Review

Engineering Approaches for Investigating Tumor Angiogenesis: Exploiting the Role of the Extracellular Matrix

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Abstract

A major paradigm shift in cancer research is the emergence of multidisciplinary approaches to investigate complex cell behaviors, to elucidate regulatory mechanisms and to identify therapeutic targets. Recently, efforts are focused on the engineering of complex in vitro models, which more accurately recapitulate the growth and progression of cancer. These strategies have proven vital for investigating and targeting the events that control tumor angiogenesis. In this review, we explore how the emerging engineering approaches are being used to unlock the complex mechanisms regulating tumor angiogenesis. Emphasis is placed on models using natural and synthetic biomaterials to generate scaffolds mimicking the extracellular matrix, which is known to play a critical role in angiogenesis. While the models presented in this review are revolutionary, improvements are still necessary and concepts for advancing and perfecting engineering approaches for modeling tumor angiogenesis are proposed. Overall, the marriage between disparate scientific fields is expected to yield significant improvements in our understanding and treatment of cancer. Cancer Res; 72(23); 1–8. ©2012 AACR.

Introduction

In order for tumors to grow and fuel their insatiable metabolic demands, ongoing formation of new blood vessels must occur, a process referred to as angiogenesis. Under nonpathologic conditions, angiogenesis is strictly regulated by matrix proteins, cell–cell connections, and the abundance of pro- and antiangiogenic factors (1). During events associated with wound healing, for example, endothelial cells (EC) transition from a quiescent to an activated state, degrading their underlying basement membrane, which, in turn, permits their migration and proliferation in the interstitial extracellular matrix (ECM; e.g., collagens and fibronectin; ref. 2). At this point, the ECs align into cord-like structures, synthesizing a new, supportive basement membrane upon which they resume their quiescent state (2). While strictly regulated under homeostasis, these events become perturbed during tumorigenesis, wherein the uncontrolled formation of neovessels ensues. A process initially described by the pioneering work of Judah Folkman (3), angiogenesis is a requisite for tumors to grow beyond 1 to 2 mm³. As such, a greater understanding of the cellular and molecular mechanisms governing tumor angiogenesis has been of interest to those wishing to abrogate the process. The ECM, as discussed in more detail below, is a critical determinant regulating angiogenesis. In this manner, engineering approaches using ECM-mimetic materials as scaffolds for cellular studies have provided invaluable insight into the events regulating tumor angiogenesis.

Throughout the years, in vitro investigations centered mainly on the use of 2-dimensional (2D) or 3-dimensional (3D) spheroid cell culture systems to elucidate the cellular and molecular events governing angiogenesis, strategies that insufficiently mimic that which takes place in vivo. To more accurately recapitulate the events promoting tumor angiogenesis, several strategies presently merge engineering principles with the life sciences. Specifically, to overcome the inherent limitations of traditional in vitro cell culture systems, 3D ECM-inspired materials have been engineered to generate scaffolds to study cell behavior in an environment that more accurately corresponds to the in vivo scenario. While such biomaterials may be derived and synthesized from biologic sources, many are fabricated from synthetic materials. Scaffolds are designed to permit a myriad of cell behaviors and function as both a therapeutic entity, delivering cells to diseased and wounded tissues, and as a platform to investigate cell activities in vitro. Focusing on the latter, one of the most important attributes of an engineered scaffold, aside from being nontoxic to the cells, is that it must permit cell attachment, typically by containing or incorporating cell-adhesive entities such as the integrin-binding Arg-Gly-Asp (RGD) motif (4). Furthermore, the scaffold must permit sufficient transport of nutrition and oxygen, as well as the movement of cells within the scaffold while maintaining sufficient mechanical strength to withstand cellular traction forces (4). In addition to these, other modifications can be addressed during scaffold
fabrication such as surface chemistry (e.g. tethering of bioactive molecules for directed cell behavior), mechanical attributes (e.g. thermal-, photo-, or chemically-induced crosslinking of polymers), and topography (e.g., introduction of cell-adhesive regions containing variations in height, width, and shape). Together, these features allow the precise control over multiple cues and can be fine-tuned to elicit the desired cell response.

In the following sections, we discuss the employment of natural ECM matrices and the engineering of new scaffolds to study the cellular and molecular events regulating tumor angiogenesis in a biomimetic environment. These approaches have yielded significant advancements in our understanding of tumor angiogenesis and are changing the way in which scientists approach complex behaviors of tumor cells in their surrounding environment. Figure 1 provides an overview of the engineering-based approaches discussed in this review and their applications for investigating the independent and coordinate interactions taking place between vascular and tumor cells.

**ECM and Its Implications in Angiogenesis and Tumor Angiogenesis**

The ECM is a noncellular, protein-rich entity that supports cell and tissue organization and controls cell quiescence, survival, growth, and differentiation (5). The ECM contributes to these diverse cellular functions by providing attachment sites for cells, sequestering bioactive molecules and providing mechanical support to overlying cells (5). Regarding angiogenesis, it has been shown that fibrillar ECM proteins (e.g., fibronectin and collagens I and III) and basement membrane proteins (e.g., laminin and collagen IV) play distinct and overlapping roles in angiogenesis (6). Recently, we have shown that the collective ECM, deposited from cocultured breast cancer cells and fibroblasts, support vascular morphogenesis of ECs (7). The most pronounced changes in EC capillary features were attributed to ECM deposited from metastatic breast cancer cells cocultured with fibroblasts, suggesting that the tumor microenvironment may differentially affect vascular morphogenesis during tumor progression (7). Given its proangiogenic functions, researchers are aiming to better understand how specific ECM proteins, as well as ECM structure and degradability, govern the cellular and molecular processes ultimately leading to tumor angiogenesis.

To recapitulate the host environment, polymerized hydrogels derived from natural protein-based (e.g., Matrigel, collagen, and fibrin) materials are used to mimic the natural ECM. The benefit associated with natural protein-based materials is that they are more representative of that which occurs in the body, containing the appropriate spatiotemporal presentation of instructive cues necessary for directing a variety of cell behaviors. Thus, a great deal of effort has been placed on using natural ECM matrices for investigating vascular cell and tumor-directed vascular cell behavior. One of the most extensively used natural ECM substrates for investigating angiogenesis is Matrigel, a soluble basement membrane extracted from mouse tumors. Comprised primarily of laminin and collagen IV, Matrigel supports organization of ECs into complex networks of vascular structures (8) and has been widely used for investigations into the discovery of antiangiogenic therapeutic targets and efficacy of newly developed antiangiogenic agents (9–11). For instance, Mammoto and colleagues (12) showed that changes in the mechanical stiffness of Matrigel, a feature of the tumor microenvironment, altered the expression of 2 Rho-activated transcription factors, Transcription Factor II-I and GATA2, known to regulate VEGF receptor 2 expression. These factors and/or their pathways may serve as potential targets for antiangiogenic therapies. In addition, it was shown that Matrigel encapsulated with immature dendritic cells (DC), as opposed to Matrigel encapsulated with mature DCs, facilitated tumor angiogenesis when implanted adjacent to subcutaneous grown tumors (13), knowledge that may lend to the development of targeted therapies. While Matrigel provides numerous benefits to evaluate the mechanisms controlling and abrogating angiogenesis, it has several drawbacks including batch–batch variations that reduce reproducibility of results, high cost, and temperature sensitivity that limit matrix handling (14). As a result, additional sources of pure populations of ECM proteins (e.g. collagen, gelatin, and fibrin) are increasingly being used as substrata for studying angiogenesis (15–17). Such pure ECM matrices allow investigators the opportunity to investigate how changes in the biologic, structural, and mechanical properties of the ECM affect capillary morphogenesis (18, 19). Pertaining to cancer, Ghosh and colleagues (20) used fibrin gels to show that tumor-derived ECs exhibit abnormal mechanosensing and angiogenesis. In addition, collagen I gels have been used to show that proangiogenic cytokines, secreted from spheroids of MDA-MB-231 breast cancer cells or OSCC-3 oral squamous carcinoma cells, promote formation of capillary sprouting and gel invasion (16, 21).

Although these and other studies have shown the use of collagen and fibrin-based scaffolds for investigating angiogenesis and tumor-induced angiogenesis, the restricted ability to modify certain chemical and mechanical properties limits the modes, the range, and the complexity of the ECM-driven cues that can be studied using such matrices. The opportunities to design a scaffold with desired physical and chemical properties is thus imperative for dissecting how various ECM-driven cues affect cell behavior.

**Engineering Approaches Using ECM to Investigate Tumor Angiogenesis**

Similar to natural ECM matrices, scaffolds from biologic or synthetic materials have been used to investigate diverse mechanisms controlling angiogenesis. Examples of ECM-mimetic hydrogels synthesized from biologic sources include alginate, hyaluronic acid (HA), collagen, gelatin, and fibrin (22). Commonly used synthetic scaffolds for studies of vascular regeneration include poly(caprolactone) (PCL), poly(ethylene glycol) (PEG), polyglycolic acid (PGA), poly-l-lactic acid (PLLA), and poly(lactic-co-glycolic acid) (PLGA; refs. 22, 23).

While these scaffolds have been primarily used for engineering of healthy vasculature for regenerative medicine applications, researchers are increasingly applying these systems to
Figure 1. Overview of engineering-based approaches used for investigating angiogenesis. Top, 3D ECM scaffolds are used to investigate tumor–EC interactions in vitro (A) and in vivo (B). Middle, micropatterning is used to evaluate vascular cell behaviors on geometrically patterned arrays of ECM (A), to investigate tumor cell behaviors on ECM proteins allowing the evaluation of (i) angiogenic cytokine secretion and (ii) cell adhesion and migration (B), and to assess tumor–EC interactions including (i) tumor cell–directed invasion of ECs into gels and (ii) ECs and tumor cells coculture in 3D ECM scaffolds (C). Bottom, microfluidic devices are used to evaluate vascular cell responses to physical and chemical gradients, delineate the tumor–EC interactions taking place within a controlled environment (A), and to study vascular morphogenesis taking place in the presence and absence of tumor cells in 3D ECM scaffolds (B). Drawing not to scale.
investigate tumor angiogenesis in vitro and in vivo (Fig. 1, top). Fischbach and colleagues (24, 25) have shown enhanced angiogenic factor secretion, interleukin 8 (IL-8), from OSCC-3 tumor cells cultured in both biologic (alginate coupled with RGD) and synthetic (PLG compounded with Matrigel) 3D scaffolds. Conditioned media from such 3D cultures promoted capillary proliferation and elongation in vitro and stimulated increased vascularity of tumors in vivo over that observed for 2D cultures (24). Others have shown that human lung tumor cells grown in 3D HA hydrogels compounded with laminin and growth factors, promoted tumor vascularization in vivo over those scaffolds not containing additional ECM or growth factor components (26). Recently, it was found that secreted factors from quiescent ECs, embedded in porous gelatin matrices, slowed the progression of lung tumor cells following their implantation adjacent to subcutaneously growing tumors (27). The authors attribute the use of the gelatin matrix to preserving the quiescent activity of the ECs, which, in turn, maintain their secretome (27). Together, these studies not only show that the 3D ECM environment participates in regulating tumor angiogenesis, but additionally illustrates the importance of choosing the appropriate ECM scaffold necessary for addressing the question at hand.

In addition to the use of bulk ECM-based biomaterials for 3D studies of tumor angiogenesis, micropatterning techniques are being used to investigate EC phenotypic changes occurring in the tumor microenvironment (Fig. 1, middle). These approaches are desirable as they permit the precise control over the presentation of instructive environmental cues, including regulation of ECM spatial orientation, topography, and mechanics to guide cell behavior (28). Several groups have established the importance of controlling ECM spatiotemporal matrix features on EC behavior. Together, these studies outline how vascular cell behavior, including the switch from growth to apoptosis (29), differentiation (30), alignment and elongation (31–34), and cord formation (35) is controlled as a result of altering the geometry, spacing, and stiffness of patterned ECM. Recent studies have shown the successful use of micropatterning for elucidating the microenvironmental features controlling tumor angiogenesis. In an effort to investigate patterns of proangiogenic factor secretion by tumor cells, single populations of squamous or prostate tumor cells were cultured on microprinted, spatially defined fibronectin regions (36). It was found that profiles of VEGF, IL-8 and basic fibroblast growth factor were markedly different based on tumor cell type and whether the cells were grown singularly, lacking cell–cell interactions or in clusters, permitting cell–cell interactions (36). This study not only illustrates how cell–cell interactions influence profiles of cytokine secretion, but also has the importance of regulating the spatial presentation of cells on ECM to investigate patterns of cell behavior. In a similar study, it was found that IL-8 secretion was increased in microprinted stamps of OSCC-3 cells embedded in collagen (37). Increased IL-8 secretion was further shown to promote tumor angiogenesis in vitro via enhanced EC migration and invasion (37). As a proof-of-principle, Chiu and colleagues (38) micropatterned tumor cells and ECs together in spatially and geometrically defined regions of fibrinogen; methodology the authors believe will not only allow for studies of tumor cell–directed EC migration and capillary formation, but will permit patterning of numerous cell types on the same substrate. Work in our lab has shown the specific adhesion of breast cancer cells on micropatterned HA surfaces (39) and endothelial progenitor cell elongation and unidirectional network assembly on micropatterned fibronectin and fibrin gel stripes (32), suggesting the role of spatial presentation of specific ECM molecules in regulating tumor and vascular cell behaviors. Overall, through control over the geometry and spacing of ECM molecules, investigators will be better equipped to address the complex interactions taking place between cancer and vascular cells during angiogenesis in the tumor environment.

Microfluidic devices are an emerging engineering-based approach enabling investigators precise control over several aspects of the tumor microenvironment (Fig. 1, bottom). An apparatus consisting of one or more micro- or nanometer-sized channels, microfluidic devices allow the investigator to regulate the physical and chemical environment of cell population(s) of interest (40). Numerous groups have capitalized on the benefits afforded from microfluidic devices to investigate vascular cell behaviors in controlled microenvironments. For instance, microfluidic devices have been used to evaluate vascular cell responses to shear stress (41) and migration in response to cytokine gradients in 2D (42) and 3D collagen gels (43). In addition, groups are using microfluidic devices to control the spatial and temporal distribution of oxygen, technology that will allow investigators the opportunity to probe cancer and vascular cells’ responses to low oxygen and to gradients of oxygen tension in a controlled microenvironment (44, 45). With specific relevance to angiogenesis, Golden and Tien used microfluidics to create a series of interconnected channels in a collagen and fibrin-containing scaffold (46). The voided channels were subsequently seeded with ECs, forming a primitive vascular network (46). Using a similar approach, a more recent publication shows the use of microfluidics for the fabrication of living and biofunctional microvascular networks within collagen gels (47).

While the aforementioned studies have clear implications for the field of vascular biology, these concepts have been recently exploited to the study of tumor angiogenesis. In an attempt to elucidate how tumor cells regulate angiogenesis, Cross and colleagues (16) used a microfluidic device to assay EC invasion and capillary and lumen formation in spatially defined micromolded collagen gels with and without tumor cells (16). It was found that ECs readily invaded collagen gels, a property attributed to tumor cell release of proangiogenic factors, producing networks of capillaries with defined lumens (16). Chung and colleagues (48) have used microfluidic devices to show that different tumor cell populations, grown with and without smooth muscle cells (SMC) in collagen gels, induced varying responses in ECs. Interestingly, in the absence of SMCs, MThn3 rat mammary tumor cells, but not U87 glioblastoma cells, induced capillary formation of ECs (48). Toward miniaturizing the tumor environment, these and others...
show the feasibility of using microfluidics for studying tumor angiogenesis.

Overall, these studies illustrate the importance of both relevant in vitro biomimetic systems and controllable microenvironments to investigate vascular–tumor cell behaviors and interactions. These advanced cell culture platforms will undoubtedly become important tools for cancer researchers interested in interrogating the role of biochemical, physical, cell-to-cell, and cell-ECM events driving tumorigenic progression.

Conclusions and Future Directions: The Next Generation of Engineered Systems

To understand the cellular, molecular, biochemical, and physical events contributing to tumor initiation and progression, one must apply models that most accurately recapitulate this in humans. Three-dimensional scaffolds allow one to interrogate the cell–cell and cell–ECM events regulating tumor angiogenesis. Micropatterning technologies allow one to control the spatial and temporal distribution of cells, enabling determination of how the geometry and topography of the confined surroundings influence tumor angiogenesis. Finally, microfluidic devices allow the investigator to precisely control the physical and chemical environment of cell population(s), enabling a better understanding of how microenvironmental insults regulate vascular cell behavior and tumor angiogenesis. While these techniques have and continue to yield great insight into tumor and vascular cell behaviors, it is foreseeable that as the technology progresses, cancer researchers will have platforms from which to gain a greater understanding of how tumors initiate, progress, and respond to treatment.

A major area of focus for cancer researchers and engineers is on the development of scaffolds that better recapitulate the complex and dynamic tumor niche. One well-known aspect of the tumor niche is that it is "stiffer" compared with its normal tissue counterparts. For example, the mechanical stiffness of breast carcinomas has been reported to be as high as 42.5 kilopascals (kPa) for high-grade invasive ductal carcinomas as opposed to 3.25 kPa reported for nonmalignant mammary tissues (49). Similarly, lymph nodes harboring metastatic tumor foci had a mechanical stiffness of 3.35 ± 1.57 g/cm (e.g., 329 Pa) versus nontumor bearing lymph nodes having a mechanical stiffness of 1.23 g/cm (e.g., 121 Pa; ref. 50). To elucidate the putative role of stiffness during tumor development, polyacrylamide substrates were used to show the role of matrix stiffness in enhancing tumor cell proliferation and migration (51, 52) and resistance to chemotherapeutics (52). Similarly, it has been found that ECM change their behavior and morphology in a manner dependent on substrate stiffness (53, 54). Hence, using scaffolds fabricated to model the stiffness of the tissue/tumor of interest will be paramount for elucidating how the mechanically stiffer tumor contributes to pathologic angiogenesis.

Engineering the mechanical stiffness of the scaffold, while decoupling it from other key properties such as cell adhesion sites, elasticity, composition, and cytokine sequestering, may allow a better understanding of how the physical environment of the tumor contributes to cell phenotypic changes governing tumor angiogenesis and progression. In an attempt to use engineering approaches to understand the role of matrix stiffness on cellular behaviors, a recent publication used a range of matrix stiffnesses of 2 different hydrogel substrates to investigate how substrate properties influence mesenchymal stem cell (MSC) differentiation (55). By covalently linking collagen I to the hydrogel surface, the authors discovered that the stem cells respond to the mechanical feedback of the collagen anchored to the substrate (55). Hydrogels of different stiffness led to differences in anchoring densities and thereby altered the mechanical feedback of the collagen (55). Decoupling matrix stiffness from cell adhesiveness, the authors suggest that this approach more accurately models how cells receive information on mechanical changes in their environment via the ECM (55). In this manner, dissociating matrix stiffness from additional matrix-related cues will be imperative for addressing if and how the stiffened tumor environment facilitates tumor angiogenesis.

Advances in the technology are also envisioned to enable the better imitation of the dynamic surroundings. Currently, most technologies capture a specific makeup of the microenvironment and allow the study of vascular behaviors in response to a given cue(s). The ability to engineer dynamic environments, in which the timing, length, and magnitude of the cues can be controlled and manipulated along the culture period, will be critical for unraveling how a complex tumor environment dictates angiogenesis. Furthermore, engineering an environment that responds to feedback provided from tumor and vascular cells and other cell types in the tumor microenvironment (e.g., fibroblasts, MSCs, immune cells, etc.) will also allow for the analysis of vascular responses to the dynamically changing cancerous surroundings. An example is the fabrication of 3D scaffolds for investigating matrix metalloproteinase (MMP)-regulated angiogenesis. MMPs are zinc-dependent endopeptidases that digest various structural components of the ECM during tissue remodeling, including angiogenesis, where they degrade the vascular basement membrane and liberate matrix-sequestered proangiogenic factors (56). In addition to their homeostatic role, MMPs have also received widespread recognition for their ability to promote tumor angiogenesis (56). As various 3D scaffolds can be coupled with MMP responsive groups, allowing controlled degradation of the scaffold for investigations of cell behavior and angiogenesis (57–60), this platform is translatable to studies of MMP-regulated tumor angiogenesis. Here, one may directly assess how the activity of one or more tumor-secreted MMPs regulates the degradation of the MMP-complexed scaffold, which, in turn, permits formation of vessels. This knowledge may allow for a greater understanding of how tumor-directed matrix-degradation controls tumor angiogenesis in a relevant 3D environment.

Merging the engineering-based approaches is envisioned to simultaneously provide the investigator with a platform from which to study tumorigenesis in a controlled environment harboring spatially defined regions of 3D cell constructs. Such platforms would enable the investigator unprecedented
control over the spatial and temporal delivery of oxygen, solutes, and potentially other cell populations to the 3D cell construct. For example, spatial control over vascular formation is essential for the generation of “desired patterns” of vascular networks. A recent study used confocal imaging combined with 2 photon laser scanning lithography to pattern hydrogels with multiple cell adhesive moieties (61). This approach enabled spatial control over 3D cell adhesion to guide the formation of well-organized vasculatures in PEG-based hydrogels (61). Another study showed the use of a cyto-compatible sacrificial template to generate vascular networks in various hydrogel systems (62). The endothelialized networks were subsequently perfused with blood, sustaining the metabolic activities of living cells within the hydrogels (62). While these innovative approaches were exploited to generate microvasculature networks of healthy tissue, it is conceivable that they may be used to recapitulate the abnormal architecture of tumor vessels. Here, the recreated tumor vessels may be used to investigate how the tortuous nature of the tumor vasculature combined with aberrant blood flow patterns within the vessel, elicit changes in surrounding tumor cell behavior.

Another example is the use of micropatterning to arrange one or more cell populations, encapsulated in the same or different biologically relevant hydrogel (63), in a microfluidic device. In this manner, one can evaluate how different tumor cell lines, representative of the various stages of tumorigenesis, differentially influence EC behavior and vascular morphogenesis in a spatially, chemically, and physically controlled environment. In addition, one may be able to ascertain how different cell types in the tumor microenvironment coordinate with tumor cells to direct capillary formation. Showing the feasibility of such a system, Huang and colleagues (64) used a microfluidic device in which they spatially patterned 3D hydrogels containing tumor cells or macrophages to investigate tumor–immune cell interactions. The authors report that Matrigel-encapsulated macrophages readily invaded collagen I–encapsulated breast cancer cells (64), providing proof-of-principle that a merged engineered platform may be successfully used to elucidate tumor cell behaviors. As complexity is added to the system, through inclusion of various cell populations and/or establishment of physical and chemical gradients, investigators will not only have a platform from which to evaluate the biologic events driving tumor angiogenesis, but will additionally have a system from which to reliably test the use of known and emerging antiangiogenic or chemotherapeutic drugs.

Altogether, recent engineering-based approaches are allowing investigators the ability to control key physicochemical and spatiotemporal features of the tumor environment. Specifically, these approaches have proven promising for investigations into vascular cell behavior in the cancerous environment. It is foreseeable that merging the technologies presented in this review will allow for unprecedented discoveries into not only tumor angiogenesis, but in the biology and treatment of tumor cells.

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