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The myofibroblast: Paradigm for a mechanically active cell

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ABSTRACT

Tissues lose mechanical integrity when our body is injured. To rapidly restore mechanical stability a multitude of cell types can jump into action by acquiring a reparative phenotype—the myofibroblast. Here, I review the known biomechanics of myofibroblast differentiation and action and speculate on underlying mechanisms. Hallmarks of the myofibroblast are secretion of extracellular matrix, development of adhesion structures with the substrate, and formation of contractile bundles composed of actin and myosin. These cytoskeletal features not only enable the myofibroblast to remodel and contract the extracellular matrix but to adapt its activity to changes in the mechanical microenvironment. Rapid repair comes at the cost of tissue contracture due to the inability of the myofibroblast to regenerate tissue. If contracture and ECM remodeling become progressive and manifests as organ fibrosis, the outcome of myofibroblast activity will have more severe consequences than the initial damage. Whereas the pathological consequences of myofibroblast occurrence are of great interest for physicians, their mechano-responsive features render them attractive for physicists and bioengineers. Their well developed cytoskeleton and responsiveness to a plethora of cytokines fascinate cell biologists and biochemists. Finally, the question of the myofibroblast origin intrigues stem cell biologists and developmental biologists—what else can you ask from a truly interdisciplinary cell?

1. Introduction

This special issue of the Journal of Biomechanics covers a variety of tissue and cell types that are subject to different mechanical challenges and that actively play various mechanical roles. In normal tissues under physiological conditions the residing cells experience specific mechanical signals within a distinct range of magnitudes. Typical examples are vascular endothelial cells and leukocytes, exposed to shear stress, epithelial cells to shearing and stretching, smooth muscle cells to stretch, striated muscle cells to stretch and compression, osteoblasts and chondrocytes to compression. In other words, most cells in the adult organism live in a 'mechanical niche' and it is generally acknowledged that this defined set of mechanical cues is crucial to maintain their identity (Discher et al., 2005; Janmey and McCulloch, 2007). Similarly, during development the constantly changing mechanical environment is a major determinant of cell fate (Krieg et al., 2008). At this stage cells are more plastic and adapt/contribute to complex mechanical patterns present in the in early embryo. Adult cells can (re-)gain some level of plasticity after tissue injury and during repair, conditions that in many respects can resemble the situation in the embryo. Tissue boundaries are disintegrated and the mechano-protective architecture of the extracellular matrix (ECM) is disturbed. In addition to this dramatic imbalance in their mechanical equilibrium cells become exposed to an overwhelming cocktail of cytokines, initially deriving from damaged and inflammatory cells (Gurtner et al., 2008).

Activated by mechanical stress and cytokines, many cells of predominantly mesenchymal origin differentiate into myofibroblasts which drive tissue repair by secreting collagen and reorganizing (contracting) the ECM (Tomasek et al., 2002). Despite the fact that acquisition of the myofibroblast phenotype is generally called 'differentiation' it may be more appropriate to consider this cell being less differentiated than its precursor and rather primitive. Primitive is here used in the positive sense of the word: 'being of origin' or 'being of simple character' and several characteristics of the myofibroblast support this point of view. Typical molecular features of the differentiated myofibroblast are neo-expression of α-smooth muscle actin (α-SMA) and of the fibronectin (FN) splice variant ectodomain (ED)-A FN. Phylogenetically and during embryogenesis, α-SMA is one of the earlier

Abbreviations: AFM, atomic force microscopy; α-SMA, α-smooth muscle actin; ECM, extracellular matrix; ED-A, extra domain A; FA, focal adhesion; FN, fibronectin; LAP, latency associated protein; LTBP, latent transforming growth factor β binding protein; MRTF, myocardin-related transcription factor; TGFβ, transforming growth factor β

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expressed muscle actins. During heart development for instance, cardiomyocytes first transiently express α-SMA, followed by α-skeletal actin, which is finally replaced by α-cardiac actin persisting in the in the adult heart muscle (Clement et al., 2007). Similarly, the ED-A FN splice variant is characteristic for embryonic development, becomes down-regulated in most adult tissues and re-appears during tissue repair in the context of myofibroblast development (Ffrench-Constant et al., 1989; Serini et al., 1998). Also on the functional level, myofibroblasts are rather poor construction workers. They effectively repair defects and re-establish mechanical tissue integrity but never truly regenerate the damaged tissue. The resulting formation of a collagenous and stiff scar leads to reduced tissue function and even organ failure if myofibroblast repair becomes chronic such as during progression of fibrosis (Hinz, 2009).

Together, these features render the myofibroblast an interesting cell type for the study of mechanobiology: (1) it is highly relevant for physiological and pathological tissue remodeling, (2) it is mechanically active and contributes to alterations in overall tissue mechanics, (3) it is mechano-sensitive and mechanically inducible, and (4) the fundamental character of the myofibroblast allows for studying basic mechanical principles and pathways.

2. A beginner's guide to the myofibroblast

The myofibroblast was discovered by Gabbiani and coworkers in the early 1970s and first shown to actively promote dermal wound contraction (Gabbiani et al., 1971). Since then, this cell has been on the rise and its importance demonstrated for many pathophysiological processes that include tissue repair and remodeling. Myofibroblast activity is beneficial for dermal wound closure and for restoring the mechanical stability of injured organs against rupture. De-regulated and chronic myofibroblast activity however generates tissue deformation by contracture and impedes organ function. Tissue contractures are clearly visible in skin hypertrophic scars such as those developing after burns (Atiyeh et al., 2005), in scleroderma (Strehlow and Korn, 1998; Varga and Abraham, 2007) and in the palmar fibromatosis of Dupuytren's disease (Tomasek et al., 1999). Myofibroblast-generated contractures are also fundamental in organ fibrosis, affecting liver (Gressner and Weiskirchen, 2006; Iredale, 2007), heart (Baudino et al., 2006; Brown R.D. et al., 2005), lung (Phan, 2002; Thannickal et al., 2004) and kidney (Liu, 2006) with often lethal consequences. Myofibroblasts are instrumental in creating tissue constrictions around solid body implants (Comut et al., 2000), they contract silicone breast implants (Rudolph et al., 1978; Siggelkow et al., 2003) and are activated by different implanted biomaterials in a fibrotic host reaction (Anderson et al., 2008; Li et al., 2007). Myofibroblasts further contribute to the evolution of atheromatous plaque after blood vessel injury (Bochaton-Piallat and Gabbiani, 2006) and play a crucial role in the stroma reaction to epithelial tumors (De Wever et al., 2008; Desmouliere et al., 2004). The finding that cancer progression is stimulated by the myofibroblast-created environment has exposed the tumor-associated myofibroblast as an important target for anti-cancer therapy (Albini and Sporn, 2007).

Another reason for the attractiveness of the myofibroblast for a broad scientific and clinical audience is the large panel of cells that can develop this phenotype upon activation. It appears that myofibroblasts can be recruited from whatever local cell type is suitable to rapidly repair injured tissue (Hinz et al., 2007). Local fibroblasts residing in different tissue locations are considered the most prominent source of myofibroblasts. However, a variety of other precursor cells contribute to the myofibroblast population depending on the nature of the injured tissue and the particular microenvironment. The incomplete list, in no particular order, includes chondrocytes, osteoblasts, hepatic stellate cells, smooth muscle cells, pericytes, fibrocytes, mesenchymal stem cells, epithelial cells undergoing epithelial-to-mesenchymal transition, and possibly astrocytes (for more a more detailed evaluation of myofibroblast precursors, see (Hinz, in press; Hinz et al., 2007 and references therein).

The amazingly heterogeneous selection of possible progenitors raises the question: What are the criteria that identify the myofibroblast? Today, neo-expression of the smooth muscle actin isoform α-SMA is the most widely used myofibroblast marker in research and clinical diagnostics. Myofibroblasts are usually negative for desmin, smooth muscle myosin heavy chain, h-caldesmon, and smoothelin, distinguishing them from normal smooth muscle cells (Schurch et al., 2007). The convenience of using α-SMA as molecular marker may have contributed to the misconception that a myofibroblast must express α-SMA to be a myofibroblast. A priori however, the definition of the myofibroblast is based on its contractile function reflected in its well chosen name. Myofibroblasts combine ultrastructural and functional features of smooth muscle (myo-) by forming contractile actin/myosin-containing stress fibers, with the extensive endoplasmic reticulum of synthetically active fibroblasts (Gabbiani et al., 1971). To highlight the fact that the contractile cytoskeleton is not a feature of normal tissue fibroblasts, we previously introduced the term 'proto-myofibroblast' for stress fiber-containing, but α-SMA-negative fibroblasts. 'Differentiated myofibroblast' designates cells with α-SMA-positive stress fibers (Tomasek et al., 2002). This distinction is more than semantic finesse because both phenotypes can co-exist *in vitro* and *in vivo* and perform different functions. For instance, in the early granulation tissue of open rat wounds, α-SMA-negative proto-myofibroblasts, identified by Phalloidin decoration of stress fibers, emerge after 6 d of healing. Proto-myofibroblasts lay down the first collagen bundles and pre-organize the provisional ECM by exerting comparably small traction forces. Consecutive appearance of α-SMA-positive differentiated myofibroblasts in 9 d-old wounds then hallmarks the contractile phase of wound closure (Fig. 1) (Hinz et al., 2001b).

3. Mechanical control of the myofibroblast phenotype—it is in the matrix

Mechanics play a pivotal role in controlling myofibroblast differentiation and function. The goal of myofibroblast activity is to rapidly re-establish tissue integrity by secreting and organizing new ECM; this process is precisely controlled through a mechanical feedback from the ECM. The provisional ECM laid down after acute tissue injury, e.g., the fibrin clot of dermal wounds, is estimated to be very compliant with a Young's modulus of 10–1000 Pa (Fig. 2). Under comparable conditions *in vitro*, such as growth on very soft two-dimensional polyacrylamide gels and in three-dimensional soft collagen gels, development of stress fibers by fibroblasts is suppressed. Fibroblasts without stress fibers form only very small and immature adhesions with the ECM that are called focal complexes or nascent adhesions (Tamariz and Grinnell, 2002; Yeung et al., 2005) (Fig. 3). The proto-myofibroblast phenotype is only produced on stiffer culture substrates exhibiting an elastic modulus of at least 3000 Pa; these cells form α-SMA-negative stress fibers that terminate in mature focal adhesions (FAs) (Figs. 1–3) (Yeung et al., 2005). A stiffness of ~18,000 Pa has been measured in 7 d-old rat wound granulation tissue which is mainly populated by proto-myofibroblasts (Figs. 1 and 2). Even stiffer culture substrates with a Young's modulus of ~20,000 Pa and higher are required to permit further myofibroblast differentiation. Expression of α-SMA in stress fibers on stiff substrates is associated with the formation of large supermature FAs (Goffin et al., 2006; Wells, 2005) (Figs. 1–3).