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Cell-matrix mechanobiology: Applications to brain tumors and design of tissue engineering scaffolds

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Abstract—**It is becoming increasingly clear that mechanical and other biophysical signals from the extracellular matrix (ECM) can powerfully influence a wide variety of fundamental cell behaviors, including proliferation, differentiation, death, and motility. This concept has significant implications both for understanding the pathophysiology of disease and the design of biointerfaces found in cellular microdevices and tissue engineering platforms. Here we briefly review recent progress from our laboratory in investigating the role of ECM-derived mechanical signals in the specific context of two systems: The growth and spread of malignant brain tumors and the design of microscale cardiac tissue engineering systems. In both cases, mechanical signals encoded in the ECM govern motility, mechanics, and/or proliferation in profound and unexpected ways and rely upon the cell's reciprocal ability to generate contractile force through myosin and its molecular regulators.**

I. Introduction

ONE of the most exciting developments in cell biology over the past two decades is the emerging recognition that living cells can sense, process, and respond to mechanical and other biophysical cues in their environment. These signals have been demonstrated to powerfully guide a wide variety of life-defining cell properties, including proliferation, death, and motility, and in the case of stem and progenitor cells, they can even guide fate choices [1,2]. This phenomenon has given rise to a new field, cellular mechanobiology, which focuses on the mechanical and mechanotransductive properties of cells and the role these properties play in physiology and disease. From an engineering perspective, cells may be regarded as actuators that can interconvert mechanical information (force) and biochemical information (intracellular signaling), and suggests that biochemical inputs will yield different cellular outcomes depending on the "mechanical microenvironment" of the cell. An important component of this mechanical microenvironment is the extracellular matrix (ECM), the biopolymeric network against which the cell adheres and exerts force; cells can both passively sense cues encoded in the microstructural and micromechanical properties of the ECM and actively create these cues by remodeling the ECM. This concept of "dynamic mechanical reciprocity" has significant implications for our understanding of disease processes in which cells interact with the ECM, as mechanical signals exchanged between the cell and ECM may contribute to pathogenesis and/or serve as a useful target for therapeutics. Moreover, these signals may be leveraged to control cell behavior in the context of tissue engineering systems and other cellular biotechnologies. For example, mechanical or topological information may be encoded into biomaterial scaffolds to be used in vivo, where the biochemical milieu may be difficult or impossible to control.

The goal of our research program is to understand the molecular-scale mechanisms that enable cells to transduce mechanical signals from the ECM and other components of the mechanical microenvironment into biochemical signals, and vice versa. We study fundamental aspects of this problem and leverage concepts from cellular mechanobiology both to gain insights into specific disease processes and to inform the design of biointerfacial technologies. Here we summarize recent progress in two specific areas of interest. First, we discuss our efforts to understand the role of ECM elasticity in controlling the structure, cytoarchitecture, motility, and proliferation of malignant brain tumor cells. Second, we describe our investigations of the role of ECM microtopography in controlling proliferation of scar-mediating fibroblasts in microengineered cardiac tissue engineering platforms.

II. The Rigidity of the ECM Regulates the Structure, Motilty, and Proliferation of Glioma Cells [3]

A. Background

Glioblastoma multiforme (GBM) is a highly malignant astrocytic brain tumor with an average survival time of approximately 15 months, even with aggressive surgical intervention, chemotherapy, and radiation therapy. This aggressiveness is derived in part from the ability of single tumor cells to infiltrate the ECM parenchyma of normal brain tissue, which it extensively remodels through a combination of metalloprotease-based digestion, secretion of new ECM proteins, and mechanical force. While considerable effort has been devoted to understanding the biochemical nature of this crosstalk between GBM tumor cells and the ECM, comparatively little is known about the mechanical components of this signaling. Narrowing this gap in our understanding is particularly important given the

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recent observations that ECM stiffness and other micromechanical inputs can control malignant transformation, tumor cell invasion, and expression of specific tumor markers (reviewed in [4]).

B. Approach and Results

To gain insight into how mechanical contributions from the ECM might influence properties of GBM tumor cells relevant to growth and invasion, we adhered a series of GBM culture models (U373-MG, U87-MG, U251-MG, SNB19, C6) to fibronectin-conjugated polymeric ECM substrates of defined mechanical stiffness and probed the role of ECM rigidity in modulating tumor cell structure, migration, and proliferation. On the most rigid ECMs (>100 kPa), GBM tumor cells adopted a phenotype in which they spread extensively, formed prominent actomyosin stress fiber bundles and mature focal adhesions, and migrated rapidly. As ECM rigidity was reduced to values approaching that of normal brain tissue (<1 kPa), tumor cells rounded and failed to productively migrate. Surprisingly, cell proliferation was also strongly influenced by ECM rigidity, with cells dividing much more rapidly on rigid than compliant ECMs. To gain insight into this relationship, we repeated these experiments in the presence of inhibitors of actomyosin-based contractility. Either direct or indirect suppression of nonmuscle myosin II-based contractility diminished this rigidity-sensitivity and, on highly compliant ECMs, rescued cell motility. These results provide support for a novel model in which ECM rigidity may provide a transformative, microenvironmental cue that acts through actomyosin contractility to regulate the invasive properties of GBM tumor cells. Taken together with prior clinical observations that GBM tumors and their surrounding stroma are stiffer than normal brain tissue, our results also suggest that these tumors may stiffen their microenvironment as they grow and spread, and that these remodeling events may deliver reciprocal mechanical inputs to cells that promote tumor invasion.

III. Contractility-dependent modulation of cell proliferation and adhesion by microscale topographical cues [5]

A. Background

One of the fundamental goals of tissue engineering and regenerative medicine is the creation of material scaffolds that present spatially- and temporally-defined, cell-specific behavioral cues. Incorporation of these cues into a scaffold may potentially enable one to pattern complex populations of cells into organized and functional tissues and organs, as well as promote the physiological activity of one cell type while simultaneously suppressing that of another. More specifically, the design goals of many tissue engineering strategies are to promote attachment and function of desired cell types while attenuating the function of cell types that contribute to scar formation, including fibroblasts. While organismal development frequently achieves this goal by establishing complex spatial and temporal gradients of soluble growth, death, and differentiation factors, this often fails in tissue engineering and regenerative medicine applications, because there is little direct control over the local soluble milieu of implanted scaffolds. Thus, the field has increasingly sought to incorporate *biophysical* cues into these scaffolds, with the hope that they might yield this microscale, cell-specific instruction. While properties such as mechanical stiffness and mesh size are now routinely considered in scaffold design, three-dimensional microstructure (microtopography) remains a relatively understudied biophysical signal that can strongly influence cell behavior.

B. Approach and Results

In a previous study, Russell, Desai, and coworkers had shown that culturing fibroblasts on microtextured scaffolds has the surprising effect of stunting proliferation [6]. To gain mechanistic insight into this observation, we fabricated arrays of 5-15 μm-tall laminin-functionalized protrusions ("micropegs"), cultured 3T3 fibroblasts on these arrays, and examined adhesion, morphology, cytoarchitecture, and proliferation. Consistent with the earlier observations, we found that cell proliferation on microtextured scaffolds was nearly twofold lower than on flat substrates, as measured by bromodeoxyuridine (BrdU) incorporation. To confirm that this effect was a direct result of micropeg adhesion, we showed that a particular cell bound to a particular micropeg was statistically less likely to proliferate than its counterpart on a flat region of the same substrate. Morphometric analysis demonstrated that micropeg-bound cells and their nuclei were more elongated than on flat substrates, and scanning electron microscopy revealed that cells adhered to micropegs adopted a three-dimensional structure, with cells "crawling" up the sides of the micropegs while retaining anchorage to the scaffold base. Together, these observations led us to hypothesize that micropeg adhesion might be altering proliferation through a mechanism that required altered cell mechanics and generation of contractile forces. To test this, we repeated the proliferation experiments in the presence of inhibitors of Rho-associated kinase and myosin light chain kinase, two regulators of myosin. Indeed, inhibition of contractility modestly but significantly attenuated the ability of the micropegs to stunt proliferation. Interestingly, we found similar results for C2C12 myoblasts, suggesting that this adhesion-dependent suppression of proliferation is at least partially independent of cell type. Together, our results support a model in which cell fate decisions may be directly manipulated within tissue engineering scaffolds by the inclusion of microtopographical structures that alter cellular mechanics.

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REFERENCES

[1] D. Discher, C. Dong, J. J. Fredberg, F. Guilak, D. Ingber, P. Janmey, R. D. Kamm, G. W. Schmid-Schonbein, and S. Weinbaum, "Biomechanics: cell research and applications for the next decade," *Ann. Biomed. Eng*., vol. 37, pp. 847-59, 2009.

[2] V. Vogel and M. P. Sheetz, "Cell fate regulation by coupling mechanical cycles to biochemical signaling pathways," *Curr. Opin. Cell Biol*., vol. 21, pp. 38-46, 2009.

[3] T. A. Ulrich, E. M. de Juan-Pardo, and S. Kumar, "The rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells," *Cancer Res.*, vol. 69, pp. 4167-74, 2009.

[4] S. Kumar and V. M. Weaver, "Mechanics, malignancy, and metastasis: the force journey of a tumor cell," *Cancer Metastasis Rev*., vol. 28, pp. 113-27, 2009.

[5] R. G. Thakar, M. G. Chown, A. Patel, L. Peng, S. Kumar, and T. A. Desai, "Contractility-dependent modulation of cell proliferation and adhesion by microscale topographical cues," *Small*, vol. 4, pp. 1416-24, 2008.

[6] S. Y. Boateng, T. J. Hartman, N. Ahluwalia, H. Vidula, T. A. Desai, and B. Russell, "Inhibition of fibroblast proliferation in cardiac myocyte cultures by surface microtopography," *Am. J, Physiol. Cell Physiol.*, vol. 285, pp. C171-82, 2003.