Editorial

Getting Physical With the Aortic Valve

Peter F. Davies, Marie A. Guerraty

Mammalian cells and tissues rely on continuous interactions with the structural, physical, and soluble chemical environment to adapt to rapidly changing conditions. This ensures the continuation of proper physiological functions, accurate developmental cues during embryogenesis, containment of chronic pathological changes, and efficient repair of acute injuries. As part of cell regulation, the mechanical status of cells is constantly undergoing adjustment through coordinated responses to changes of intracellular tension imposed by internal and external forces and by encounters with extracellular matrices of varying stiffness.[1,2] The role of extracellular proteins in mechanoregulated cell biology has emerged over the last decade in studies of fibrosis—the differentiation of myofibroblasts that express contractile α-smooth muscle actin (α-SMA) organized into stress fibers. Myofibroblast differentiation requires sustained mechanical tension that in turn is dependent on the stiffness of the extracellular matrix as sensed through integrin adhesion sites in the cell membrane.[3] A critical soluble protein required for myofibroblast differentiation is transforming growth factor-β (TGF-β)[4] that is secreted from myofibroblasts and, in an autocrine loop, can stimulate the cell via TGF-β receptors. However, secreted TGF-β binds to the extracellular matrix via a fibronectin splice variant (TGF-β latent complex), making it unavailable to the cell and preventing differentiation.[5] In 2007, Wipff et al[6] showed that TGF-β availability depends on the matrix stiffness and that its sequestration is reversed when the matrix is stiff rather than soft. The underlying mechanism is that intracellular cytoskeletal tension (inherent to all anchorage-dependent cells) is increased by the deformation-resistant stiff matrix against which it pulls. As a result, the TGF-β complex, attached to the matrix as well as to the cell membrane, undergoes force-induced conformational change, releasing TGF-β from the latent complex.[6,7] The free molecule then interacts with the cell to promote α-SMA synthesis through TGF receptor binding, phosphorylation, and activation of Smads. Thus intracellular and extracellular mechanical properties converge with critical soluble proteins at the cell/matrix interface to regulate cell differentiation. This elegant integrated regulatory mechanism in myofibroblasts applies to wide-ranging examples of fibrosis in injury repair (eg, granulation tissue contractility[8]) and chronic inflammatory diseases, including atherosclerosis and calcified aortic valve disease (CAVD), pathologies that are usually associated with significant localized changes of matrix stiffness.

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In this edition of the journal, Chen et al[9] add a new mechanics-related cross-talk mechanism to TGF-β-induced myofibroblast differentiation through their investigations of swine valve interstitial cells (VICs). Increased myofibroblast differentiation of VICs is a prominent feature of CAVD lesions.[10] In humans and swine, CAVD occurs preferentially and more severely on the aortic side of the valve and is preceded by valve thickening. The aortic valve functions in a spatially heterogeneous hemodynamic environment that is particularly complicated on the downstream aortic side,[11] where the subendothelial fibrosa layer is stiffer than the ventricularis region located on the opposite side.[12] Consequently, the mechanical environment of the fibrosa is both intrinsically stiffer and extrinsically subject to more irregular outside forces than the ventricularis. TGF-β1/Smad signaling is activated in CAVD[13]; Chen et al[9] noted the coexisting activation of TGF-β1 and Wnt/β-catenin pathways in valves and proposed that they may be related both to each other and to spatially related matrix stiffness within the tissue. Importantly, they showed that although Smad phosphorylation and nuclear translocation are critical to TGF-β1-induced myofibroblast differentiation, Smad proteins alone are insufficient to activate transcription,[14] implying a requirement for cross-talk between TGF signaling and other signaling pathways. Shafer and Towler (2009)[15] recently showed convergence of Wnt3a and TGF-β1/Smad signaling in the regulation of SM22α. Could the canonical Wnt/β-catenin pathway satisfy this cross-talk requirement in VIC differentiation and furthermore be linked to local matrix mechanical properties that mediate mechanotransduction signaling?

In primary cultures of swine VICs isolated from normal valve leaflets and grown on plastic, by using multiple interventions, β-catenin was shown to be required for TGF-β1-induced VIC myofibroblast differentiation through TGF-β type 1 receptor. TGF-β1 and Wnt3A synergistically increased β-catenin nuclear translocation, promoting greater myofibroblast differentiation and α-SMA transcription than TGF-β1 alone. Furthermore, using collagen I–coated polyacrylamide gels that mimic the stiffnesses of fibrosa and ventricularis, Chen et al[9] demonstrated that TGF-β1-induced β-catenin activation and subsequent myofibroblast differentiation occurred only on the stiffer matrix that mimics fibrosa. To extend the cell studies to compromised tissue, immunocytochemistry of diseased valves from swine fed a hypercho-

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lesterolemic diet expressed abundant SMA/pSmad, TGF-β1, and Wnt/β-catenin in the fibrosa, in contrast to normal valves, where expression was undetectable. Collectively, the data suggest that regions of the fibrosa where the matrix is of greater stiffness (>22 kPa) are permissive for VIC myofibroblast differentiation through β-catenin selective mechanisms and may play a role in CAVD susceptibility at those locations.

In providing greater mechanistic insights into extracellular regulation of intracellular biology, the study by Chen et al[9] identifies several important future investigations including the mechanisms by which TGF-β1 activates β-catenin and the subsequent regulation of α-SMA transcription by β-catenin. The study also highlights the important role of biomechanics in adjusting the outside-in/inside-out cytoskeleton/integrin/extracellular matrix signaling axis, although shifts in matrix composition were not investigated. As VICs differentiate to myelofibroblasts and collagen I is replaced by basement membrane collagen IV, might the engagement of different integrins become important?

Whether the myofibroblast differentiation is a contributor to CAVD remains unclear. A recent investigation[16] of global endothelial gene expression in swine aortic valves during early CAVD unexpectedly revealed the induction of gene expression pathways that are predicted to be exclusively protective to the fibrosa side but cause no significant changes to the endothelial transcription profile on the ventricular side. It should not therefore be assumed that the myofibroblast differentiation of VICs in the fibrosa is pathological only because it maps with early CAVD sites; it may initially be a protective adaptation but one that later contributes to pro-pathological TGF-β/Wnt-regulation of bone morphogenetic protein/osteogenic and calcification pathways. The studies by Chen et al represent an interesting context-specific example of mechanisms that may be generalizable to other contexts of mechanobiology, as reflected by many articles that link matrix stiffness to cell behavior in a variety of tissues. More examples of cross-talk in TGF-β signaling pathways will likely be uncovered, some promiscuous and others cell-specific, as is the case in TGF-β chemical signaling[5] and intracellular endothelial mechanotransduction.[17] Mechanical and chemical signaling are so intimately entwined to maintain cells within an acceptable functional range that being mechanically regulated and being growth factor induced are frequently mutually dependent.

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