mice and metastasis in patients: melanomas that are destined to form distant metastases in patients spontaneously form distant metastases in the mice (‘efficient’ metastasizers), while melanomas that do not form distant metastases in patients metastasize more slowly in mice (‘inefficient’ metastasizers) (Science Translational Medicine 4:159ra149). This demonstrates there are intrinsic differences among melanomas from different patients in their potential to metastasize.

Using this assay, we discovered that metastasizing melanoma cells experience high levels of oxidative stress and that the rare melanoma cells that successfully metastasize undergo reversible metabolic changes that increase their capacity to survive oxidative stress, including increased dependence on the folate pathway (Nature 527:186). Treatment of xenografted mice with anti-oxidants increases the frequency of circulating melanoma cells in the blood as well as metastatic disease burden, demonstrating that oxidative stress is one factor that limits distant metastasis. These data suggest that rather than treating cancer with anti-oxidants, which have promoted cancer initiation and progression in large clinical trials, that we should devise pro-oxidant therapies that inhibit cancer progression by exacerbating oxidative stress. A major focus of my lab is understanding the mechanisms cancer cells use to cope with oxidative stress, with a view to developing therapies that inhibit these mechanisms.

A recent discovery is that metabolic differences among melanomas, and even among melanoma cells from the same tumor, confer differences in metastatic potential by modulating the ability to cope with oxidative stress. We observe differences in Monocarboxylate Transporter 1 (MCT1) expression among melanomas that correlate with differences in metastatic potential and differences in survival among patients. The main physiological function of MCT1 is to transport lactate in and out of cells. We found that MCT1 function promotes melanoma metastasis by reducing oxidative stress. Efficiently and inefficiently metastasizing patient-derived xenografts similarly metabolized isotopically-labeled glucose and glutamine; however, efficient metastasizers took up more lactate. Efficient metastasizers expressed higher levels of MCT1 and MCT1 inhibition reduced lactate uptake in vivo. MCT1 inhibition had little effect on the growth of primary subcutaneous tumors but substantially depleted circulating melanoma cells and reduced metastatic disease burden. MCT1 inhibition suppressed the oxidative pentose phosphate pathway and increased reactive oxygen species (ROS) levels. Anti-oxidant treatment rescued the effect of MCT1 inhibition on metastasis. MCT1high and MCT1low cells from the same melanomas had similar capacities to form subcutaneous tumors, but MCT1high cells formed more metastases after intravenous injection. Metabolic differences among cancer cells thus confer differences in metastatic potential as metastasizing cells depend upon MCT1 to manage oxidative stress. MCT1 inhibition is thus a pro-oxidant therapy.
Abstracts

that might impair disease progression in patients with high risk stage II and III melanomas.

Microbiota as a key modulator of the tumor microenvironment

Romina S. Goldszmid

Inflammatory Cell Dynamics Section, Cancer and Inflammation Program, CCR, NCI, Bethesda, MD.

Cancer has historically been viewed as a disease determined by genetic and environmental factors, however, it is now clear that inflammation affects all stages of the disease: initiation, progression and metastasis formation. The inflamed tumor microenvironment is in part sustained by infiltrating myeloid cells such as macrophages, monocytes, dendritic cells, and neutrophils. In cancer, as in infection, these cells can induce adaptive immune responses, but in cancer they mainly promote the tumor’s immune evasion, progression, and metastasis. Moreover, the role of distinct myeloid cell populations in response to cancer therapy remains unclear. We and others have previously uncovered a role for commensal microbes in controlling the response to cancer immuno- and chemotherapy. In this presentation, we will discuss the role of the microbiota in regulating the composition and function of the myeloid cell compartment in the tumor microenvironment and the role of these cells in the response to cancer therapy. Targeting myeloid cells in the tumor microenvironment represents a powerful approach to manipulate the outcome of cancer therapy; therefore, a clear understanding of their regulation and functional organization may lead to rational novel cancer immunotherapeutic approaches.

The Role of the Gut and Tumor Microbiome in Response to Cancer Therapy

Wargo Jennifer

MD Anderson Cancer Center, Houston, TX (missing)

From early dissemination to manifest metastasis: Theoretical and practical challenges of an unsolved problem.

Christoph A. Klein

Experimental Medicine and Therapy Research, University of Regensburg and Fraunhofer Institute of Toxicology and Experimental Medicine, Regensburg, Germany

Mutation, selection and adaptation are - by convention - thought to occur primarily within, and to a lesser degree, outside the primary tumor. However, we previously noted in breast cancer and melanoma that metastatic dissemination occurs often early and that advanced tumor stages seed relatively fewer cells. This indicates that metastatic founder cells may lodge and evolve considerable periods of time outside the primary tumor. However, it generates a plethora of questions: How do cancer cells survive at distant sites? From which disseminated cancer cells descent metastatic colonies? Can genomic evolution occur during dormancy in quiescent cells? Does the environment trigger progression of early-disseminated cancer cells or do late-arriving cancer cells take over incipient metastatic colonies? We try to address these questions by analysing single disseminated cancer cells isolated at various time points of disease. Our data suggest that available models and endpoint analyses - such as the comparison of primary tumours and metastases - are insufficient to reflect disease dynamics in patients. Therefore, we have to carefully consider the clinical and evolutionary stage of an individual disease when we try to address the underlying mechanisms.

Neutrophil extracellular traps produced during inflammation awakens dormant cancer cells

Mikela Egeblad

Cold Spring Harbor Laboratory
Cold Spring Harbor, NY

Every year, ~40,000 women in the US who had been successfully treated for primary breast cancer nonetheless have metastatic recurrence. Metastasis requires four key steps: 1) tumor cells leave the tumor; 2) tumor cells enter a new tissue; 3) disseminated tumor cells (DTCs) re-initiate proliferation; and 4) an inflammatory microenvironment is established to support the growing metastasis. Steps 1-2 are rarely amenable to intervention, as they usually occur before the primary tumor is detected and treated. However, we may be able to target steps 3-4 to ultimately reduce the occurrence of metastasis and its associated mortality. Our research on neutrophil extracellular traps (NETs) has provided novel insights into how inflammation can cause a) DTCs to re-initiate...
proliferation, and b) result in a metastasis-supporting inflammatory microenvironment. NETs are released by neutrophils to the extracellular space in response to infections and inflammation, and they consist of meshes of genomic DNA with ~40 associated proteins, including proteases and high mobility group box 1 (HMGB1). We recently reported that lung inflammation, induced by either tobacco smoke exposure or bacterial lipopolysaccharide (LPS), drove quiescent DTCs to re-initiate proliferation. This resulted in metastases that killed mice with experimental lung inflammation in <4 weeks, while control mice survived >8 months without metastasis. Using intravital imaging, we found that DTCs that exit quiescence after lung inflammation are surrounded by NETs. We further discovered that proteases on NETs cleave the basement membrane protein laminin, generating a b1-integrin-activating epitope that caused quiescent DTCs to re-initiate proliferation. Thus, NET-associated proteases drive step 3 of the metastatic process. Our new data now show NETs also drive a complex, feed-forward-loop between NETs, macrophages, and fibroblasts leading to the creation of a highly inflammatory microenvironment. We propose that this NET-induced microenvironment is critical for sustaining the proliferation and survival of the metastasizing cancer cells. By dissecting the mechanisms by which NETs promote re-initiation of proliferation of quiescent DTCs and generation of a sustained inflammatory microenvironment, we have identified several points of potential intervention to prevent dormant cell awakening and prolong the survival of cancer patients.

A novel chromatin directed vulnerability in BRCA mutated cancers

Priyanka Verma, Junwei Shi and Roger A Greenberg

Department of Cancer Biology, Basser Center for BRCA, Perelman School of Medicine, University of Pennsylvania, 421 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA.

The DNA damage response encompasses acute and delayed signaling events that culminate in repair of genomic lesions and the production of inflammatory cytokines that attract immune responses. This multifaceted DNA damage response is critical to cancer etiology and response to therapy, particularly in light of the realization that DNA damaging therapies can synergize with immune checkpoint blockade to eradicate tumors. A central aspect of the DNA damage response is the induction of myriad homology directed DNA repair mechanisms that use templated DNA synthesis to execute either high fidelity restoration of lesions to their ground state or inherently error prone mechanisms that result in loss of genome integrity. This prominence of repair mechanism utilization is illustrated in the setting of homologous recombination deficiency due to mutation within a network of genes centered around the breast and ovarian cancer suppressor proteins. This BRCA network is required for high fidelity DNA repair by homologous recombination. While deficiency in this canonical homology directed DNA repair pathway is confers cancer susceptibility, it also creates vulnerability to agents that target orthogonal repair mechanisms. Poly(ADP) Ribose Polymerase (PARP) inhibitors are approved to treat homologous recombination deficiency breast and ovarian cancer, with demonstrated improvements in progression free survival in tumors with BRCA mutations. The efficacy of PARP inhibitors depends on expression of the PARP1 enzyme, which becomes trapped on chromatin and necessitates intact BRCA dependent HR for repair in S-phase. In the absence of BRCA1, toxic use of nohomologous endjoining repair mechanisms creates dicentric chromosomes that result in a loss of viability upon passage through mitosis. The intersection of these repair pathways in PARPi response leads to resistance mechanisms that entail either loss of PARP1 expression or deficiency in nohomologous endjoining repair. These are thought to account, at least in part, for failure of ~50% of BRCA mutated cancers to respond to PARPi. Ideally, additional targets could be identified that circumvent PARPi resistance regardless of mechanism and restore efficacy in BRCA mutated cancers.

This presentation will describe our unpublished results that implicate the chromatin remodeling protein CHD1L as a novel therapeutic target in BRCA mutated cancers. Functional domain directed CRISPR-Cas9 screens were used to identify CHD1L as a vulnerability in BRCA1 and BRCA2 mutated cells. Our findings demonstrate that CHD1L loss is synthetic lethal in combination with mutation to either BRCA1 or BRCA2, while conferring extreme PARPi hypersensitivity regardless of resistance mechanism. Models to conceptualize these findings will be presented as well as their implications for harnessing DNA damage responses to enhance therapy in homologous recombination deficient cancers.

Charting the Genetic Architecture of the DNA Damage Response

Durocher Daniel

Lunenfeld-Tannenbaum Research Institute
Mount Sinai Hospital, Toronto, Canada
The orchestration of DNA repair is of fundamental importance to the maintenance of genomic integrity and tumor suppression. DNA damage must be detected in the context of the varied chromatin landscape, its presence must be communicated throughout the cell to alter many ongoing processes, and the machinery that will mend the lesion must be recruited to the damage site. In my presentation, I will discuss our recent efforts in mapping genome maintenance pathways using genome-scale CRISPR/Cas9 screens in human cells. I will highlight how these screens can be used to identify new genome stability factors, characterize drug responses and provide new insights into the genetic architecture of the genome stability network by identifying potentially actionable synthetic lethal genetic interactions. I will argue that somatic genetic screens in human cells are powerful tools to study the DNA damage response and its integration within other cellular pathways.

Maintaining telomeres in ALT cancers

Simon J. Boulton
Tha Francis Crick Institute, London, UK

In non-malignant somatic cells, telomeres undergo progressive shortening after DNA replication, which eventually results in replicative senescence and checkpoint-driven cell death. In contrast, tumour cells achieve replicative immortality by activating one of two distinct telomere maintenance mechanisms; cancers either re-express telomerase or induce Alternative Lengthening of Telomeres (ALT), which involves recombination between telomeres. ALT is utilised in 10-15% of all tumours and is associated with poor prognosis due to their complex karyotype and lack of targeted therapies. Currently, the mechanisms underpinning ALT induction and maintenance remain poorly understood. Here, we show that ALT recombination requires coordinate regulation of the SMX resolvasome and BTR complex to ensure the appropriate balance of resolution and dissolution activities at recombining telomeres. Critical to this control is SLX4IP, which accumulates specifically at ALT telomeres and interacts with SLX4, XPF and BLM. Loss of SLX4IP results in a hyper-ALT phenotype that is incompatible with cell viability following concomitant loss of SLX4. Inactivation of BLM is sufficient to rescue toxic telomere aggregation and synthetic lethality in this context, suggesting that SLX4IP favours SMX-dependent resolution by antagonising promiscuous BLM activity during ALT recombination. The clinical importance of SLX4IP in the ALT process is highlighted by its inactivation in a subset of ALT positive osteosarcomas. Collectively, our findings uncover an SLX4IP-dependent regulatory mechanism critical for telomere maintenance in ALT cancer cells.

The role of non coding RNA in genome integrity

Fabrizio d’Adda di Fagagna1,2
1IFOM Foundation, Milan, 20139, Italy; 2Istituto di Genetica Molecolare, National Research Council, Pavia, 27100, Italy.

The DNA damage response (DDR) is a signaling pathway physiologically activated in cancer initiation (Di Micco et al Nature 2006) and ageing (d’Adda di Fagagna et al. Nature 2003, Fumagalli et al. Nature Cell Biology 2012). More recently, we reported that DNA double-strand breaks (DSBs) trigger the synthesis of damage-induced long non-coding RNA (dlincRNA) that can be processed into shorter DNA damage response RNAs (DDRNAS) (Francia et al Nature 2012). Such transcripts are essential for full DDR activation and their inhibition by antisense oligonucleotides (ASO) allows site-specific inhibition of DNA damage signalling and repair (Michelini et al Nature Cell Biology 2017; D’Alessandro et al Nature Communications). We will discuss our progress in understanding the mechanisms of dlincRNA synthesis by the different factors associated with the RNA pol II holoenzyme in cells and in a novel reconstituted in vitro system, challenging the distinction between a DNA lesion and a transcriptional promoter. In addition, we will show how sequence-specific DDR inhibition can be achieved in vivo by targeting dlincRNA and DDRNA and we will discuss its potential therapeutic uses.

Cancer cell of origin, tumor heterogeneity and EMT transitional state

Cédric Blanpain
WELBIO, Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles (ULB), 1070 Bruxelles, Belgium

Different theories have been proposed to explain tumour heterogeneity including the cancer cell of origin. Here, we developed new genetically engineered mouse models allowing lineage tracing together with oncogenic activation in different cell lineages of the skin epidermis and the mammary gland and assessed whether
the cancer cell of origin controls tumour heterogeneity. I will present evidence that the cancer cell of origin controls tumour heterogeneity and the underlying molecular mechanisms by which the cell of origin control tumor differentiation, stemness, EMT, resistance to therapy and metastasis. These results have important implications for our understanding of the mechanisms controlling tumor heterogeneity and the development of new strategies to block tumor initiation, progression, metastasis and resistance to therapy. This work is supported by the ERC, WELBIO, FNRS, TELEVIE, and the Fondation Baillet-Latour.

Tumors As Wounds That Never Heal: A YAP/TAZ Perspective

Stefano Piccolo

Dip. Medicina Molecolare Università di Padova

Enhanced YAP/TAZ activity is emerging as common trait of multiple solid tumors in humans. Strikingly, in mouse models, adult organs lacking YAP/TAZ are unable to develop tumors without overt side effects for normal tissue homeostasis, making YAP/TAZ prime candidates for cancer therapy. That said, YAP/TAZ have physiological functions during tissue repair, being essential for organ regeneration after injury. YAP/TAZ are typically inactive in normal tissues but potently induced by mechanical and physical cues that the cell receives from its microenvironment, such as extracellular matrix stiffness and topology, and 3D architectural features of the tissue. I will focus on new discoveries on the mechanisms by which YAP/TAZ are controlled by mechanotransduction, and on YAP/TAZ as reprogramming factors in epithelial cells. Indeed, in normal tissues YAP/TAZ activation turns more differentiated normal cells into cells endowed with stem-like properties; in tumors, YAP/TAZ convert non-stem tumor cells into cancer-stem cells, increasing tumor aggressiveness and fueling metastasis. I will also expand on these mechanisms, providing new hints for therapeutic intervention.

Principles of Gene Regulation by Single-Cell RNA Sequencing

Nikolaus Rajewsky

The Berlin Institute for Medical System Biology, Berlin, Germany


William Sellers

Broad Institute of MIT, Cambridge, MA

The Cancer Cell Line Encyclopedia, a large-scale collection of well annotated cell lines, has provided a rigorous backbone upon which to study genetic variants, candidate targets, small molecule and biologic therapeutics and to identify new marker driven cancer dependencies. With the goal of more fully understanding molecular features that contribute to cancer phenotypes including drug response we have expanded the cell line characterizations to include genetic, RNA splicing, DNA methylation, histone H3 modification, miRNA expression, metabolomic and reverse phase protein-array data for 1,072 cell lines from various lineages and ethnicities. Integrating these data with functional characterization including drug sensitivity, shRNA knockdown and CRISPR/Cas9 knockout data reveals potential new cancer drug targets and associated biomarkers. In addition, the unbiased association analysis linking cancer metabolome to genetic alterations, epigenetic reprogramming, and gene dependency provides a detailed description of metabolic consequences of the cancer dysregulated genome.

Importantly, in the metabolic space we observed distinct patterns of cell autonomous synthesis and secretion of kynurenine, an immune-suppressive metabolite. Furthermore, we found significant variation in the ability to suppress kynurenine with IDO1 selective inhibitors. Finally, by manipulating amino acid availability in large-scale screens of >500 barcoded cell lines, we demonstrated that aberrant ASNS hypermethylation sensitizes subsets of gastric and hepatocellular cancers to asparaginase therapy. Together, this dataset and an accompanying public data portal provide a resource to accelerate cancer research.

References:

Metabolic limitations of cancer progression

Matthew G. Vander Heiden
Koch Institute for Cancer Research, Massachusetts Institute of Technology, Cambridge MA USA

Complex regulatory mechanisms enable cell metabolism to match physiological state. The major pathways cells use to turn nutrients into energy and to synthesize macromolecules have been elucidated; however, there remain many unanswered questions regarding how metabolism supports cancer cell proliferation and thus how best to target metabolism for cancer treatment. Of note, many existing cancer therapies target metabolic processes that are thought to be essential in all cells. This raises a key question of how drugs which target these processes show differential effectiveness to treat cancer as well as a therapeutic window between cells. We have characterized the factors that drive sensitivity to disruption of specific metabolic processes, and find that both cancer cell extrinsic and intrinsic factors dictate metabolic vulnerabilities. Further, this leads to different cells being more reliant on limiting nutrients for different metabolic processes, creating a framework to understand how cancers become differentially sensitive to agents which target otherwise essential pathways.

Addressing cancer heterogeneity: personalized cancer vaccines

Catherine J. Wu, M.D.
Professor of Medicine
Chief, Division of Stem Cell Transplantation and Cellular Therapies
Department of Medical Oncology
Dana-Farber Cancer Institute and Harvard Medical School, Boston MA USA

The recent successes and challenges of cancer immunotherapy have motivated intense investigation of the molecular and cellular determinants of therapeutic response. The generation of broad computational and analytic tools to directly probe human samples has led to the emergence of systematic approaches to meet this challenge. At the heart of productive anti-tumor immune responses is the interaction of the T cell and the antigen presenting cell, with recognition of antigen by the T cell receptor (TCR); these interactions are further impacted by heterogeneous immune cell populations within the tumor microenvironment. While the search for immunogenic tumor antigens has been the subject of decades-long studies, multiple lines of evidence have convincingly demonstrated tumor neoantigens as an important class of immunogenic tumor antigens. Neoantigens arise from amino acid changes encoded by somatic mutations in the tumor cell and have the potential to bind to and be presented by personal HLA molecules. Using next-generation sequencing approaches, we can now systematically identify mutations leading to amino acid changes that can be potentially recognized immunologically through the implementation of neoantigen discovery pipelines. In recent studies, we have demonstrated that neoantigen load is associated with clinical outcome to immune-based therapies, and neoantigens can be safely and feasibly targeted to generate customized cancer vaccines. We have been undertaking pilot clinical trials to develop personal cancer vaccines in melanoma and glioblastoma that utilize synthetic long peptides as delivery approach for this therapy. Recent results and new directions will be discussed.
ABSTRACTS OF POSTERS

In-vivo reprogramming of postmitotic neurons induces medulloblastoma

Giuseppe Aiello CIBIO
PhD Student: Giuseppe Aiello Armenise-Harvard Laboratory of Brain Disorders and Cancer Centre for Integrative Biology - CIBIO (University of Trento)

It is widely accepted that the “cell of origin” of tumors has to possess a proliferative capacity. Particularly for brain cancer, the transition of neural progenitor to differentiated postmitotic neurons is considered irreversible in physiological and pathological conditions. Therefore, postmitotic neurons have not been considered as suitable cell of origin of brain cancer. Here, we show that neurons reprogramming may occur upon Shh activation and it leads to Medulloblastoma (MB) formation in vivo. The Shh MB is a cerebellar tumor, found in infants and adults that is thought to originate from cerebellar granule neuron progenitors. More recently, it was discovered that the two different forms of SHH MB are distinguished by different transcriptome/methylation levels suggesting that the adult SHH MB may originate from a different cell of origin. Relying on these data, we use a conditional Cre-Lox recombination system that recapitulate the human adult medulloblastoma pathogenesis in mice and demonstrate that the post-migratory mature granule neurons can be reprogrammed in-vivo. This process leads to Shh medulloblastoma and the tumor formation is restricted to the cerebellum. Thus suggesting that cerebellar granule neurons have defined characteristics that allow the reprogramming process and cancer formation, upon specific mutational hits. Our novel model of cancer development could explain the human SHH medulloblastoma onset in adult individuals where granule neuron progenitors are no more present. We strongly believe that our model represents an important starting point to study other tissues where postmitotic cells might originate cancer and therefore this will open a new field in cancer and stem cell biology.

Pharmacological activation of TRPM8 channel overcomes innate resistance to standard-of-care therapies in prostate cancer

Alessandro Alaimo1, Marco Lorenzoni1, Paolo Ambrosino2, Arianna Bertossi1, Alessandra Bisio1, Alice Macchia1, Eugenio Zoni1, Sacha Genovesi1, Francesco Cambuli1, Veronica Foletto1, Dario De Felice1, Maria Virginia Soldovieri2, Francesco Gandolfi1, Gianluca Petris1, Anna Cereseto1, Maria Caterina Mione1, Marco Durante5, Alvaro Villarroel1, Mattia Barbareschi1, Arkaitz Carracedo1, George Thalmann4, Alessandro Romanel1, Maurizio Tagliatela1, Marianna Kruthof-de Julio4,9 and Andrea Lunardi1

1Department of Cellular and Computational Integrative Biology (CIBIO), University of Trento, Trento, Italy.
2Department of Health Sciences, University of Molise, Campobasso, Italy.
3CIC bioGUNE, Bizkaia Technology Park, Derio, Spain.
4Urology Research Laboratory, Department of Urology and Department of Clinical Research, University of Bern, Bern, Switzerland.
5Trento Institute for Fundamental Physics and Applications (TIFPA), INFN, University of Trento, Trento, Italy.
6Instituto Biofisika, University of the Basque Country (CSIC-UPV/EHU), Leioa, Spain.
7Unit of Surgical Pathology, Santa Chiara Hospital, Trento, Italy.
8Department of Neuroscience, University of Naples “Federico II”, Naples, Italy.
9Department of Urology, Leiden University Medical Centre, Leiden, The Netherlands.

Corresponding Author
Andrea Lunardi, Armenise-Harvard Laboratory of Cancer Biology & Genetics, Department of Cellular and Computational Integrative Biology (CIBIO), University of Trento, Via Sommarive 9, Povo-Trento, 38123, Italy. Phone: 39-0461-285288; E-mail: andrea.lunardi@unitn.it

Discovery of novel ‘druggable’ targets is a primary goal in cancer translational research. Transient Receptor Potential subfamily M member 8 (TRPM8) is a cation channel almost exclusively expressed by the luminal compartment of the prostate epithelium in the human body. Primarily associated with calcium homeostasis, TRPM8 levels rise in primary and metastatic prostate cancer (PCa), which makes it a possible oncogenic factor and a suitable target of potential clinical interest.

Herein, we show that increased TRPM8 levels in
prostate cells favor calcium uptake and activation of pro-survival calcium/caldesmon-dependent kinase II (CaMKII). Nevertheless, by combining a multidisciplinary approach to an in vitro genetic platform modelling prostate tumorigenesis, we demonstrate that potent TRPM8 agonists synergize with X-ray treatments to induce massive apoptotic response in radioresistant pre-malignant and malignant preclinical prototypes of primary prostate lesions. As well, TRPM8 activation enhances docetaxel and enzalutamide efficacy in eradicating hormone naïve metastatic PCa cells. Overall, our findings identify TRPM8 as a valuable target for the treatment of PCa and provide a solid rationale to pursue the clinical testing of TRPM8 agonists in combination with standard-of-care therapies in PCa patients.

Establishment and Analysis of Patient-Specific Group 3 Medulloblastoma Mouse Models

Claudio Ballabio - PhD Student
Armenise-Harvard Laboratory of Brain Disorders and Cancer

Department of Cellular, Computational and Integrative Biology - CIBIO
University of Trento
Trento (TN), Italy

Brain cancer is now the deadliest form of childhood cancer in the United States. In particular, Group 3 Medulloblastoma (MB) is the pediatric brain tumor with highest morbidity and mortality. Patients with Group 3 MB currently have the worst outcome and nearly 50% are metastatic at the time of diagnosis. However, the cellular and molecular mechanisms underlying Group 3 MB are still unknown. What is still lacking in the field is the possibility to obtain tumors by direct genetic modification of mice and to be able to recapitulate the growth and metastasis formation of Group 3 MB.

Exploiting in-vivo transfection of mouse cerebellar cells with CRISPR-Cas9 and PiggyBac transposase systems, we tested different combination of putative oncosuppressors and putative oncogenes, derived from human Medulloblastoma NGS data, for their ability to induce Group 3 MB in mice. Surprisingly, concomitant overexpression of c-Myc and other transcription factors in mouse cerebellum is able to induce MB in few months. The newly generated mouse model is able to fully recapitulate human Group 3 MB.

Using this proposed patient-specific model, we were able to unravel the molecular aspects of Group 3 medulloblastoma tumorigenesis and identify molecules inhibiting tumor growth in a targeted manner.

Cell-autonomous and cell non-autonomous downregulation of the tumor suppressor DAB2IP by microRNA-149-3p promotes cancer progression by microenvironment remodeling.


The dynamic crosstalk established between tumor cells and surrounding stroma cells is crucial in carcinogenesis (Quail and Joyce, 2013). In this context, signaling modulators and adaptors that dictate intrinsic cell responses to microenvironmental cues play a fundamental role, often underestimated. The tumor suppressor DAB2IP belongs to this category: it is a cytoplasmic Ras-GAP and adaptor protein that negatively modulates multiple oncogenic pathways, including canonical WNT signaling, VEGF signaling via PI3K/Akt, TNF signaling via NF-κB, and Androgen Receptor (AR) activity (Bellazzo et al., 2017). Not surprisingly, its expression is frequently reduced by gene methylation in several tumors, including breast and prostate cancer. In addition, various post-transcriptional mechanisms of DAB2IP inactivation have been reported; in particular, due to the long 3'UTR sequence of its main transcript, DAB2IP is a strong candidate for microRNAs (miRNAs)-mediated regulation (Bellazzo et al., 2017). Performing an high-throughput screening of a large collection of human miRNAs, we have identified miR-149-3p as a negative modulator of DAB2IP (Bellazzo et al., 2018). By efficiently downregulating DAB2IP, miR-149-3p enhances NF-κB signaling activation in prostate cancer cells, promoting cell motility, and improving the secretion of pro-inflammatory and pro-angiogenic factors. Importantly, we found that the inhibition of endogenous miR-149-3p restored DAB2IP tumor suppressive functions, and efficiently reduced tumor growth and dissemination of prostate cancer cells in vivo (Bellazzo et al., 2018). DAB2IP loss can also affect the behavior of endothelial cells surrounding the tumor: in fact, conditional DAB2IP knockout in vascular endothelial cells was shown to potentely support formation of a pre-metastatic niche, facilitating tumor growth and dissemination in mouse models of melanoma and breast cancer (Ji et al., 2015). We discovered that cancer cells can reduce DAB2IP levels in neighboring endothelial cells and fibroblasts by exosome-mediated secretion of miR-149-3p, stimulating their proliferation and motility, and potentially remodeling the tumor microenvironment. Notably, experiments with miR-149-3p in-
hibitors clearly indicate that additional signals released by cancer cells also contribute to DAB2IP downregulation in nearby endothelial cells and fibroblasts, and these need to be uncovered. These results have various implications; they contribute to clarify the complex mechanisms that govern the dynamic crosstalk between tumor cells and surrounding stroma, and they may suggest promising therapeutic strategies in cancer. Indeed, approaches aimed to upregulate DAB2IP levels would offer the unique therapeutic opportunity to act on a single protein that can negatively modulate multiple oncogenic signals, both in cancer cells and in stromal cells. In this perspective, factors involved in cell non-autonomous downregulation of DAB2IP might be optimal targets to develop pharmacologic strategies to limit cancer progression by potentiating DAB2IP functions in multiple cellular components of the tumor tissue.


A novel platform to study and target undruggable Ewing onco-chimeras.

Arianna Bertossi, Rubens Begaj, Sacha Genovesi, Veronica Fioletto, Dario De Felice, Alessandro Alaimo, Marco Lorenzoni, Andrea Lunardi

Università di Trento, CIBIO, via Sommarive 9, 38123, Povo (TN), Italy

Cancer in children is relatively rare, yet it is the leading cause of death by disease in developed countries. Development of efficient therapeutic strategies in the past years has drastically reduced the lethality of several common types of pediatric and juvenile forms of cancer such as leukemia and lymphoma, but such a tremendous success does not include sarcoma’s treatment, whose mortality rate remains the same as twenty years ago. Ewing sarcoma is an aggressive type of bone tumor representing 1% of all childhood cancers, and its initial oncogenic event is represented by a balanced chromosomal translocation originating a chimeric oncoprotein, which in 90% of the patients derives from the fusion between EWS and FLI1 genes. Ewing sarcoma’s treatment is currently based on the use of generic chemo-agents such as doxorubicin, vincristine, cyclophosphamide, and daunorubicin, and novel targeted treatments are urgently required. The identification of novel drugs through preclinical studies is however heavily influenced by the model enrolled in the studies. Faithful preclinical models able to recapitulate the biological characteristics of the human disease should be employed to improve the efficiency of the preclinical tests and to decrease the percentage of subsequently failing clinical trials. Currently, however, no faithful preclinical model of Ewing Sarcoma is available. Therefore, first objective of this study will be the development of reliable models able to faithfully recapitulate Ewing sarcomagenesis by inducing the expression of Ewing oncoproteins in the correct cellular microenvironment, which is considered to be the mesenchymal stem cell. As exclusive hallmark of tumor cells and driving force of the disease, oncochimeras are ideal targets in medical oncology. Among them, however, transcription factor oncoproteins such as Ewing sarcoma oncoproteins are classified as “undruggable” from a conventional pharmacological point of view, lacking in their structure convenient targeting pockets, and even though much is known regarding the oncogenic functions of different chimeras, the success rate at which this advanced knowledge has been translated into effective therapies is pitifully low. Second objective of this study will therefore be the enrollment of the generated models in preclinical screenings to identify those molecular mechanisms whose pharmacological tuning will tear down Ewing sarcoma lethality by modulating oncocimera’s stability. By succeeding, our project will definitely break down the ancient dogma postulating the undruggability of oncogenic chimeras belonging to the class of transcription factors, unveil new therapeutic strategies towards the eradication of Ewing sarcoma, and, finally, provide a pivotal platform easily exportable to all those tumors driven by “undruggable” oncogenic chimeras.

Contribution of centriole appendages to PIDDosome activation

Matteo Burigotto, Alessia Mattivi, Chiara Valentini, Daniele Migliorati, Giovanni Magnani, Alexander Schmidt, Martin Offterdinger, Andreas Villunger, Luca Fava
Failure in the physical separation of two cells at the end of cell division (i.e. cytokinesis) is one of the most common malfunctions of the cell division cycle, predisposing cells to neoplastic transformation. Cytokinesis failure is invariably followed by a reduction in the propensity of cells to commit to additional cell cycles. Fava et al. demonstrated that this p53-dependent cell cycle arrest depends on the activation of the PIDDosome, a multiprotein complex comprising PIDD1, RAIDD and Caspase-2 (Fava et al, 2017). Moreover, centrosome abundance appears crucial for determining the cellular behavior in response to cytokinesis failure and PIDD1 physically associates with the centrosome.

A genetic screen for centrosomal proteins revealed the PIDD1 position within the epistatic map of centriole appendage proteins. Moreover, delocalization of PIDD1 from the centrosome results into compromised PIDDosome activation and p53-dependent cell cycle arrest. Taken together, our data shed light on the role of the PIDDosome as a centrosome counting entity.


Targeting tumor-associated macrophages in osteosarcoma: depletion versus re-direction

Adriana Salvaggio1, Valeria Cancila2, Cristina Guiducci3, Claudio Tripodo3, Katia Scotlandi4, Mario P. Colombo1 and Claudia Chiodoni1

The pro-tumorigenic role of tumor-associate macrophages (TAM) has been widely demonstrated in several tumor types. On the contrary, very few data exist on the activity of TAM and of other immune cells in osteosarcoma (OS), the most common primary bone tumors in young adolescents and children.

We have recently demonstrated in immuno-competent OS mouse models that trabectedin, a marine-derived chemotherapeutic agent, exerts a potent anti-tumor activity that is further enhanced by the combination with anti-PD-1 antibody. Besides directly affecting neoplastic cells, trabectedin modifies the tumor immune landscape by recruiting T lymphocytes at the tumor site. However, contrary to what expected from the literature, the treatment did not affect TAM.

To better investigate the role of TAM in OS, we performed a “proof-of-concept” co-injection experiment with OS cells mixed with macrophages differentiated in vitro toward classical M1 or M2 phenotype, or left undifferentiated (M0). While the presence of M1 macrophages inhibited OS, neither M0 nor M2 macrophages affected significantly tumor growth. This result indicated that the presence of TAM per se does not influence OS growth and suggested that their re-direction toward a M1 phenotype could exert therapeutic activity. To clarify this issue, mice bearing OS tumors on both flanks were treated locally, only in one lesion, with either liposome-encapsulated clodronate to deplete TAM, or with SD101, a synthetic oligonucleotide with immunostimulatory CpG motifs. Despite clodronate reduced TAM infiltration, tumor growth inhibition was limited, on both flanks; on the other hand, SD101 efficiently halted the growth of both treated and untreated lesions. TAM number was not affected by SD101 treatment, but they showed a significant reduction in the expression of the M2 marker CD206. Additionally, tumor infiltration by CD8 T cells was enhanced in both treated and untreated tumors by SD101, but unaffected by clodronate. Overall these preliminary results support the hypothesis that re-directing the phenotype of TAM in OS could be therapeutically more efficient than their direct elimination.

The glutamine addiction of multiple myeloma cells shapes the metabolic microenvironment of the bone marrow niche impairing osteoblastic differentiation

Martina Chiu, Denise Toscani, Giuseppe Taurino, Emanuela Vicario, Fabrizio Accardi, Nicola Giuliani and Ovidio Bussolati
Department of Medicine and Surgery (DiMeC), University of Parma, Italy

Metabolic alterations of cancer cells are final-
ized to satisfy the increased nutrient needs due to uncontrolled proliferation but also impact on normal cells of tumor microenvironment. Glutamine addiction of multiple myeloma (MM) cells (Bolzoni, Chiu et al., Blood 2016; 128:667-679) leads to decreased glutamine levels in the bone marrow (BM) plasma of patients. Osteolytic bone lesions are a hallmark of active MM, and several mechanisms have been implied in their pathogenesis. However, the role of MM-related alterations of glutamine metabolism in bone lesions has not been investigated yet. Bone lesions in MM are characterized by loss and impaired differentiation of osteoblasts. Interestingly, Glutamine Synthetase (GS), the only enzyme able to catalyze intracellular glutamine synthesis, is down-regulated during osteoblast differentiation. Moreover, it has been recently demonstrated that the inhibition of glutamine metabolism decreases bone mass in mice (Yu et al., Cell Metabolism 2019; 29, 966-978). Thus, we hypothesized that the low-glutamine microenvironment imposed by MM plasma cells negatively affects osteoblastogenesis.

Human MM cell lines (HMCLs), immortalized BM mesenchymal stem cells (MSC) and osteoblastic cell lines (HOBIT and HOB-01) were grown in DMEM supplemented with 2 mM Gln and 10% fetal bovine serum. Changes in extracellular Gln were measured with a commercial kit or with mass spectrometry, and amino acid uptake was assessed as previously described (Bianchi et al. Neuroscience. 2008; 151:1042-1052). The expression of osteoblastic markers (ALP, COL1A1 and RUNX2), ALP activity and positivity to ALP staining were then assessed during osteoblast differentiation.

As expected from data obtained ex vivo, HMCLs consumed large amounts of glutamine (750 ± 50 nmol/10^6 cells/day) and exhibited fast initial uptake of the amino acid. When co-cultured with MSC or osteoblasts, HMCLs accelerated the depletion of extracellular glutamine (+25%/day, compared to monocultures of MSC/osteoblasts), promoted GS expression in MSC and hindered viability of osteoblasts but not of MSC. Consistently, osteoblasts were more sensitive to Gln depletion (EC_{50} of 240 ±15 μM for HOBIT cells and 300± 5 μM for HOB-01 cells) than MSC (EC_{50} 80 ± 20 μM). The expression and the activity of the concentrative glutamine transporter SNAT2 (SLC38A2) were induced during osteoblastogenesis, while the expression of other transporters, such as ASCT2 and SNAT1, was unchanged. SNAT2 induction was also associated with the increased expression of Glutaminase 1 (KGA, long-transcript form), suggesting higher glutamine demand and consumption in differentiating osteoblast precursors. In agreement with the experimental hypothesis, MSC osteoblastic differentiation was substantially impaired in the absence of glutamine; moreover, the decrease of extracellular glutamine concentration from 0.6 mM (the average physiological BM plasma concentration) to 0.4 mM (the average concentration in the BM plasma of MM patients) was sufficient to cause a significant reduction of osteoblastic markers as well as of ALP activity and staining.

These preliminary results suggest that glutamine addiction of MM contributes to osteoblast impairment in the bone lesions of the MM BM niche.

**JMJD6-mediated Regulation of Estrogen Receptor in Breast Cancer and its role in Tamoxifen response**

**B. Cioni, D. Lecis, E. Fontanella and M.P. Colombo**

**Department of Molecular Immunology, Via Giacomo Venezian, 1, 20133 Milano MI, Istituto Nazionale Tumori**

**Introduction**

Breast cancer (BCa) is one of the most common cancer types in women worldwide, and estrogen receptor (ER) is expressed in the vast majority of these tumors. ER-positive BCa relies on estrogen levels for the development and progression of the disease, as activation of ER signaling regulates the expression of critical genes involved in proliferation and migration of tumor cells. Thus, anti-hormonal treatment, through the inhibition of ER action, is the mainstay therapeutic option for high-risk ER-positive breast cancer patients. It is well known that modulation of chromatin conformation via epigenetic modifications regulates transcription of many genes, including hormone receptors (HRs). The bifunctional arginine demethylase and lysyl-hydroxylase, JMJD6, is an epigenetic modifier often associated with tumor progression in many tumors, including BCa. A crucial role of JMJD6 is to demethylate arginine residues 3 and 2 of histone 4 (H4R3) and 3 (H3R2), respectively, thus affecting structural conformation of the chromatin and transcription and function of HRs.

**Hypothesis and aims**

We hypothesized that modulation of the methylation status of these histones by JMJD6 is critical for the regulation of ER expression and signaling in BCa, thus possibly affecting proliferation and metastatic potential of tumor cells. On the other hand, we expect that increased ER expression would improve efficacy of hormone therapy in BCa patients. This project aims to explore the molecular and functional role of JMJD6 in regulating ER signaling and response to Tamoxifen in BCa cell lines and in suitable mouse models. Ultimately, we aim to validate our findings in hormone treated and not treated BCa patients cohorts.
Results
Our preliminary data revealed that CRISPR-Cas9 JMJD6 knock-out (KO) ER-positive MCF7 showed a strong increased methylation of H4R3, as well as increased ER expression, and expression of ER-responsive genes, RARA and GREB1. In agreement with this, proliferation of JMJD6 KO cells was strongly increased compared to wild-type (WT) control. As expected, stimulation with estradiol (E2) further enhanced proliferation of both JMJD6 KO and WT MCF7 cells, however, Tamoxifen treatment equally suppressed proliferation of both cell types. Furthermore, JMJD6 KO MCF7 cells showed increased migration when stimulated with E2 due to loss of E-cadherin expression, nevertheless, Tamoxifen treatment successfully suppressed cell migration. Importantly, increased ER expression and improved response to Tamoxifen was also observed in ER-low BT474 BCa cell line that poorly respond to Tamoxifen treatment in WT condition, suggesting that inhibition of JMJD6 levels can improve response to hormone therapy by modulating ER levels. Supporting this hypothesis, we found that reduced levels of JMJD6 were significantly correlated with longer overall survival of BCa patients treated with hormone therapy in the METABRIC database compared to hormone treated patients expressing high levels of JMJD6, suggesting that JMJD6 could be a predictive marker for hormone therapy response in these patients.

Conclusion
Our data suggest that inhibition of JMJD6 in BCa patients could potentially improve response to hormone therapy by increasing ER expression, thus providing a rational for the development of JMJD6 inhibitors.

New platform for the direct profiling of microRNAs in biofluids

Detassis Simone

Department of Cellular, Computational and Integrative Biology - CIBIO
University of Trento

Circulating microRNAs have been identified as potential biomarkers for early detection, prognosis and prediction of several diseases. Their use in clinical diagnostics has been limited by the lack of suitable detection techniques. Most of the current technologies suffer from requiring complex protocols, not yet able to deliver robust and cost-effective assays in the field of clinical diagnostics. In this work, we report the development of a breakthrough platform for profiling circulating microRNAs. The platform comprises a novel silicon photomultiplier-based reader in conjunction with a chemical-based method for nucleic acid detection. Accurate microRNAs profiling without extraction, pre-amplification or pre-labeling of target is now achievable. We designed and synthesized a set of reagents that combined the chemical-based method with a chemiluminescent reaction. The signals generated were read using a novel, compact silicon photomultiplier-based reader. The platform sensitivity was determined by measuring known concentrations of hsa-miR-21-5p spike-ins. The limit of detection was calculated as 4.7 pmol/L. The platform was also successfully used to directly detect hsa-miR-21-5p in eight non-small cell lung cancer plasma samples. Levels of plasma hsa-miR-21-5p expression were also measured via TaqMan RT-qPCR.

Ultrasonisitive detection of cancer biomarkers from blood circulating NBI-isolated vesicles.

VG. D’Agostino1, M. Notarangelo1, C. Zucal1, L. Pasini2, H. Beltran3, F. Demichelis1, A. Provenzano1, A. Quattrone1.

1 Centre for Integrative Biology (CIBIO), University of Trento
2 Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola
3 Department of Medicine, Weill Cornell Medicine, New York, NY, USA

Extracellular vesicles (EVs) are secreted membranous particles intensively studied for their potential cargo of diagnostic markers. An efficient and high-throughput study of EVs in the routine clinical practice is needed to understand their clinical utility. We designed the nickel-based isolation (NBI) procedure to rapidly isolate EVs to preserve their integrity and original dispersity in solution. Then, we combined it with ultrasensitive homogeneous assays, such as amplified luminescent proximity homogeneous assay (alpha) or droplet digital PCR (ddPCR), to detect known cancer biomarkers from retrospectively analysed oncological patients.

By applying alpha-NBI, we detected picomolar concentrations of prostate-specific membrane antigen (PSMA) in fractions of EVs isolated from the plasma of prostate cancer patients, discriminating them from control subjects. Directly from oil-encapsulated EVs for digital PCR, we identified somatic BRAF and KRAS mutations from the plasma of metastatic colorectal cancer (CRC) patients, matching 100% of concordance with tissue diagnostics and higher sensitivity and specificity compared with immune-enrichment of tumor-derived EVs. We propose NBI-combined approaches as a further tool to develop liquid biopsy studies and gain advantages from the possibility of probing tumor heterogeneity from circulating EVs.
The successful integration of a unique chemical-based method for nucleic acid detection with a novel silicon photomultiplier-based reader created an innovative product (ODG platform) with diagnostic utility, for the direct qualitative and quantitative analysis of microRNA biomarkers in biological fluids.

The SWI/SNF complex is a mechanism-regulated inhibitor of YAP and TAZ

Inactivation of ARID1A and other components of the nuclear SWI/SNF protein complex occurs at very high frequencies in a variety of human malignancies, suggesting a widespread role of the SWI/SNF complex in tumour suppression. We show that ARID1A-containing SWI/SNF complex (ARID1A-SWI/SNF) operates as an inhibitor of the pro-oncogenic transcriptional activators YAP and TAZ (also known as WWTR1)1. YAP and TAZ are necessary to mediate the effects of the inactivation of the SWI/SNF complex, such as cell proliferation, acquisition of stem cell-like traits and liver tumorigenesis2.


* co-first authors

Pro-tumoral role of Complement activation in murine tumor models

Elena Magrini1, Sabrina Di Marco1, Chiara Perruchini1, Fabio Pasqualini1, Andrea Ponzetta1, Maria Luisa Barbagallo1, Colombo Piergiuseppe1, Ferdinando Canan1, Kevin Berthenet1, Antonio Infortzato1, Sebastien Jaillon1, Andrea Doni1, Alberto Mantovani1, and Cecilia Garlanda1,2,3

1 IRCCS - Humanitas Clinical and Research Center, Rozzano, Milan, Italy
2 Humanitas University, Milan, Italy
3 Cancer related inflammation (CRI) plays a fundamental role in fueling tumor appearance and development. Although the important contribution of complement activation to inflammation, its role in CRI still remains understudied. Recently our group demonstrated the pro-malignant role of complement activation in models of mesenchymal carcinogenesis, induced by 3-methyloanthrene (3-MCA), and epithelial inflammation-driven skin carcinogenesis, induced by dimethylbenz-α-anthracene/terephthalic acid treatments (DMBA/TPA). Our results showed that mice deficient for the central complement component C3 were protected from tumor development. Further experiments revealed that C3-cleavage products were deposited on vessels and tumor cells of tumor tissues, while they were absent in normal subcutaneous tissues. The C3 deposition on tumor cells was also observed in vitro, both on 3-MCA-derived sarcoma and on different murine cancer cell lines. Further, in vivo experiments suggested that complement activation was mainly due to the activation of the classical pathway. Then, we investigated C3-downstream mechanism(s) of protection in three different murine tumor models. We observed that C3AR- but not C5aR1- and C5L2-deficient mice were protected from tumor growth in a transplantable model of sarcoma (MN-MCA1), as well as in the 3-MCA-induced carcinogenesis model, suggesting that the C3a/C3AR axis was most likely responsible for the phenotype showed by C3-deficient mice in sarcoma models. However, in the DMBA/TPA model we observed that the C5a/C5aR1 dependent signaling was mainly responsible for the phenotype showed by C3-deficient mice, since C5aR1-/- mice were protected from tumor development, and that the underlying mechanism involved the hematopoietic compartment Our results indicate that complement activation occurs in tumor and contributes to tumor development, although the mechanism/s implicated could be different in the mouse models.

Targeted NGS in pharmacogenes to identify novel rare variants related to fluoropyrimidines toxicity

Ecca F.1,2, De Mattia E.1, Serra F.1, Roncato R.1, Dreussi E.1, Romanato L.1, Buonadonna A.4, De Paoli A.3, Berretta M.4, Mini E.4, Nobili S.5, Toffoli G.1, Cecchin E.1

1 Exper and ClinPharmaco, Centro Di Riferimento Oncologico- National CancerInstitute, Aviano 2 PhD School, University of Udine 3 INSERM U 1065, C3M, Team: Control of Gene Expression Nice, France. 4 Medical Oncology Dpt, Centro Di Riferimento Oncologico - National CancerInstitute, Aviano 5 Radiation Oncology Dpt, Centro Di Riferimento Oncologico - National CancerInstitute, Aviano 6 Section of Internal Med, Dpt of Experimental and Clin Med, University of Florence
Metabolic re-programming by malat1 depletion in prostate cancer cell lines and organotypic slice cultures

S. Nanni 1, A. Aiello 2, C. Salis 1, A. Re 2, C. Cencioni 2, L. Bacci 1, F. Pierconti 1, F. Pinto 3, D. Pugliese 1, C. Ripoli 1, R. Ostano 4, E. Iorio 5, P. Bassi 1, 2, C. Grassi 1, 2, G. Chiorino 2, A. Pontecorvi 1, 3, C. Gaetano 2 and A. Fasetti 1

1 Università Cattolica del Sacro Cuore Rome, Italy;
2 National Research Council-IBCN, Rome, Italy;
3 Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy;
4 Fondazione Edo ed Elvo Tempia, Biella, Italy;
5 Istituto Superiore di Sanità, Rome, Italy;
6 Istituti Clinici Scientifici Maugeri, Pavia, Italy.

BACKGROUND: Prostate cancer (PCa) is the most common malignancy in men worldwide with an increased global burden. The continuous improvement of next-generation sequencing methods shed light on the direct involvement of multiple non-coding RNA (ncRNA) transcripts in cell biology and homeostasis, cancer growth and progression as well as in other pathophysiological contexts. Contextually, cancer metabolism recently emerged at the upfront edge of anticancer research with the promise to develop novel therapeutic approaches. Interestingly, very recent evidences linked long (>200nt) ncRNAs (lncRNAs) to metabolism opening up to novel therapeutic strategies based on lncRNAs. Therefore, the study of their effects on metabolism might unravel overlooked pathways to target in an anticancer perspective.

HYPOTHESIS & AIM: Although MALAT1 has been one of the earliest identified IncRNAs and its role in cancer as promoter of tumour progression and metastasis is well defined, little or no information is available about its direct involvement in mitochondrial metabolism regulation. In this light, our working hypothesis is that MALAT1 might play a pivotal role in PCa as a metabolically active signal integrator. For this reason, the main goal of our study is to investigate whether MALAT1 might represent a metabolically active target for PCa inhibition in Organotypic Slice Cultures (OSCs), an ex vivo human model suitable for gene expression, gene targeting, and drug testing analyses. 

EXPERIMENTAL DESIGN: Our experimental strategy is made of a multi-pronged approach aimed at defining a novel role of MALAT1 in PCa metabolism. Specifically, we characterized MALAT1-dependent transcriptome in human PCa cells with aggressive/metastatic phenotype and in OSCs and identified putative MALAT1-dependent metabolic enzymes and metabolites that were further investigated by NMR-based metabolomics analysis before and after ex-vivo gene targeting.
Mechanisms of telomere maintenance in zebrafish brain tumors: correlation with pediatric glioblastoma

Aurora Irene Idilli1, Emilio Cusanelli1, Francesca Pagani2, Emanuela Kerschbamer1, Francesco Berardinelli3, Marialuisa Cayuela4, Pietro Luigi Poliani2, Marina Mione1

1 Centre for Integrative Biology (CIBIO), University of Trento, Trento - Italy
2 Department of Molecular and Translational Medicine, University of Brescia School of Medicine, Brescia, Italy
3 Department of Science, University of Rome “Roma Tre”, Rome, Italy
4 Department of Surgery, CIBERehd, University Hospital ‘Virgen de la Arrixaca’ and Instituto Murciano de Investigación Biosanitaria (IMIB), 30120 Murcia, Spain.

Alternative lengthening of telomeres (ALT) occurs in pediatric brain tumours and may develop as a result of chromosomal instability promoted by altered histone H3 modifications in subtelomeric regions and/or in association with ATRX mutations/downregulation. However, development of ALT may require additional genetic and epigenetic changes, at present largely unknown. Here we have investigated whether ALT develops as a result of the coordination between DNA damage accumulation and chromatin status at telomeres in brain cancers. We used a model of juvenile zebrafish brain tumour based on the conditional expression of human RAS oncogene in brain progenitor cells (Mayrhofer et al. 2017). Zebrafish brain tumours have long and irregular telomeres, undergo sister chromatid exchange and are positive for C-Circles, suggesting that ALT mechanisms are predominant. Following ALT phenotype, DNA damage at telomeres was significantly higher than in control cells. We found that during the progression of tumours from single cancer initiating clone to a full tumour, downregulation of tert through hypomethylation of its promoter precedes the development of ALT and is linked to an increase of Terra expression. To study the contributions of telomerase in the development of ALT, we established the same model in a background of co-overexpressed tert and terc. The tumour generated were similar in location but less aggressive respect tumours without tert/terc overexpression. The analysis of telomere and ALT biomarkers showed an ALT rescue in the function of tert and terc over-expression. We also found a reduction of telomeric DNA damage, downregulation of genes of the pre-replication complex and the status of heterochromatin of telomeres was re-established in telomerase positive brain tumours. We suggest that the activity of telomerase can reduce the replication stress at telomeres by regulation of telomeric heterochromatin. Finally, we characterized telomere maintenance mechanisms in a cohort of 20 human brain tumours, where we measured telomeric DNA damage in association with telomeric chromatin. Besides reporting the first in vivo genetic model of ALT+ brain tumour, this study identifies telomeric maintenance mechanisms as significant drivers of the coordination between DNA replication and chromatin status at telomeres in brain cancers.

Adenocarcinoma-neuroendocrine transition of castration resistant prostate cancer depends on SPARC down-regulation in stromal accessory cells

Claudia Enriqueza, Valeria Cancilab, Renata Ferria, Roberta Sulsentia, Irene Fischettia, Ivano Ariolia, Claudio Tripodob, Maria P. Colomboa and Elena Jachetti

a Molecular Immunology Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, via Amadeo, 42, 20133 Milan, Italy
b Tumor Immunology Unit, Department of Health Sciences, University of Palermo, Italy

Adenocarcinoma-neuroendocrine transition of castration resistant prostate cancer can occur in a relevant subset of patients as a mecha-
Pro-tumorigenic role of ETS-related gene (ERG) in pre-cancerous prostate lesions

Marco Lorenzoni, Alessandro Alaimo, Francesco Cambulli, Veronica Foletto, Sacha Genovesi, Michela Zaffagni, Dario De Felice, Arianna Bertossi, Alessandro Romanel, Mattia Barbareschi, Marianna Kruithof-de Julio, Marco Gaspari and Andrea Lunardi

1 The Armenise-Harvard Laboratory of Cancer Biology & Genetics, Department of Cellular, Computational and Integrative Biology, University of Trento, Italy
2 Laboratory of Bioinformatics and Computational Genomics, Department of Cellular, Computational and Integrative Biology, University of Trento, Italy
3 Unit of Surgical Pathology, Santa Chiara Hospital, Trento, Italy
4 Urology Research Laboratory, Department of Urology and Department of Clinical Research, University of Bern, Bern, Switzerland
5 Department of Clinic and Experimental Medicine, Magna Graecia University of Catanzaro, Italy
6 Present Address: Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, NY, USA
7 Corresponding Author: Andrea Lunardi_andrea.lunardi@unitn.it

Prostate Cancer (PCa) is the second most common cancer in men. While several genomic, genetic and molecular alterations characterizing human PCa have been functionally associated with tumor onset, progression and resistance to therapy, the role of many other molecular events remains still unclear. By combining prostate organoids technology with genetic engineering and CLICK-chemistry coupled Mass Spectrometry approaches, we identified a panel of secreted proteins regulated by ETS-related gene (ERG). Based on the nature of the identified factors, ERG activity in pre-cancerous human prostate lesions may prime prostate cells and the surrounding stroma to create a tolerant and supportive environment during the initial steps of tumorigenesis.

Cross-talk with lung epithelial cells regulates Sfrp2 expression enabling disseminated breast cancer cell latency

Marco Montagner, ChrisTape, Erik Sahai

1 Tumour Cell Biology Laboratory, Francis Crick Institute, London, UK
2 Department. of Molecular Medicine, University of Padua, Padova, Italy
3 University College London, UK

The process of metastasis is highly complex. In the case of breast cancer, there are frequently long timespans between cells leaving the primary tumour and the growth of overt metastases. During this period, cancer cells persist in an indolent or latent state before transitioning back to an aggressive growth. Possible reasons for disease indolence include interplay with myeloid and fibroblastic cells in the tumour microenvironment and ongoing immune surveillance. However, the signals causing actively growing cells to enter into an indolent state, and enabling them to survive for extended periods of time, are not well understood. In this project, we propose that the behavior of indolent breast cancer cells in the lung is determined by their interactions with alveolar epithelial cells. In vivo data, as well as a newly developed lung organotypic system, revealed that lung epithelial cells promote the formation of fibronectin (FN) fibrils by indolent cells that
drive integrin-dependent pro-survival signals. Combined in vivo RNA sequencing and drop-out screening identified Secreted frizzled-related protein 2 (Sfrp2) as a key mediator of this interaction. Sfrp2 is induced by lung epithelial cells and promotes FN fibril formation, integrin activation and survival, while blockade of Sfrp2 expression reduces the burden of indolent disease. Treatment of mice with an integrin-specific inhibitor, reduces the survival of disseminated indolent breast cancer cells in vivo, suggesting FDA-approved integrin-inhibitors as a valuable adjuvant therapies to eradicated these cells and prevent metastasis.

F-actin dynamics regulates mammalian organ growth and cell fate maintenance

Pocaterra et al
J Hepatol 2019

Department of Molecular Medicine - University of Padova

In vitro, cell behavior can be potently regulated by the mechanical properties of cells and of their microenvironment. Cells sense these features through integrin receptors and focal adhesions and counteract external forces by developing internal pulling forces via their actomyosin cytoskeleton, in turns regulating intracellular pathways, including the transcriptional coactivators YAP/TAZ. Whether mechanical cues are relevant for in vivo regulation of adult organ homeostasis, and whether this occurs through YAP/TAZ, remains largely unaddressed. We developed Capzb conditional knockout mice and obtained primary fibroblasts to characterize the role of Capzb in vitro. In vivo, functional analyses were carried out by inducing Capzb inactivation in adult hepatocytes. We found that the F-actin capping protein CAPZ restrains actomyosin contractility: Capzb inactivation alters stress fiber and focal adhesion dynamics leading to enhanced myosin activity, increased traction forces, and increased liver stiffness. In vitro, this rescues YAP from inhibition by a small cellular geometry; in vivo, it induces YAP activation in parallel to the Hippo pathway, causing extensive hepatocyte proliferation and leading to striking organ overgrowth. Moreover, Capzb is required for the maintenance of the differentiated hepatocyte state, for metabolic zonation, and for gluconeogenesis. In keeping with changes in tissue mechanics, inhibition of the contractility regulator ROCK, or deletion of the Yap1 mechanotransducer, reverse the phenotypes emerging in Capzb-null livers. These results indicate a previously unsuspected role for CAPZ in tuning the mechanical properties of cells and tissues, providing a missing genetic evidence for mechanical properties as potent and specific regulator of liver organ growth, cell-fate and tissue metabolism in vivo. More generally, it indicates for the first time that mechanotransduction has a physiological role in maintaining liver homeostasis in mammals. Our genetic system will thus open the possibility for testing, in the future, the effective and functional role of mechanotransduction in multiple other tissues and in altered context, such as cancer development.

Histone chaperone ANP32E as a proto-oncogenic factor in MYC-driven tumorigenesis

Vittoria Poli1, Luca Fagnocchi1, Alessandra Fasciani1, Miriam Gaggianesi2, Alice Turdo3, Matilde Todaro3, Alessio Zippo1

1 Laboratory of Chromatin Biology & Epigenetics, Center for Integrative Biology (CIBIO), University of Trento, 38123, Trento, Italy.
2 Department of Surgical Oncological and Stomatological Sciences, University of Palermo, Palermo, 90127, Italy.
3 DiBIMIS, University of Palermo, Palermo, 90127, Italy.

Breast cancer consists of highly heterogeneous tumors, whose driver oncogenes result difficult to be uniquely defined. We have recently reported the central role of MYC in initiating and sustaining a step-wise epigenetic reprogramming process in mammary luminal epithelial cells (IMEC) toward a stem cell-like condition, which favors cell transformation and tumor initiation. Among the chromatin players that may synergize with MYC, we identified the H2A.Z-specific chaperone ANP32E, whose expression is induced in MYC-transformed IMEC (t-IMEC). Interestingly, analysis of the TCGA dataset showed that ANP32E alteration is specifically enriched among basal-like breast cancers characterized by MYC deregulation and this combination correlates with a worst prognosis. These observations suggested a possible cooperation between MYC and ANP32E in tumor formation and maintenance. Of note, ANP32E overexpression in t-IMEC was associated with increased tumorigenic potential both in vitro and in vivo. Considering that MYC overexpression is cause of replication stress (RS) and that ANP32E-mediated eviction of H2A.Z is required for DNA-damage repair (DDR), we investigated whether their simultaneous deregulation could further impact on RS and result in DNA damage accumulation. Of note, t-IMEC overexpressing ANP32E (t-IMEC-ANP32E) are characterized by increment of transcriptional R-Loops and high-
Bone marrow hematopoietic adaptation to distant breast cancer begins early in transformation in association with deregulated circulating microRNAs

Tiziana Ada Renzi1, Claudia Chiodoni1, Valeria Cancila2, Milena Perrone3, Andrea M. Tomirotti1, Sabina Sangaletti1, Laura Botti1, Matteo Dugo1, Matteo Milani1, Lucia Bongiovanni2, Maurizio Marrale4, Claudio Tripodo2 and Mario P. Colombo1

1 Molecular Immunology Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.
2 Tumor Immunology Unit, Department of Health Sciences, Human Pathology Section, University of Palermo School of Medicine, Palermo, Italy.
3 Platform of Integrated Biology - Bioinformatics, Department of Applied Research and Technology Development, Fondazione IRCCS Istituto Nazionale dei Tumori Milan, Italy.
4 Department of Physics and Chemistry, University of Palermo, Palermo, Italy.

During tumorigenesis, newly transformed cells initiate an active cross-talk with bystander cells, mostly of bone marrow (BM) origin, to establish a pro-tumorigenic microenvironment. We hypothesized that signs of this cross-talk can be identified in the BM at the very early phases of cancer development, being finalized to the instruction of a tumor-promoting hematopoiesis. In the MMTV-NeuT model of spontaneous mammary carcinoma we showed that gene expression profiling of the bone marrow along disease progression indicates modifications in the hematopoietic compartment already at early disease stages that become more relevant with tumor progression. The transcriptional profile of the adapted hematopoiesis revealed the induction of programs related with innate immunity and responses to danger signals, alongside the down-modulation of adaptive immune response. These transcriptional reprogramming is paralleled by an expansion of the myeloid populations at the expense of erythroid and B lymphoid fractions. The finding of such modifications in the BM microarchitecture even at earlier stages of cancer development (high-grade dysplasia/in situ cancer stage) provided the first evidence of the BM acting as a very early sensor of peripheral transformation. Moreover, we profiled plasmatic microRNAs at late and early stages of tumor progression and found, already at early time points, differentially expressed microRNAs, which could potentially be used as early biomarkers of cancerogenesis. In conclusion, our data lay a first demonstration that BM hematopoietic adaptation to cancer is not confined to a general immunosuppressive state associated with advanced cancers, rather it represents an early
Anti-CSPG4 DNA vaccination reveals potential therapeutic effects for the treatment of CSPG4+ tumors: a comparative oncology study

Federica Riccardo1, Giuseppina Barutello1, Lidia Tarone1, Maddalena Arigon1, Davide Giacobino2, Selina Iussich2, Sergio Occhipinti2, Angela Petito2, Soldano Ferrone4, Paolo Buracco2, Federica Cavallo1

1 Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Torino, Torino, Italy
2 Department of Veterinary Sciences, University of Torino, Grugliasco, Italy
3 Center for Experimental Research and Medical Studies (CERMS), Città della Scienza di Torino, Torino, Italy
4 Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Among the most interesting targets for immunotherapeutic approaches, the Chondroitin Sulfate Proteoglycan (CSPG4) stands out, with low expression in healthy tissues, high expression in several solid tumors and a key role in cancer progression. Because of the translational power of dogs as pre-clinical models for human malignancies and the CSPG4 over-expression by both human and canine malignant melanoma (MM), we demonstrated the safety and the clinical effectiveness of a xenogeneic human (Hu)-CSPG4 DNA vaccine in client-owned canine patients with stage II-III surgically resected CSPG4+ MM. However, Hu-CSPG4 vaccine was barely effective in activating human T cells from healthy donors in vitro. Based on these results, we aimed to increase the translational power of our approach and to extend it for the treatment of other CSPG4+ tumors besides MM.

As a step forward in this direction, we generated a hybrid plasmid, derived in part from the dog (Do)-CSPG4 sequences and in part from the human (Hu)-CSPG4, strongly immunogenic in mice. In canine patients, the procedure was safe and induced antibodies against both Hu- and Do-CSPG4, with a higher affinity and anti-tumor potential as compared to Hu-CSPG4. Clinically, HuDo-CSPG4 was effective in increasing the overall survival of vaccinated canine MM patients as compared to controls. Data obtained in vitro with T cells from human healthy donors suggested HuDo-CSPG4 is more immunogenic than Hu-CSPG4. Moreover, we started to investigate CSPG4 role in both canine and human osteosarcoma (OSA) to eventually propose CSPG4 DNA vaccination as an innovative comparative therapy for OSA treatment, too. We found a strong correlation between CSPG4 over-expression and a worse prognosis in both human and canine OSA patients. The potentiality of CSPG4 immune-targeting for OSA treatment was demonstrated by the ability of anti-CSPG4 monoclonal antibodies (mAbs) to significantly inhibit both canine and human CSPG4+ OSA proliferation, migration and osteospheres generation. In addition, anti-CSPG4 mAbs potentiated the anti-proliferative effect of doxorubicin. Interestingly, sera derived from canine MM patients enrolled in our previous veterinary trials, were also able to inhibit OSA cells tumorigenic potential in both adherent and non-adherent conditions.

Overall, these results provide the rationale to propose HuDo-CSPG4 vaccination for the treatment of canine CSPG4+ tumors, to be successfully translated in a human setting.

A novel integrative strategy to prevent colorectal cancer within the diet-host-microbiota triangle: from organoids to human in vivo reality

Josep Rubert, Andrea Lunardi

CIBIO - Department of Cellular, Computational and Integrative Biology. University of Trento (Italy). Via Sommarive 9, 38123 Povo (Trento)

Colorectal cancer (CRC) is one of the most common cancers in the western world. Several hundreds of thousands people are diagnosed annually with CRC and over half of patients die or have comorbidities. Research has suggested that dietary patterns, dysbiosis and microbial metabolites may play a pivotal role in, leading to increasing interest among scientists. However, despite the fact that microbial metabolites play a crucial role in many biological cases, adequate tools for deciphering the relationship between diet-microbiome-host are not yet available. TRIANGLE aims to provide new insight into the mechanisms by which microbial metabolites may prevent CRC. The first objective is targeted at designing in vitro models mimicking human organogenesis and tumorigenesis to evaluate the role of microbial metabolites. To accomplish this, human colon organoids/tumoroids will be established. The second objective is to identify microbial metabolites that can act as cancer-preventive agents. These
metabolites will be produced with a gastrointestinal model inoculated with faeces from both healthy and CRC patients and determined by mass spectrometry. Released microbial metabolites from a food model will then be tested in the colon organoids/tumoroids, and metabolite signatures of organoids will be studied. Metabolomics analysis and 3D cell assays of colon organoids/tumoroids will provide valuable new insights into the mechanisms by which nutrient-gene interactions influence colon stem cell niche and CRC, and will open up new possibilities for CRC understanding and prevention. In summary, the results from TRIANGLE through the integration of microbial metabolites (diet), gut microbiota and colon organoids (host), will be highly multidisciplinary, and will undoubtedly set the stage towards the identification of mechanisms contributing to CRC understanding and will ultimately pave the way to phytochemical use and prevention.

Acknowledgement
This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement N. 794417.

A screen for drugs that affect brain tumor development and microglia recruitment in zebrafish

Valeria Savoca1, Isabella Pesce1, Dirk Sieger2 and Marina Mione1

1 Centre for Integrative Biology, University of Trento, Via Sommarive, 9, Trento 38123, Italy
2 Centre for Discovery Brain Sciences, The University of Edinburgh, The Chancellor’s Building, 49 Little France Crescent, Edinburgh EH16 4SB, United Kingdom

One of the hallmarks of brain cancer is the formation of an immune-microenvironment infiltrated with tumor associated macrophages (TAMs), which contribute to establish a status of chronic inflammation. Thus, the production of high amount of immune inhibitory cytokines or inflammatory mediators leads to an increase in tumor cells proliferation and invasion. The most frequent and aggressive form of brain tumor, glioblastoma multiforme (GBM), is resistant to standard of care therapies because of its heterogeneity, infiltration properties and immune suppressive microenvironment, therefore innovative therapies are being investigated, among which immunotherapies, whose aim is to alleviate GBM-associated immune suppression and to boost anti-tumor immune responses. In this project we use a zebrafish model of brain tumor to investigate the immune microenvironment of healthy and tumoral brains and the effect of compound treatments both on brain tumor development and immune cells, mainly microglia. Leukocytes, including macrophages and microglia are normally present in the brain at homeostatic conditions at larval and adult stages. Both in the larval and adult tumor model an increase in the number of immune cells in the region expressing the HRAS oncogene, together with an increased percentage of amoeboid-like active microglia was observed.

Given that an altered epigenetic landscape is very common in brain tumors, we have screened zebrafish larvae with a library of compounds targeting epigenetic factors and, as a result, 3 hits were found to have an effect in slowing down the development of brain tumors, but only one, an HDAC inhibitor, led to changes in microglia morphology. In order to characterize gene expression changes in tumor cells after treatment with the HDACi, we have optimized the TRAP (Translating Ribosomes Affinity Purification) technique to specifically pull down translating ribosomes in the cytoplasm of oncogene-expressing brain cells. The translatome of HDACi treated tumor cells was compared with the transcriptome from FACS sorted zebrafish brain tumor cells; from the analysis of the differentially translated or transcribed genes, the translatome emerged as more precisely reflecting the biological phenotype that had been observed after HDACi treatment. Pathway analysis of the differentially translated genes revealed that the drug treatment caused the down-regulation of many factors involved in purine metabolism, which could explain the decrease in brain tumor growth in the zebrafish model treated with HDACi.

Taken together, these results reinforce the knowledge that the zebrafish provides a useful model to investigate the effects of HDACi in brain tumors and associated microglia.

miRNAs as potential predictive biomarkers of metastases in thin and thick primary cutaneous melanomas

Virginia Valentini1, Veronica Zelli1, Emanuela Gaggiano2, Valentina Silvestri1, Piera Rizzolo1, Agostino BucaI1, Stefano Calvieri2, Pasquale Frascione2, Pietro Donati2, Antonio Giovanni Richetta2 and Laura Ottini1

1 Department of Molecular Medicine, "Sapienza" University of Rome, Rome, Italy;
2 Department of Internal Medicine and Medical Specialties, Unit of Dermatology, “Sapienza" University of Rome, Rome, Italy;
3 Department of Oncological and Preventative Dermatological, San Gallicano Dermatological
Institute, IRCCS, Rome, Italy; 4 Laboratory of Cutaneous Histopathology, San Gallicano Dermatologic Institute, Rome, Italy

Background: The early detection of primary cutaneous melanomas at high-risk of metastatic dissemination is essential to improve clinical management and outcomes of melanoma patients. The high potential of miRNAs as diagnostic, prognostic and therapeutic biomarkers in melanoma is well established. The aim of this study was to characterize miRNAs expression profile in relation to metastatic process of cutaneous melanoma and correlate miRNAs expression with clinical and pathological factors.

Materials and Method: Expression levels of six miRNAs, known to be involved in metastatic process (miR-145-5p, miR-150-5p, miR-182-5p, miR-203-3p, miR-205-5p and miR-211-5p), were analyzed by quantitative Real-Time PCR in a series of 32 metastatic and non-metastatic primary cutaneous melanomas, including thin and thick melanomas. Eight samples of metastases were also examined. Associations between miRNA expression levels and clinical-pathologic characteristics of primary tumors were also evaluated. All statistical analyses were performed with the R software (www.r-project.org.).

Results: A lower miR-205-5p expression was observed in metastases when compared with primary metastatic melanomas (p=0.04). Furthermore, a progressive downregulation of miR-205-5p expression was observed from loco-regional metastasis to distant metastasis. Significantly lower miR-145-5p and miR-203-3p expression levels were found in cases with Breslow thickness >1mm (p = 0.002 and p = 0.005, respectively), high Clark level (p = 0.007 and p = 0.005, respectively), ulceration (p = 0.00001 and p = 0.0002, respectively) and mitotic rate ≥1/mm² (p = 0.02 and p = 0.001 respectively).

Conclusion: Our findings add insights into the characterization of miRNAs expression profile of thin and thick melanomas, pointing to miR-205-5p as potential marker of distant metastases and to miR-145-5p and miR-203-3p as markers of aggressiveness in primary tumors.

Study supported by AIRC-IG21389 to L.O.

Polyunsaturated fatty acids reduces in vitro tumor growth of colorectal cancer patient-derived organoids

Vara-Messler M.1, Di Blasio L.2, Monica V.3, Somale D.2, Chiaverina G.1, Puliafito A.1, Palmiero M.1, Peracino B.3, Primo L.1

1 Department of Oncology at Candiolo IRCCS. University of Torino, Torino, Italy.

Posters
Pezcoller Foundation–AACR International Award for Extraordinary Achievement in Cancer Research

2019 Program guidelines and nomination instructions

NOMINATION DEADLINE
August 1, 2019

NOMINATION PROCESS
Nominations may be submitted by any individual, whether an AACR member or nonmember, who is currently or has previously been affiliated with any institution involved in cancer research, cancer medicine, or cancer-related biomedical science. Self-nominations are prohibited. Nominators must maintain strict confidentiality of their nominations and all nominations must be submitted online to myaacr.aacr.org. Paper nominations will not be accepted. Eligible nominations must include the following nomination materials:

• A letter of recommendation written in English (Max: 1,000 words) that comprehensively describes the nominee’s major scientific achievement(s) in basic cancer research and/or their significant contributions to translational cancer research. This letter must also outline the nominee’s current research activity and indicate how this research holds promise for continued substantive contributions to the cancer field.

• A brief scientific citation (Max: 50 words) highlighting the major scientific contribution(s) justifying the award candidate’s nomination.

AWARD ELIGIBILITY AND CRITERIA
The prestigious Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research was established in 1997 to recognize a scientist of international renown who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research. Eligible candidates must continue to be active in cancer research, have a record of recent, noteworthy publications, and be conducting ongoing work that holds promise for continued substantive contributions to progress in the field of cancer. The Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the award in the event that their investigations are intimately related in subject matter and have resulted in work that is worthy of the award and a joint nomination. Cancer researchers affiliated with institutions in academia, industry, or government involved in cancer research, medicine, or cancer-related biomedical science anywhere in the world are eligible. Institutions and/or organizations are not eligible to receive the award.

AWARD SELECTION PROCESS
All eligible nominees will be considered by a prestigious Pezcoller Foundation-AACR International
Award for Extraordinary Achievement in Cancer Research Selection Committee consisting of an international cohort of renowned cancer leaders appointed by the AACR President in consultation with the Pezcoller Foundation Council.

The Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research Selection Committee will consider all nominations as they have been submitted and are restricted from combining submitted nominations, adding new nominees, or otherwise making alterations to any submitted nomination.

Once chosen, the primary and alternate award recipient selections made by the Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research Selection Committee shall be sent to the AACR Executive Committee and the Pezcoller Foundation Council for final consideration and ratification.

Selection of the award winner will be made on the basis of the candidate’s scientific accomplishments. No regard shall be given to race, gender, nationality, religion or political preference.

Selected award recipients will receive an unrestricted grant of €75,000, a commemorative award, and be invited to present a scientific lecture in conjunction with the AACR Annual Meeting immediately following their selection.

THE AWARD RECIPIENT
The winner of the Pezcoller Foundation-AACR International Award for Extraordinary Achievement in Cancer Research will present an Award lecture at the AACR Annual Meeting 2020 in San Diego, CA (April 24-29, 2020). The winner will also present the Twenty-third Annual Pezcoller Foundation-AACR International Award for Cancer Research Lecture, just prior to the official Award ceremony to be held in Trento, Italy in May 2020. Should the recipient be unable to participate in either event, the award must be forfeited and will instead be presented to the selected Award alternate.

In the rare event that there are dual winners of the Award, the monetary award will be shared equally between both recipients while the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.
The AACR has been proud to offer Scholar-in-Training Awards to enable the participation of meritorious early-career scientists at the Annual Meeting 2019. Since its inception in 1986, the AACR Annual Meeting Scholar-in-Training Award program has provided more than 4,580 grants to young investigators and has received support from more than 55 cancer research foundations, corporations, individuals and other organizations dedicated to the fight against cancer. Scholar-in-Training Awards are highly competitive and recognize outstanding young investigators presenting meritorious proffered papers at the AACR Annual Meeting.

2020 AACR-Pezcoller Foundation Scholar-in-Training Awards
The Pezcoller Foundation supports these awards to enhance participation in the programs and activities of the AACR by early-career investigators residing in Europe and to provide these outstanding Scholar-in-Training Awardees with an opportunity to share their research findings with the international cancer research community at the AACR Annual Meeting.

Selections are made by the criteria from Pezcoller (i.e. European scientists with at least one awardee representing Italy) and based on the meritorious score of the submitted abstract and application. Any questions can be directed to sita@aacr.org

Picture:
2019 Scholar-In-Training Awardees with President Gallignoni in Atlanta, March 31, 2019