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Summary

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It's with great pleasure that I announce that the recipient of the 2011 Pezcoller Foundation-AACR Award for Cancer Research is Pier Paolo Pandolfi, MD, PhD -Director, Cancer Genetics Program, Associate Director, Cancer Center Beth Israel Deaconess Medical Center and George C. Reisman Professor of Medicine, Harvard Medical School

The Selection Committee met in Philadelphia on November 11, 2010 and was chaired by William G. Nelson, M.D., Ph.D. ,Director S.K. Comprehensive Cancer Center, Johns Hopkins, Baltimore. The members of the Committee were James P. Allison, Memorial Sloan-Kettering C.C. New York; Anna Bagnato, Regina Elena Cancer Center Roma; Anne-LiseBorresen-Dale, Institute for Cancer Research University of Oslo; Alfredo Fusco, Università di Napoli; Moshe Oren, Weizmann Inst. of Science, Rehovat - Israel; Janet D. Rowely, Un. Of Chicago Med. Center; Gabriella Sozzi, Istituto Nazionale Tumori di Milano with Ex-officio members: Margareth Foti, PhD CEO of American Association for Cancer Research and Gios Bernardi, M.D. President of the Pezcoller Foundation.

Pandolfi was recommended as the recipient for his work in the field of cancer genetics and mouse models for cancer. His groundbreaking work is outstanding and has contributed to new therapies for treating cancer. The research carried out in Dr.Pandolfi's laboratory in the mid1990s led to an understanding of the molecular mechanisms and the genetics underlying the pathogenesis of acute promielocytic leukemia (APL). Importantly, his laboratory generated mouse models for the various subtypes of APL and showed that specific drug combinations were effective for specific subtypes of APL. These results supported conclusions that were emerging from clinical studies with these same drugs in China and led to clinical trials in the US and Europe, which have ultimately provided cures for most types of APL over the past 10 years.

In addition to this work, Dr.Pandolfi has made major contributions to our understanding of the role of PTEN deletion in solid tumors. He showed that deletion of PTEN in the mouse prostate resulted in prostate tumors that became more aggressive in the context of deletion of other tumor suppressor genes (p53 or p27). Importantly, he showed that deletion of PTEN leads to senescence and that loss of p53 circumvents the senescence, explaining the synergy of these two tumor suppressor genes. More recently, Dr. Pandolfi has made major breakthroughs in identifying microRNAs that target PTEN. In an extremely interesting paper published in Nature, Dr. Pandolfi showed that a PTEN pseudo gene acts as a tumor suppressor by binding to microRNAs that would otherwise target PTEN. This paper suggests that pseudo genes, which were previously thought to have no function, are actually playing critical roles in cellular regulation.

Pandolfi was introduced at the 2011 AACR Annual Meeting in Orlando where he delivered to a large audience the Pezcoller Lecture: "The Non-Coding Revolution: A Coding-Independent Function of Gene and Pseudogene mRNAs Regulates Tumour Biology".

The Award was given to Pandolfi on May 6, 2011 with a solemn ceremony in the prestigious reception hall of the Buonconsiglio Castle in Trento, Italy. In the same week he gave the "Korsmeyer Lecture" in Padova at VIMM to honor the memory of the late Stanley Korsmeyer who received the Pezcoller-AACR in 2004. A few days later I introduced him in Rome at the "Regina Elena" Cancer Institute where he gave the "Tecce Lecture".

This issue of the Journal is dedicated to the 23rd Pezcoller Symposium entitled "Engineering in Cancer Research" which will be held in Trento from June 16 to June 18, 2011.

The meeting program is been co-organized by Peter Friedl (U Nijmegen), Jeff Hubbell (EPFL-Lausanne), David Livingston (Dana Farber Cancer Institute) and with the collaboration of Enrico Mihich (Dana Farber Cancer Institute).

The topic will extend across a significant segment of scientific activity in this field from a mechanistic analysis of how mechanical forces affect cellular signaling and other key cell behaviors to a discussion of engineering science contributions to an analysis of the cellular microenvironment and cellular immunity, to nanotechnology, valuable cell capture, and therapeutic delivery, to the study of animal cancer models and, finally, to biosensing, intravital microscopy, and specific molecular imaging as modalities that can enrich the deconvolution of tumor cell and whole tumor behavior.

The speakers are: Anton Berns, The Netherlands Cancer Institute, Amsterdam, NL; Philippe Bousso, Pasteur Institute, Paris, F; Dennis Discher, University of Pennsylvania, Philadelphia, PA; Mauro Ferrari, The Methodist Hospital Research Institute Houston, TX; Peter Friedl, Nijmegen Centre for Molecular Life Sciences (NCMLS) Nijmegen, NL; Jeffrey Hubbell, Ecole Polytechnique Federale de Lausanne (EPFL) Lausanne, CH; Paul Janmey, Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, PA; Rakesh Jain, Massachusetts General Hospital, Boston, MA; Tyler Jacks, MIT David H Koch Inst for Integrative Cancer Research, Boston, MA; Kazunori Kataoka, University of Tokyo, Japan; David Livingston, Dana Farber Cancer Inst, Boston, MA; Enrico Mihich, Dana Farber Cancer Institute Boston, MA; David Mooney, Harvard University, Cambridge, MA; Michal Neeman, The Weizmann Institute of Science, Rehovot, Israel; Stefano Piccolo, University of Padova, Italy; Cynthia Reinhart-King, Cornell University, Ithaca, NY; Michael Sixt , Institute of Science and Technology, Klosterneuburg, A ; Joachim Spatz, Max Planck Institute for Metals Research, Stuttgart, D; Melody Swartz, Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, CH; Mehmet Toner, Harvard Medical School, Massachusetts General Hospital, Cambridge MA; Viola Vogel, Swiss Federal Institute of Technology (ETH), Zurich, CH; Ralph Weissleder, Center for Systems Biology, Massachusetts General Hospital, Cambridge MA; Valerie Weaver, Center for Bioengineering & Tissue Regeneration, University of California at San Francisco, CA; David Weitz, Harvard University, School of Engineering & Applied Sciences, Cambridge, MA.

The abstracts of this symposium are in the following pages.

Gios Bernardi
Editor and Pezcoller Foundation President

Picture on the front page, "2011 Pezcoller Foundation-AACR International Award for Cancer Research" ceremony in Trento. From the left: A. Andreatta, Mayor of Trento; U. Rossi, local Minister for Public Welfare and Health; G. Bernardi, President of the Pezcoller Foundation; P.P. Pandolfi, the winner; M. Foti, CEO of AACR; A. De Poli, interpreter.

23rd Pezcoller Symposium

Engineering influences in cancer research

Trento, Italy, June 16-18, 2011

ABSTRACTS OF ORAL PRESENTATIONS

Tumor cell heterogeneity and cell-of-origin of small cell lung cancer in mice

Kate Sutherland, Joaquim Calbo, Min-chul Kwon, Natalie Proost, & Anton Berns
The Netherlands Cancer Institute, Amsterdam.

Small cell lung cancer (SCLC) is one of the most lethal human malignancies, due to its high metastatic potential and chemo-resistance upon relapse. Using the mouse model for SCLC, we found that the tumors are often composed of phenotypically different cells, characterized by mesenchymal and neuroendocrine markers. These cells had a common origin as they shared specific genomic aberrations. Crosstalk between mesenchymal and neuroendocrine cells can endow the neuroendocrine cells with metastatic capacity, illustrating the potential relevance of tumor cell heterogeneity in dictating functional tumor properties. Interestingly, these neuroendocrine cells can convert into the mesenchymal component by Ras pathway activation, although additional concurrent changes are likely required. This raises the question of the cell-of-origin of this tumor.
To investigate this, we inactivated *Trp53* and *Rb1* in distinct cell types in the adult lung by targeting *Cre*-recombinase expression to Clara (CC10 positive) cells, neuroendocrine (CGRP positive) cells, and alveolar type 2 (SPC positive) cells using adenoviral vectors. Using these cell-type-restricted Adeno-*Cre* viruses we could show that inactivation of *Trp53* and *Rb1* can efficiently transform CGRP and SPC-positive cells leading to SCLC, albeit SPC positive cells at a lesser efficiency. In contrast CC10-expressing clara cells were largely resistant to transformation. The results indicate that NE cells serve as the predominant cell of origin of SCLC. The consequences of these observations for the treatment of SCLC will be discussed.

Visualizing CTL and NK cells cytotoxic activity in solid tumors

Jacques Deguine, Béatrice Breart, Fabrice Lemaître and Philippe Bousso
Institut Pasteur, Unité des Dynamiques des Réponses Immunes, Paris, France.

Cytotoxic T cells and NK cells (CTLs) are key players of anti-tumor immune responses. CD8 T cells can recognize and lyse cells expressing tumor antigens while NK cells can kill tumor cells that have dowregulated MHC class I molecule and/or upregulated ligands for NK cell activating receptor. To counteract the host immune response, tumors rely on a wide array of mechanisms to escape destruction by infiltrating cytotoxic effectors. Despite our fundamental knowledge on the interplay between the immune system and tumor microenvironments, we have a poor understanding on the efficiency with which intratumoral immune effectors find, interact

and kill their targets. Clearly, a better understanding of how these events occur and change during the course of tumor development will help identify why immune responses often failed to clear tumors. We will discuss how intravital two-photon imaging combined with fluorescent to probes to track tumor cell apoptosis in real time can provide quantitative informatation in tumor cell killing in situ. Moreover, we will present recent data showing that NK cells and CTL use strikingly different dynamics during tumor cell regression.

From extracellular matrix mechanics and nuclear rigidity in cell fates to flexibility and 'self' recognition in anti-cancer therapy

Dennis E. Discher, Dept. Chemical & Biomolecular Eng., Physics and Cell & Molecular Biology Graduate Groups, University of Pennsylvania, Philadelphia, PA.

From tissue matrices to viruses, biology is filled with remarkable polymeric structures that motivate mimicry with goals of both clarifying and exploiting biological principles. Several distinct systems will be described from our explorations of flexibility and 'nano'-shape in bio-function and recognition. First, crosslinked tissue matrices are measured and then imitated in their elasticity with polymeric hydrogels, demonstrating the potent influence of matrix elasticity on basic processes such as stem cell differentiation [1] while motivating diverse studies of nuclear structure-function [2] and conformational flexibility with novel Mass Spec proteomic approaches [3]. Second, filamentous viruses have inspired development of worm-like polymer micelles that reveal nano-therapeutics deliver better if flexible and non-spherical in shape [4]. The results have motivated deeper insight into 'self' compatibility in circulation [5] with lessons from cancer. The results collectively illustrate the potential for a productive interplay between materials biophysics and biomolecular engineering.

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Dennis E. Discher is the Robert D. Bent chaired Professor at the University of Pennsylvania in Engineering and Applied Science and in the Graduate Groups of Cell & Molecular Biology and Physics. He received a Ph.D. from the University of California, Berkeley and San Francisco in 1993 for studies in molecular and cell biophysics, and was a US National Science Foundation International Fellow at the University of British Columbia until 1996. He has coauthored more than 150 publications that range in topic from biochemical physics of protein folding to self-assembling polymers applied to disease and matrix effects on stem cells, with papers appearing in *Science, Cell,* and various *Nature* journals. Additional Honors and Service include a Presidential Early Career Award for Scientists and Engineers from the US-National Science Foundation, the Friedrich Wilhelm Bessel Award from the Humboldt Foundation of Germany, and membership on the Editorial Board for *Science*.

Nanotechnology-Enabled Individualized Medicine

Mauro Ferrari, Ph.D., President and CEO, Ernest Cockrell Jr Distinguished Endowed Chair, The Methodist Hospital Research Institute, Houston, Texas.

The advent of novel engineering technologies affords unprecedented advances toward long-elusive objectives of medical research. Individualized medicine responds to the

basic but generally unattainable question of identifying the right therapy, reaching the right therapeutic target in the body at the right time, and securing immediate feedback as for its efficacy and undesired collateral effect. Finally, individualized medicine appears to be a credible general objective in cancer and other fields of medicine, owing to the integration of classical disciplines of clinical medicine, methods of molecular biology, and novel technology platforms.
Nanotechnologies are of great interest in the context of the drive toward individualized medicine, and may prove to be the necessary catalyst for its large-scale implementation.
In this talk I will present nanoporous-silicon-based approaches for the individualization of medical intervention: multistage vectors for the preferential localization of therapeutic agents; therapeutic monitoring nanotextured chips for the proteomic and peptidomic content profiling of biological samples; nanochannel delivery systems for intelligent time-release from implants, and bionanoscaffolds for tissue regeneration.
While novel nanoplatforms engender direct clinical applications, at the same time they afford the formulation of novel frameworks and hypotheses for the basic understanding of pathological processes. In particular, multistage particulates are the probes that afford the exploration of a new perspective of cancer, that is, that a unifying aspect of the canonical 'hallmarks of cancers' all relate to dys-regulation of mass transport at scales including the molecular, cellular, microenvironmental, and systemic. These considerations are the starting point for "Transport OncoPhysics".
The majority of this presentation will be dedicated to the theme of Transport Oncophysics, and in particular will address how engineered nanoconstructs such as our multi-stage vectors can be employed to deliver therapy and imaging contrast agents in an individualized manner. The principal innovation is that the individualization of treatment is attained by individualizing the vector to the target lesion, and more specifically to the characteristics of blood flow in the neo-vascular bed supplying the lesion, as determined by radiological imaging. Examples will be provided, including the delivery of therapeutic siRNA in murine models of ovarian cancer, of cytotoxic agents in orthotopic mouse models of breast cancer, and of MRI contrast agents of enhanced relaxivity.

Infrared multiphoton microscopy to visualize cancer invasion and response to molecular therapy

Peter Friedl
Microscopical Imaging of the Cell, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

The tumor microenvironment contributes to cancer invasion, growth and survival and thereby impacts tumor responses to therapy. Multiphoton microscopy (MPM) has become the method of choice for investigating cell structure and function in tissues and organs, including the invasion and progression of cancer lesions. Using a novel approach of infrared-excited (IR-)MPM at wavelengths above 1080 nm that enhances deep and non-toxic tissue microscopy in orthotopic fibrosarcoma xenografts, we here show deep collective invasion strands of several hundred connected cells. Invasion was fast (up to 200 µm per day), non-destructive and independent of ß1 and ß3 integrins. Despite normoxia, perivascular invasion strands were resistant to high-dose hypofractionated irradiation which otherwise was sufficient to induce regression of the tumor main mass. This invasion-associated radioresistance was sensitive to the simultaneous inhibition of ß1 and ß3 integrins by RNA interference or combined anti-ß1/αV integrin antibody treatment caused byproliferation arrest, anoikis induction ablating both tumor lesion and invasion strands. Thus, collective invasion is an important invasion mode in solid tumors into a microenvironmentally priviledged perivascular survival niche which conveys radioresistance by integrin-dependent signals. Consequently, combining anti-integrin therapy with hypofractionated irradiation may be amenable to clinical cancer treatment of locally destructive and otherwise radioresistant tumor lesions.

Modulating Cellular and Immune Responses with Protein-based and Synthetic Materials

Jeffrey A. Hubbell and Melody A. Swartz, Institute for Bioengineering, Ecole

Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.

In this presentation, two examples of materials engineering at the nanoscale will be provided, one on templating signalling from parallel cell-surface receptor systems with the recombinant variants of the extracellular matrix protein fibronectin (FN), and one on using nanomaterials to modulate the immune microenvironment within lymph nodes that drain tumors.

Templating cell signalling. The extracellular matrix plays an important role in modulating cellular behaviour in many environments, including in tumors. Although FN is usually considered as an adhesion molecule, it had been previously demonstrated to bind to VEGF-A165 and to modulate its signaling by templated co-association of VEGF-R2 and integrins. To explore these interactions, we have engineered a fibrin-binding domain of FN, containing the 12^{th}-14^{th} type III repeat (FN III 12-14, which was known to bind VEGF-A). In studies of the FN III 12-14, we determined that the growth factor (GF) binding activity of this domain was rather promiscuous, binding to GFs from the platelet-derived growth factor, fibroblast growth factor, transforming growth factor-b and neurotrophin families. Overall, 25 new binding interactions were demonstrated and thus, GF binding may be one of FN's main physiological functions. The FN III 12-14 domain resides approx. 8 nm from the major integrin-binding domain of FN, the FN III 9-10 domain, which binds principally integrin a5b1. We explored synergistic signaling between this integrin and the receptors for the growth factors VEGF-A165, PDGF, and BMP-2 in contexts of inducing angiogenesis (in response to VEGF and PDGF) and mesenchymal stem cell migration and osteogenesis (in response to BMP-2 and PDGF), and observed synergy both *in vivo* and *in vitro* only when the growth factors were templated by FN III 9-10/12-14, i.e. a fusion protein of the two domains appropriately spaced, but not FN III 9-10 + FN III 12-14, i.e. by two separate proteins of the two domains. Although our main examples are morphogenesis in the context of regenerative medicine, it seems likely that morphogenetic influences in tumors could also be in play.

Nanomaterials for modulating immune interactions. We have developed polymeric nanomaterials, subviral in size, that are rapidly cleared into the lymphatics that drain the injection site. The materials comprise a hydrophobic core and are able to sequester and slowly release hydrophobic drugs. To explore the extent to which the environment within the lymph nodes draining a tumor can be modulated, we encapsulated the chemotherapeutic paclitaxel, which is released over several days. When injected intradermally into a tissue bed that drains to the same lymph node as a tumor (but not injecting so as to drain into the tumor itself), tumor immune responses can be dramatically altered. Tumor growth is blunted, and the number of tumor antigen-specific CD8 T cells is elevated by this draining lymph node-directed chemotherapy, even though systemic doses are far too low to demonstrate any direct antitumor effects. This effect may be mediated through the activity of paclitaxel to activate TLR-4, as the node-directed chemotherapy dramatically upregulates dendritic cell activation within the tumor-draining lymph node.

Developing a molecular biography of lung cancer

Tyler Jacks
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Adenocarcinoma of the lung progresses through multiple stages including tumor initiation, expansion in the lung, and metastasis of the primary tumor to distant organs. Dissection of this process requires quantitative and tractable models that recapitulate the human disease at the genetic and histological level. Over the past several years, we have employed gene targeting technology to create a series of mouse models of non-small cell lung cancer that share genetic and pathological features of the cognate disease in humans. Tumors in these models are initiated through somatic activation of oncogenic K-ras, either through a spontaneous recombination event or via Cre-mediated recombination. Amplification of the oncogenic signaling is associated with progression to adenocarcinoma in this model. The p53 tumor suppressor limits tumor progression in this model. Restoration of the *Trp53* locus has pinpointed the adenoma-

adenocarcinoma transition as the stage in which p53 function is critical for tumor suppression. Expression of oncogenic *Kras* in the absence of p53 leads to the development of distant metastases after long latency. Gene expression and DNA copy number analyses of metastasis-derived cell lines and primary lung tumors have uncovered a metastasis associated gene expression signature and recurrent genomic alterations. Integration of these datasets, other genome-wide analyses and human tumor data are providing insights into the molecular mechanism and biology of lung cancer progression and metastasis. In addition, working with colleagues from the laboratory of Sangeeta Bhatia, we have used extracellular matrix (ECM) arrays to search for patterns of ECM components that distinguish adhesion by metastatic and non-metastatic cell lines. With the Bhatia and Anderson laboratories, we are using nanotechnology-based approaches to attempt to deliver to lung cancers in vivo members of the mir-34 family of microRNAs, which are regulated by p53 and are capable of inducing p53-like responses in cancer cells.

"Normalizing tumor microenvironment to treat cancer: From mathematical model to mouse to man"

Rakesh K. Jain, Ph.D. Andrew Werk Cook Professor of Tumor Biology
Harvard Medical School Director, Edwin L. Steele Laboratory for Tumor Biology
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For more than three decades, Dr. Jain has championed the notion that a solid tumor is like an aberrant organ – comprised of cancer cells and host cells embedded in an extra-cellular matrix – nourished by blood vessels and drained by lymphatic vessels. To unravel the complex biology of this aberrant organ, he and his multi-disciplinary team of engineers, physicists, biologists and oncoloists developed an array of cutting-edge and innovative imaging technologies and sophisticated mathematical and animal models. Using these tools, they showed that blood and lymphatic vessels as well as matrix associated with tumors are abnormal and how these abnormalities can create a hostile tumor microenvironment (e.g., hypoxia, high interstitial fluid pressure). They also revealed consequences of these abnormalities – specifically, how these abnormalities fuel malignant properties of a tumor as well as prevent treatments from reaching and attacking tumor cells. Dr. Jain then proposed a novel concept that "normalizing" tumor vessels and matrix would allow cancer therapies to penetrate the mass and to function more effectively. He then went on to show first in mice and then in cancer patients that anti-angiogenic drugs - originally deigned to destroy tumor vessels - could, paradoxically, also "normalize" them, creating a window of opportunity to attack the cancer most effectively. This concept is also opening doors to treating other vascular diseases, such as age-related wet macular degeneration, a leading cause of blindness, and neurofibromatosis-2, which can lead to deafness. More recently, he has shown that the drugs approved by the FDA for lowering hypertension can "normalize" the collagen matrix and improve the delivery of molecular and nanomedicine.

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Control of cell function by extracellular matrix mechanics

Janmey, P.A., Univ. Phensilvanya, Philadelphia, PA.

Many cell types are sensitive to mechanical signals. One striking example is the modulation of cell proliferation, morphology, motility, and protein expression in response to substrate stiffness. Changing the elastic moduli of substrates alters the formation of focal adhesions, the formation of actin filament bundles, and the stability of intermediate filaments. The range of stiffness over which different primary cell types respond can vary over a wide range and generally reflects the elastic modulus of the tissue from which these cells were isolated. Mechanosensing also depends on the type of adhesion receptor by which the cell binds, and therefore on the molecular composition of the specific extracellular matrix.

The viscoelastic properties of different extracellular matrices and cytoskeletal elements also influence the response of cells to mechanical signals, and the unusual non-linear elasticity of many biopolymer gels, characterized by strain-stiffening leads to novel mechanisms by which cells alter their stiffness by engagement of molecular motors that produce internal stresses. The molecular mechanisms by which cells detect substrate stiffness are largely uncharacterized, but simultaneous control of substrate stiffness and adhesive patterns suggests that stiffness sensing occurs on a length scale much larger than single molecular linkages and that the time needed for mechanosensing is on the order of a few seconds.

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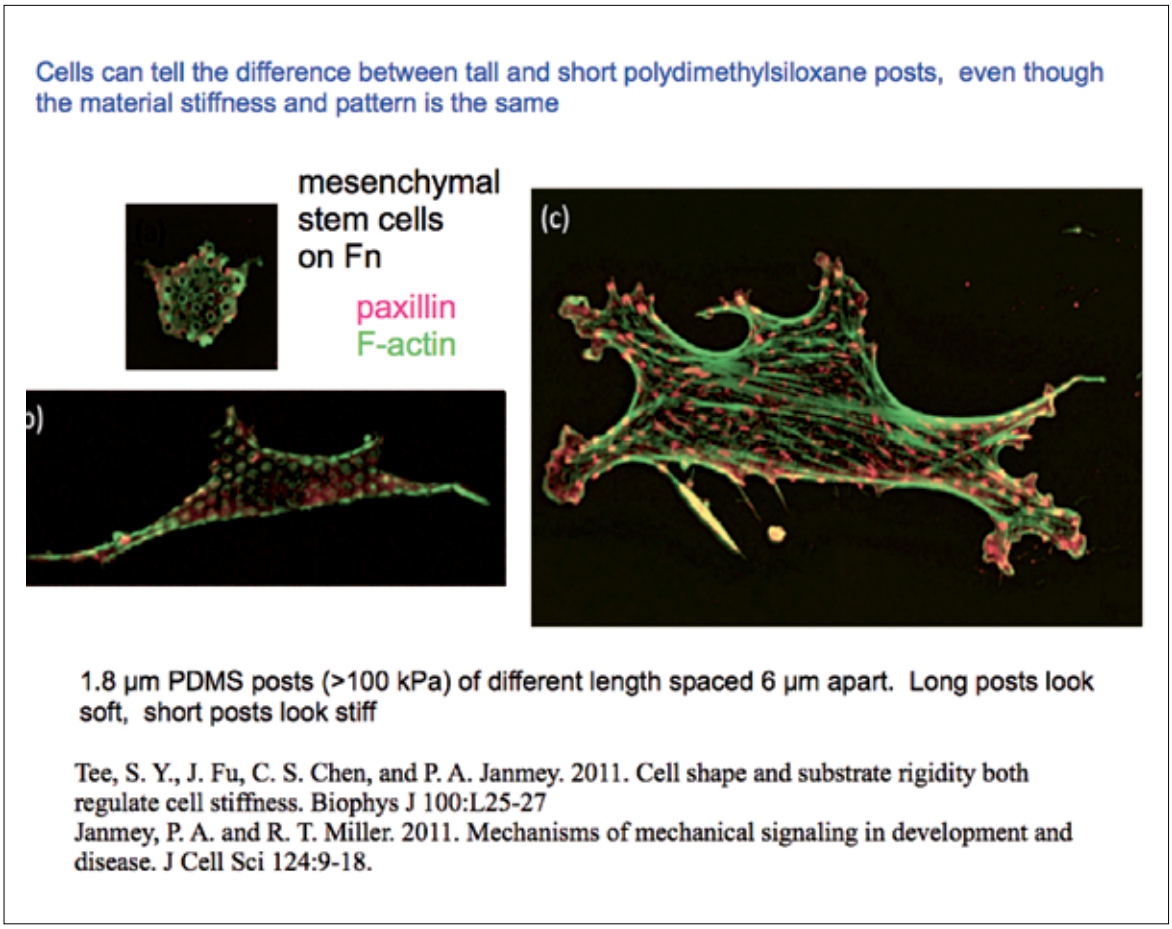

Bioengineering and Clinical Applications of Circulating Tumor Cell (CTC) Chip

Mehmet Toner, PhD
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Viable tumor-derived circulating tumor cells (CTCs) have been identified in peripheral blood from caner patients and are probably the origin of intractable metastatic disease. The reliable isolation of CTCs using the microfluidic chip in metastatic cancer offers the possibility of monitoring patient response and changes in tumor genotypes during the course of treatment.
However, the ability to isolate CTCs as a potential alternative to invasive biopsies as a source of tumor tissue for detection, characterization and monitoring of cancer patients have proven to be difficult due to the exceedingly low frequency of CTCs in circulation. We previously demonstrated the effectiveness of a microfluidic device, the CTC-Chip, in capturing rare CTCs using antibody-coated micro-posts under laminar flow conditions. More recently, we developed a second-generation chip based on high throughput microfluidic mixing approach, the herringbone-chip, or 'HB-chip', which provides an enhanced platform for CTC isolation. The HBchip design applies passive mixing of blood cells through the generation of micro-vortices to dramatically increase the number of interactions between target CTCs and the antibody-coated chip surface. We first analyzed the fluid flow and the chemical kinetics of the CTC capture using theoretical modeling and cell line studies. We then applied the microfluidic platforms to blood samples obtained from metastatic lung, prostate, breast, colon, and pancreatic cancer patients. These studies with patient blood showed very high sensitivity and specificity of the microchip.
We also tested the microchip in a cohort of patients with metastatic cancer undergoing systemic treatment and showed the temporal changes in CTC numbers correlated well with the clinical course of disease as measured by standard radiographic methods. To further show the utility of the CTC-chip, we isolated CTCs from patients with metastatic non-small-cell-lung cancer and identified the expected EGFR activating mutation in CTCs. We also detected the T790M mutation, which confers drug resistance, in CTCs collected from patients with EGFR mutations who had received tyrosine kinase inhibitors. More recently, we applied microchip to isolate CTCs from blood specimens of patients with either metastatic or localized prostate cancer. Remarkably, the low shear design of the HB-chip revealed micro-clusters of CTCs in a subset of patient samples. Microscopic CTC aggregates may contribute to the hematogenous dissemination of cancer. Currently, the work is focused on dissemination of the technology to multiple clinical centers as well as the development of novel tools for high sensitivity detection of CTCs for early detection of cancer.

Polymers to control immune cell trafficking

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Cancer vaccines typically depend on extensive manipulation of cells in the laboratory, and subsequent cell transplantation leads to large-scale cell death, poor lymph node homing and limited efficacy. We propose that materials mimicking aspects of microbial infection may instead be used to directly control immune cell trafficking and activation in the body.[1] This possibility was addressed using a macroporous system fabricated from poly(lactide-co-glycolide), an FDA-approved biodegradable polymer, to first provide a sustained delivery of an inflammatory cytokine, GM-CSF, to create a gradient in the surrounding tissue to recruit host dendritic cells (DCs). The subsequent local presentation of CpG oligonucleotides and cancer antigens to DCs residing in the material was used to activate the resident DCs, and induce antigen-presenting DCs to home to the lymph nodes. This system generated specific and protective anti-tumor immunity in a prophylactic melanoma model,[2] and demonstrated complete regression of distant and established melanoma tumors

in a therapeutic model.[3] These materials show broad promise as vaccine systems, and demonstrate the principle that appropriately designed polymeric delivery systems may be designed to replace current cell therapies.

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Molecular Imaging as a tool for revealing the control of tumor angiogenesis

Michal Neeman
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Despite the relatively low success of controlling tumor progression by suppression of angiogenesis, it is clear that remodeling of the vasculature plays an important role in cancer progression and response to therapy. In order to understand the role of blood and lymphatic vessels in tumor physiology, it is important to visualize them in context of the living organism, as their structure and function are tightly linked, spatially and temporally to changes in the surrounding cells. Over the last years molecular imaging expanded our capabilities in visualizing processes beyond the classical parameters of the vasculature such as blood volume and flow, vessel permeability and perfusion. New tools emerged for monitoring the expression of specific genes, the changes in cell surface markers and the alteration in differentiation, proliferation, migration and survival of endothelial and perivascular cells.
The extracellular matrix is altered significantly during angiogenesis. Molecular imaging tools reveal changes in collagen deposition and it's degradation by MMPs, the extravasation of fibrinogen and cross linking of fibrin to form a provisional matrix. The high molecular weight hyaluronan is degraded by tumor derived hyaluronidase, to convert it from anti to pro-angiogenic fragments. Maturation of vessels can be revealed by gain of vasoreactivity, and leads to increased survival of the vessels. In tumors, part of the properties of mature vessels are gained by recruitment of fibroblasts and myofibroblasts, which can be followed by imaging. Recruitment of this supporting stroma cells significantly alters vascular morphology and physiology.

Role of YAP/TAZ in mechanotransduction

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Cells perceive their microenvironment not only through soluble signals but also in term of physical and mechanical cues, such as extracellular matrix (ECM) stiffness or confined adhesiveness. By mechanotransduction systems, cells translate these stimuli into biochemical signals controlling multiple aspects of cell behavior, including growth, differentiation and cancer malignant progression; but how rigidity mechanosensing is ultimately linked to activity of nuclear transcription factors

remains poorly understood. Here we report the identification of the *Yorkie*-homologues YAP and TAZ as nuclear relays of mechanical signals exerted by ECM rigidity and cell-shape. This regulation requires Rho activity and tension of the acto-myosin cytoskeleton but is independent from the Hippo/LATS cascade. Crucially, YAP/TAZ are functionally required for differentiation of mesenchymal stem cells induced by ECM stiffness and for survival of endothelial cells regulated by cell geometry; conversely, expression of constitutive active YAP overrules physical constraints in dictating cell behavior. These findings identify YAP/TAZ as sensors and mediators of mechanical cues instructed by the cellular microenvironment.

Cellular Forces in Angiogenesis

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To adhere and migrate, most cells exert traction stresses against their extracellular matrix. In addition to driving cell movements, cellular traction stresses also aid in the ability of cells to establish and maintain cell-cell contact within tissues. Here we show that cellular force generation plays a critical role in establishing the balance between cell-cell and cell-matrix adhesion necessary for the formation of vasculature. Using engineered matrices mimicking the stiffness of the normal and tumor tissue microenvironments in concert with measurements of cellular traction stresses, we have discovered that increases in the stiffness of the extracellular matrix contribute to loss of endothelial cell-cell connectivity and decreased network formation. Matrix stiffening drives increased cell-matrix adhesion and contractility resulting in weakening of endothelial cell-cell contacts, whereas more compliant matrices drive the formation of tight cell-cell junctions and the assembly of endothelial cells into network structures. These results indicate that tissue stiffening, like which occurs during tumor progression, may contribute to the disorganized vasculature that is a hallmark of growing tumors. The significance of the effects of matrix stiffening on angiogenesis will be discussed.

Mechanics of leukocyte locomotion

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The organization principle of the immune system is based on high speed cell motility. Accordingly, immune cells migrate up to 100 times faster than mesenchymal and epithelial cell types. Although the biophysical migration mode of such fast cells is still poorly investigated some principles are emerging and it is now well established that leukocytes do not strictly rely on transmembrane adhesion receptors when they crawl through the interstitial environment, which is usually a 3D scaffold of extracellular matrix molecules. Instead leukocytes are able to directly transduce force by deformations of the cell body. Using in vitro and ex vivo imaging approaches we now demonstrate that deformation based migration is not the default strategy of leukocyte locomotion but rather part of a plasticity program that allows the cells to instantaneously switch between adhesion receptor dependent and independent migration. We find that invasion of dense matrices and crawling over stiff surfaces relies on adhesion while migration in the confined space of an interstitium does not and that leukocyte can shift back and forth between these modes without altering their proteome. We find that apart from the geometry of the extracellular environment also the distribution of the guidance cue can dictate the locomotion strategy and immobilized cues preferentially cause adhesive migration whereas soluble cues trigger adhesion independent movement.

Induction of cellular responses by molecularly defined nanopattern

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Introduction:
Integrin based adhesion has been shown to participate in numerous processes in living

cells, which sense, via their adhesions, multiple environmental cues, integrate them, and develop a complex, multi-parametric response. However, due to their intrinsic molecular complexity the specific functional roles of different components of the adhesion site are still poorly understood *(1)*.

Methods:
The specific system investigated in this project is the extracellular matrix (ECM) adhesion system, whereby cells sense the chemical and physical properties of the surrounding environment, via specific receptors of the integrin family. The cellular responses to these interactions are complex and diverse, and include changes in the cells' shape and dynamics, reorganization of the cytoskeleton, and regulation of long-range and long-term signaling processes that affect cell viability, differentiation and proliferation (*1*). By working toward this aim, a specific molecular understanding of how living cells interpret complex chemical and physical cues presented to them by their immediate environment will be achieved. The stimulating environments used in these experiments will be either natural matrices, made of fibronectin- or vitronectin-, or synthetic matrices of varying degrees of stiffness or integrin ligand spacing, building on our past experience in nanoscopically defined model cell adhesion matrices (*2, 3)*.

Results:
Many cell functions are known to depend both on the elasticity of their environment and on the distribution of available cell adhesion ligands. So far an independent control of these two parameters in cell adhesion experiments *in vitro* has been impossible. Here we present a method which allows individual control of substrate stiffness, ligand density and spacing by fabrication of nanoscopically controlled biomolecule anchors using micelle nanolithography, followed by transfer onto elastic polyethleneglycole polymers. To evaluate cell adhesion on the substrates, live cell imaging and single cell force microscopy were used. We show that on surfaces with a Young's modulus larger than 8 kPa, cell adhesion can be manipulated by varying ligand density, whereas on softer surfaces cell adhesion is consistently deficient, irrespective of ligand presence (Fig. 1).

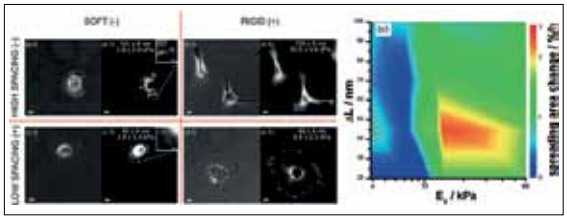

Fig. 1. Morphology of cells as affected by the physical state of the extracellular matrix (molecular spacing and substrate stiffness as indicated by insets): Phase contrast (I) and fluorescence micrographs (II) of rat embryonic fibroblasts stably transfected with paxillin fused to yellow fluorescent protein (REF-YFP-paxillin) on elastic nanopatterned substrates under cell culture conditions 24 hours after seeding. Scale bars (a)-(d): 10 µm. Insets: Focal contact and focal adhesion formation on different substrates. Scale bars: 2 µm. (e) Signaling of cells as affected by variations of the physical state of the synthetic extracellular matrix (DL=molecular spacing of ligands, Eg=substrate stiffness): cellular spreading rate given in color code.

Discussion & conclusions:
Hence, our results demonstrate that substrate elasticity and ligand density is independent from each other with respect to cell adhesion response. It is also shown that the cellular spreading rate is maximized with physical substrate parameters that are between ≈ 12 and 30 kPa stiff, and 35-65 nm in ligand spacing presentation.

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Acknowledgements:
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Tumor-associated lymphatics: Hijacking mechanisms of peripheral tolerance

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Lymphatic vessels drain fluid, proteins and macromolecules, and immune cells from the periphery to the lymph nodes (LNs). In addition to transporting activated dendritic cells to mount adaptive immune responses in the LN, lymphatic drainage is brings soluble antigens from the periphery to LN-resident immature

dendritic cells and B cells. It is also the most common site for cancer metastasis. Despite its importance, the role of tumor-associated lymphatic vessels and their drainage to the LN in regulating host immune responses to the tumor is poorly understood. We show that tumor expression of VEGF-C, the most potent lymphatic growth factor, promotes pro-tumor immune tolerance by several mechanisms. For one, it enhances drainage to the draining LN, where tumor antigens along with suppressive cytokines bathe the LN and could affect B and T cell education there. Second, tumor VEGF-C upregulates CCL21 in the tumor stroma and surrounding lymphatic vessels, which itself drives immune tolerance as we previously demonstrated. Third, VEGF-C drives peritumoral lymphangiogenesis, which can modulate the myeloid cell repertoire towards more suppressive phenotypes. These changes resulted in increased infiltrations of regulatory T cells and myeloid-derived suppressor cells, and increased levels of regulatory cytokines. Interestingly, VEGF-C-expressing tumors were impervious to prior immunization against tumor antigen, unlike control-transfected tumors, which were hindered by the vaccine-induced immune response. Together, these findings suggest that lymphatic drainage serves to maintain peripheral tolerance and that tumors may hijack such mechanisms to escape host immunity.

Mechanoreciprocity Regulates Tumor Progression

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Tumors are initiated by oncogenic transformation that is frequently accompanied by a desmoplastic response characterized by extracellular matrix (ECM) deposition, cross-linking and MMP-dependent remodeling. Oncogenically-transformed cells are also frequently more contractile than normal cells and we showed that oncogenic transformation induces cell contractility through engagement of RhoGTPases. The consequence of these pathological changes is that tumors are frequently stiffer than normal tissue. We have been studying the consequence of extrinsic (ECM stiffening) and intrinsic (cell contractility) tension in the malignant progression of a variety of cancers including breast, skin, pancreatic and brain. Thus far our studies have established a positive association between ECM stiffening, tissue fibrosis and the invasive phenotype of breast, pancreatic and skin cancers. We showed that inhibiting ECM tension, through pharmacological or antibody-mediated inhibition of lysyl oxidase-mediated collagen cross-linking, reduced tissue fibrosis and prevented breast and skin tumor proliferation and invasion. We also demonstrated that reducing tissue tension not only enhanced tumor latency and decreased tumor incidence but also significantly repressed lung metastasis. Organotypic models employing ECMs with calibrated stiffness further demonstrated that ECM cross-linking and stiffness collaborate with oncogenes to promote the invasive behavior of a modified epithelial tissue and does so by increasing cell contractility to promote focal adhesion assembly and enhance growth factor signaling to PI3 kinase, ERK and Wnt. Indeed, we found that the enhanced epithelial cell contractility itself induces ECM remodeling and stiffening thereby fostering a vicious feed forward mechanism that enhances tumor growth and invasion and compromises tissue integrity. Consistently, in both breast and skin cancer, inhibiting integrin or focal adhesion activity prevented oncogene-initiated tumor progression and ECM remodeling and stiffening. More recently we found that Ras transformation sensitizes epithelial cells to mechanical cues and chronically increases cell tension by elevating the expression of integrin alpha 5 and fibronectin to potentiate cell force generation, and induce ECM remodeling that directs the persistent migration and invasion of tumor cells into the interstitial matrix. Collectively the work illustrates the dynamic and reciprocal association between oncogenic transformation and intrinsic and extrinsic tension in tumor progression and metastasis. (Work was supported by grants from the DOD BCRP W81XWH-05-1-330, NIH U54CA143836-01, R01 CA138818-01A1, 1U01 ES019458-01, CA151925-01 and 2R01CA085492-11A1 to VMW)

MicroNMR for Rapid and Multiplexed Molecular Analysis of Scant Human Cancer Cells

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Detection of cancer cells using image-guided interventions presents a valuable source of information for molecular diagnostics but conventional means of analysis remain limited. Recently, we have developed a quantitative micro-Nuclear Magnetic Resonance (NMR) system, capable of rapid, multiplexed analysis of cancer cells. The focus of this presentation will be to review this new technology and to summarize the latest experimental and clinical data. Thus far, our results show that cancer diagnostic accuracies with microNMR surpass those obtained using conventional analyses of cells and exosomes. This point-of-care technique is poised to significantly enhance current molecular diagnostic capabilities.

Cell Volume and Cell Stiffness: The Role of the Extra-Cellular Matrix Mechanics

David A. Weitz, Harvard Univ., School of Engineering & Applied Sciences, Cambridge, MA.

This talk will discuss new results that probe the volume of cells as a function of the stiffness of the substrate on which they are grown, and will discuss the consequences of these changes. In addition, results on the mechanics of the major constituent of the extra-cellular matrix, reconstituted gels of collagen, will be presented.

Supramolecular Nanomedicines for Targeted Cancer Therapy

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Polymeric micelles are one of the polymer self-assemblies comprised of distinct two domains of the hydrophilic shell and the core for loading a variety of drugs, from low molecular weight anticancer drugs to macromolecular nucleotides (Fig 1), and thus are promising nano-scale drug carriers. Currently, several micellar anticancer drugs are in Phase I to II clinical trials. Those studies have evidenced the practical utility of micellar formulations in systemic drug delivery, thereby promoting further developments of polymeric micelles as nanocarriers for various compounds relevant to diagnosis and therapy of intractable diseases. Engineering of block copolymers has allowed the fine-tuning in physicochemical and biological performances of polymeric micelles, such as size, stability, longevity in the blood stream, targetability for specific tissues, intracellular trafficking, and drug-releasing profiles. Herein, the strong potentials of polymeric micelles are demonstrated especially for the above-mentioned critical challenges with experimental evidences.

Based on the enhanced permeability and retention (EPR) effect found in solid tumors, polymeric micelles with both the size smaller than ~100 nm and the longevity in the blood stream have been evidenced to efficiently accumulate in several tumor models. On the other hand, recent studies have suggested that the tumor tissue penetration of macromolecules strongly depended on their size (or molecular weight) and also the types of tumor models. In particular, a hypovascular pancreatic tumor (BxPC3) was found to significantly impede the tissue penetration of a PEG-modified liposomal carrier with the size of ~100 nm, resulting in the poor therapeutic outcome. In this regard, (1,2-diaminocyclohexane)platinum(II) (DACHPt)-loaded micelles with varying diameters (from 30 to 100 nm) were constructed from PEG-polyglutamate (PGlu) block copolymers to examine the singular effect of micellar size on the tumor tissue penetration and the antitumor activity. Despite the similar profile in plasma clearance in the tested micelles, only 30-nm micelles were able to deeply penetrate the BxPC3 tumor and to achieve significant antitumor activity. In contrast, all the micelles were active against a colon tumor (C26) as they deeply penetrated the tissue. These results suggest that polymeric micelles with fine-tuning in their size possesses the strong advantage for the treatment of hypovascular tumors.

The PEG-PGlu/DACHPt micelles were further

found to overcome the oxaliplatin (the prodrug form of DACHPt)-resistance of cancer cells. The micelles exerted the similar IC_{50} value in cell viability between normal and resistant colorectal cancers, whereas IC_{50} in free oxaliplatin was drastically elevated in the resistant cancer. The resistance against oxaliplatin is believed to occur in the cytoplasmic detoxification systems, such as metallothionein and methionine synthase. Free platinum drug formulation can directly penetrate the cellular membrane, and then travel the cytoplasm to reach the cell nucleus as the target site. In this case, major portions of drugs are most likely to be entrapped in the detoxification systems. In contrast, macromolecular drugs including the micellar formulation are known to be internalized by cells through the endocytic pathway, followed by the microtubule tracking from the early endosomes to late endosomes, where luminal pH (~5.5) is significantly lower than extracellular pH (7.4). This lowered pH was confirmed to appreciably facilitate DACHPt release from the micelle, allowing DACHPt release selectively in the perinuclear region. Hence, it was concluded that the DACHPt micelles exerted the strong cytotoxic effect against the resistant cell by bypassing the detoxification systems through the endocytic pathway, which may contribute similarly to the circumvention of the ATP-dependent efflux pumps for overcoming a variety of resistant mechanisms.

How Forces Can Tune the Chemical Display of Extracellular Matrix Fibers

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Since the physical and biochemical properties of extracellular matrix provide critical differentiation cues to cancer cells, it is of major importance to deepen our understanding how rigidity factors of the stroma are altered during cancer progression, how the composition and architecture of extracellular matrix is changing, and at the level of mechanotransduction, how the stretching or perhaps cleavage of extracellular matrix fibers by myofibroblasts and cancer cells might alter the biochemical display of ECM fibrils. Integrated information at all these levels is require to understand and perhaps interfere with the reciprocal interplay of cancer cells with their microenvironments.

Our research has focused on developing experimental and computational tools to elucidate the detailed mechanisms how the functional display of the extracellular matrix protein fibronectin can be switched on or off by stretching it, and how this regulates the architecture of newly deposited collagen.

We thereby utilize FRET probes to visualize the extent to which ECM fibrils are stretched by cells in living 2D and 3D cell cultures. Stretch assays are employed to reveal the mechanical strain at which binding sites are activated or deactivated. Steered molecular dynamic simulations are finally essential to identify structural intermediates in the forced unfolding pathway of proteins and thus gain an insights into the mechano-regulated mechanisms involved. Deciphering how proteins can serve as mechano-chemical signalling switches is not only essential to learn how cancer cells probe and respond to their environments, but it has also far reaching implications in tissue engineering, systems biology and medicine.

ABSTRACTS OF POSTERS

Impact of carbamylation and glycation of collagen type I on tumor cell migration

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Carbamylation and glycation are well known as nonenzymatic posttranslational modifications that occur throughout the lifespan of proteins *in vivo*. Due to its longevity, collagen type I, largely present in connective tissues, becomes a prominent target for such modifications. Protein carbamylation and glycation have been implicated in normal aging and in a number of pathologies like diabetes, renal diseases and cancer. In addition, recent studies suggest a possible link between increased cancer risk and diabetes or end-stage renal disease. Here, we investigate the impact of carbamylation and glycation of collagen on the proliferation and migration capacities of the highly invasive human fibrosarcoma cell line HT1080. For this purpose, collagen type I extracted from rat tail tendons was used for *in vitro* carbamylation with cyanate or glycation with ribose. The amount of carbamylation and glycation was evaluated respectively via HPLC and spectrofluorimetry, and only the maximum levels of carbamylated and glycated collagens were considered. Tumor cell behavior was then evaluated on coatings of native, carbamylated or glycated collagen. Our results show that the proliferation of HT1080 cells on modified collagens did not differ from that on native form after 1, 2 and 3 days of culture. Concerning tumor cell adhesion, the glycated collagen delayed the adhesion time of seeded cells whereas the carbamylated form had no effect. Using time-lapse videomicroscopy, the migration ability of HT1080 was studied by quantifying single cell speed. Only, the glycated collagen strongly inhibited cell speed migration. We next investigated the effect of these collagen modifications on the organization of actin and vinculin, two proteins involved in cell locomotion. On glycated collagen, cells revealed a dramatic loss of actin stress fibers where disorganized F-actin was principally localized at the rim of the cell. Disturbance of actin integrity was accompanied with a disorganization of vinculin that was also localized at the cell periphery. On the other hand, the cells on carbamylated collagen maintained a fully organized actin network. The impact on the focal adhesion kinase (FAK), which plays a crucial role in focal adhesion formation, was also evaluated. Only glycated collagen induced a significant inhibition of the expression level of FAK, whereas both collagen modifications provoke a differential inhibition of its phosphorylation state that is mainly taking place with glycation. In conclusion, only glycation is demonstrated as an important factor affecting tumor cell migration. This impact has to be certainly considered in order to better understand the link between diabetes, aging and cancer incidence.

Genotoxic stress-dependent, p53-directed post-transcriptional controls of the VEGFR-1, FLT1 gene.

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We recently established that a C>T single nucleotide polymorphism (SNP) in the promoter of the Vascular Endothelial Growth Factor Receptor-1 (VEGFR-1), commonly known as FLT1, generates a half-site p53 response element (RE-T) that results in p53 responsiveness of the promoter. It was also

shown that p53 is required but not sufficient for FLT1 transactivation and that there is cooperative interaction with ligand-bound Estrogen Receptors (ERs) *via* two ER half-site (EREs) located near the RE-T. ER levels, specific ligands and genotoxic stresses were shown to influence the coordinated regulation of the FLT1-T promoter.
We have now asked whether activation of p53 by genotoxic stress could also regulate FLT1 at post-transcriptional levels. The FLT1 transcript can undergo alternative splicing resulting either in a membrane-bound-(mFLT1) or in a truncated soluble-form (sFLT1) that appears to act as decoy receptor, where a portion of intron 13 is retained in the mRNA introducing a premature STOP codon and an alternative 3'UTR. We measured by qPCR the expression levels of mFLT1 and sFLT1 transcripts in cell lines differing for the promoter SNP and p53 status: HCT116 (C/T, p53 $wt^{+/+}$or p53 $null^{-/-}$), GIMEN (C/T, p53 wt), MCF7 (C/C, p53 wt). Surprisingly, we observed that the relative abundance of mFLT1 and sFLT1 was differentially affected by doxorubicin treatment (doxo) in that mFLT1 was up-regulated in C/T heterozygous p53 wt cells, as expected, while sFLT1 levels did not change or were even reduced, depending on the cell line. Notably, ectopic expression of p53 in $HCT116^{-/-}$ led to the same specific increase in mFLT1/sFLT1 expression ratio. On the contrary, treatment with 5FU, while effective at activating p53, led to a selective strong upregulation of sFLT1. This effect was seen also in the MCF7 C/C cell line, but appeared to be dependent on the presence of p53 wt, based on results in $HCT116^{-/-}$.
As the mFLT1 and sFLT1 transcripts contain different 3'UTRs, we investigated using luciferase-based reporter vectors whether these regulatory sequences could underlie the differential response to p53 activation. Results indicated that doxo treatment in $HCT116^{+/+}$ selectively increased mFLT1-3'UTR stability. 5FU had no differential impact in this assay. We also developed an FLT1 minigene containing exon 13, exon 14 and part of the intron 13 as intervening sequence in a dual luciferase vector. Interestingly, in $HCT116^{+/+}$ cells transfected with the minigene treatment with 5FU but not doxo led to a shift in the splicing pattern toward sFLT1. Treatment with Nutlin, a molecule disrupting p53-MDM2 interaction, has a similar impact as 5FU. The effect of genotoxic stress-dependent, p53-directed changes in FLT1 transcripts' balance on FLT1-induced signalling is currently being evaluated, using the specific ligand PlGF. Collectively our emerging results are revealing that, in response to specific stimuli p53 can regulate not only FLT1 transcription, but also its mRNA maturation and stability.

The matricellular protein SPARC oppositely regulates inflammation and fibrosis in bleomycin induced lung damage with implication for malignant transformation.

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Although cigarette smoking has long been indicated as the predominant cause of lung cancer, other risk factors have been identified. Among these, chronic parenchymal inflammation leading to fibrosis has also been linked to increased lung cancer risk. It has been hypothesized that inflammation might concur to transformation but the absence of spontaneous mouse models render the comprehension of the underlying mechanisms quite challenging. In the context of bleomycin-induced pulmonary fibrosis we have demonstrated that the matricellular protein SPARC distinctly regulates inflammation and collagen deposition depending on its cellular origin. Reciprocal $Sparc^{-/-}$ and WT bone marrow chimeras revealed that SPARC in host fibroblasts is required to induce collagen fibrosis. $Sparc^{-/-}$>WT chimeras showed exacerbated inflammation and fibrosis due to the inability of $Sparc^{-/-}$ macrophages to down-regulate TNF production because of impaired response to TGFb1 . Accordingly, the use of bone marrow cells expressing a dominant negative form of TGFb-RII under the monocyte-specic CD68 promoter, as a decoy, phenocopied $Sparc^{-/-}$ donor chimeras. Despite the exacerbated fibrosis in $Sparc^{-/-}$ >WT BM chimeras no malignant transformation

was observed. We reasoned that a second event is likely required to favor malignant tranformation in the context of unhealing inflammation and fibrosis. To this end we increased the irradiation dosage during bone marrow transplantation. According to our hypothesis this event was sufficient to induce epithelial dysplasia ranging from low-grade to high-grade/in situ adenocarcinoma in wt> wt combination whereas in case of *Sparc*$^{-/-}$ as donor malignant transformation and invasive lung were the most evident outcome. Therefore, under the same radiation and BLM stimuli, a hampered capacity to control inflammation because of SPARC deficiency greatly increases lung carcinogenesis. This model provides a solid backbone for studies focusing on the contribution of chronic inflammation to lung carcinogenesis and points to matricellular proteins as key regulators of this process.

Rab5 couples migratory protrusions and pericellular proteolysis during tumor cell invasion.

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Cells, and in particular tumor cells, can adopt different modes of cell motility. The ability to switch between diverse modes of migration enables tumors to adapt to micro-environmental conditions and to metastasize. The critical pathways and cellular processes underlying the plasticity of tumor cell motility have only begun to be identified. An appealing hypothesis, supported by recent evidence, is that endocytosis, originally thought of as a device to internalize nutrients and membrane-boundmolecules, is a connectivity infrastructure (which we call “the EndocyticMatrix”) of different cellular networks necessary bound molecules ion of various cellular programs. A primary role of the Endocytic Matrix is the delivery of space- and time-resolved signals to the cell, and is thus essential for the execution of polarized functions during 3D cell migration and invasion. Here, we will focus on the endocytic and signaling functions of Rab5, a smallGTPases essential for endosome biogenesis. We will discuss experimental evidence that support the general paradigm that intracellular trafficking, controlled by Rab5, is needed to re-direct molecules to restricted regions of the plasma membrane and to couple the formation of Rac-dependent migratory protrusions with proteolytically active adhesive site, ultimately mediating tumor cell invasion in 3D matrices.

Cell mobility and metastatic spreading: a study on human neoplastic cells using optical tweezers

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The primary causes of death in cancer patients are local invasion and metastasis but their mechanisms are not yet completely understood. Metastatization is accompanied by alterations of the cytoskeleton and membrane structure leading to changes in their biomechanical properties[1]. In this study we analyzed by means of Optical Tweezers the mechanical properties of two different breast adenocarcinoma cell lines corresponding to different metastatic potential. OT were used to grab the plasma membrane by a 1,5 um silica bead and form a plasma membrane tether. We measured the force exerted by the cell membrane on the bead and drew the force-elongation curves. Fitting data in the Kelvin Body model [2]we found out the values for the viscoelastic parameters influencing the pulling of the membrane tethers.
The first cell line analyzed, MCF-7, associated to a low metastatic potential showed tether stiffness of 153 pN/um in average.
The second cell line, MDA-MB 231, poorly differentiated with a high metastatic potential had a tether stiffness of 36pN/um in average, that is a four times lower value. These results seems to confirm the hypotesis that metastasis prone cells are softer than less aggressive cancer cells, and support the use of OT for

these measurements for its sub-pN force resolution and because cells are manipulated without damage.

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Correlation between immunological findings and in vivo microscopy can assist evaluation of treatment effectiveness in mouse melanoma hyperthemia.

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BACKGROUND: Microwave induced hyperthermia (MWHT) is suitable for inducing changes in the microenvironment and architecture of tumors. Both direct (cellular damage) and indirect (heating diffusion) effects of MW can be used to modulate the tumor microenvironment immunity. The evolution of 1- and 2-photon laser scan confocal microscopy is promising for direct investigations of patient tissues *in vivo*. We investigated the possibility to correlate both tissue architecture and immunological changes in the view of better tumor-tailored treatments. MATERIALS, ANIMALS, AND METHODS: B16F10 melanomas developed in C57BL/6 mice were treated, under general anesthesia, with MWHT (max. 44°C x 20 min) using an appositely developed superficial applicator controlled by computerized feed-back thermometry (singular treatment). At scheduled times, treated animals still under general anesthesia were examined in confocal microscopy for tumor changes by combining reflection mode (RM), second harmonics generation (SHG) and autofluorescence methods. Immunological tests were performed at the same time schedule (FACS analysis, cytotoxicity, WB, cytokine arrays). RESULTS: The *in vivo* imaging evidenced both structural and, possibly, functional changes (e.g. tumor stroma collagen organization and inflammatory edema) after MWHT. They correlated with: 1) HSP-70 production; 2) temporary increase of cytokines and chemochines involved in inflammation, chemoactraction and activation of NK cells, monocytes and neutrophyls (TNF-α, MCP-1, IFN-γ) and modulatory activities (IL-10, IL-17, sTNF RI); 4) variations of MHC class I expression; 4) changes in immune cell subpopulation proportions; 5) rolling of leucocytes and clotting in the peripheral capillaries of treated tumor. Animals that presented the structural changes shower a reduced tumor growth according to the heat dose. CONCLUSIONS: Changes of immunity and *in vivo* confocal microscopy imaging can correlate to MWHT effects on target tissues, opening perspectives for future clinical treatment-monitoring and tumor-tailored applications. Acknowledgements: Grants 310/08/H077, GAAV IAA500200510, GAAV IAA500200509, and and IAA500200917 (CZ), Institutional Research Concepts No. AV0Z50200510 and AV0Z50110509; ARPA F oundation, Pisa (IT)

Colonic environment, immunity, cancer: a lesson from germ-free vs conventionally reared rats.

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BACKGROUND: The gut microbial environment is considered to play an important role both

in the colorectal tumor development and in the modulation of the mucosal immunity. Studies on animals reared in germ-free (GF, without any intestinal microflora) versus conventional (CV, with regular microflora of the bowel) conditions can assist to better enlightening the influence of microflora and the existence of bacteria-independent patterns on carcinogenesis and anticancer immune response.
Moreover, the comparison between the continuously activated immunity of CV animals vs the less stimulated mucosal immunity of GF animals can offer a new model to evaluate regulatory mechanisms of inflammation and their contribution to modeling the tissue structures. We investigated the gut of germ-free and conventionally reared rats to identify elements possibly influencing immune responses (especially anticancer). MATERIAS, ANIMALS AND METHODS: In our study we applied confocal imaging methods for living or fresh tissue examination in healthy and cancer induced AVN-Wistar rats following our model (Vannucci et al. Int J Oncol. 25(4):973-81, 2004). We also compared the immunity (lymphocyte subpopulations, cytotoxicity, etc) in healthy and cancer bearing animals. The induction of chronic colitis by dextran sodium sulfate orally administered in 3% aqueous solution produced structural changes in the mucosa and an acceleration of cancer development when azoxymethane (AOM) was subsequently administered (9 mg/kg bw 1x4 weeks) vs AOM-only treated animals . RESULTS: The GF rats present a different intestinal anatomy with larger bowel and thinner intestinal wall than conventional rats. Confocal microscopy on fresh unstained tissue revealed for the first time a different pattern of complexity in the collagen mucosal stroma that undergoes variations associated to K-ras, TGF-beta, VEGF expression in inflammation and cancer induced animals, with differences between the parts of the large bowel. Moreover, different infiltration pattern of the interstitial spaces by immune cells was confirmed. Healthy animals presented a naturally higher number of NK and NKT cells, as well as increased CD8+ cell subpopulation in cancer resistan animals when compared to the CV animals. Second harmonic generation revealed important modifications of the collagen architecture in the mucosal stroma associated to the inflammatory and carcinogenic stimulations. Conclusions: The observations in GF rats suggest that different antigenic challenge, the absence of the "physiologic inflammation" related to the presence of commensal microflora in the gut, and consequently different metabolism of the intestinal content may influence both structure and immune function of the bowel. The different stromal assessment may create different niches for colonocyte and immune cell maturation, with effects on local and systemic immunity. Chronic inflammation and carcinogenesis can condition the stromal architecture with possible effects on immune cell trafficking and recognition of the pathological tissue. Acknowledgements: grants of the GAAV IAA500200509 and IAA500200917, IRC AV0Z50200510 (CZ); ARPA Foundation, Pisa, and Ido and Cristina Gragnani Fund, Prato,Italy.

A Pin1/mutant p53 axis promotes aggressiveness in breast cancer

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TP53 missense mutations dramatically influence tumor progression, however their mechanism of action is still poorly understood. Here we demonstrate the fundamental role of the prolyl isomerase

Pin1 in mutant p53 oncogenic functions. Pin1 enhances tumorigenesis in a Li-Fraumeni mouse model and cooperates with mutant p53 in Ras-dependent transformation. In breast cancer cells, Pin1 promotes mutant p53 dependent inhibition of the anti-metastatic factor p63 and induction of a mutant p53 transcriptional program to increase aggressiveness. Furthermore, we identified a transcriptional signature associated with poor prognosis in breast cancer and, in a cohort of patients, Pin1 overexpression influenced the prognostic value of p53 mutation. These results define a Pin1/mutant p53 axis that conveys oncogenic signals to promote aggressiveness in human cancers.

Pezcoller Foundation-AACR

Call for 2012 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 eleven physicians and scientists dedicated to the conquest of cancer and now has over 25,000 laboratory, translational, clinical and epidemiological scientists engaged in all areas of cancer research in the United States and in more than 60 other countries around the world. The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. 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 2012), and the memorial Korsmeyer lecture at the VIMM in Padua and will receive the award in a ceremony at the Foundation's headquarters in Trento, Italy (May, 2012). The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: September, 12, 2011

Questions about the nomination process: Monique P. Eversley, Program Coordinator, Scientific Achievement Awards - American Association for Cancer Research, 17th Floor, 615 Chestnut Street, Philadelphia, PA 19106-4404 - Tel. +1 (267) 646-0576; e-mail: monique.eversley@aacr.org - www.aacr.org

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