Commentary & View

Regulation of cancer invasiveness by the physical extracellular matrix environment

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Long-term clinical outcomes are dependent on whether carcinoma cells leave the primary tumor site and invade through adjacent tissue. Recent evidence links tissue rigidity to alterations in cancer cell phenotype and tumor progression. We found that rigid extracellular matrix (ECM) substrates promote invasiveness of tumor cells via increased activity of invadopodia, subcellular protrusions with associated ECM-degrading proteinases. Although the subcellular mechanism by which substrate rigidity promotes invadopodia function remains to be determined, force sensing does appear to occur through myosin-based contractility and the mechanosensing proteins FAK and p130Cas. In addition to rigidity, a number of ECM characteristics may regulate the ability of cells to invade through tissues, including matrix density and crosslinking. 3-D biological hydrogels based on type I collagen and reconstituted basement membrane are commonly used to study invasive behavior; however, these models lack some of the tissue-specific properties found in vivo. Thus, new in vitro organotypic and synthetic polymer ECM substrate models will be useful to either mimic the properties of specific ECM microenvironments encountered by invading cancer cells or to manipulate ECM substrate properties and independently test the role of rigidity, integrin ligands, pore size and proteolytic activity in cancer invasion of various tissues.

In multicellular organisms, cells must sense and respond to multiple cues for proper functioning within tissues. Although most experimental research has focused on the regulation of cellular processes by external chemical signals, there is increasing recognition that mechanical forces also regulate critical cellular functions. Indeed, rigidity of the extracellular environment has been shown to regulate such diverse processes as muscle cell differentiation, stem cell lineage fate, breast epithelial signaling and phenotype, and fibroblast motility.1 5

In breast cancer, accumulating evidence suggests a role for tissue rigidity in promoting both the formation and invasiveness of tumors. Mammographic density of breast tissue has been correlated with increased cancer risk and included in models to predict the likelihood of in situ and invasive breast cancers.6 Histologically, dense breast tissue has increased stromal collagen content and in vitro analyses have shown that cancerous breast tissue is much stiffer than normal tissue (as represented by values for the elastic or Young’s moduli).3,7 In addition, experimentally increased expression of collagen fibrils in a mouse mammary model of spontaneous breast cancer was recently shown to promote tumor formation, invasion and metastasis.8 Therefore, both clinical and animal data suggest a correlation between tissue density and cancer aggressiveness, and mechanical factors appear likely to play a role in this process.9

A well-established mechanism by which extracellular matrix (ECM) rigidity signals can drive phenotypic transformations is through mechano-signal transduction (mechanotransduction) pathways in which external forces are transmitted via integrin receptors at linear focal adhesion structures to cytoskeletal and signaling proteins inside the cell. Actomyosin contractility leads to stretching and activation of proteins such as talin, p130Cas and potentially focal adhesion kinase (FAK).10 12 For example, stem cell lineage was found to be dependent on formation of cellular focal adhesions and actomyosin contractility in response to substrate tensile properties.2 Mammary epithelial cells grown on compliant matrices will differentiate and polarize to form lactating 3-D structures that resemble in vivo acini but fail to do so on stiff matrices due to increased cytoskeletal contractility.3 Activation of mechanotransduction molecules, such as FAK, Rho and ROCK, are required for the rigidity-induced phenotype changes.5 5 Using polyacrylamide (PA) gel systems, Yu-li Wang’s group found that rigid substrates induce fibroblast and epithelial cells to migrate away from each other instead of aggregating to form tissue-like structures.13 This transformation in phenotype is characteristic of the epithelial to mesenchymal transition and thought to be crucial for tumor cell migration.14

A critical feature of tumor aggressiveness is the ability to invade across tissue boundaries, through degradation of ECM. The subcellular structures responsible for this invasive activity are...
thought to be invadopodia: actin-rich, finger-like cellular protrusions that proteolytically degrade local ECM. These structures are characteristic of invasive cells and have been implicated in tumor cell metastasis due to their association with ECM degradation.15 Similar structures, podosomes, are formed in src-transformed cells, as well as normal cells such as osteoclasts and dendritic cells that need to degrade matrix and/or cross tissue boundaries.16 In addition to mediating ECM degradation, podosomes have been postulated to function as adhesion structures, since well-characterized adhesion proteins localize to podosomes and many podosome-expressing cells no longer express focal adhesions.17 Furthermore, podosomes have been shown to be essential for chemotactic motility and transendothelial migration, although not for chemokinetic motility.18,19

We recently found that ECM rigidity increases both the number and activity of invadopodia, and this effect was dependent on the cellular contractile machinery (Fig. 1A).20 Consistent with a role for mechanotransduction in this process, we found localization of the active, phosphorylated forms of the mechanosensing proteins FAK and p130Cas in actively degrading invadopodia and an increase in invadopodia-associated degradation in breast cancer cells overexpressing FAK and p130Cas. These results suggest that in breast cancer, increases in tissue rigidity may directly lead to increased cellular invasiveness and tumor progression.

The localization of phosphorylated FAK and p130Cas at invadopodia and the requirement for actomyosin contractility in our study suggests that invadopodia have the potential to act as mechanosensing organelles. This concept is supported by our finding that ~40% of breast cancer cells cultured on rigid substrates had rings of myosin IIA surrounding invadopodia (Fig. 1B)21 and the recent finding that similar podosome structures can exert local traction forces.21 In addition, a few studies have implicated integrin activity in invadopodia function as well as localized β1 and β3 integrins to invadopodia.22-25 However, whether invadopodia can serve as tension-generating adhesion structures is controversial, in part because of the presence of both focal adhesions and invadopodia in many cancer cells (Fig. 1C).

Regulation of invadopodia and podosome function is also not straightforward. Although our data,20 along with results from Collin et al.,21 suggests that mechanical tension promotes invadopodia and podosome activity, in some systems podosome formation is promoted by a loss rather than a gain of cytoskeletal tension.
That is, local cytoskeletal relaxation has been shown to promote podosome formation coincident with focal adhesion dissolution in both vascular smooth muscle cells treated with phorbol ester and neuroblastoma cells. A yin-yang activity between focal adhesions and podosomes has been known for many years, whereby activation of src kinase leads to both disassembly of focal adhesions and formation of podosomes. However, the role of tension in this process is unclear, particularly since activation of src kinase occurs downstream of mechanical stimuli and should promote podosome/invadopodia activity, yet loss of tension apparently induces biological activities dependent on src kinase (focal adhesion disassembly and podosome formation). For invadopodia, the role of tension is even less clear. Basic characterization studies need to be performed to establish molecular and structural differences between invadopodia and focal adhesions and to measure force profiles at the two structures. Since invadopodia have much smaller diameters compared to podosomes (50–100 nm vs. ~1 μm, respectively), the latter task of determining traction forces may be difficult due to resolution limitations in measuring potentially tiny substrate displacements. The standard identification of invadopodia, by association of actin-rich puncta with sites of degradation of fluorescent ECM, adds another technical limitation since the thickness and fluorescence of the ECM matrix used to identify proteolytic activity may hinder visualization of embedded fluorescent beads in the underlying PA gel (displacement of beads is typically used to calculate traction forces). Thus, an important future direction should be the development of new in vitro experimental systems that have manipulable substrate properties and allow simultaneous identification of subcellular forces and proteolytic activity.

The cellular response to rigidity is often characterized using PA gels with tunable stiffness in the range spanning that of normal and cancerous breast tissue (elastic moduli = 100–10,000 Pa). PA gels will likely continue to be invaluable tools for understanding cellular responses to rigidity. However, this system is inherently simple and cannot fully replicate cellular events occurring in a complex in vivo ECM microenvironment. Given that invading breast cancer cells are likely to experience different microenvironments as they cross through the basement membrane (BM) and into neighboring collagenous stromal tissue (Fig. 2), biological hydrogels such as reconstituted basement membrane (Matrigel) and type I collagen gels are often utilized to mimic these ECM substrates. However, both of these models lack many of the chemical, physical, and mechanical characteristics of tissues found in vivo and have been recently questioned as suitable models for studying cancer cell invasion. Type I collagen gels have a fibrillar architecture but a low density and high porosity and frequently lack crosslinking sites. Although Matrigel contains many of the biochemical components of the BM, it is tumor-derived and the major component is laminin-1, which is only abundant in fetal tissues. By contrast, the major component of normal BM is type IV collagen. In addition, Matrigel is a solubilized preparation that lacks crosslinks and a fibrillar component. Both sparse collagen gels and Matrigel are quite compliant with Young's moduli of ~1,000 and ~200 Pa, respectively; therefore, without further manipulation these substrates lack the rigidity required to mimic tumor-associated ECM.

In order to invade neighboring stromal tissue, carcinoma cells must first breach the BM, a complex, interwoven meshwork composed of type IV collagen, laminin, nidogen/entactin, and various proteoglycans and glycoproteins. The highly ordered and crosslinked type IV collagen network is regarded as the limiting barrier to cancer cell invasion since it forms pores on the order of 100 nm that are too small for passage of cells without proteolytic degradation of the BM. In addition to degradation, decreased BM synthesis may contribute to the initial steps of cancer invasion by altering the balance between BM formation and remodeling. Once cancer cells cross the BM, they encounter stromal collagen tissue. In tumors, this desmoplastic stroma is frequently fibrotic due to increased ECM deposition and crosslinking by carcinoma-associated fibroblasts. Although controversial, cancer cells are thought to use a nonproteolytic, amoeboid mode to traverse this connective tissue; therefore, different modes of migration may be necessary to traverse BM or stromal collagenous matrices (Fig. 2). However, the amoeboid phenotype has been described using either sparse collagen gels without crosslinks or Matrigel. In vivo, the
process of invading through tumor-associated stromal collagen is likely to depend on the pore size, the crosslinking status, and whether cells are migrating collectively or individually.34,43

In light of these concerns and many others, there has been a push for more physiologically relevant in vitro models that represent closer approximations of BM or stromal collagen tissue. Successful models, whether natural or synthetic, must be able to mimic the composition, architecture and mechanical properties of the in vivo environment as well as support cell culture in ex vivo conditions. Natural substrates can be produced by cultured cells, such as the epithelial basement membranes synthesized by MDCK cells.37 Alternatively, organotypic models derived from biological specimens have recently been utilized to study invasion. These materials can be based on processed biological tissue, such as detergent-extracted mouse embryo sections,44 homogenized involution matrix,38 and decellularized human dermis,45 or native tissue such as chick chorioallantoic membrane46 and explanted peritoneal or mammary tissue.34,37 In addition, the field of tissue engineering has already provided novel hybrid scaffolds and advanced tissue culturing methods that can be utilized for cancer research.47 Biological materials developed for clinical use in tissue reconstruction and regeneration, such as small intestinal submucosa and urinary bladder matrix, are attractive candidates as new in vitro models since they maintain their tissue-like properties as well as specific spatial arrangements of biologically active molecules.48,49 These tissue-derived scaffolds are composed of well-defined structural and functional proteins, originally produced by cells in vivo, and can represent closer approximations of BM or stromal collagen tissue. A push for more physiologically relevant in vitro models that mimic the composition, architecture and mechanical properties as well as specific spatial arrangements of biologically active molecules is likely to depend on the pore size, the crosslinking status, and whether cells are migrating collectively or individually.34,43

In summary, the physical microenvironment is increasingly recognized as a major influence on cellular phenotype. Recent data emphasizes the importance of mechanical factors in tumor progression, including cellular invasiveness. Exciting future directions include understanding how stromal and BM environments affect cellular invasiveness at multiple scales, including subcellular and molecular regulation of ECM degradation in response to ECM rigidity and the role of proteases in crossing diverse tissue barriers. The development of novel model systems with appropriate biological and physical properties will facilitate all of these goals.

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