Fibrosis: recent advances in myofibroblast biology and new therapeutic perspectives

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Introduction and context
Tissue destruction by organ fibrosis contributes to the lethal outcomes associated with heart, lung, liver, kidney, and skin diseases. The cell responsible for the detrimental fibrotic tissue contractures is the myofibroblast, which has a phenotype characterized by excessive production of collagenous extracellular matrix (ECM) and tensile force [1]. The concept that the myofibroblast plays a pivotal role in the establishment of fibrotic conditions has paved the way for a new approach in the understanding of the mechanisms of these pathologic situations [2]. In particular, it has become accepted that mechanical force generation by myofibroblasts, which in turn depends on the neo-expression of α-smooth muscle actin (α-SMA) in stress fibers of these cells, regulates essential phenomena for tissue remodeling, such as cytokine synthesis and ECM component production [2]. The myofibroblast participates in a variety of phenomena, including embryologic development, organ fibrosis, and the stroma reaction to epithelial tumors [1]. This widespread occurrence suggests that the term myofibroblast describes a functional status rather than a fixed cell type. This assumption has been supported by recent findings indicating that myofibroblasts originate from a spectrum of cellular sources depending on the physiological or pathological situation [1]. Myofibroblast origin and its tissue environment should be considered when planning new therapeutic strategies that aim at decreasing myofibroblast number or activity.

Major recent advances
The list of cells from which myofibroblasts can derive has grown impressively during the last years. It includes local fibroblasts, epithelial cells, endothelial cells, smooth muscle cells, pericytes, hepatic perisinusoidal cells, mesenchymal stem cells, and bone marrow-derived cells known as fibrocytes [1,3]. Most attention has been given to the fibrocyte as a possible myofibroblast precursor [4] and the phenomena of epithelial- and endothelial-mesenchymal transition as myofibroblast sources, particularly during lung and kidney fibrosis [5,6]. Transition of epithelial cells all the way to the myofibroblast phenotype is inducible in culture and regulated by different signaling pathways [7,8]. However, the relative contribution of myofibroblast precursors remains to be determined. As one would intuitively expect, it appears likely that in most situations local fibroblasts represent the major source of myofibroblasts [1]. The local derivation of myofibroblasts from mesenchymal rather than epithelial or endothelial cells has recently been documented in a model of renal interstitial fibrosis by means of genetic lineage tracing [9].

At present there is no accepted therapy for fibrotic diseases [10]. A number of previous and recent antifibrotic strategies attempt to interfere with myofibroblast formation by targeting key factors in the differentiation process (Figure 1). It is well established that myofibroblast

Abstract
The crucial role of the myofibroblast in wound healing and fibrosis development is well established. This review discusses the mechanisms of myofibroblast action and the new findings that may develop into therapeutic strategies during the next few years.

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differentiation and organ fibrosis are predominantly controlled by transforming growth factor β1 (TGF-β1) [11] and the ED-A (extra domain A) found in cellular fibronectin [12]. Moreover, several cytokines and chemokines (and their receptors), as well as coagulation factors and ECM components, have been implicated in this process [13-18]. It is likely that the heterogeneity of the myofibroblast origin requires specific factors and specific mechanical conditions in each situation.

Fibrosis is usually diagnosed when tissue destruction is already progressing, and it is possible that therapies will have to target the resident myofibroblast population. For this, aiming at the contractile apparatus is allegedly the most straight forward and promising strategy to inhibit myofibroblast function (Figure 1). A new direction, which has proven experimentally successful, is based on the observation that intracellular delivery of the α-SMA amino-terminal sequence Ac-EEED inhibits the incorporation of this protein in myofibroblast stress fibers, thus reducing force production as well as collagen type I synthesis by myofibroblasts in vitro; moreover, it significantly inhibits experimental wound contraction in vivo [19]. Due to its relative specificity for α-SMA-expressing myofibroblast stress fibers, this peptide appears as a good candidate for topical (e.g., burn scars) and systemic (e.g., organ fibrosis) administration.

Another promising strategy to induce myofibroblast disappearance is to stimulate them to go into apoptosis. Two major intracellular pathways have been identified that act pro-survival (or anti-apoptotic) for the myofibroblast: focal adhesion kinase signaling in cell ECM adhesions and phosphatidylinositol 3-kinase (PI3K)-AKT signaling. Focal adhesion kinase activation protects myofibroblasts from going into apoptosis in response to the loss of cell adhesion, a phenomenon called anoikis [20]. TGF-β1 and endothelin-1 have been shown to independently

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Figure 1. Targeting the myofibroblast

Potential antifibrotic therapies can interfere with the chemical and mechanical factors that lead to myofibroblast formation from different precursor cells (shown here for fibroblasts). Alternatively, or additionally, specific features of the differentiated myofibroblast can be targeted to induce myofibroblast regression and/or apoptosis. α-SMA, alpha-smooth muscle actin; ECM, extracellular matrix; ED-A, extra domain A; IST-9; fibronectin antibody; TGF-β1, transforming growth factor β1.
activate the PI3K-AKT pathway and thereby render myofibroblasts apoptosis-resistant [21]. Development of protein kinase inhibitors as specific inducers of myofibroblast apoptosis is an exciting new avenue in fighting fibrosis [22]. Other strategies could include myofibroblast-specific delivery of apoptosis-inducing drugs as applied in a mouse model of liver fibrosis [23].

Inducing myofibroblast disappearance does not necessarily include their killing; interrupting the auto/paracrine production of active TGF-β1 leads to myofibroblast de-differentiation, at least in vitro [24]. However, attempts to use general inhibitors of TGF-β1 have been relatively unsuccessful, showing that fibrosis development is a more complex phenomenon than expected [25-28]. The limitation of such global strategies is the interference of the beneficial effects of the pleiotrophic TGF-β1, such as controlling homeostasis of epithelial, vascular, endothelial, and immune cells. Therefore, more promising strategies may be to prevent latent TGF-β1 activation in a cell-type-specific manner rather than blocking already active TGF-β1. TGF-β1 is secreted together with LAP (latency-associated peptide), forming a large complex with latent TGF-β1 binding protein 1 (LTBP-1) in the ECM [29]. Epithelial cells activate latent TGF-β1 via integrin αvβ6 [30,31], which requires ECM binding mediated by LTBP-1 [29]. Inhibition of the epithelium-specific αvβ6 integrin protects from lung, kidney, and bile duct fibrosis [32-35]. In addition, integrins αvβ3, αvβ8, and αvβ5 play a role in latent TGF-β1 activation by fibroblastic cells, either directly or in a process involving proteases [11,36-40]. We have described a novel mechanical mechanism of latent TGF-β1 activation for myofibroblasts that, literally, pulls on the large latent complex using the integrin αvβ5 [41].

Future directions

Blocking specific integrins is a promising future strategy to control the development of myofibroblasts in fibrotic disorders (Figure 1). In addition to blocking the latent TGF-β1-activating integrin αvβ5 [41-44], inhibition of integrin α3 [8,45], α11, [46] αvβ3 [44,47], and β1 [48] were shown to block myofibroblast development and may be developed into future therapies. The latter integrins are all implicated in myofibroblast mechanoperception and transduction. It becomes increasingly clear that myofibroblast differentiation crucially depends on mechanical factors such as ECM stiffness and intracellular tension. Mechanical stress determines the stress fiber localization of α-SMA [49], modulates α-SMA promoter activity and protein expression in a process that involves the myocardin-related transcription factor [7,48,50], and modulates the bioactivity of TGF-β1 [41]. Hence, releasing myofibroblasts from stress – for example, using the Ac-EEED peptide [51] – will have a profound and long-term effect on myofibroblast persistence and may even induce myofibroblast apoptosis [52].

Abbreviations

α-SMA, alpha-smooth muscle actin; ECM, extracellular matrix; PI3K, phosphatidylinositol 3-kinase; LTBP-1, latent transforming growth factor β1 binding protein 1; TGF-β1, transforming growth factor β1.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

The work of BH is supported by grants from the Swiss National Science Foundation (grant# 3100A0-113733/1), the Heart and Stroke Foundation Ontario (grant# NA7086), and the Canadian Institutes of Health Research (grant# 210820).

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