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Matrix Elasticity, Cytoskeletal Tension, and TGF- β : The Insoluble and Soluble Meet

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Abstract

Soluble growth factors are potent regulators of normal and pathological processes. Mechanical factors are emerging as similarly important, but there has been no obvious mechanism linking the different factors. A recent report now demonstrates that cell-generated mechanical tension results in release of active transforming growth factor- β from stiff extracellular matrix, providing a mechanism for differentiation and maintenance of myofibroblasts in processes like fibrosis. More broadly, the work suggests that matrix stiffness could regulate the equilibrium between storage and release of a host of matrix-bound growth factors.

The link between the tension generated by a cell and its physical and soluble microenvironments has long been a conundrum. Referring to muscle and its secreted soluble products, it was hypothesized by Starr more than a century ago that

“...the active shortening of the fibre is due to an increase in the surface tension of the substance of the cell, caused by an increase in the proportional amount of the products of decomposition. Equilibrium is restored—after the stimulus which hastened the chemical changes has ceased—by a part of the products of decomposition finding their way into the blood-current....” (1)

The problem has only broadened over the past 50 years with the discovery that most nonmuscle cells express muscle-like assemblies of actin and myosin—appropriately called stress fibers—and thereby also generate contractile tension (2). Understanding the interplay between cell contractility and cellular microenvironment became additionally complex with the recognition that the extracellular matrix serves not only as a scaffold for cells, but also as a local depot for growth factors that are potent regulators of cell tension (3). Hinz and co-workers now integrate these observations by demonstrating that cell-generated tension—depending on matrix elasticity—physically shifts the equilibrium between matrix binding and release of transforming growth factor- β (TGF- β), resulting in maintenance of the contractile phenotype (4).

Cells sense their surroundings not through sight or sound but through the “smell” or “taste” of various soluble factors and the “touch” or “feel” of insoluble mechanical cues. Matrix elasticity is one of those cues, with an abundance of research over the last decade demonstrating that

matrix mechanics alters the phenotype of a wide range of cells including fibroblasts (5-9), normal or cancerous epithelial cells (8,10), neurons (11-13), myocytes (14,15), osteoblasts (16), and even stem cells (17). Although individual cell types vary in their responses, increased matrix stiffness generally results in increases in protein phosphorylation, stress fiber assembly, and cellular stiffness. The elastic modulus E of solid tissues quantifies the stress (force) required to strain (lengthen) a tissue and ranges from about 0.1 to 1 kPa for especially soft tissues, such as brain, fat, and liver, to ~10 kPa for static muscle and ~20 to 100 kPa for stiff, noncalcified scars, tendons, and cartilage (18-20). E has the same units as pressure, force per area, so that increasing E can be intuitively understood as being similar to lifting a heavier weight. The actin-myosin cytoskeleton of a cell establishes stress such that cells pulling on matrices of different stiffness respond as one would in lifting different weights, easily sensing even a twofold increase in either case. In comparison, cells grown directly on glass or plastic are doing isometric “exercise,” as one would in pulling or pushing on a weight (or wall) that simply does not budge. At the tissue rather than cellular level, increases in tissue stiffness can often be felt by palpation and occur frequently enough that sclerosis—which means hardening in Greek—aptly describes a wide range of clinical conditions, including atherosclerosis, multiple sclerosis, scleroderma, and osteosclerosis, to name a few.

The TGF- β s are matrix-associating, protein growth factors with important roles in embryogenesis, malignancy, and fibrosis. Interestingly, myofibroblasts—contractile cells expressing the α -smooth muscle isoform of actin (α -SMA)—are involved in all three processes. Gabbiani, Hinz, and colleagues previously proposed that myofibroblast differentiation from fibroblasts proceeds by a two-step process (21). In step one, the differentiation to a “protomyofibroblast” that expresses cellular β -actin filaments requires mechanical tension. In step two, the differentiation to contractile, mature myofibroblasts with α -SMA—containing stress fibers requires continued tension as well as TGF- β and a particular fibronectin splice variant (EDA). Myofibroblasts secrete TGF- β in an autocrine fashion, which stimulates them as part of the processes of wound healing and tissue fibrosis to deposit additional matrix material, including fibrillar collagens, EDA-fibronectin, and growth factor-binding proteoglycans. Other TGF- β superfamily members play similarly important roles in matrix-related processes. Myostatin, for example, inhibits myogenesis, and the functions of the bone morphogenetic proteins (BMPs) include regulation of bone formation. Thus, although the details of the relation have until now been a mystery, it is clear that there is a critical link between TGF- β , matrix, and mechanics.

The first demonstration that TGF- β exerts differential effects on cells depending on matrix stiffness came from culturing contractile myofibroblasts in collagen gels (5). With floating (soft) gels of $E \sim 8$ kPa [calculated from equations in (22)], myofibroblasts lacked stress fibers and their α -SMA slowly decayed, whether TGF- β was present or not. In comparison, cells growing within anchored (stiff) gels with $E \sim 20$ kPa or grown on top of even stiffer collagen-coated plastic proved highly responsive to TGF- β , increasing α -SMA abundance and stress fiber organization. Interestingly, unless TGF- β is present, the collagen-specific integrin α_2 subunit is lost from cells cultured in or on stiff gels. Although this early work suggested that the effects of TGF- β were coupled to matrix elasticity and significantly modulated transcript levels as well as synthesis or degradation of cytoskeletal proteins and integrins, molecular mechanisms were hypothesized to involve tension-dependent signaling within the cell but remained unexplored.

TGF- β is secreted from cells as part of a latent complex that adheres to matrix proteins such as fibrillin, proteoglycans, and fibronectin (23-25), giving rise to a matrix reservoir of the growth factor that may function as an extracellular sensor (26). One component of the latent complex, the latency-associated protein (LAP), interacts directly with integrins (specifically $\alpha_v\beta_5$, although other integrins might also contribute) and thus provides a cellular handle on

extracellular stores of TGF- β (Fig. 1). The important finding is that the cell exerts tension on the latent complex through this integrin handle, causing conformational changes likely similar to those occurring in other structural proteins under force (27)—and releasing sequestered TGF- β in an active form to diffuse and bind cell surface receptors. This model of Hinz and co-workers depends on matrix elasticity. A soft matrix with $E \leq 5$ kPa preferentially deforms under the stresses applied by cells, leaving the latent complex intact and TGF- β sequestered even after treatment with a contraction agonist, whereas a stiff matrix with $E \gg 10$ kPa resists deformation, resulting in distortion of the latent complex and the release of active TGF- β . The growth factor is thus sequestered like perfume in a tightly sealed bottle that requires pulling the lid off to release the aromatic molecules inside. Matrix stiffness, cell tension, and TGF- β release are therefore all required to increase the abundance of cytoskeletal α -SMA. The feed-forward, load-limiting mechanism (Fig. 1) promotes and maintains myofibroblast differentiation (and therefore continued fibrogenesis) in the continuous remodeling that occurs as soft wounded tissue is pulled together in healing, scar formation, and even in pathological fibrosis. The model also suggests—as Starr might have predicted in 1886—that interruption of either leg of the stiffness/TGF- β cycle might provide a therapy leading to dedifferentiation of myofibroblasts and regression of disease.

Hinz and his group have provided an example of the biological relevance of matrix mechanics that has implications beyond the ones given above. Matrix stiffness could be an underlying mediator of TGF- β -driven processes, such as the epithelial-to-mesenchymal transition in development and cancer cell metastasis. Fibronectin dynamics might be influenced by the mechanosensitive release of TGF- β given that stretch can expose fibronectin self-assembly sites (28,29) and that TGF- β affects both fibronectin synthesis and assembly (30,31). An intriguing possibility is that EDA-fibronectin, which is required in addition to TGF- β and matrix tension for myofibroblast differentiation, might be particularly sensitive to changes in stiffness. In addition, the epithelial cell response to injury results in inflammation and up-regulation of the integrin $\alpha_v\beta_6$, and like the myofibroblast integrin $\alpha_v\beta_5$ studied by Hinz's group, $\alpha_v\beta_6$ binds the latent complex and activates release of active TGF- β (32). Although the role of mechanics in $\alpha_v\beta_6$ -mediated TGF- β activation has not been examined, matrix elasticity could provide an important link between inflammation, on the one hand, and normal wound healing and pathological fibrosis on the other.

Finally, it is appealing to speculate that mechanics-driven mechanisms might also be responsible for the release of other growth factors sequestered in the matrix. Matrix elasticity has been found to direct stem cell lineage specification and to specifically regulate transcription of BMPs and myostatin (GDF8) (17). Such effects might explain why osteogenesis is seen in some stiff scars, including myocardial infarcts that—in therapeutic interventions—are followed by stem cell injection (33). Whether extracellular reservoirs of other matrix-bound growth factors, including FGFs (fibroblast growth factors), IGFs (insulin-like growth factors), and EGF (epidermal growth factor), could be similarly mechanically responsive is not yet known. For some growth factors, proteolysis of associated binding proteins appears to be required for activation, and force could be key to accelerating conformational exposure of proteolytic sites for growth factor release. The scheme outlined by Wipff *et al.*, although specific for TGF- β , might therefore reflect a general mechanistic link between soluble and mechanical factors.

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Fig. 1.

Mechanical tension results in TGF- β release and maintenance of the myofibroblast phenotype through a “feed-forward” loop. Myofibroblasts behave differently on soft versus stiff matrices. On soft extracellular matrices (ECMs), cell-generated tension on the TGF- β latent complex (through α -SMA stress fibers and integrins) results in deformation of the ECM. The latent complex remains intact, keeping TGF- β sequestered in the matrix and preventing its release (**left**). Without release of TGF- β , there is no active TGF- β to bind to its receptor and Smads remain cytoplasmic. Existing α -SMA degrades and cells synthesize less new α -SMA, resulting in loss of the contractile, myofibroblast phenotype. When myofibroblasts are on stiff matrices, cell-generated tension meets resistance from the matrix, with deformation of the TGF- β latent complex and release of soluble TGF- β (**right**). This bioactive TGF- β binds its receptor, resulting in phosphorylation and nuclear localization of the Smads with synthesis of ECM, the TGF- β latent complex itself, and α -SMA, which maintains the myofibroblast phenotype or even makes it more contractile.