The Rigidity Connection: Matrix Stiffness and Its Impact on Cancer Progression

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The extracellular matrix (ECM) has always been studied in the context of the structural support it provides tissues. However, more recently, it has become clear that ECM proteins do more to regulate biological processes relevant to cancer progression: from activating complex signaling pathways to presenting soluble growth factors. In 2009, Ulrich and colleagues provided evidence that the physical properties of the ECM could also contribute to glioblastoma tumor cell proliferation and invasion using tunable hydrogels, emphasizing a role for tumor rigidity in central nervous system cancer progression. Here, we will discuss the results of this landmark article, as well as highlight other work that has shown the importance of tissue stiffness in glioblastoma and other tumor types in the tumor microenvironment. Finally, we will discuss how this research has led to the development of novel treatments for cancer that target tumor rigidity.

See related article by Ulrich and colleagues, Cancer Res 2009; 69:4167–74

The extracellular matrix (ECM), which provides structure and support to our tissues, is a major component of all solid tumors. Recent proteomic efforts have led to the in-depth characterization of ECM proteins: glycoproteins, collagens, proteoglycans, and their associated proteins, ECM regulators, secreted factors, and ECM-affiliated proteins encoded by more than 1,000 genes (www.matrisome.org). The ECM regulates many of the different cancer hallmarks, such as proliferation, cell survival, and invasion. Originally, effects of the ECM were focused on biochemical signaling, via the activation of cell surface receptors by domains in the ECM proteins themselves. Further studies into the ECM, facilitated by a range of engineering approaches, have demonstrated that the density, rigidity, and geometry of the ECM change as tumors form and grow. Understanding how these biophysical properties impact tumor cell phenotypes within organs containing different ECM composition and rigidity requires interdisciplinary approaches.

Ulrich and colleagues set out to investigate the impact of tissue rigidity on glioblastoma multiforme (GBM) properties, a high-grade astrocytoma that is a fast-growing tumor with median survival of 14 months (1). The aggressive invasion of tumor cells into the surrounding tissue is a hallmark of GBM, and understanding the effect of ECM on tumor invasion is critical to ultimately developing impactful treatment for these patients. The authors fabricated polyacrylamide substrates with independently defined mechanical and biochemical properties with functionalized fibronectin and with rigidities spanning the range between normal and tumor tissue (from 0.08 to 119 kPa), compared with glass (1,000 kPa). They found that decreasing the rigidity of their gels decreased the spreading area of the human GBM cell line U373-MG, along with a dissipation of stress fibers and focal adhesions, decreasing proliferation without inducing apoptosis. Furthermore, decreasing rigidity also reduced migration speed, switching cells to a "stick-slip" movement, where cells would extend as the leading edge advanced, with the trailing edge abruptly detaching and snapping forward to catch up. Finally, inhibition of nonmuscle myosin II (NMMII) or ROCK on soft ECMs very quickly rescued the phenotype, leading to increased spreading and migration (**Fig. 1**). This study provided important evidence for the role of tissue rigidity in GBM progression.

Building on this work, there have been a number of studies dissecting the relationship between tissue rigidity and GBM progression (2). An important step forward has been the development of brain-mimetic hydrogels, where the ECM composition more closely resembles the native ECM of the brain, which is unique to other organs in the body. Brain ECM is low in fibrous proteins like collagen and fibronectin and basement membrane proteins, but rich in hyaluronic acid (HA). The Kumar lab designed two-dimensional and three-dimensional (3D) HA-based hydrogels with independently tunable stiffness and biochemical ligand density and demonstrated structural and phenotypic properties similar to those found in the brain. HA-based hydrogels have been used to further dissect how different rigidities such as the softer ones found in the peritumoral region versus the stiff ones found in the tumor impact properties of tumor cells. Using similar 3D brain mimetic gels, Sohrabi and colleagues found that changes in the stiffness of the GBM local environment can influence cell metabolism, which determines whether cells exhibit proliferative or invasive behaviors (3). Together, these studies demonstrate the versatility of 3D tunable hydrogels to recapitulate the rigidity of the tumor-containing brain ECM and their importance in dissecting the impact of tumor stiffness on tumor cell properties and mechanisms driving them. Studies by the Weaver lab using mouse models of GBM have shown that the stiff GBM environment activates integrin mechanosignaling that drives a mesenchymal, more stem cell-like and aggressive phenotype (4). These findings, along with others in the field, suggest that strategies that aim to alter the biophysical priorities of the ECM could offer novel therapeutic approaches to reduce mortality due to GBM.

The fact that ECM proteins were not just pretty fibrils was also becoming clear in the context of other tumor types, leading to new approaches to manipulate and study the effects of tissue rigidity. Around the same time as the landmark study from Ulrich

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Cancer Res 2024;84:958–60

doi: 10.1158/0008-5472.CAN-24-0394

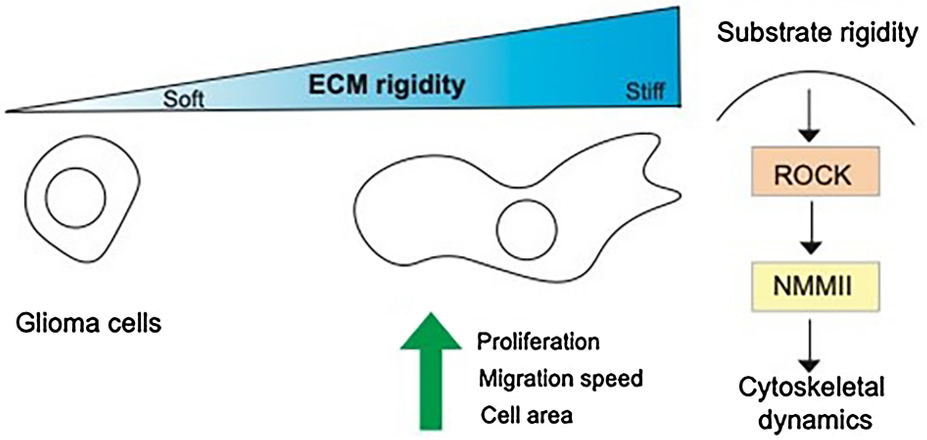

Figure 1.
The study from Ulrich and colleagues utilized engineered ECM substrates of differing stiffness to describe the impact of ECM rigidity on multiple aspects of tumor cell behavior. This study also highlighted the potential of targeting mechanosensitive pathways in tumor cells to decrease their invasive capacity.

and colleagues, Provenzano and colleagues investigated the impact of collagen I density and matrix stiffness on breast tumor cells, the most abundant ECM protein in breast tissue (5). They found that stiffness drives an invasive phenotype via 3D adhesion signaling and hyperactivation of the Ras–MAPK pathway, leading to growth of mammary epithelial cells *in vitro* and *in vivo* and activation of a proliferative gene signature that can predict breast cancer patient outcome. Denais and colleagues brought a new perspective to the study of tissue stiffness, delving into how the mechanical stress generated by increased tissue stiffness impacts cells during invasion. Using a microfluidic device that mimics the size of interstitial pores through which cancer cells pass during invasion, they found that increased tissue stiffness leads to transient nuclear envelope rupture accompanied by DNA damage (6). These studies highlight different mechanisms by which tissue stiffness can impact tumor cell properties.

The ECM is a major component of the tumor microenvironment, which is rich in many stromal and immune cells. Tissue stiffness has been shown to impact the recruitment of immune cells to the primary tumor, restricting the potential impact of immunotherapy in dense and rigid tumors. Nicolas-Boluda and colleagues demonstrated that tumor stiffness can be reversed by inhibiting the collagen cross-linking enzyme LOX, which improves T-cell migration and infiltration to tumor cells and enhances the efficacy of treatment targeting PD-1 on tumor growth in five preclinical mouse models (7). In the context of metastasis, the leading cause of cancer-related deaths, tissue stiffness can also play a role. Shen and colleagues found that highly activated fibroblasts located in liver metastases of colorectal primary tumors, characterized by αSMA and phospho-MLC2, increase the stiffness of the local ECM, which leads to increased angiogenesis via activation of YAP/TAZ signaling in endothelial cells (8). Inhibition of the renin-angiotensin system effectively deactivated the fibroblasts and reduced ECM deposition, increasing the efficacy of antiangiogenesis drug bevacizumab. These studies highlight the role of the stroma, in particular fibroblasts, in regulating local ECM stiffness, which can in turn play a critical role in regulating the composition of cells present in the tumor microenvironment.

Given the extensive role of ECM rigidity on tumor and stromal cells, many studies have now shown that stiffness can contribute to the development of resistance to approved drugs, suggesting that therapeutic approaches that can regulate tissue rigidity may pave the way for the treatment of therapy-resistant cancers. Recently, Chitty and colleagues suggested that targeting tissue stiffness with a combination of LOX inhibitor could improve response to chemotherapy in pancreatic ductal adenocarcinoma (PDAC; ref. 9), an aggressive cancer with a low 5-year survival rate known to exhibit a dense fibrotic stroma caused by excessive ECM deposition and cross-linking. They demonstrated that a first-in-class pan-LOX inhibitor PXS-5505 alters LOX activity and decreases collagen stiffening induced by cancer-associated fibroblasts. Treatment of mice containing PDAC tumors with PXS-5505 and gemcitabine extends mouse survival by decreasing fibrillar collagen, improving the drug delivery to tumors and reducing liver metastasis. These results indicate that treatments targeting tissue stiffness have the potential to advance the treatment of refractory cancers that exhibit drug resistance or are metastatic. There are numerous clinical trials aimed at disrupting the effects of the stiff ECM on tumor cells, either by targeting mechanosensors within the tumor cells, such as integrins at the cell surface or downstream FAK, or by normalizing the tumor microenvironment by inhibiting TGFβ signaling to target CAFs (10).

The ECM is a key player in the tumor microenvironment, whose biochemical and physical properties can regulate many of the hallmarks of cancers. Ulrich and colleagues study was significant in that it shed light on how ECM rigidity can impact tumor cell proliferation and invasion and emphasized the importance in incorporating engineering experimental approaches to better understand the role of the ECM in cancer progression. Leveraging *in vitro* approaches that allow for the precise manipulation of the complex physical properties of the ECM is critical to understanding its effects, as ultimately, the development of novel strategies to regulate ECM properties *in vivo* will provide new opportunities to enhance our arsenal against the most deadly cancers.

Authors' Disclosures

No disclosures were reported.

Acknowledgments

The authors' research is supported by NIH DP2CA271387 and R01CA255742.`

Received February 2, 2024; accepted February 5, 2024; published first April 1, 2024.

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