The Pezcoller Foundation

Journal

Summary

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Editorial

It's a great pleasure to report that the recipient of the 2016 Pezcoller Foundation-AACR International Award for Cancer Research is Joan Massagué Ph.D., Director, Sloan Kettering Institute, Alfred P. Sloan Chair Memorial Sloan Kettering Cancer Center, New York, NY.

The Selection Committee met in Philadelphia on November 30th, 2015 and was chaired by Hans Clevers, MD, PhD, FAACR, professor of Molecular Genetics at the Hubrecht Institute, Utrecht, NL. The other members of the Committee were: Eduard Batlle, ICREA Research professor, Istitute for Research in Biomedicine, Barcelona, E – Silvia Giordano, MD, Full Professor University of Turin, Candiolo, Italy – Jedd Walchok, MD, PhD, Lloyd J Old/ Ludwig Cjair in Clinical Investigation, Memorial Sloan Kettering Cancer Center, New York, NY - Caroline Dive PhD, Deputy Director & CEP Group Leader CRUK Manchester Institute, Manchester, UK – Bradley E. Bernstein, MD, PhD, Associate Professor Depatment of Pathology Massachusetts General Hospital,. Boston, MA - William C. Hahn, MD, PhD Chief Division of Molecular and Cellular Oncology, Dana Farber Cancer Institute, Boston MA – Matthew G. Vander Heiden, Md,PhD, Associate Professor of Biology MIT Koch Institute for integrated Cancer Research, Cambridge, MA.

Dr. Joan Massagué was recommended as the recipient of the Award for his seminal discoveries in TGF-ß biology, now considered fundamental to our understanding of cellular physiology. His pioneering efforts were the first to delineate the TGF-ßsignaling pathway and its mechanism of action from receptor activation to the regulation of key target genes. Furthermore Dr. Massagué's studies demonstrated how TGF-ß can be both a growth suppressor and promoter of metastasis.
Dr. Massagué's stellar work has illuminated vital aspects of developmental biology, tissue homeostasis, and cancer metastasis.
Dr. Massagué has been recognized with myriad honors throughout his career, including the 2009 AACR G.H.A. Clowes Memorial Award, the 2008 AACR Distinguished Lectureship in Breast Cancer Research, the Pasarow Prize, and the Frontiers Prize in Biomedicine from the BBVA Foundation, and elected membership to the U.S. National Academy of Sciences and National Academy of Medicine, and the Spanish Royal Academies of Medicine and of Pharmacy.

Dr. Massagué received his doctorate from the University of Barcelona in his native Spain and completed a postdoctoral fellowship at Brown University in Providence, Rhode Island. Before joining the faculty at Memorial Sloan Kettering in 1989, he was an associate professor of biochemistry at the University of Massachusetts Medical School.

Dr. Massagué has been an active member of the AACR since 1990. He served on the AACR Board of Directors (2009-2012) and is currently a scientific editor of Cancer Discovery.

Dr. Massagué has presented the Pezcoller Lecture, "Latent Metastasis," Sunday, April 17, 5:30 p.m., in New Orleans Theater B of the Ernest N. Memorial Convention Center. Afterwards the award was solemnly given in the prestigious hall of the Buonconsiglio Castle in Trento on May 13. In this occasion Joan Massagué was introduced by Jose Baselga, 2015-2016 President of AACR.
Greetings from the American Association of Cancer Research were given by Patrice Morin, PhD, Senior Director, Scientific Review and Grants Administration.

The day before the ceremony in Trento Dr. Massagué gave a lecture at Ci.Bio of the University of Trento. The title was "TGF-ß signaling from Cytostasis to Metastasis". He related that the effects of the TGF-ß signal transduction pathway depend on the cellular context. For example, TGF-ß mediates differentiation in stem cells, cell cycle arrest in epithelial progenitor cells, death in pre-malignant cells, and metastasis in carcinoma cells. We delineated the TGF-ß signaling pathway and investigated the molecular basis for its contextual action. TGF-ß activates Smad transcription factors that regulate master differentiation genes in embryonic stem cells, and mediate cytostasis through CDK inhibitors in epithelial progenitor cells. However, in pre-malignant pancreatic cells, TGF-ß induces an epithelial identity program and, simultaneously, an epithelial-to-mesenchymal transition. This

contradictory combination is lethal, triggering death of the pre-malignant cells by apoptosis. Carcinoma progression requires the loss of this tumor suppressor effect, which can occur through mutation of Smad4 or other means. With this, carcinoma cells can repurpose TGF-β signaling for metastatic dissemination through various mechanisms. These insights shed light on how TGF-β action is switched from cytostatic to pro-apoptotic to pro-metastatic during tumor evolution, and sheds light on TGF-β as a key mediator of normal physiology and disease, and as a potential target for therapy."

This issue of our Journal is dedicated to the 28th Pezcoller Symposium entitled "Initial Steps on the Route to Tumorigenesis" to be held in Trento from June 20 to June 21, 2016 co-chaired by David Livingston, Mariano Barbacid, Alberto Bardelli, Massimo Loda, Enrico Mihich, Stefano Piccolo, Eugenia Piddini.
This Symposium will focus on current knowledge of what molecular and biological steps must be taken for a normal cell in a given organ to acquire neoplastic potential and, eventually, full malignancy. From recent work of multiple speakers, it is clear that permission to enter a pathway to tumorigenesis requires cells to pass through multiple physiological 'gates'. The nature of these control events and how to detect whether or not future cancer cells have successfully overcome them will be prime foci of this meeting. Ideally, an ever richer and more accurate understanding of these steps could lead to rational, new approaches to molecular cancer prevention.

Session I, Tumor Cell communication, competition and Immunogenicity chaired by Eugenia Piddini, The Gurdon Institute, University of Cambridge, United Kingdom
Session II, Epigenetics and Cell Plasticity, chaired by Stefano Piccolo, University of Padova, School of Medicine, Italy
Session III, Early Steps in Cancer Development, chaired by Mariano Barbacid, Centro Nacional de Investigaciones Oncologicas, Madrid, Spain
Session IV, New Approaches to Detecting Early Steps in Cancer Development, chaired by Massimo Loda, Dana Farber Cancer Institute, Boston, MA

The invited participants are:
Barbacid Mariano, Centro Nacional del Invetsigaciones Oncologicas, Madrid, Spain
Batlle Eduardo, Institute for Research in Biomedicine, Barcelona, Spain
Bardelli Alberto, Institute for Cancer Research And Treatment, University of Turin, Italy
Davoli Teresa, Harvard Medical School, Boston, MA
Dranoff Glenn, Novartis Institute for Biomedical Research, Cambridge, MA
Benjamin Ebert, Brigham and Women's Hospital, Boston, MA
Esteller Badosa Manel, DIBELL Department of Cellular Medicine, Barcelona, Spain
Freedman Matthew, Dana Farber Cancer Institute, Boston, MA
Hich Marjtxell, The Gurdon Institute, University of Cambridge, UK
Knoblich Jürgen, IMBA Institute of Molecular Biotechnology, Vienna, Austria
Livingston David, Dana Farber Cancer Institute, Boston, MA
Loda Massimo, Dana Farber Cancer Institute, Boston, MA
Marais Richard, Cancer Research Manchester Institute, Manchester, UK
Moreno Eduardo, University of Bern, Switzerland
Moscat Jorge, Sanford-Burnham Medical Discovery Institute, La Jolla, CA
Piccolo Stefano, University of Padova, School of Medicine, Dept of Molecular Medicine, Italy
Piddini Eugenia, The Gurdon Institute, University of Cambridge, UK
Ponder Sir Bruce, Cambridge Cancer Center, Cambridge, UK
Rodewald Hans Reimer, Deutsche Krebsforschungszentrum, Heidelberg, Germany
Van de Watering Marc, Hubrecht Institute, Utrecht, The Nederland
Wolpin Brian, Dana Farber Cancer Institute, Boston, MA
Yilmaz Omer Hidir, The David Koch Institute, Cambridge, MA
Vogelstein, Bert, The Johns Hopkins University, Baltimore, MD

The abstracts of the symposium are in the following pages.

Gios Bernardi M.D.
Editor and President Emeritus

Picture on front page: 2016 Pezcoller Foundation-AACR International Award for Cancer Research
From the left Gios Bernardi, President Emeritus – Davide Bassi, President – Joan Massagué, winner – José Baselga, President AACR – Cristina Massa, interpreter – Patrice Morin, AACR Director of Awards and Grants

28th Pezcoller Symposium

Initial steps on the route to tomorigenesis

Trento, Italy, June 20-21, 2016

ABSTRACTS OF ORAL PRESENTATIONS

Mechanisms of Protective Tumor Immunity

Glenn Dranoff, M.D.
Novartis Institutes for Biomedical Research, Cambridge, MA

The cytokine granulocyte-macrophage colony stimulating factor (GM-CSF) enhances anti-tumor CD4+ and CD8+ anti-tumor effector T cell responses and is a key component of Provenge and TVEC, two recently FDA approved immunotherapies. However, analysis of mice rendered deficient in GM-CSF established an additional critical role for the cytokine in immune homeostasis through the maintenance of FoxP3+ regulatory T cells (Tregs). These opposing activities complicate the therapeutic application of the cytokine in cancer treatment, as potent anti-tumor immunity is associated with a high ratio of tumor infiltrating T effectors to Tregs. The impact of GM-CSF on immune responses is mediated through myeloid cells, particularly dendritic cells and monocyte/macrophages. To learn more about the mechanisms underlying the dual roles of GM-CSF in immunity, we dissected downstream signaling pathways. Our investigations uncovered unexpected roles for peroxisome proliferator-activated receptor gamma (PPAR-g) and mTOR in modulating the ability of GM-CSF stimulated myeloid cells to induce T effectors and Tregs. Small molecules targeting these proteins augmented T effectors and attenuated Tregs, which resulted in increased anti-tumor effects in vivo. These studies highlight the complex role of GM-CSF in immunity and illustrate the potential for combining targeted agents with immunotherapy to improve tumor control.

Cell competition, progenitor turnover, and t cell acute lymphoblastic leukemia

Hans-Reimer Rodewald, Katrin Busch, Vera Martins, Csilla Lengyl, and Thorsten Feyerabend.
Division of Cellular Immunology, German Cancer Research Center, Heidelberg, Germany

Fate mapping experiments have delineated the flux emanating from hematopoietic stem cells and the routes leading to myeloid and lymphoid lineages in vivo. However, the pathways of progenitors from the bone marrow into the thymus remain poorly understood. Thymus grafting experiments have shown that the thymus is constantly, or at least frequently and periodically, colonized by new T cell progenitors arriving from the bone marrow. Under normal conditions, intrathymic T cell progenitors have short life spans (in the order of days), and the short residency in the thymus of each wave of progenitors was considered a cell intrinsic property. Using a series of mouse mutants and thymus grafting experiments, we find that the life span of T cell progenitors in the thymus is regulated, at least in part, in trans by a process termed cell competition. 'Young' competing progenitors entering the thymus from the bone morrow constantly replace 'old' thymus-resident progenitors. In contrast, in the absence of competing progenitors from the bone morrow, thymus-resident progenitors persist, demonstrating that the life span of T cell progenitors is regulated at least partially by cell competition between young and old cells. By comparison of competitive versus non-competitive states, key roles for interleukin 7 receptor (IL7R) signal

'utilization' were revealed; IL7R signaling provides a stronger anti-apoptotic state to young compared to old progenitors. We also identified cell competition-responsive gene expression in T cell progenitors. Long-term disruption of progenitor replacement leads to progenitor import-independent productive T cell development, a process termed thymus autonomy. Autonomous T cell development is maintained by self-renewal of intrathymic progenitors, and this state frequently (in greater than 50% of the cases) leads to the emergence of T cell acute lymphoblastic leukemia (T-ALL). This model of murine T-ALL bears clinical, pathological, cellular and molecular hallmarks of the human disease, notably activating mutations in *Notch1*. We are developing a new mouse model to visualize the emergence of T-ALL-characteristic *Notch1* mutations in living cells during leukemogenesis in vivo. Collectively, cell competition is a tumor suppressor mechanism in the thymus and its disruption drives T cell progenitors into leukemogenesis. Cell competition may also more generally play a role in the development of cancer in other tissues that are maintained by stem and progenitors cells, such as epithelia in the skin and the gut. In this model of tumorigenesis, when competition is interrupted, extended life span of progenitors leads to the acquisition of mutations which may initially be selecting for cells with highest self-renewal capacity to maintain tissue integrity. This process eventually also predisposes cells for malignant transformation.

The emerging role of cell competition in cancer formation from Drosophila studies

Eugenia Piddini
The Gurdon Institute - University of Cambridge, UK

Tumour-host interactions play an increasingly recognized role in modulating tumour growth. Thus, understanding the nature and impact of this complex bidirectional communication is key to identify novel successful anti-cancer strategies.

It has been suggested that tumour cells might compete with and kill neighbouring host tissue and that this event might be important for tumour cells to clear space, fuelling their expansion. Our recent work using *Drosophila* intestinal tumour models indeed shows that tumor cells compete with and kill host cells. Surprisingly, we observe that preventing this process is sufficient to block tumour growth. Overall our data suggest a model whereby, by generating an environment permissive for tumour growth, cell competition acts as a key driver of tumourigenesis, providing a novel angle to counter tumour expansion.

Regulating the cellular composition of our bodies using fitness fingerprints

Eduardo Moreno
University of Bern

Humans are able to detect fitness decay in other colleagues by looking at the graying of the hair or the wrinkles in their faces. Work from my laboratory in the last few years has shown that cells can also detect fitness levels of neighboring cells using a molecular code. Those "fitness fingerprints" can be used to mediate cell selection by recognizing and eliminating less fit cells during ageing (Merino et al., Cell, 2015) and cancer (Levayer et al., Nature 2015).

Cross-talks at the tumor microenvironment by autophagy adapters

Jorge Moscat
Cancer Metabolism and Signaling Networks Program, SBP NCI-Cancer Center, La Jolla, California 92037, USA

Hepatocellular carcinoma (HCC) is the most common form of human primary liver cancer and is considered the third leading cause of cancer death in the world. More than 28,000 different somatic mutations have been identified in HCC, which makes the design of better therapeutic strategies based on cancer-linked genetic alterations extremely challenging. However, inflammation and metabolic stress caused by components of the tumor epithelium can result in non-genetic vulnerabilities that can be potentially exploited for the design of innovative therapeutic approaches. In most cases, HCC develops in conjunction with chronic hepatitis, fibrosis, cirrhosis, inflammation and stromal activation, being the key mediators of a hepatic microenvironment conducive to tumorigenesis. Therefore, HCC is a prototypic example of the importance of inflammation and the tumor microenvironment in cancer initiation and progression. p62/SQSTM1 is autophagy substrate and signaling protein that accumulates in premalignant liver diseases and HCC. Although p62 was proposed to participate in formation of benign adenomas in autopha-

gy-deficient livers, its role in HCC initiation was not explored. Here we show that parenchymal p62 is necessary and sufficient for HCC induction in mice and that its high expression level in non-tumor human liver predicts rapid HCC recurrence after curative ablation. High p62 expression is needed for activation of NRF2 and mTORC1, c-Myc induction and protection of HCC-initiating cells from oxidative stress-induced death. On the other hand, we have recently identified that p62 is downregulated in the stroma of several types of tumors, including HCC and prostate cancer. Results highlighting the dual role of p62 as a tumor promoter in the epithelium and as tumor suppressor in the microenvironment will be presented, and its potential use of this duality for the design of new anti-cancer therapies will be discussed.

Cancer Epigenomics: From Knowledge to Applications

Manel Esteller
Institut d'Investigatiò Biomedica de Bellvitge Barcelona, Spain

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of *MGMT* and *GSTP1* hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasys in neoplasia, but without forgetting the novel advances in other human disorders. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic or genetic drugs.

Using Drosophila neuroblasts as a model for stem cell biology and tumorigenesis

Jürgen A. Knoblich
IMBA Institute, Vienna, Austria

Stem cells are of crucial importance for tissue development, maintenance and regeneration in our body. They also play a critical role in tumor formation and growth. We are using Drosophila neuroblasts as a model system to understand how stem cell proliferation is controlled and how defects in the control mechanisms can lead to tumor formation.
Drosophila neuroblasts undergo repeated rounds of asymmetric cell division, generating one neuroblast and one more differentiated cell. The asymmetric segregation of the cell fate determinants Numb, Prospero and Brat is responsible for establishing distinct fates in the two daughter cells. When any of those determinants are mutated, neuroblasts overproliferate, leading to the formation of lethal transplantable brain tumors. We have used Drosophila to identify new tumor suppressors that have helped us to characterize the molecular mechanisms leading to stem cell immortalization in tumors at a level of detail currently not possible in vertebrate models.

Connecting Intestinal Stem Cells to Colorectal Cancer

Eduard Batlle
ICREA & Oncology Program. Institute for Research in Biomedicine (IRB Barcelona). The Barcelona Institute of Science and Technology. Barcelona. Spain

The inner layer of the intestinal tube, the intestinal epithelium, is in a constant process of renewal. Hundreds of millions of terminally differentiated intestinal cells are replaced by new cells every day during the life of an adult organism. This tremendous regenerative power is ultimately sustained by a small population of intestinal stem cells. It is believed that alterations in the biology of ISCs account for the pathophysiology of various large-bowel disorders, including colorectal cancer (CRC). We discovered that most human CRCs are constituted by cell populations with

phenotypes similar to either colon stem cells or differentiated cells organized into well-defined compartments. We showed that CRCs shares a common cell hierarchy with the intestinal mucosa and the acquisition of an intestinal stem cell gene program is a central process during disease relapse after therapeutic intervention. Here I will present our latest data on the mechanisms employed by CRC stem cells to regenerate a new tumor at the metastatic site. We have discovered that metastasis relies on a tumor cell non-autonomous program expressed in the microenvironment. This stromal program confers a survival advantage to the disseminated CRC stem cells during the initial phase of metastasis.

The Mutations Responsible for Human Tumorigenesis

Bert Vogelstein
Ludwig Center and Howard Hughes Medical Institute at Sidney Kimmel-Johns Hopkins Cancer Center, Baltimore, MD

Now that most of the genetic alterations responsible for human tumorigenesis have been discovered, several questions can be addressed in more informed ways than previously possible. One major question concerns the origins of these mutations: are they the result of environmental, inherited, or naturally-occurring, replicative processes? A second fundamental question concerns the number of driver gene mutations: how many are required to convert a normal cell to a malignant cell capable of lethal, widespread metastasis? A third concerns genetic heterogeneity: how genetically different are cancer cells within and among different lesions of the same patient? And a fourth, particularly important question relates directly to patients: how can knowledge of cancer genetics best be used to reduce cancer deaths in the future? Tentative answers to each of these four questions, based on a combination of data assembled by geneticists, molecular biologists, epidemiologists, and mathematicians, will be presented.

Network based approaches to understanding early events in cancer development

Bruce Ponder
CRUK Cambridge Institute, Cambridge CB2 0RE, UK

Genome-wide association studies have identified many loci, inherited variation of which affects risk of cancer. The effects at any individual locus are small. It is therefore important to understand the combined effects of many loci in interaction with each other and with the environment. Our hypothesis is that the effects on risk will mostly be expressed through the normal tissues from which the cancers arise. Patterns of gene expression in normal tissue may then provide an integrated readout of genes and their interaction with exposure. As the GWAS loci are linked with cancer risk, they provide an anchor to causality.

If the effects of the multiple GWAS loci impact on only a few pathways or mechanisms, the problem will be more tractable than if they are diverse. Using existing data, we created a transcription factor-centric gene regulatory network for breast cancer, and showed that eQTLs related to the top 72 GWAS loci are enriched within a cluster of 36 overlapping regulons, centred on ESRI, FOXA1 and GATA3, involved in estrogen signalling and already implicated in breast cancer. Further analysis resolved two opposing groups of transcription factors, related respectively to ER+ and ER- breast cancer. Analysis of regulon activity provided a measure of 'ER+-ness' that had greater predictive value for outcome or treatment response than ER status alone.

These results, although not relating directly to early disease, suggest that a network approach is feasible and can provide useful results. We are now extending the analysis to lung cancer, where our hypothesis is that the 15% of smokers who develop lung cancer do so in part because their genetic background influences their airway response to smoke injury. We will use regulatory networks as a framework, on which to map lung GWAS eQTLs; genes differentially expressed in the airway of patients who are current or former smokers, with and without cancer; genes differentially expressed in bronchial epithelial cells in response to challenges in vitro; and genes differentially expressed between normal, dysplastic and cancerous epithelium in the same patient. We hope that the results will provide insight into the regulatory mechanisms that are perturbed in lung cancer development.

Targeting early events in K-Ras driven lung adenocarcinoma

Mariano Barbacid
Molecular Oncology Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Madrid, Spain.

KRAS oncogenes have been implicated in about one fifth of all human cancers including

lung adenocarcinoma, one of the tumors with worse prognosis. Unfortunately, identification of suitable therapies to treat these tumors remains elusive and patients are still treated with cytotoxic compounds approved more than two decades ago. The recent discovery that lung tumors display significant levels of clonal heterogeneity (see Govindan, Science, 2014) adds another complexity hat needs to be addressed in order to design rational and efficacious therapies. In an attempt to provide potential solutions to these issues, we decided to search for novel therapeutic targets that may contribute to the early stages of lung tumor development, hoping that these targets will be present in all tumor cells –including cancer initiating cells and cancer stem cells– and not only in limited populations of evolving clones. To this end, we took advantage of our genetically engineered mouse (GEM) tumor model, K-Ras$^{+/LSLG12Vgeo}$ that co-expresses a color marker (β-galactosidase) with the K-Ras^{G12V} oncoprotein. This property allowed us to identify and isolate K-Ras^{G12V}-expressing lung cells from the earliest possible stages of tumor development (Mainardi et al., PNAS, 2014). Using this experimental approach we isolated hyperplastic areas (~300 cells) and established their transcriptional profile. Surprisingly, we identified two independent, well-defined signatures even at this early stage, suggesting the existence of at least two different types of cancer initiating cells. Whereas one of the signatures resembled that of normal lung alveolar tissue, the other was similar to that present in aggressive K-Ras driven lung tumors (Sweet-Cordero et al., Nat. Genetics, 2005), suggesting, although not proving, that the latter hyperplastic areas represent the precursor lesions of the malignant lung tumors. The top variant gene in this "aggressive" signature was Myc, an oncogene known to be induced by oncogenic Ras signaling. Since the Myc protein is not an obvious druggable target, we focused our interest in another highly overexpressed gene, Ddr1, that encodes a druggable tyrosine protein kinase receptor. Genetic analysis using mice devoid of Ddr1 revealed that this receptor plays a key role in tumor initiation and progression, providing that the tumors retained p53 activity. Pharmacological inhibition of Ddr1 mimicked these genetic results. More importantly, when we concomitantly inhibited Ddr1 and Notch signaling, a downstream mediator of Ddr1 activity, we observed significant anti-tumor effects even in aggressive K-Ras^{G12V}; p53 mutant adenocarcinomas. Indeed, the therapeutic activity of the combined Ddr1+Notch inhibitors was superior to that observed with standard chemotherapy regiments with significant lower toxic side effects. Importnatly, DDR1 is overexpressed in most human lung tumors. Hence, our results could be easily validated in the clinic by (i) establishing by IHC techniques whether DDR1 is expressed in all tumor cells and (ii) by establishing optimal therapeutic strategies with novel and more potent DDR1 and NOTCH inhibitors. Moreover, characterization of other druggable genes identified in the "aggressive" transcriptional signature should provide additional targets to potentiate the putative therapeutic activity of DDR1 and NOTCH inhibitors in patients suffering from *KRAS* mutant lung cancer.

Altered systemic metabolism and early pancreatic adenocarcinoma

Brian Wolpin
Dana Farber Cancer Institute, Boston, MA

Pancreatic cancer is the third leading cause of cancer mortality in the United States and fifth leading cause in Europe. More than 80% of patients present with advanced disease at diagnosis, and the overall five year survival rate is less than five percent. A defining feature of pancreatic ductal adenocarcinoma (PDAC) is its relationship with altered systemic metabolism. The risk of developing PDAC is increased among individuals with chronic obesity, hyperglycemia, and hyperinsulinemia. Furthermore, weight loss and new-onset diabetes commonly develop within the 1-2 years before diagnosis, and advanced PDAC is strongly associated with development of tissue wasting and muscle loss (sarcopenia). Our work suggests that alterations in whole-body metabolism can be detected in patients with pancreatic cancer prior to diagnosis. In fact, the metabolic alterations identified have a phenotype consistent with early cancer cachexia, suggesting the presence of a sub-clinical catabolic state. Ongoing work seeks to understand the mechanisms by which pancreatic tumors cause host metabolic changes. Furthermore, studies in large human populations are in progress to define how best to harness these detectable metabolic alterations for use in population-based risk stratification programs and for identifying patients at risk for progressive sarcopenia, which impairs patient quality of life and survival. Given the late stage at presentation for most patients with PDAC, detecting early disease is a priority to reduce the high rates of mortality from this malignancy.

The role of aneuploidy during tumorigenesis

Teresa Davoli,
Harvard Medical School, Boston MA

Since Boveri's postulates in 1914, aneuploidy has been recognized as a hallmark of human cancer, however, no comprehensive theory exists to explain its patterns and its role in tumor evolution. We have developed Tumor Suppressor and Oncogene (TUSON) Explorer, a computational method for the prediction of tumor suppressor genes (TSG) and oncogenes (OG) through the analysis of the pattern of somatic mutations in cancer. By integrating our prediction of TSGs and OGs with information of copy number alterations, we found that the distribution on chromosomes and the potency of TSGs and OGs can predict the frequency of arm-level and chromosome-level deletions and amplification (Davoli et al., 2013). We propose that it is the decrease or increase in the gene dosage of TSG and OG genes in deletion and amplification to determine the recurrent patterns of aneuploidy selected during tumor evolution. More recently, we have performed gene expression analysis on tumor samples, comparing tumors with different levels of aneuploidy. We have found that tumors with high levels of aneuploidy show increased markers of proliferation as well as a strong decrease in infiltrating immune cells, especially CD8+ T cells. Notably, we show that aneuploidy predicts patients' survival in two clinical trails of immunotherapy in melanoma patients. Compared to neoantigen load, aneuploidy represents and independent and stronger predictor of survival. A combined score including aneuploidy and neoantigen load allows superior prediction of survival after immunotherapy. We propose that aneuploidy contributes to two important cancer hallmarks, proliferation and cancer immune evasion.

Inherited Variation and Cancer

Matthew Freedman
Dana Farber Cancer Institute, Boston MA

Our laboratory investigates the role of inherited variation and its influence on cancer-related phenotypes such transformation and response to therapeutic agents. My talk will focus on the impact of germline genetics in oncology.

Sequencing of the human genome and subsequent characterization of genetic polymorphisms has provided a high-resolution map of inter-individual variation. This information serves as the basis for genome wide association studies (GWAS). Over the past decade, hundreds of large cancer GWAS have successfully identified variants associated with cancer risk. An unexpected outcome of these studies is that the vast majority of variants (>90%) reside outside of known protein coding regions. Because of linkage disequilibrium and the lack of a genetic code to interpret non-protein coding variants identifying the actual causal variants driving cancer pathogenesis has been elusive. Thus, our group has developed an integrated end-to-end pipeline termed CAUSEL to enable the functional evaluation of variants discovered through GWAS and to establish mechanisms driving disease pathogenesis.

Inherited variation also plays roles beyond disease risk. It can influence disease aggressiveness as well as response to chemotherapy. We performed a study to evaluate the prevalence of germline BRCA2 variants in prostate cancer and to determine the responsiveness of prostate tumors harboring these variants to platinum-based chemotherapy. The BRCA2 gene was sequenced in a total of 848 men across three clinical stages of prostate cancer. Deleterious mutations were significantly enriched in men with metastatic disease unselected for family history or age. Our data also revealed that BRCA2 carriers responded to carboplatin-based chemotherapy compared to non-carriers. Notably, platinum-based chemotherapy is not typically used for prostate cancer and a large phase III trial with a platinum agent did not show significant benefit. Our data motivate BRCA2 sequencing in men with high-grade prostate cancer in larger, prospective studies and its use as a predictive marker for platinum-based chemotherapy in various clinical settings. These results demonstrate the power of interrogating prior datasets with new technologies and ideas to rapidly actualize the goals of precision medicine.

Dietary control of stem cells in physiology and disease

Omer Yilmaz
The Koch Institute for Integrative Cancer, Cambridge, MA

Organismal diet has a profound impact on tissue regeneration, aging, and disease in mammals. However, the mechanisms through which diet perturbs stem and progenitor cell biology and leads to diseases, such as cancers are poorly understood. With the rise of obesity throughout the world – more than 1 in 3 adults are obese in the US – understanding

the relationship between diet, stem cell biology, and cancer incidence takes on great importance. From epidemiologic data, it has been long observed that obesity correlates with augmented cancer incidence in humans. Yet, little is known about how diets that lead to obesity such as a high fat diet (HFD) regulate adult stem and progenitor cell function and how a HFD influences the vulnerability of these cells to undergo oncogenic transformation to form tumors.
I will present our findings of how a pro-obesity HFD alters the biology of intestinal stem cells (ISCs) and progenitors, and how such changes contribute to the early steps of intestinal tumorigenesis.

The role of ultraviolet light in melanoma initiation

Richard Marais PhD
The CRUK Manchester Institute, Manchester, UK

Melanoma is a potentially deadly skin cancer. Epidemiological studies have linked melanoma to exposure to ultraviolet radiation (UVR) from sunlight and from artificial tanning devices. Advances in genome sequencing techniques have provided enormous insight into the genetics of melanoma. We now know that the *BRAF* gene is mutated in about 45% of cutaneous melanomas arising on hair bearing skin and *NRAS* is mutated in another ~20% of cases and it appears that these different driver oncogenes have distinct relationships to UVR exposure. Specifically, *BRAF* mutant melanomas tend to arise in younger individuals on regions of the body that are exposed intermittently to UVR such as the back and trunk ("recreational" exposure), whereas *NRAS* mutant melanomas tend to arise in older individuals on regions that experience chronic exposure to UVR, such as the face and neck ("habitual" exposure).

It is difficult to study the gene-environment relationships in melanomagenesis in the outbred human population; intensity and pattern of UVR exposure need to be intuited from questionnaires, and the genetic diversity of the human population provides an additional level of complexity. Thus, to study the interaction between specific oncogenes and UVR, we have developed mouse models of melanoma driven by common melanoma oncogenes. We find that UVR exposure accelerates both *BRAF* and *NRAS* melanomas, but as in humans, in mice the *NRAS*-driven tumours require more life-time accumulation than the *BRAF*-driven tumours. Moreover, we see clear differences in the cooperating oncogenes in *BRAF* and *NRAS*-driven tumours, and we note that the tumours that arise bear the cardinal pathological features of human melanomas driven by oncogenic BRAF and NRAS. Moreover, our studies have revealed new therapeutic targets in these tumours that we are able to validate in samples taken from melanoma patients. Thus, our mouse models not only mimic the genomic and pathophysiological features of human melanoma, they provide insight into how treatment of this disease can be optimised through precision medicine protocols.

Clonal hematopoiesis

Benjamin Ebert, MD, PhD
Brigham and Women's Hospital, Boston MA

Clonal hematopoiesis of indeterminate potential (CHIP) is a common, age-associated condition. CHIP is defined by the presence of clonal, somatic mutations in the blood. We have characterized the presence of clonal, somatic mutations in exome sequencing data from peripheral blood DNA from over 60,000 individuals and examined the phenotypic consequences of these mutations. The mutations identified in CHIP are the same as those found in hematologic malignancies such as myelodysplastic syndrome and myeloproliferative neoplasms. Indeed, the mutations identified in CHIP, including mutations in *DNMT3A*, *TET2*, and *ASXL1*, are lesions that are commonly acquired early in the genetic ontogeny of hematologic malignancies, prior to the development of overt disease. Consistent with the concept that CHIP is a pre-malignant state, CHIP is associated with an increased risk of hematologic malignancy. Individuals with CHIP have a 50-fold increase in the risk of hematologic malignancies, and approximately 0.5 to 1% of individuals with CHIP progress to hematologic malignancy per year. Individuals with CHIP, generally bearing just a single mutation in a driver gene, do not have altered blood counts, but do have an elevated red blood cell distribution width (RDW). Clonal mutations are present in terminally differentiated blood cells and have the potential to alter the phenotype and functional properties of blood cells. Most strikingly, individuals with CHIP have a 40% increase in overall mortality which appears to be due to an elevated risk of cardiovascular disease. Some aspects of the aging phenotype of hematopoiesis may be related to the acquisition of clonal, somatic mutations.

Tumor evolution and cell differentiation

Francesca Demichelis
Centre for Integrative Biology (CIBIO), University of Trento, Italy

Understanding treatment resistance is emerging as a critical hurdle for precision medicine in cancer care. We exploit single base resolution data and allele-specific analysis to reconstruct tumor evolution charts and to quantify intra- and inter-tumor molecular heterogeneity. These approaches proved useful in nominating genomic events likely to occur during early steps in prostate cancer development and to identify potential mechanisms of resistance to AR (androgen receptor) directed therapies. Specifically, we found evidence of the emergence of an alternative 'AR-indifferent' cell state through divergent clonal evolution as a mechanism of treatment resistance in advanced disease. Through serial plasma samples analyses, we then delineated patients' tumor dynamics from baseline through AR-directed therapy management.

Organoid cancer models

Marc van de Wetering
Hubrecht Institute, Utrecht, The Netherlands

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. We originally defined Lgr5 as a Wnt target gene, transcribed in colon cancer cells. Two knock-in alleles revealed exclusive expression of Lgr5 in cycling, columnar cells at the crypt base. Using lineage tracing experiments in adult mice, we found that these Lgr5+ve crypt base columnar cells (CBC) generated all epithelial lineages throughout life, implying that they represent the stem cell of the small intestine and colon. Lgr5 was subsequently found to represent an exquisitely specific and almost 'generic' marker for stem cells, including in hair follicles, kidney, liver, mammary gland, inner ear, tongue and stomach epithelium.

Single sorted Lgr5+ve stem cells can initiate ever-expanding crypt-villus organoids, or so called 'mini-guts' in 3D culture. The technology is based on the observation that Lgr5 is the receptor for a potent stem cell growth factor, R-spondin. Similar 3D cultures systems have been developed for the Lgr5+ve stem cells of human stomach, liver, pancreas, prostate and kidney. Using CRISPR/Cas9 technology, genes can be efficiently modified in organoids of various origins.

Adult progenitors and organoid cultures for the study of human biology and disease

Meritxell Huch(1,2,3)
1) Gurdon Institute-Wellcome Trust/ Cancer Research UK, CB2 1QN United Kingdom
2) Department of Physiology, Developmental Biology and Neuroscience, University of Cambridge,
3) the Wellcome Trust-Medical Research Council Stem Cell Institute

In vitro 3D cultures are emerging as novel systems to study tissue development, organogenesis and stem cell behaviour ex vivo. For several endoderm- and ectoderm-derived organs, embryonic stem cells (ESCs) grown in 3D self-organize and acquire the right tissue patterning to develop into the corresponding embryonic buds and organs. Tissue-resident adult stem cells (AdSCs) grown in 3D maintain their properties of self-renewal and multipotency, while preserving their genetic integrity and lineage commitment. Here we will present our novel culture system where mouse and human liver stem/progenitor cells can be indefinitely expanded in vitro (for >1 year), into "liver organoids". The expanded cells are highly stable at the chromosome and structural level, while single base changes occur at very low rates. The cells can readily be converted into functional hepatocytes in vitro and upon transplantation in vivo. Organoids from α1-antitrypsin deficiency and Alagille Syndrome patients mirror the in vivo pathology. Similarly, organoids derived from liver cancer patients faithfully reproduce the pathophysiology of the disease. Clonal long-term expansion of primary adult stem/progenitor cells opens up experimental avenues for disease modeling, toxicology studies, regenerative medicine and gene therapy.

ABSTRACTS OF POSTERS

BARD1δ acts as growth inhibitor in normal cells and oncogenic driver in cancer by compromising mitotic spindle and telomere structures

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Key words: BARD1, growth repression, centrosome amplification, telomere alteration, shelterin, genetic instability, cervical cancer, genome permutator.

BARD1 acts with BRCA1 as tumor suppressors in DNA repair and cell cycle control. Expression of high levels of a deletion-bearing isoform of BARD1, BARD1δ, was specifically correlated with tumor aggressiveness and progression. To understand BARD1δ's oncogenic activity, we investigated its cellular functions *in vitro* and *in vivo* in cell cultures, transgenic mice, and cells from breast cancer patients. We found that BARD1δ overexpression in cells of various origins induces proliferation arrest. Immunohistochemistry revealed the specific upregulated expression of BARD1δ in terminally differentiated cells in healthy tissues, but its absence from proliferating cells.
BARD1δ overexpression was associated with multipolar spindles and telomere and chromosome aberrations in cell cultures *in vitro* and in transgenic mice carrying the BARD1δ transgene. BARD1δ binds more efficiently than wild type BARD1 to γ-tubulin and to telomere binding proteins and affects altered turnover and aberrant localization of these proteins. Impaired γ-tubulin turnover causes mutipolar spindles, and dislocalization of telomere binding proteins results in telomere alteration and loss; these defects lead to the observed cell cycle arrest in BARD1δ overexpressing cells. In the absence of G2/M check point controls, BARD1δ expressing cells continue to divide and accumulate genetic instability. BARD1δ therefore acts as genome permutator, thus an inducer of carcinogenesis and driver of continuous uncontrolled proliferation of cancer cells.

MEF2s: true oncogenes or "re-thought" tumor suppressors?

Eros Di Giorgio, M. Faggiani, E. Franforte, R. Picco, V. Cutano, C. Brancolini
Dept of Medical and Biological Sciences, University of Udine.

MEF2 (MEF2A, B, C, D) transcription factors (TFs) regulate many homeostatic processes, spanning from differentiation to stress-adaptation. MEF2's activities are therefore strictly regulated in a time-dependent manner; the transcriptional repressors class IIa HDACs (HDAC4, 5, 7, 9) are intimately involved in this fine tuning as they act as molecular switches that shut down the activity of MEF2 whenever is needed.
In many cancer types the physiological cycle of turning on and off of MEF2 factors is subverted. This loss of control impacts both on the pathogenesis and the progression of the disease. However, on the basis of loss-of-function experiments, MEF2 factors are classified sometimes as oncogenes and sometimes as tumor suppressors. With this project we aim to address this apparent paradox.
We have recently demonstrated that in healthy fibroblasts the activation of MEF2 is modulated during the cell cycle and reaches a peak in G1 and decreases starting from the S-phase. At least two mechanisms contribute to this regulation: the phosphorylation and activation of the TAD taking place in G0/G1 transition and the proteasome-mediated degradation of MEF2 proteins that is regulated by the CDK4/SKP2 pathway. In a feed-back manner MEF2 proteins regulate the progression through the cell-cycle by binding a regulatory enhancer region of CDKN1A gene and promoting its transcription. Beside this cyclic regulation of MEF2 there is the repression exerted by class IIa HDACs that seems not to be affected by the cell cycle but by other extra- and intra-cellular stimuli.
We figured out that soft-tissue sarcomas and leiomyosarcomas (LMSs) in particular are tumors characterized by a strong repression of MEF2, which is achieved through an increase both in the proteasomal-mediated degradation of MEF2 proteins and in the repression exerted by HDAC4 and HDAC9. MEF2 degradation prevails in a cellular model of low-grade tumors, while in a model of high-grade LMSs MEF2 proteins are stably converted into transcriptional repressors of specific genome

loci. In this latter case the knock-down (KD) of MEF2D and MEF2A causes the de-repression of some MEF2-regulated genes and, surprisingly, has a tumor suppressive effect. By crossing the transcriptome profile of MEF2D/MEF2A KD with ChIP data we figured out that MEF2 exerted these peculiar repressive activities essentially on some genomic loci close to the TSS.
With this work we suggest a re-thinking about the role of MEF2s in cancer. In particular we are persuaded that the biological significance of loss-of-function experiments should be interpreted taking into account the biological mechanism underlying the phenomenon.

Circadian clock disruption on the route to gastric carcinoma

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Background:
Circadian rhythms are regular sequences of events, which occur about every 24 hours (from latin: *circa diem*). Sleep-wake cycles, cycling of body temperature, rhythms in hormone secretion, heart rate, blood pressure, excretion and many physiological parameters are all circadian phenomena controlled by biological clocks, endogenous self-sustained mechanisms able to synchronize with environmental cues such as light and temperature, but also social cues as physical activity and feeding behaviour. Cogwheels of the circadian clock are proteins, whose production and degradation are controlled by interlocked feed-back loops. These, so called, *clock genes* may affect cancer susceptibility through effects on biological pathways that regulate DNA damage and repair, carcinogen metabolism and/or detoxification, cell-cycle and apoptosis. It has been estimated that 2–10% of all mammalian genes are clock-controlled, indicating extensive circadian gene regulation. Furthermore, some pioneering work in the field of molecular cancer epidemiology has demonstrated that genetic variants in the clock genes are potential risk factor for many types of cancer such as breast, colorectal, prostate, pancreatic and ovarian.
Gastric cancer is the eighth most common cause of cancer death, with median age of diagnosis 70 years for men and 74 years for women. Surgical resection is still the method of choice and surgical trials are focused on identifying prognostic factors for groups of patients undergoing curative surgery. TNM classification does not offer a clear prognosis with patients in the intermediate stages and their suitability for adjuvant chemotherapy. Recent studies have explored that defects in circadian pathway genes increase susceptibility of developing gastric cancer and poor prognosis.
Scope:
Correlation analysis of Single Nucleotide Polymorphisms of circadian pathway genes with the risk of developing gastric carcinoma and identification of new independent prognostic factors for gastric carcinoma.
Materials and Methods:
We used the Biobank patient management database of Clinica Chirurgica 1°, Azienda Ospedaliera Padova to extract data of 60 patients (age range between 40-87years), that underwent gastric carcinoma surgery between 1992 and 2012. The peripheral blood samples obtained from these patients during the pre operative stage were used to extract DNA and were analysed by Allelic Discrimination PCR for the following SNPs: rs1801260, rs3749474 (CLOCK), rs3027178 (PERIOD 1), rs934945 (PERIOD 2) and rs7302060 (TIMELESS). Univariate survival analysis was carried out to know the effect of single polymorphism of circadian pathway genes on the overall survival of gastric cancer patients followed by Multivariate analysis with respect to the TNM stages.
Results:
The recessive genetic model for Clock rs1821260 was statistically significant (Hazard ratio 0.49% and p value 0.047), which reduced the risk of tumor relapse by 50% with a diagnostic accuracy of almost 69% (C-index). When this result was combined with the sex, age and TNM stages of the patients in a multivariate analysis, the Hazard Ratio becomes 0.37 reducing the death risk by 63%, with a diagnostic accuracy of 75% (C-index).
Conclusions:
Clock polymorphism rs1821260 has a protective effect with respect to gastric carcinoma. Patients who are carriers of this polymorphism have a minor risk of developing gastric carcinoma and even if they develop, possess a better prognosis with respect to those who do not possess this SNP in their germline. With a simple pre operative blood sample SNP analysis, the patients can be grouped as rs1821260 positive and negative. Instead of initiating chemotherapy to all the patients, this polymorphism can be taken into consideration as an independent prognostic factor for selecting patients, along with TNM staging and performance status of patients, to be subjected to adjuvant chemotherapy for gastric cancer. Future work will be focused on clock gene polymorphisms and risk of developing gastric carcinoma.

MAPK pathway mutations

and DNA hypomethylation are involved in early step of oxidative DNA damage driven colorectal carcinogenesis

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Carcinogenesis of colorectal cancer (CRC) is a multistep process with known perturbations to the genome and/or epigenome of normal colonic epithelial that drive it to malignant transformation from hyperproliferative epithelium to adenoma and then to cancer.
Screening for the detection of CRC has been largely improved but the discrimination of high risk adenomas is still challenging, both in hereditary syndromes and in sporadic cases.
To date, there is a strong need to establish clinically useful biomarkers for risk assessment and early detection of CRC.
MUTYH associated polyposis (MAP), a hereditary colorectal cancer syndrome due to biallelic MUTYH germline mutations and associated with an inactive BER pathway, is characterized by a rapid progression marked by adenomas and cancers with specific c.34G>T transversions (G12C) on K-RAS gene and chromosomal instability phenotype (CIN).
DNA global hypomethylation is recognized to be an early causal event in colorectal carcinogenesis and recently active DNA demethylation processes have been shown to be mediated by BER system.
We aimed at characterizing the interplay between BER, which counteracts DNA oxidative damage, and DNA methylation levels in the progression of colorectal tumorigenesis proving its role as biomarker of higher risk.
To this purpose K-RAS and N-RAS status was checked on 49 adenomas from MAP and 60 control patients (familial adenomatous polyposis (FAP) and sporadic) as well as on 10 MAP cancers by using pyrosequencing and mass spectrometry (MALDI-TOF) techniques; the samples were also tested for DNA methylation levels with global LINE-1 and local L1-MET assays.
K-RAS activating mutations were more frequently detected in MAP adenomas (37%) than in FAP (23%) or sporadic (25%) adenomas. In addition, N-RAS mutations were observed only in MAP adenomas (8%).
Taking into consideration both K-RAS and N-RAS genes, the overall frequency of MAP mutated adenomas increased to 45% and it was significantly higher than the frequency observed in FAP adenomas (p=0.04).
K-RAS p.G12C mutation was found in 77.5% of the MAP mutated adenomas; by contrast, this mutation was totally absent in FAP and sporadic adenomas that were enriched for the more common p.G12V, p.G13D and p.G12D mutations.
As for the MAP CRCs, 90% showed K-RAS alterations; among these, 89% displayed K-RAS p.G12C and 33% also a PI3KCA mutation.
MAP adenomas exhibited a significant higher frequency of hypomethylated samples (38,8% and 51,1% of cases with LINE-1 and L1-MET analyses respectively) compared to FAP adenomas (16,7% and 18,2% of cases) and sporadic lesions (8,3% and 4.2% of cases) (p=0.007 and p=0.0001).
Interestingly, MAP cancers showed a frequency of 60% and 90% hypomethylated samples with two assays. This finding suggest that DNA hypomethylation could have a specific role in MAP progression.
Our results showed that both well-known specific molecular mutations of MAPK genes and DNA hypomethylation characterize early MAP carcinogenesis. Although there was no correlation between two mechanisms, both appear to be independent and could strengthen each other in the presence of oxidative stress injuries.

Targeting potential metabolic drivers of Triple-Negative Breast Cancer aggressiveness

Valentina Piano[1], Sharon Louie[2], Daniel K. Nomura[2] and Andrea Mattevi[1]
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Mortality due to breast cancer is usually attributed to metastatic spread of the disease to other organs, thus precluding resection as a treatment method. However, studies over the past decade have uncovered certain breast cancer types associated with poor prognosis and chemotherapy-resistance, such as estrogen/progesterone/HER2 receptor-negative, so called triple-negative breast cancers (TNBCs). There are currently no therapies that target this malignant population of breast tumors[1].
We fused our competence in structural biology and metabolomic analysis to use an innovative chemoproteomic approach, which relies on reactivity-based chemical probes to map dysregulated metabolic enzymes in TNBC cells.

Through this profiling effort, we uncovered alkyl-dihydroxyacetone phosphate (ADPS) and glutathione transferase P1 (GSTP1) as significantly up-regulated enzymatic targets in TNBCs.
ADPS is a peroxisomal enzyme performing the key step in ether phospholipids synthesis[2]. Although this is still a rather neglected area of lipid metabolism, it has been demonstrated that ADPS over-expression is correlated to cancer cells aggressiveness, by elevating ether phospholipids levels. In fact, high levels of ether phospholipids favor cells ability to proliferate and migrate, by shifting the equilibrium of fatty acids usage toward the synthesis of ether linked oncogenic lipids.
On the other hand, GSTP1 belongs to a superfamily of enzymes with diversified functions. These enzymes are also known to decrease the pharmacologic activity of a wide range of structurally unrelated drugs through their conjugation with glutathione.
Surprisingly, we discovered that GSTP1 over-expression exerts an effect on the glycolytic metabolism, by modulating glyceraldehyde-phosphate dehydrogenase (GAPDH) activity through a mechanism completely unexplored. Indeed, we demonstrated that GSTP1 lowers GAPDH feedback inhibition by scavenging 1,3-bisphosphoglycerate, catalyzing a new enzymatic activity not yet characterized.
Our work showed that genetic ablation of this two promising targets, ADPS and GSTP1, led to a lowering of TNBC cell survival and *in vivo* tumor growth, through impairing lipid and glycolytic cancer metabolism[3,4].
Therefore, we put forth in developing molecules inhibitor of these newly identified targets finally open the doors for the future progress of anti-cancer therapies against this incurable form of breast tumor. In particular, we established a medium-throughput small-molecules screening and succeeded in discovering the very first ADPS inhibitors, effective in depleting breast cancer cells aggressiveness by specifically lowering ether phospholipids levels.

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Nenci, S., et al. (2012) Precursor of ether phospholipids is synthesized by a flavoenzyme through covalent catalysis. Proc. Natl. Acad. Sci. U.S.A. 109, 18791-18796.
Piano, V., et al. (2015) Discovery of Inhibitors for the Ether Lipid-Generating Enzyme AGPS as Anti-Cancer Agents. ACS Chem. Biol. 150904102314006-9.
Louie, SM., et al. (2016) GSTP1 is a driver of triple-negative breast cancer cell metabolism and pathogenicity. In press at Cell Chemical Biology.

Alteration of RNA PolII dynamics account for BET inhibition efficacy

Elisa Donato, Ottavio Croci, Sabò Arianna, Heiko Muller, Marco Morelli, Mattia Pelizzola, Stefano Campaner
IIT@SEMM (Milano)

Myc is an attractive molecular target for cancer therapy since its misregulation, due to genomic rearrangement or alteration of upstream regulatory pathways, is essential for tumor formation and maintenance. Unfortunately, Myc is intrinsically resilient to direct pharmacological targeting. To overcome this issue, alternative approaches have been explored. In the last years, independent groups showed that BET proteins inhibition leads to Myc downregulation in Multiple Myelomas and Acute Myeloid Leukemias, with consequent cell cycle arrest and tumor regression.
In order to extend our understanding of the mechanism of action of BETi, we evaluated global transcriptional alteration and chromatin profiles in Burkitt's Lymphomas in response to BET inhibitors (JQ1).
Our results demonstrate that JQ1 efficacy is dependent on global alteration of RNA PolII dynamics. Yet, despite a pervasive eviction of BRD4 from chromatin and a global effect on RNA PolII observed following BETs inhibition, the transcriptional alterations are restricted to a subset of genes for which transcription elongation is a rate-limiting step.
These observations highlight the role of BETs protein in regulating gene expression and transcriptional elongation and provide a rationale to explain how broad inhibition of elongation may lead to a selective transcriptional response.

Function and Mechanism of Action of the Polycomb Repressive Complexes in the Homeostasis of Adult Intestinal Cells

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Polycomb group proteins (PcG) are among the most important gatekeepers that ensure the correct establishment and maintenance of cellular identity in metazoans. This occurs by sequentially modifying chromatin through the activity of two Polycomb Repressive Complexes (PRC1 and PRC2) that deposit H2A ubiquitylation and H3K27 methylation respectively, in order to maintain repression of

their target genes. Although the development of PRC2 inhibitory compounds is becoming a very promising strategy for specific cancer treatment, the controversial role of PcG proteins, acting as oncogenes or tumor suppressors in a tissue/cancer specific manner, prompt us to further investigate their role in maintaining adult tissue homeostasis. Using different genetic models, we have found that both PRC1 and PRC2 are required for maintaining intestinal epithelia homeostasis. Using high-throughput transcription and location analysis, we have dissected the direct transcriptional pathways regulated by these complexes. PRC1 is required to maintain ISCs by sustaining Wnt transcriptional activity also in the presence of oncogenic b-catenin mutation. PRC2 has a dual role; by repressing Ink4a/Arf expression it maintains cell proliferation, by repressing master regulators of secretory lineage it restricts goblet cell proliferation. Globally our data reveal a crucial role of PcG proteins in maintaining intestinal cell identity and plasticity.

Modeling prostate tumorigenesis with 3D organoid cultures

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Prostate cancer (PCa) is one of the most frequent forms of tumor in men, characterized by a high degree of variability among individuals in terms of both clinical and genomic features. Improving the current understanding of the molecular mechanisms underlying PCa is considered crucial for the design of more accurate screenings, patient stratification strategies and targeted therapies. Recent advances in PCa genome profiling have revealed a vast spectrum of recurrent genetic alterations, the functional role of which largely remains to be elucidated. However, such research has been hampered by long-standing difficulties in establishing accurate models, with the result that up to now nearly all studies have been performed on a few metastatic or transformed cell lines, whose relevance, in particular for modeling primary tumor development, is limited.
Here, by combining recent advances in establishing primary prostate cultures and transgenic technologies, we report our progress in generating 3D androgen responsive mouse prostate organoids with inducible expression of gene fusion (found in about 50% of PCa) and fine-tunable repression of the key onco-suppressor gene (associated with in approximately 25% of primary PCa, and observed with increased frequency and severity at advanced stages).
We envisage that generation of such models will provide a novel route for more accurate investigations of prostate tumorigenesis, enabling the application of high-throughput molecular profiling, gene editing and drug testing for the study of the mechanisms involved in tumor aggressiveness and resistance to therapy.

Systematic Identification of Epigenetic Alteration Across Human Cancers

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2 Department of Medical Genetics, University of Lausanne, Lausanne, Switzerland

Epigenetic alterations have been associated with all stages of tumorigenesis. DNA methylation mediates transcriptional silencing thus determining lineage differentiation and regulating cellular functions. In cancer, increased DNA methylation at transcriptional start sites (TSS), or lack of it, plays a critical role in cell de-differentiation and repression/activation of tumor suppressors/oncogenes. Here, we developed a novel approach to systematically evaluate aberrant DNA methylation at gene TSS in tumors, based on their pooled distribution of methylation levels in matching healthy tissue. For aberrantly methylated genes, we assess functional effects on mRNA expression and statistical significance of recurrence within and across multiple cancer types.
We applied our approach to the comprehensive methylome dataset from The Cancer Genome Atlas counting over 10,000 samples from 33 tumor types with DNA methylation levels assessed at 450K sites.
Our work provides a systematic framework to identify recurrent epigenetic events in cancer, thus complementing similar approaches for somatic mutations and copy number alterations in detecting cancer drivers. Importantly, discovering functional DNA methylation events contributing to tumor development, together with strategies to reverse DNA methylation, promises new possibilities for cancer therapy.

SAMHD1 a new gatekeeper of genome stability

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Sterile alpha motif and HD-domain containing protein 1 (SAMHD1) is a triphosphohydrolase that degrades DNA precursors (dNTPs). It is expressed in almost all human tissues and in the immune system where it acts as a restriction factor that makes myeloid cells refractory to lentiviral infections. Mutations in the SAMHD1 gene are linked to the autoimmune disorder Aicardi-Goutières Syndrome (AGS) and to various types of cancer. These lines of evidence suggest the involvement of SAMHD1 in the innate immune response and in cancer development through the control of dNTP homeostasis even if the physiological functions of SAMHD1 in normal uninfected cells remain to be fully defined.
In proliferating cells SAMHD1 is phosphorylated on Thr-592 by the S-phase regulated kinases Cdk2 and Cdk1complexed with cyclin A2. The crystal structure of the protein and in vitro assays suggest that Thr-592 phosphorylation downregulates the dNTP hydrolase activity of SAMHD1 but how this modification controls its function in vivo is unclear.
Our group investigates the role of SAMHD1 in the enzyme network regulating the concentrations of dNTPs in uninfected mammalian cells. We observed that the level of SAMHD1 protein is inversely related to cell proliferation (being more abundant in resting or differentiated cells than in cycling cells). We demonstrated that SAMHD1 is a major regulator of dNTP turnover preventing the overproduction of dNTPs in mammalian cells both during proliferation and quiescence.
In the present study we used human fibroblasts derived from AGS patients bearing different SAMHD1 gene mutations and monocytic THP-1 cells that have high constitutive expression of endogenous SAMHD1 or a knock-out derivative (THP-1 KO). Both in fibroblasts and monocytic cells the absence of SAMHD1 induces a marked expansion of all 4 dNTP pools (in particular the dGTP and dATP pools) and changes their relative proportions leading to pool imbalance. In quiescent cultures of AGS fibroblasts this effect is still more pronounced. Despite the abnormal levels of dNTPs, the AGS fibroblasts and THP-1 KO cells grow in culture equally well as the matched controls. In subpopulations of elutriated THP-1 cells highly enriched in G1, S or G2/M phase we found that in the absence of SAMHD1 the dNTP levels increase most in G1 indicating that the enzyme exerts its activity especially when DNA synthesis does not occur.
We investigated the correlation between cell cycle progression and SAMHD1 expression level and its phosphorylation on Thr-592. In wild type THP-1 cells the amount of SAMHD1 protein remains relatively unchanged in the different phases of the cell cycle. The phosphorylation on Thr-592 is absent in G1-phase while in S- and G2/M-phase all the protein was phosphorylated. Cell cycle synchronization experiments and in vitro kinase assays showed that the phosphorylation starts already at the G1/S transition by the cyclin E/Cdk2 complex. In proliferating cells overexpressing SAMHD1 by transient transfections the protein is largely phosphorylated and dGTP pool size decreases slightly, suggesting that SAMHD1 activity maybe downregulated by phosphorylation when the cells need dNTPs for nuclear DNA synthesis. We propose a gatekeeper function of SAMHD1 for the stability of the cell genetic information, strictly dependent on the cell-cycle regulation of the enzyme.

Call for 2017 Pezcoller Foundation-AACR International Award for Cancer Research

The prestigious Pezcoller Foundation-AACR International Award for Cancer Research was established in 1997 to annually recognize a scientist:
- who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research;
- who continues to be active in cancer research and has a record of recent, noteworthy publications;
- whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.

The Award is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the Award when their investigations are closely related in subject matter and have resulted in work that is worthy of the Award. In the rare event that there are dual winners of the Award, the cash award will be shared equally between them, and the AACR Executive Committee will determine which of the two co-recipients will present the Pezcoller-AACR Award Lecture at the AACR Annual Meeting.

Candidates for the Award will be considered by a prestigious international Selection Committee of renowned cancer leaders appointed by the President of the AACR and the Council of the Pezcoller Foundation. The Committee will consider all nominations as they have been submitted; the Committee may not combine submitted nominations, add a new candidate to a submitted nomination, or otherwise make alterations to the submitted nominations. After careful deliberations by the Committee, its recommendations will be forwarded to the Executive Committee of the AACR and the Council of the Pezcoller Foundation for final consideration and determination.

Selection of the Award winner will be made on the basis of the candidate's scientific accomplishments. No regard will be given to race, gender, nationality, or religious or political view.

The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Previously the Pezcoller Foundation gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science, in collaboration with the ESO-European School of Oncology.

The American Association for Cancer Research (AACR) was founded in 1907 by a group of 11 physicians and scientists interested in research, "to further the investigation and spread the knowledge of cancer." Today, the AACR accelerates progress toward the prevention and cure of cancer by promoting research, education, communication, and collaboration

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

Because of the commitment of the Pezcoller Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of the Award. This will

strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting (April 1-5, 2017) in Washington DC and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy (May 5, 2017). The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: August 10, 2016
Questions about the nomination process:
Monique P. Eversley, M.S., Senior Coordinator,
Scientific Review and Grants Administration
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28th Pezcoller Symposium

INITIAL STEPS ON HE ROUTE TO TUMORIGENESIS

Trento, Italy • June 20 - 21, 2016

PROGRAM

MONDAY, JUNE 20, 2016

8:00 Registration

8:35 Davide Bassi **Welcome**

8:45 David Livingston **Focus & Goals**

Session I, Tumor cell communication, competition and immunogenicity
Chair/Moderator: Eugenia Piddini

09.00 Glenn Dranoff
Mechanisms of Protective Tumor Immunity
09.25 Discussion
09.40 H.Reimer-Rodewald
Cell competition, progenitor turnover and T cell acute lymphoblastic leukemia
10.05 Discussion
10.20 *Coffee Break*
10:35 Eugenia Piddini
The emerging role of cell competition in cancer formation from Drosophila studies
11.00 Discussion
11:15 Eduardo Moreno
Regulating the cellular composition of our bodies using fitness fingerprints
11.40 Discussion
11.55 Jorge Moscat
Cross-talks at the tumor microenvironment by autophagy adapters
12.20 Discussion
12.35 *Lunch*

Session II, Epigenetics and Cell Plasticity
Chair/Moderator: Stefano Piccolo

14.00 **Keynote Address**
The Road to Cancer: A Genetic Perspective
Introduction: Alberto Bardelli
Speaker: Bert Vogelstein, M.D. (by video connection)
15.00 Manel Esteller
Cancer Epigenetics: From Knowledge to Applications
15.25 Discussion
15.40 Juergen Knoblich
Forever young: Loss of temporal identity immortalizes stem cells during brain tumor formation
16.05 Discussion
16.20 Eduard Battle
Connecting Intestinal Stem Cells to Colorectal Cancer
16.45 Discussion
17.00 Poster session
17.40 Adjourn
19.30 Symposium Dinner

TUESDAY, JUNE 21, 2016

Session III, Early Steps in Cancer Development
Chair/Moderator: Mariano Barbacid

08.00 Bruce Ponder
Gene network approaches
08.25 Discussion
08.40 Mariano Barbacid
Understanding early events in K-Ras driven tumors
09.05 Discussion
09.20 Brian Wolpin
Altered systemic metabolism and early pancreatic adenocarcinoma
09.45 Discussion
10.00 Teresa Davoli
The role of aneuploidy during tumorigenesis
10.25 Discussion
10.40 *coffee break*
10.55 Matthew Freedman
Genetic and epigenetic insights into prostate tumorigenesis
11.20 Discussion
11.35 Omer Yilmaz
Dietary control of stem cells in physiology and cancer
12.00 Discussion
12:15 Richard Marais
The role of ultraviolet light in melanoma initiation
12:40 Discussion
12:55 *Lunch*
13:55 Ben Ebert
Clonal hematopoiesis: a precursor to hematologic malignancies
14.20 Discussion
14:35 Francesca Demichelis
Tumor evolution and cell differentiation
15.00 Discussion

Session IV, New Approaches to Detecting Early Steps in Cancer Development
Chair/Moderator: Massimo Loda

15.15 H.Van de Wetering
Organoid cancer models
15.40 Discussion
15.55 Meritxell Huch
Adult progenitors and organoid cultures for the study of human biology and disease
16.20 Discussion
16.35 Poster Discussion
17.45 David Livingston
Concluding remarks
17.55 Adjourn

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