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A Stiff Blow from the Stroma: Collagen Crosslinking Drives Tumor Progression

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Matrix stiffness is an important microenvironmental cue that regulates cell growth, motility, and differentiation. In a recent *Cell* report, Weaver and colleagues implicate lysyl-oxidase-mediated collagen crosslinking as a contributor to tumor matrix stiffening, which leads to enhanced integrin signaling and invasive behavior in tumors.

Cells in most tissues interact with an elastic microenvironment that provides not only chemical signals but also inputs of a physical nature. The mechanical properties of a cell's microenvironment can play a significant role in governing cellular behaviors. For example, matrix stiffness can direct differentiation of mesenchymal stem cells into distinct lineages, consistent with differences in tissue compliance (Engler et al., 2006). Matrix stiffness has also been implicated in tumorigenesis; both tumor cells and adjacent stroma display increased stiffness compared to normal tissues (Paszek et al., 2005). Tumor-associated stroma is composed of several cell types including adipocytes, fibroblasts, and immune cells, as well as a variety of extracellular matrix (ECM) proteins, such as collagen and fibronectin. What causes the increase in tumor stromal stiffness and how stromal stiffness contributes to tumor progression have become central questions in examining tumorigenesis from the mechanical-chemical perspective. The recent report by Weaver and colleagues (Levental et al., 2009) provides some answers to these questions.

Elevated deposition of fibrillar collagen, the most abundant ECM protein in the stroma, has been particularly associated with an altered stroma during breast tumorigenesis, correlating with increased mammographic density and greater breast cancer risk (Provenzano et al., 2006). It was discovered that increased collagen gel density or concentration increases matrix stiffness and disrupts mammary morphogenesis in 3D culture systems (Paszek et al., 2005; Provenzano et al., 2009). Furthermore, reduction of collagen degradation in an engineered mouse model increased stromal collagen density in mammary tissues and the incidence of mammary tumors (Provenzano et al., 2008). Evidence therefore pointed to collagen as a significant contributor to the changes in cellular mechanical microenvironment that accompanies tumor progression.

As the links between stromal collagen density, matrix stiffness, and tumor progression solidify, the underlying molecular mechanisms are slowly unraveling. The report by Weaver and colleagues contributes further insights by establishing a correlation between collagen crosslinking and matrix stiffness in vivo and by implicating the ECM-crosslinking enzyme lysyl oxidase (LOX) as a culprit driving stiffness-associated tumor progression (Levental et al., 2009). By conditioning mammary fat pads with fibroblasts expressing constitutively active LOX, the authors artificially increased collagen crosslinking in mouse mammary stroma and found that not only were the tissues stiffened, but the conditioned glands also promoted growth and invasion of normally noninvasive mammary epithelial cells (Figure 1). Conversely, inhibition of LOX was found to reduce breast tumor progression in a mouse model. Importantly, it was demonstrated that LOX-mediated tumor progression is probably due to extracellular LOX modifications of the tumor microenvironment rather than intracellular LOX signaling in the tumor cells.

The implication that LOX alters the tumor microenvironment and promotes tumor progression follows previous evidence linking LOX in tumorigenesis. LOX protein expression is elevated in many types of tumors, is associated with poor prognosis, and is shown to be involved in recruiting inflammatory stromal cells that contribute to tumor progression (Erler et al., 2009). These findings together support the potential therapeutic value in targeting LOX for cancer treatment. Questions still remain, however, regarding the origin and regulation of LOX expression and secretion, as well as its mechanism of action during tumorigenesis. Weaver and colleagues described elevated LOX in mouse mammary tumor stroma and used LOX-expressing fibroblasts to promote tumor progression, yet LOX was reported to be secreted by tumor cells themselves in response to hypoxia (Erler et al., 2009). Aside from collagen, LOX can also crosslink the ECM protein elastin and has been found to interact with fibronectin, another stromal ECM component that can modulate matrix stiffness (Payne et al., 2007). Intriguingly, LOX may also play intracellular roles in tumorigenesis and may in fact play a dichotomous role as a tumor suppressor (Payne et al., 2007); its action in crosslinking the ECM may oppose the role of matrix metalloproteases (MMPs), which promote tumor progression via matrix degradation. LOX is likely to collaborate with MMPs to generate a dynamic microenvironment that mechanically and chemically influences tumorigenesis.

Matrix remodeling represents only one of a complex set of processes that govern tumor development. Another critical insight revealed by the study of Weaver and colleagues is that increased matrix stiffness alone cannot promote tumor invasion. Acini cultured in collagen-I-containing basement membrane gels stiffened by ribose-induced collagen crosslinking displayed disrupted morphology but no

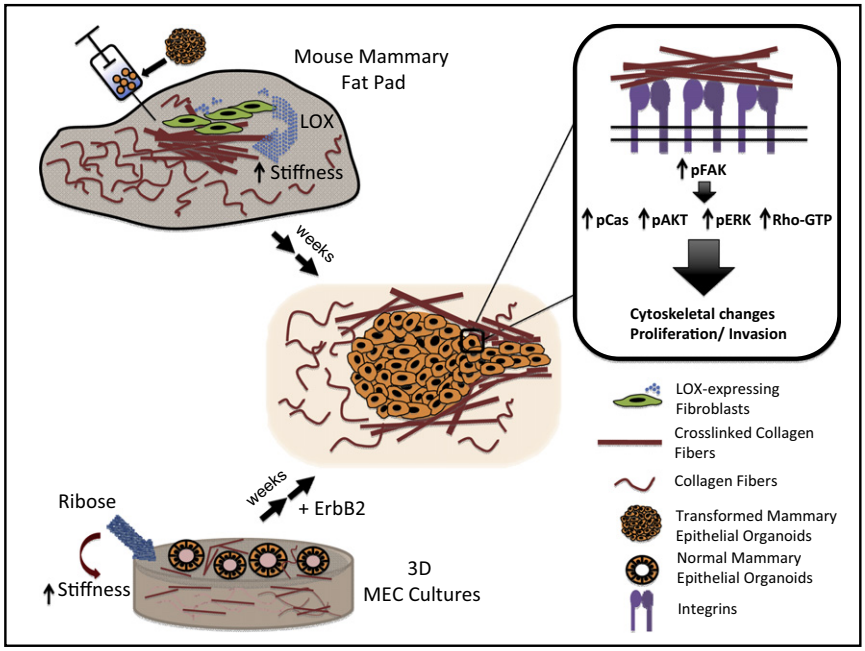

Figure 1. Increased Collagen Crosslinking Increases Matrix Stiffness and Promotes Tumor Invasion In Vivo and In Vitro

Weaver and colleagues found that preconditioning of the mammary fat pad with LOX-expressing fibroblasts increases matrix stiffness and enhances the growth and invasion of Ha-Ras premalignant mammary organoids. Similarly, crosslinking of collagen gels with ribose results in invasive structures in 3D mammary epithelial cell (MEC) cultures induced to express the activated oncogene ErbB2. Studies in this and previous reports demonstrate that collagen crosslinking-mediated matrix stiffening increases integrin clustering, which leads to phosphorylation of focal adhesion kinase (pFAK) and Crk-associated substrate (pCas), as well as activation of the AKT, ERK, and Rho pathways. Not shown are other cell types and ECM proteins present in the mammary tissue, which may also play important roles in tumorigenesis but are not discussed in Levental et al. (2009).

invasive structures develop unless the oncogene ErbB2 was activated (Figure 1). By using a mutant variant of β1-integrin that clusters constitutively to mimic the effects of stiff matrix, the authors similarly observed that invasion occurred only with Ha-Ras-transformed mammary epithelial cells and not with normal cells. Tumor progression and malignancy therefore require cooperation between the mechanical microenvironment and the intrinsic cell states.

How such cooperation is orchestrated is not well understood. Mechanical properties of the microenvironment are sensed by integrin family receptors that connect ECM proteins outside the cell to the actin cytoskeleton inside the cell (Chen, 2008). Increased tension from stiffer matrix induces integrin clustering, the development of focal adhesions, and the activation of multiple downstream signaling pathways. Consistent with this, Weaver and colleagues showed that tumors developing in stroma that contains LOX-crosslinked collagen display elevated integrin signaling, including phosphorylation of focal adhesion kinase (FAK) and Cas (Levental et al., 2009). They also found that the PI3-kinase substrate AKT is hyperactivated in stiffer tumors.

FAK phosphorylation is known to signal the Rho pathway, which influences cell contractility by mediating cytoskeletal processes such as focal adhesion and actin stress fiber formations (Chen, 2008). The Weaver laboratory had previously found that Rho activity is increased in cells on stiffer 2D substrates (Paszek et al., 2005), a result corroborated recently by the Keely laboratory with evidence from 3D mammary epithelial cell cultures (Provenzano et al., 2009). In parallel, enhanced FAK activation on stiffer matrix can also increase the phosphorylation of extracellular signal-regulated kinase (ERK) via Ras activation (Paszek et al., 2005; Provenzano et al., 2009). ERK can control cell migration, invasion, proliferation, and differentiation through modulation of acto-myosin contraction as well as induction of transcriptional programs, some of which have recently been found to respond differentially to stiff and soft microenvironments (Provenzano et al., 2009). Similarly, AKT can also modulate multiple cellular programs that regulate motility, growth, proliferation, and metabolism (Manning and Cantley, 2007). Importantly, the Rho, ERK, and AKT pathways are all strongly implicated in cancer. The intersection between mechanotransduction pathways with oncogenic signaling pathways is probably not coincidental; in fact, they may be synergistic in promoting tumor malignancy. For example, stiffness-dependent ERK activation may augment chronic activation of the ERK pathway in tumor cells that overexpress ErbB2 or Ras, leading to the invasive behavior seen by Weaver and colleagues. Elucidating the crosstalk between mechanotransduction and oncogenic signaling will be an important next step toward understanding the complex reciprocal interactions between tumors and their microenvironment.

Thus, while Weaver and colleagues pinpoint one microenvironmental factor—LOX-mediated collagen crosslinking—as a contributor to stromal stiffening that leads to breast tumor progression, their work also promotes an understanding of cancer not only as dysregulated cellular signaling and behaviors, but also as a context-dependent pathology where tumor cells interact with and respond to their physical microenvironment. Understanding tumor-stroma interactions, especially how extracellular mechanical and chemical signals integrate with intrinsic genetic and epigenetic alterations in tumor cells to regulate tumor progression, will be crucial in combating cancer.

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