Cell tension, matrix mechanics, and cancer development

Oncologists often diagnose cancer based on a change of tissue stiffness sensed by palpation, yet cancer researchers generally focus on biochemical signaling mechanisms. Tumors are more rigid because they have a stiffer extracellular matrix. A new study shows that this alteration of matrix mechanics activates integrins, which not only promotes mitogenic signaling through Erk but also cell contractility through Rho, which can further increase matrix stiffness. This establishes a positive feedback loop that switches on the malignant phenotype in mammary epithelial cells. This mechanical "autocrine loop" brings solid-state mechanotransduction on a par with oncogenic signaling pathways in malignant transformation.

Clinicians often diagnose tumors based on differences in tissue rigidity sensed by palpation, and pathologists have long known that cancer involves distinct changes in the extracellular matrix (ECM) that normally holds together cells within distinct tissue patterns. Although this matrix was initially viewed as a host barrier to tumor invasion, past studies have suggested that changes of ECM structure or mechanics, such as whether the matrix is stiff enough to resist cell traction forces, might actively contribute to tumor formation (Ingber et al., 1981). Alterations of mechanical properties can, in fact, influence tumor development, as illustrated by experiments that show that a rigid piece of metal or plastic can trigger cancer formation when implanted in the body, whereas tumors do not form when the same material is introduced as a powder (Bischoff and Bryson, 1964). Normal cells also need to attach to a rigid matrix and physically stretch to proliferate (Folkman and Moscona, 1978), whereas malignant cells lose this "shape dependence" (Wittelsberger et al., 1981). But how can the mechanics of a material alter cell growth, destroy tissue architecture, and induce cancer formation?

Only now, as a result of the vast amount of accumulating knowledge about how cells sense mechanical signals and convert them into changes in cellular biochemistry, are we in a position to unite cellular mechanotransduction with oncogenic signaling. Integrins have been shown to modulate signaling by the EGF receptor (EGFR), and to control the differentiation and transformation of mammary epithelial cells cultured on ECM gels, but not on rigid planar substrates (Wang et al., 1998). These transmembrane ECM receptors also act as mechanoreceptors (Wang et al., 1993) and mediate mechanotransduction by transferring forces to specialized anchoring structures, known as focal adhesions, that both link integrins to the cytoskeleton and orient much of the cell's signaling machinery (Bershadsky et al., 2003). However, this is not a one-way process. Cell traction forces generated in the actin cytoskeleton are exerted on these same sites, and thus integrins and focal adhesions are maintained in a state of isometric tension. External

Figure 1. A mechanical autocrine loop that may contribute to cancer development

Increases of rigidity in the matrix that better resist cell tensional forces activate integrins, promote focal adhesion assembly, and stimulate the Rho/ROCK pathway which enhances cell contractility, thereby further increasing matrix stiffness. Because of the crosstalk between the integrin/Rho pathway and the canonical growth factor receptor/Erk mitogenic signaling cascade, this self-sustaining positive feedback loop may stabilize the undifferentiated proliferative phenotype of mammary epithelial cancer cells and lead to neoplastic disorganization of tissue architecture.

stresses also can feed back to increase tension generation by activating the small G protein Rho and its target Rho-associated kinase (ROCK), which controls myosin light chain phosphorylation. Rho is mitogenic and can stimulate cell cycle progression in the absence of cell spreading (Roovers and Assoian, 2003). Thus, because changes of ECM stiffness alter the cellular force balance, a mechanics-based positive feedback control loop exists that can impact cell proliferation.

In this issue of *Cancer Cell*, Paszek et al. (2005) explore the role of bidirectional force transfer across integrins in the context of differentiation and tumor formation. Using an electromechanical indentor to directly measure tissue mechanics, they found that explanted mouse mammary tumors are stiffer than healthy mammary gland. And, they showed that undifferentiated EGFR-transformed mammary tumor cells that display elevated Erk activity also exhibit higher Rho activity. They then cultured normal mammary epithelial cells on ECM gels that varied in mechanical compliance over the range displayed by the normal and cancer tissues they measured in vivo. Not only did the stiff (force-resisting) ECM gels promote expression of the undifferentiated malignant phenotype, but Rho activity was also higher in these cells. When constitutively active RhoV14 was overexpressed in normal mammary cells adherent to a soft matrix, they acquired the malignant properties of the cells on the rigid gels: they generated more force, disrupted cell-cell junctions, spread, increased
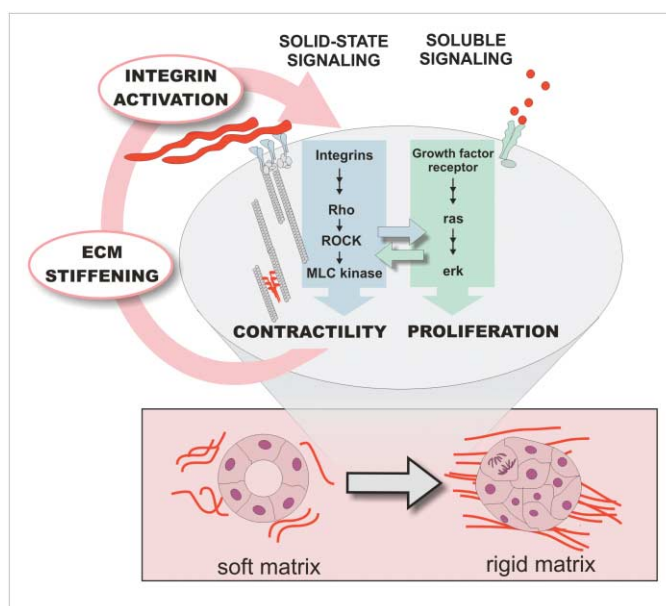
proliferation, and lost acinar organization. The dedifferentiated phenotype was reversed by blocking tension generation through pharmacological inhibition of ROCK or myosin II, suggesting that the transforming effect was not due to pleiotropic biochemical Rho signaling, but instead was specifically caused by Rho-dependent tension. Interestingly, the malignant phenotype of RhoV14 expressing cells was also normalized by inhibition of Erk, which similarly reduced force generation, pointing to the intertwining between the mitogenic EGFR/Erk and mechanotransducing Rho/ROCK pathways. Stress-induced aggregation of integrins and subsequent focal adhesion formation appeared to mediate all these effects, as suggested by studies in which cells were transfected with a mutated form of $\beta 1$ integrin which spontaneously self-associates in the cell membrane; these cells acted as if they were cultured on rigid substrates, even when plated on highly flexible ECM gels.

The ability of ECM mechanics and cell tension to contribute to cancer formation is intriguing. Increased stiffness of the ECM as observed in tumors in vivo may promote integrin clustering, Erk activation, and Rho-mediated contractility. A rise of cell tension will further increase ECM stiffness by tensing or realigning ECM components, thereby creating a deadly, self-sustaining positive feedback loop. Because Rho crosstalks with the mitogenic pathways, this self-maintained tensed state will stabilize the proliferative phenotype as a discrete behavioral program. This is the solid-state version of an autostimulatory loop known for soluble signals (Figure 1), such as the autocrine secretion of growth factors by tumor cells. A physical cue devoid of chemical specificity may therefore switch cells between entirely different phenotypes, even between normal and cancerous states, perhaps by initiating a cascade of multiple switches that simultaneously trigger the leap from one self-stabilizing "attractor" state to another within the genome-wide cell regulatory network (Huang et al., 2005). This mechanism also may explain why continued culturing of normal cells for many passages on rigid plastic dishes often leads to spontaneous transformation in vitro.

Thus, cancer can no longer be viewed solely as a result of dysregulation of intracellular signaling pathways. This regulatory activity of ECM mechanics puts cell fate regulation and its pathological derailment that leads to neoplasia back into the context of solid-state tissue properties. Increased understanding of the molecular basis of mechanotransduction may lead to identification of an entirely new class of molecular targets for anticancer therapy.

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Selected reading

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