# Mechanisms of Myofibroblast Differentiation

Sem H. Phan, Department of Pathology, University of Michigan, Ann Arbor, MI

A distinct subpopulation of fibroblasts in fibrotic tissue is characterized by expression of α-smooth muscle actin (α-SMA) and consequently referred to as myofibroblasts. These cells express high levels of extracellular matrix and exhibit contractile properties that may be significant in pathological alteration of mechanical properties of affected fibrotic tissues. Moreover they express high levels profibrogenic cytokines such as transforming growth factor β (TGFβ). These are key properties of tissues undergoing fibrosis, thus insights into the genesis of these cells should advance understanding of the pathogenesis of fibrotic diseases. There is compelling evidence, especially in vitro that myofibroblasts are derived from appropriately stimulated (e.g. with TGFβ) resident tissue fibroblasts, which normally do not express α-SMA. Recent studies also implicate epithelial and endothelial cells as potential additional sources through a process referred to as epithelial or endothelial-mesenchymal transition (EMT). An additional potential source is the circulating "fibrocyte". The relative contributions by these mechanisms to the overall myofibroblast population remain uncertain, especially in vivo. The mechanisms involved in myofibroblast differentiation from these diverse cell types are likely to have different components, although there may be similarities with respect to downstream TGFβ signaling, since this is a common agent found to be effective in inducing differentiation in all these cell types.

Give that α-SMA is a key marker of myofibroblast differentiation, an obvious focus for studies into this process is directed at regulation of expression of this gene. Since TGFβ is a potent inducer of differentiation, most studies have focused on how it affects α-SMA gene expression. Aside from involvement of the canonical Smad signaling pathway, there is evidence that MAP kinase signaling may also be involved. Studies in smooth muscle cells have implicated CArG elements, E-boxes and a purine rich motif in regulating gene expression. The corresponding transcription factors such as serum response factor (SRF) and transcription enhancer factor-1 (TEF-1) have been identified. Additionally a Smad binding element (SBE), a TGFβ hypersensitivity region (THR), and a TGFβ control element (TCE) are present in the α-SMA promoter and found to be essential for induction of α-SMA expression in response to TGFβ treatment. Additional transcription factors, such as p53, Krüppel-like factors, C/EBPβ, Sp1 and Sp3 have been found to be important transcriptional regulators of α-SMA gene expression, although their specific importance in myofibroblast differentiation may not be identical to that in smooth muscle cells. There is evidence that some of these factors may interact with each other to affect their binding to DNA and/or activity on gene expression. The involvement of additional factors has not been ruled out. Certain repressive factors, such as gut Krüppel-like factor (GKLF), Nkx2.5, YB-1, NFκB, PPARγ and the liver-enriched inhibitory protein (LIP) isoform of C/EBPβ suggest that differentiation may be due, at least in part, to a de-repression phenomenon. Finally additional factors have been shown to have regulatory effects on myofibroblast differentiation in both fibroblasts and non-fibroblastic cell types, such as in EMT. These include the Notch signaling pathway, which appears to be important in EMT. The role of epigenetic regulation in myofibroblast differentiation adds another layer of complexity to this process. The importance of histone acetylation and DNA methylation in regulating myofibroblast differentiation is increasingly being recognized. In summary the mechanisms underlying myofibroblast differentiation are multifactorial and complex.

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