Tumor Engineering: The Other Face of Tissue Engineering

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Advances in tissue engineering have been accomplished for years by employing biomimetic strategies to provide cells with aspects of their original microenvironment necessary to reconstitute a unit of both form and function for a given tissue. We believe that the most critical hallmark of cancer is loss of integration of architecture and function; thus, it stands to reason that similar strategies could be employed to understand tumor biology. In this commentary, we discuss work contributed by Fischbach-Teschl and colleagues to this special issue of Tissue Engineering in the context of 'tumor engineering', that is, the construction of complex cell culture models that recapitulate aspects of the *in vivo* tumor microenvironment to study the dynamics of tumor development, progression, and therapy on multiple scales. We provide examples of fundamental questions that could be answered by developing such models, and encourage the continued collaboration between physical scientists and life scientists not only for regenerative purposes, but also to unravel the complexity that is the tumor microenvironment.

IN 1993, VACANTI AND LANGER cast a spotlight on the growing gap between patients in need of organ transplants and the amount of available donor organs; they reaffirmed that tissue engineering could eventually address this problem by "applying principles of engineering and the life sciences toward the development of biological substitutes."¹ Mortality figures and direct health care costs for cancer patients rival those of patients who experience organ failure. Cancer is the second leading cause of death in the United States (Source: American Cancer Society) and it is estimated that direct medical costs for cancer patients approach \$100B yearly in the United States alone (Source: National Cancer Institute). In addition, any promising therapy that emerges from the laboratory costs roughly \$1.7B to take from bench to bedside.²

Whereas we have indeed waged war on cancer,³ the training grounds have largely consisted of small rodents, despite marked differences between human and mouse physiology,⁴ or plastic dishes, even though just like our tissues and organs most tumors exist within three-dimensional proteinacious milieus. One could argue that this is comparable to training for a desert war in the arctic! In this special issue of tissue engineering, Fischbach-Teschl and colleagues build a strong case for engineering complex cultures analogous to normal organs⁵ to tractably model aspects of the human tumor microenvironment^{6,7} that simply cannot be reproduced with traditional two-dimensional cell culture techniques and that cannot be studied in a controlled fashion *in vivo*.^{8,9} This idea has gained considerable traction of late as concepts presented and convincingly shown years ago^{10–12}

have only now begun to be appreciated. Perhaps, then, it is time to organize those who wish to build complex tumor models to study cancer biology under a common umbrella. Accordingly, we propose that tumor engineering be defined as the construction of complex culture models that recapitulate aspects of the *in vivo* tumor microenvironment to study the dynamics of tumor development, progression, and therapy on multiple scales. Inherent in this definition is the collaboration that must occur between physical and life scientists to guide the design of patterning techniques, materials, and imaging modalities for the study of cancer from the sub-cellular to tissue level in physiologically relevant contexts.

To date, the most successful tissue engineering approaches have employed methods that recapitulate the composition, architecture, and/or chemical presentation of native tissue. For instance, induction of blood vessel growth for therapeutic purposes has been achieved with sequential release of vascular endothelial growth factor (VEGF) and platelet-derived growth factor to induce and stabilize blood vessels.¹³ This approach imitates that which occurs during physiological angiogenesis as a result of heterotypic interactions between endothelium and stroma.¹⁴ Employing such biomimetic strategies has already led to success in cancer research. Studying tumors in 3D has proven far more accurate in reproducing *in vivo* growth characteristics and chemotherapeutic resistance than 2D approaches.^{11,15–21} A number of animal studies and co-culture experiments have identified also the importance of interactions with other nonmalignant cell types—such as endothelial cells,²² fibroblasts,^{11,21,23} adipocytes,²⁴ leukocytes,^{25–27} and circulating progenitors^{28,29}—to

support and sustain tumor growth, invasion, and metastasis (for reviews, see Refs.^{9,30}). Reproducing not only the “dynamic reciprocity”³¹ but also the “dynamic cooperativity” between these constituents in a spatially, temporally, and functionally accurate fashion presents quite a challenge for engineering tumors (Fig. 1).

So, why do it? The reason is to ask important fundamental questions that cannot easily be answered *in vivo* or on tissue culture plastic for the reasons mentioned above. For instance, despite all the promise that anti-angiogenic strategies hold to treat tumors,^{32,33} recent biological studies have cast some doubt on how to limit the ability of a tumor to recruit vasculature without adverse side effects such as thrombosis, hemorrhaging, or even selecting for metastatic subpopulations of tumor cells.^{34–36} In this light, could we engineer a model to address how tumor cells may behave as a function of progression status and distance from the microvasculature? Could we further identify extracellular matrix (ECM), proteases, and other molecules that could promote or suppress these effects?

The methods proposed by Fischbach-Teschl and Stroock to mimic the microvasculature by patterning perfused, endothelialized tubes within hydrogel matrices⁶ could be utilized to answer the first of these questions. Variations of this technique have been demonstrated before,^{37,38} but Fischbach-Teschl and Stroock specifically propose to integrate tumor cells and other cell types into this culture platform to study factors that regulate the angiogenic switch. We would further add that exerting spatial control by patterning tumor cells³⁹ from distinct stages of a progression series at in-

creasing distances from the microvasculature would also be necessary to determine how distance from the microvasculature and progression state alters invasiveness, secretion profiles of angiogenic factors, and/or the ability of tumors to recruit bone-marrow-derived cells (flowed through the microvasculature) that contribute to neovessel formation. Coupled with a fluorescent reporter of hypoxia and real-time imaging, this approach could identify stage- and microenvironment-specific events that bias whether tumors rely on invasion, angiogenic factor secretion, and/or recruitment of circulating progenitors to increase their proximity to a vascular supply.

A variety of proteins, glycoproteins, and polysaccharides compose the ECM, and determining how the composition and arrangement of these molecules influence tumor cell behavior is key to answering the second question posed above. Microenvironment arrays, such as those developed by LaBarge *et al.* in our laboratory,⁴⁰ allow hundreds of factors to be patterned in a combinatorial fashion to determine specific environs that influence a cellular output such as VEGF promoter activity. Once specific combinations are uncovered, the proteins identified with this arrayed approach could then be coupled to a synthetic ECM such as poly(ethylene) glycol or alginate⁴¹ to validate their activities in models that yield functional outputs. Synthetic ECMs have already been employed to identify how dimensionality and cell adhesion (via the fibronectin RGD sequence) influence the secretion of angiogenic factors,^{42,43} and Verbridge *et al.* take this a step further by microtuning the thickness of alginate gels to further specify how hypoxia modulates this response.⁴⁴ By de-

FIG. 1. Dynamically reciprocal and cooperative interactions occur within the tumor microenvironment. This schematic demonstrates just a subset of the complex interactions that should be considered when engineering tumor models. In this case, a loss of myoepithelial tumor suppressive functions results in mammary carcinoma penetrating the surrounding basement membrane sheath that separates the epithelium from stroma. As tumor cells invade in response to an oxygen/nutrient gradient, they not only generate proteolytic fragments that influence cell behavior (not shown), but also secrete a variety of factors that activate mesenchymal cells to a myofibroblast phenotype (darker mesenchymal cells), recruit and alter blood vessels, and attract and activate leukocytes (e.g., macrophages). In turn, soluble and insoluble factors generated from the now active stroma greatly influence receptor ligation and clustering on the surface of tumor cells (see zoomed-in depiction). These changes and others (such as those resulting from physical interactions with ECM and other cells) are transduced via signaling molecules and cytoskeletal components to the nucleus to alter gene expression. Transcriptional changes within the tumor cell affect production and secretion of ECM components and ECM remodeling enzymes (e.g., matrix metalloproteinases) and alter cytoskeletal tension. Resulting short- and long-range signaling could further sustain activation and recruitment of stromal components (e.g., through physical forces, F), perpetuating this cycle and ultimately resulting in tumor growth and/or dissemination. Not shown here are the systemic effects caused by remodeling of the primary tumor microenvironment that also influence secondary tumor sites in other organs. ECM, extracellular matrix.

coupling three-dimensionality, cell-ECM interactions, and hypoxia in this manner, they demonstrate that cell-ECM interactions critically mediate secretion of the angiogenic cytokine interleukin-8 in an oral squamous cell carcinoma cell line as well as a glioblastoma cell line, and further demonstrate that hypoxia is the key regulator of VEGF production in these cells.⁴⁴ These results predict heterogeneity within solid tumors insofar as angiogenic factor secretion is concerned, and further demonstrate why targeting only one such factor is not sufficient to sustainably inhibit tumor growth. The exploration of how motifs from other ECM components bias the angiogenic switch is also necessary. To this end, specific sequences of the basement membrane protein laminin-111 responsible for imparting proper organization to mammary epithelial cells also have been identified using a synthetic approach.⁴⁵ These signals are lost with breast cancer progression, and the concomitant loss of tissue organization induces VEGF expression and activates the initial steps of the angiogenic cascade.⁴⁶ Utilizing this type of systematic workflow could uncover novel soluble and insoluble factors that regulate the angiogenic switch in a variety of tumor types.

The fundamental questions posed above are but two of many that could benefit from creative approaches applied by the coming tumor engineers. As tissue engineers attempt to mimic developmental or wound healing programs for regenerative purposes, it is important to realize that tumors hijack these same programs to facilitate their growth, survival, and dissemination.^{30,47,48} Accordingly, many of the techniques developed to facilitate the *de novo* creation of viable tissues can also be exploited to create models of the tumor microenvironment to study how dynamic interactions within the tissue influence tumor initiation, progression, and resistance to therapy. As such, tumor biology may become an important additional focus for bioengineers in the future.

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