Meeting Report

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

Peter Friedl1, Jeff Hubbell2, David Livingston3, and Enrico Mihich3

Abstract

The cross-disciplinary focus of the meeting highlighted recent progress in physical and genetic analysis and engineering of cancer disease models. As the 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 mechanistic 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, as well as novel nanotechnologies for anticancer drug delivery. Cancer Res; 72(4); 1–4. ©2012 AACR.

Rakesh K. Jain (Massachusetts General Hospital, Boston, MA) gave the keynote address on normalization of tumor microenvironment to treat cancer as shown by mathematical models and experimentation in mouse and 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 on tumor cells and vice versa. Relaxin decreases collagen production and increases responses. Angiogenic drugs can also "normalize" tumor microenvironment. More recently, antihypertensive 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-homologs 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 into biochemical signals controlling multiple aspects of cell behavior. YAP and TAZ are sensors of cell architecture and mediators of mechanical cues from microenvironment.

Joachim Spatz (Max Planck Institute for Intelligent Systems and 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 polyethylene glycol (PEG) 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, showing 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 spectrometric 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 anticancer agents such as paclitaxel compared with 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; nonlinear 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 granulocyte macrophage colony–stimulating factor creating gradient in surrounding tissues and recruiting host dendritic cells (DC). 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 adhesion receptor–independent migration modes. He further showed that the distribution of important guidance cues (i.e., a chemo-kine) can determine whether leukocytes migrate in the adhesive versus nonadhesive mode.

Valerie Weaver (University of California at San Francisco, San Francisco, CA) indicated that oncogenic transformation is frequently accompanied by ECM deposition, crosslinking, and matrix metalloproteinase (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 showed that ECM crosslinking 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 1,080 nm that enhances deep and nontoxic 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. A combination of anti-integrin therapy with irradiation may be a viable treatment option in the clinic for locally destructive and otherwise radioresistant tumor lesions.

Melody Swartz (École Polytechnique Federale de Lausanne, Lausanne, Switzerland) indicated that tumor expression of VEGF-C promotes protumor 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 biologic 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 approximately 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 approximately 100 nm 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 agent localization, nanotextured chips for proteomic and peptidomic content profiling of biologic samples, nanochannel delivery systems for intelligent time release from implants, and bionanoscaffolds for tissue regeneration. Multistage 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 neovascular bed supplying the lesion. Other topics discussed in this presentation included delivery of therapeutic siRNA in murine ovarian cancer models and of cytotoxic agents in orthotopic mouse breast cancer models, and the role of MRI contrast agents in these treatment approaches.

Jeffrey Hubbell (Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland) discussed 2 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 variants and one on using nanomaterials to modulate the immune microenvironment within tumor-draining lymph nodes. Fibronectin was shown to modulate its signaling by coassociation of VEGF-R2 and integrins. A fibrin-binding domain of fibronectin was engineered which bound platelet-derived growth factors (PDGF), fibroblast growth factor, TGFβ, and neurotrophin families. Overall, 25 new binding interactions were shown, implicating growth factor binding as one of main physiologic functions of fibronectin. The binding domain is close to the major fibronectin 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 show direct antitumor effects.

Mehmet Toner (Harvard Medical School, Cambridge, MA) introduced novel microfluid chips for the isolation of circulating tumor cells (CTC) in metastatic cancer allowing the monitoring of patient response and changes in tumor genotypes during treatment. The CTC-Chip captured rare CTCs using antibody-coated microposts 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 (NSCLC), T790M mutation, which confers drug resistance, was detected in CTCs from patients. CTCs were also isolated from the 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 pathologic features of human NSCLC. 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. K-ras 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 nanofabricated devices, patterns of ECM components were identified that distinguish adhesion by metastatic and nonmetastatic 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. Cross-talk between mesenchymal and neuroendocrine cells can endow neuroendocrine cells with metastatic capacity. Neuroendocrine cells can convert into a 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, Trp53 and Rb1 inactivation in CGRP-expressing cells causes SCLC with high efficiency. Likewise, inactivation of 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 deactivates others. Deciphering how proteins serve as mechanochemo signaling switches sheds new light on the question of 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 2-photon imaging combined with fluorescent probes provided direct

www.aacrjournals.org Cancer Res; 72(4) February 15, 2012 OF3

Published OnlineFirst January 11, 2012; DOI: 10.1158/0008-5472.CAN-11-3080
visualization and quantitative information on tumor cell killing \textit{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 crosslinking to form a provisional matrix were seen. The high-molecular-weight hyaluronan is degraded by tumor-derived hyaluronidase from anti- to proangiogenic 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 (nuclear magnetic resonance) for rapid molecular analysis of scant human cancer cells. The NMR instrument is extremely small, the size of the palm of the 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 showed that cancer diagnostic accuracies with microNMR surpass those obtained using conventional analyses.

**Summary**

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

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

Received September 13, 2011; accepted December 4, 2011; published OnlineFirst January 11, 2012.
Twenty-Third Annual Pezcoller Symposium: Engineering Influences in Cancer Research

Peter Friedl, Jeff Hubbell, David Livingston, et al.

Cancer Res Published OnlineFirst January 11, 2012.

Updated version
Access the most recent version of this article at:
doi:10.1158/0008-5472.CAN-11-3080

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2012/01/05/0008-5472.CAN-11-3080.DC1

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.