Twenty-Third Annual Pezcoller Symposium: Engineering Influences in Cancer Research

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Abstract

The cross-disciplinary focus of the meeting highlighted recent progress in physical and genetic analysis and engineering of cancer disease models. As central theme, mechanical forces affecting cell signaling, growth, differentiation and metastasis were discussed with emphasis on the tumor microenvironment and cellular immunity, taking into account novel nanotechnology, biosensing and intravital microscopy tools to monitor animal cancer models and human cancer. Emerging themes were the role of extracellular matrix imposing mechanical mechanisms on tumor cell function including microenvironmental cues controlling the movement of tumor and immune cells, advanced genetic animal models for cancer that better recapitulate human disease, and preclinical and clinical molecular imaging of tumor architecture and stiffness, and novel nanotechnologies for anticancer drug delivery.

Findings Presented

The 23\textsuperscript{rd} annual Pezcoller Symposium titled “Engineering Influences in Cancer Research” was held in Trento, Italy, on June 16-18, 2011.

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Rakesh K. Jain (Massachusetts General Hospital, Boston, MA) gave the keynote address on normalization of tumor microenvironment to treat cancer as demonstrated by mathematical model and experimentation in mouse and in humans. Blood and lymphatic vessels and matrix associated with tumors are abnormal; these abnormalities can fuel tumor progression and prevent drugs from reaching tumor cells. In vivo imaging of tumors allowed the study of the effects of microenvironment (ME) on tumor cells and vice versa. Relaxin decreases collagen production and increases responses. Angiogenic drugs can also “normalize” tumor ME. More recently, anti-hypertensive drugs were shown to “normalize” the collagen matrix and improve the delivery and efficacy of therapeutics.

Stefano Piccolo (University of Padova, Italy) discussed the role of Yorkie-homologues YAP and TAZ in mechanotransduction and control of cell differentiation and growth. Cells perceive their microenvironment through soluble signals and physical and mechanical cues, such as extracellular matrix (ECM) stiffness. By mechanotransduction systems, cells translate these stimuli into biochemical signals controlling multiple aspects of cell behavior. YAP and TAZ are nuclear relays of mechanical signals exerted by ECM cell-shape: ECM stiffness causes their localization in nucleus, ECM relaxation their localization in cytoplasm. 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. Indeed, YAP/TAZ are sensors of cell architecture and mediators of mechanical cues from microenvironment.

Joachim Spatz (Max Planck Institute for Intelligent Systems & University of Heidelberg, Germany) indicated that cell signaling depends on the interplay of cell adhesion ligand spacing and elasticity of their environment. Individual control of substrate stiffness, single ligand density and spacing was obtained by using micelle nanolithography on elastic polyethleneglycole polymers of different degrees of stiffness. Through live cell imaging and single cell force microscopy it was found that substrate elasticity and ligand density are independent from each
other with respect to cell adhesion response which was displayed in a phase diagram for adhesion.

David Weitz (Harvard University School of Engineering and Applied Sciences, Cambridge, MA) discussed the relationships between cell volume and stiffness and the role of ECM. Cell stiffness and shape depend on substrate stiffness. The size of the nucleus also changes with changes in cell size as does the motion of particles within cells. Cells exert forces through ECM which affect motion and deformation.

Dennis Discher (University of Pennsylvania, Philadelphia, PA) discussed the effects of matrix stiffness on migration and differentiation. Crosslinked tissue matrices were imitated in their elasticity with polymeric hydrogels, demonstrating the potent influence of matrix elasticity on basic processes such as stem cell differentiation. Nuclear structure-function and conformational flexibility were focused upon with novel mass spectromatic proteomic approaches, and the plasticity of the nucleus in stem cell differentiation was highlighted. In parallel studies with a more therapeutic focus on flexibility, Worm-like polymer micelles that easily deform were shown to better deliver anti-cancer agents such as taxol compared to nanospherical shapes. The interplay between materials biophysics and biomolecular engineering was thus illustrated with diverse examples.

Paul Janmey (University of Pennsylvania, Philadelphia, PA) indicated that cell proliferation, morphology, motility, and protein expression are modulated in response to substrate stiffness. This can vary and reflects the elastic modulus of the tissue from which these cells were isolated. The viscoelastic properties of different ECM and cytoskeletal elements influence the cell responses to mechanical signals; non-linear elasticity of biopolymer gels leads to novel mechanisms by which cells alter their stiffness through engaging molecular motors producing internal stresses. Hepatic stellate cells convert into a spread phenotype, depending
upon the stiffness of ECM. The simultaneous control of substrate stiffness and adhesive patterns suggests that stiffness sensing occurs on a scale much larger than single molecular linkages and that the time needed for mechanosensing is on the order of a few seconds.

Cynthia Reinhart-King (Cornell University, Ithaca, NY) indicated that cellular traction stresses play a critical role in establishing the balance between cell-cell and cell-matrix adhesion and that establishing this balance is a necessity during angiogenesis. Matrix stiffening, a frequent hallmark of solid tumor progression, drives increased endothelial cell-matrix adhesion and weakening of endothelial cell-cell contacts; more compliant matrices decrease cell-substrate binding and increase the formation of tight cell-cell junctions and the assembly of cells into network structures. Tissue stiffening may thus contribute to the disorganized vasculature in growing tumors.

David Mooney (Harvard University, Cambridge, MA) proposed that materials mimicking aspects of microbial infection may directly and beneficially control immune cell trafficking and activation. A macroporous system fabricated from poly(lactide-co-glycolide), was used to provide sustained delivery of GM-CSF creating gradient in surrounding tissues and recruiting host dendritic cells (DCs). The local presentation of CpG oligonucleotides and cancer antigens to DCs was sufficient to activate resident DCs and induce them to home to the lymph nodes; complete regression of distant and established melanomas were seen in a therapeutic model. Thus, appropriately designed polymeric delivery systems may replace current cell therapies.

Micheal Sixt (Institute of Science and Technology, Vienna, Austria) indicated that immune cells migrate much faster and are more flexible than epithelial and mesenchymal cell types. This is partially due to their ability to instantaneously shift between adhesion receptor dependent and independent migration modes. He further demonstrated that the distribution of
important guidance cues (i.e., a chemokine) can determine if leukocytes migrate in the adhesive vs. non adhesive mode.

Valerie Weaver (University of California at San Francisco, San Francisco, CA) indicated that oncogenic transformation is frequently accompanied by ECM deposition, cross-linking and MMP-dependent remodeling. Tumors are frequently stiffer than normal tissue. The consequences of extrinsic ECM stiffening and cell contractility malignant progression of breast, skin, pancreatic and brain cancers were studied. Positive association between ECM stiffening, tissue fibrosis and invasive phenotype was shown. Inhibiting ECM tension prevented breast and skin tumor proliferation and invasion, and also repressed lung metastasis. Employing ECMs with calibrated stiffness demonstrated that ECM cross-linking and stiffness collaborate with oncogenes to promote the invasive behavior of an epithelial tissue: increased cell contractility promoted focal adhesion assembly and enhanced growth factor signaling. In breast and skin cancer, inhibiting integrin or focal adhesion activity prevented oncogene-initiated tumor progression and ECM remodeling. Ras sensitized epithelial cells to mechanical cues and elevated α5 integrin and fibronectin expression and thereby potentiated cell force generation. Induced ECM remodeling thus directs the persistent migration and invasion of tumor cells into interstitial matrix.

Peter Friedl (Nijmegen Centre for Molecular Life Sciences, Nijmegen, The Netherlands) outlined the use of infrared-excited multiphoton microscopy (IR-)MPM at wavelengths above 1080 nm that enhances deep and non-toxic tissue microscopy: in orthotopic fibrosarcoma xenografts fast collective invasion of several hundred connected cells was shown. Invasion-associated radioresistance of perivascular strands was sensitive to the simultaneous inhibition of β1 and β3 integrins. Combining anti-integrin therapy with irradiation may be amenable to clinical treatment of locally destructive and otherwise radioresistant tumor lesions.
Melody Swartz (Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland) indicated that tumor expression of VEGF-C promotes pro-tumor immune tolerance; it enhances interstitial fluid drainage to the draining LN, where tumor antigens along with suppressive cytokines could affect B and T cell education. Tumor VEGF-C led to increased infiltrations of regulatory T cells and myeloid-derived suppressor cells, and increased levels of regulatory cytokines. VEGF-C expressing tumors were impervious to immunization against tumor antigen, and cytotoxic T cells were deleted in the tumor and draining lymph node. Thus, tumors may use VEGF-C to hijack the normal lymphatic functions of peripheral tolerance to escape host immunity.

Kazunori Kataoka (University of Tokyo, Tokyo, Japan) indicated that currently micellar anticancer drugs are in Phase I to II clinical trials. 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 have been determined. A hypovascular pancreatic tumor significantly impeded the tissue penetration of a PEG-modified liposomal carrier with the size of ~100 nm. To overcome impaired delivery, (1,2-diaminocyclohexane)platinum(II)-loaded micelles with varying diameters were constructed from PEG-polyglutamate (PGlu) block copolymers and the effect of micellar size on tumor tissue penetration and antitumor activity was examined. Only 30-nm micelles penetrated the pancreatic tumor and achieved significant antitumor activity, but all the micelles tested were active against colon tumor (C26) following its penetration. These micelles were found to overcome oxaliplatin resistant tumors. Consequently, polymeric micelles smaller than ~100nm accumulate in tumor lesions, whereby tumor tissue penetration depends on their size and also on the tumor model.

Mauro Ferrari (The Methodist Hospital Research Institute, Houston, TX) discussed multistage vectors for therapeutic agents localization; nanotextured chips for proteomic and
peptidomic content profiling of biological samples; nanochannel delivery systems for intelligent
time-release from implants, and bionanoscaffolds for tissue regeneration. Multi-stage vectors
can deliver therapy and imaging contrast agents in individualized manners. Treatment
Individualization is attained by individualizing vectors to the characteristics of blood flow in the
neo-vascular bed supplying the lesion. The delivery of therapeutic siRNA in murine ovarian
cancer models cytotoxic agents in orthotopic mouse breast cancer models, and of MRI contrast
agents were discussed.

Jeffrey Hubbell (Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland)
discussed two examples of modulating cellular and immune responses with protein-based
synthetic materials, one on signaling from parallel cell-surface receptor systems with
recombinant ECM fibronectin (FN) variants, and one on using nanomaterials to modulate the
immune microenvironment within tumor draining lymph nodes. FN was shown to modulate its
signaling by co-association of VEGF-R2 and integrins. A fibrin-binding domain of FN was
engineered which bound platelet-derived GFs, fibroblast growth factor, transforming growth
factor-β and neurotrophin families. Overall, 25 new binding interactions were demonstrated,
implicating GF binding as one of FN’s main physiological functions. The binding domain is close
to the major FN integrin-binding domain. Synergistic signaling between this integrin and the
VEGF-A165, PDGF, and BMP-2 receptors was explored for angiogenesis (in response to VEGF
and PDGF) and mesenchymal stem cell migration and osteogenesis (in response to BMP-2 and
PDGF): synergy was seen only when the growth factors were templated by a fusion protein of
the two domains appropriately spaced. Polymeric nanomaterials were developed that are
rapidly cleared into lymphatics draining the injection site and paclitaxel was encapsulated. When
injected intradermally into a tissue bed draining to the same lymph node as a tumor, tumor
growth was inhibited and the number of tumor antigen-specific CD8+ T cells was elevated even
though paclitaxel systemic doses were too low to demonstrate direct antitumor effects.
Mehmet Toner (Harvard Medical School, Cambridge, MA) introduced novel microfluid chips for the isolation of circulating tumors cells (CTCs) in metastatic cancer allowing to monitor patient response and changes in tumor genotypes during treatment. The CTC-Chip captured rare CTCs using antibody-coated micro-posts under laminar flow conditions. A chip based on high throughput microfluid mixing provided enhanced CTC isolation. In patients with metastatic cancer temporal changes in CTC numbers correlated well with disease clinical course. In patients with metastatic non-small-cell-lung cancer T790M mutation, which confers drug resistance was detected in CTCs from patients. CTCs were also isolated from blood of patients with metastatic or localized prostate cancer.

Tyler Jacks (Massachusetts Institute of Technology, Boston, MA) discussed the molecular analysis of lung cancer progression using a mouse model of the disease. These models share both genetic and pathological features of human non-small cell lung cancer. Tumors are initiated through somatic activation of oncogenic K-ras and oncogenic signaling amplification is associated with progression to adenocarcinoma. p53 limits tumor progression in this model. Kras expression in the absence of p53 leads to the development of distant metastases. A metastasis-associated gene expression signature was uncovered, which highlighted the importance of the down-regulation of the transcription factor Nkx2.1. Furthermore, using nonafabricated devices, patterns of ECM components were identified that distinguish adhesion by metastatic and non-metastatic cell lines.

Anton Berns (The Netherlands Cancer Institute, Amsterdam, The Netherlands) indicated that mouse small-cell lung carcinoma (SCLC) tumors are often composed of phenotypically different cells, characterized by mesenchymal and neuroendocrine markers. Crosstalk between mesenchymal and neuroendocrine cells can endow neuroendocrine cells with metastatic capacity. Neuroendocrine cells can convert into mesenchymal component by Ras activation. Trp53 and Rb1 in distinct cell types of the adult lung were inactivated by targeting Cre-
recombinase expression to Clara (CC10 positive) cells, neuroendocrine (CGRP positive) cells, and alveolar type 2 (SPC positive) cells using adenoviral vectors. As mechanism, \textit{Trp53} and \textit{Rb1} inactivation in CGRP expressing cells causes SCLC with high efficiency. Likewise, inactivation of \textit{trp53} and Rb in alveolar type II cells gave rise to SCLC but with much lower efficiency whereas inactivation in CC10-expressing Clara cells only rarely caused SCLC. Thus, neuroendocrine cells serve as the predominant cell of origin of SCLC.

Viola Vogel (Swiss Federal Institute of Technology, Zurich, Switzerland) discussed how forces can tune the chemical display of ECM fibers, how rigidity of the stroma might be altered during cancer progression, how fibroblasts alter ECM composition and architecture as function of time, and how the stretching or cleavage of ECM fibers alters the display of ECM fibrils. The display of the ECM fibronectin can be switched on or off by stretching it, and this regulates the architecture of newly deposited collagen matrix. Stretch assays revealed how the mechanical strain can activate binding sites and deactivate others. Deciphering how proteins serve as mechano-chemical signaling switches sheds new light onto the question how cancer cells probe and respond to their environments.

Philippe Bousso (Pasteur Institute, Paris, France) discussed the mechanisms by which tumor microenvironment inhibits CTL and NK killing within tumors. Intravital two-photon imaging combined with fluorescent probes provided direct visualization and quantitative information on tumor cell killing in situ.

Michal Neeman (The Weizmann Institute of Science, Rehovot, Israel) indicated that molecular imaging monitors specific genes expression, changes in cell surface markers and alterations in differentiation, proliferation, migration and survival of endothelial and perivascular cells. ECM is altered during angiogenesis; changes in collagen deposition and its degradation by MMPs, fibrinogen extravasation and fibrin cross linking to form a provisional matrix were
seen. The high molecular weight hyaluronan is degraded by tumor derived hyaluronidase from anti to pro-angiogenic fragments. In tumors, part of the properties of mature vessels are gained by fibroblasts and myofibroblasts recruitment which significantly alters vascular morphology.

Ralph Weissleder (Massachusetts General Hospital, Cambridge, MA) outlined the use of a newly developed quantitative microNMR for rapid molecular analysis of scant human cancer cells. The NMR instrument is extremely small, the size of the palm of a hand. Markers and combinations of markers indicated variabilities of tumor cells obtained directly from patients with great accuracy and sensitivity. Temporal variability of markers could also be detected. The results show that cancer diagnostic accuracies with microNMR surpass those obtained using conventional analyses.

Summary

Discussed were the role of ECM related mechanical mechanisms in altering tumor cell function, mechanics-induced signaling from the microenvironment in affecting ECM assembly, and the movement of tumor and immunological systems cells, lung cancer mouse models and new advances in molecular imaging and the effectiveness of nanotechnologies for anticancer drug delivery.
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