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Myofibroblasts

Biao Hu and **Sem H. Phan**
Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Abstract

Purpose of review—Interest in the myofibroblast as a key player in propagation of chronic progressive fibrosis continues to elicit many publications, with focus on its cellular origins and the mechanisms underpinning their differentiation and/or transition. The objective of the review is to highlight this recent progress.

Recent findings—The epithelial origin of the myofibroblast in fibrosis has been challenged by recent studies, with the pericyte suggested as a possible precursor instead. Additional signaling pathways, including Notch, Wnt, and hedgehog, are implicated in myofibroblast differentiation. The importance of NADPH oxidase 4 was highlighted recently to suggest a potential link between cellular/oxidative stress and the genesis of the myofibroblast. Recent observations on the importance of lysophosphatidic acid in fibrosis suggest that this may be due, in part, to its ability to regulate myofibroblast differentiation. Finally, there is increasing evidence for the role of epigenetic mechanisms in regulating myofibroblast differentiation, including DNA methylation and miRNA regulation of gene expression.

Summary—These recent discoveries open up a whole new array of potential targets for novel antifibrotic therapies. This is of special importance given the current bleak outlook for chronic progressive fibrotic diseases, such as scleroderma, due to lack of effective therapies.

Keywords

epithelial–mesenchymal transition; epigenetic regulation; fibrosis; myofibroblast

INTRODUCTION

A key feature of myofibroblasts is expression of α-smooth muscle actin (α-*SMA*) [1■]. They also express other marker genes depending on their anatomic localization and their degree of activation [1■]. Their de-novo emergence in response to tissue injury along with their ability to express high levels of extracellular matrix and fibrogenic cytokines [1■,2] make them key players in the subsequent repair process and wound healing [1■,2,3]. The purpose of this review is to highlight the latest information on the origin and regulation of myofibroblast differentiation, function, and fate in the past year.

Correspondence to Dr Sem H. Phan, Department of Pathology, University of Michigan Medical School, 4830 BSRB, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA. Tel: +1 734 647 8153; fax: +1 734 936 1938; shphan@umich.edu.

Conflicts of interest
The authors report no conflicts of interest.

ORIGIN OF MYOFIBROBLASTS

Myofibroblasts are rarely found in normal tissue except for some specialized regions [2,4]. However, a large number of myofibroblasts appear *de novo* in response to tissue injury, with gradual disappearance by apoptosis upon successful repair [1■]. However their persistence is associated with chronic fibrosis that usually progresses to loss of function of the affected organs [1■]. At least three major cellular sources have been proposed for the myofibroblasts that emerge *de novo* in fibrosis.

RESIDENT FIBROBLASTS OR PERICYTES

Fibroblasts are present in virtually all tissues and organs, albeit in limited numbers under normal conditions [4]. In-situ activation of normally quiescent resident fibroblasts in response to extracellular triggers, such as Transforming Growth Factor β1 [5–7], Wnt [5,8], Jagged/Notch [9■,10], Fizz1 [10], and hedgehog [11■■] are well documented. Direct evidence is obtained from in-vitro tissue culture experiments in which de-novo expression of α-SMA was observed when isolated tissue fibroblasts are appropriately stimulated [5,6,8,9■,11■■]. Transgenic models utilizing elegant gene reporter strategies to define specific myofibroblast lineages determine that these cells are resident fibroblast-like cells or pericytes located exclusively in the perivascular interstitium and not derived from an epithelial source [12,13]. This finding is consistent with a previous kinetic study [14] in which de-novo α-SMA expression in pulmonary fibrosis is first found to localize to the adventitia of blood vessels and airways.

BONE MARROW-DERIVED PROGENITORS

The ability of bone marrow-derived cells to localize and populate distal tissue sites has been demonstrated by bone marrow transplantation studies [15–18], but their ability to differentiate into myofibroblasts is controversial. One study [19] suggests that bone marrow-derived cells contribute to more than 20% of the myofibroblasts in pancreatic injury. Another study [20] suggests derivation from $CD14^+$ monocytes, although the myofibroblast phenotype is lacking in contractile function. In contrast, other studies [15,17,21,22] cannot demonstrate significant contribution of bone marrow-derived cells to the myofibroblast population in lung, liver, kidney, and skin. The basis for these discrepant results remains unclear.

EPITHELIAL AND ENDOTHELIAL ORIGIN OF MYOFIBROBLASTS

Epithelial cells may undergo dedifferentiation and express mesenchymal markers through a process called epithelial–mesenchymal transition (EMT) [23]. Originally proposed in the fibrotic kidney as a source of myofibroblasts, EMT has subsequently been similarly implicated in fibrosis affecting other organs. The importance of endothelial cells as a source of myofibroblasts via EMT has also been suggested using similar approaches [24]. However, despite this abundant evidence, especially *in vitro*, the in-vivo significance of these processes remains uncertain. Although epithelial cells with myofibroblast features can be identified in cultured epithelial cells, the evidence for EMT *in vivo* is equivocal and sometimes contradictory [13,25–27,28■■]. In a recent study [28■■] using inducible cell

lineage-specific transgenic alleles in a model of pulmonary fibrosis, the authors are unable to show the epithelial origin of myofibroblasts. Moreover, they cannot demonstrate the pericyte as a myofibroblast progenitor but instead suggest other heterogeneous stromal cells as the likely source for myofibroblasts in this model of pulmonary fibrosis [28■■]. In human studies [29–31], a small number of epithelial cells with mesenchymal and myofibroblast markers have been described in biopsies from patients with lung allograft rejection oridiopathic pulmonary fibrosis (IPF). However, another study [32] cannot demonstrate the presence of cells with both epithelial (E-cadherin, ICAM-1, LEA, CD44v9, or SP-A) and myofibroblast markers (α-SMA or vimentin) in lung tissue sections from patients with IPF or nonspecific interstitial pneumonia. As with the controversy on the bone marrow origin of the myofibroblasts, the basis for these discrepancies is not clear and likely will engender further future studies on this topic.

REGULATION OF MYOFIBROBLAST DIFFERENTIATION

Regulation of myofibroblast differentiation is primarily investigated in terms of the regulation of myofibroblast marker genes, especially the key marker of differentiation, the α-*SMA* gene [1■]. The DNA sequence and promoter analysis have identified a series of cis-acting elements and their corresponding trans-acting factors [1■]. Many of them function in combinatorial fashion as reviewed previously [1■]. The list of factors and their interactions capable of regulating myofibroblast differentiation continue to grow, and recent progress will be discussed in the following sections. They will be organized on the basis of signaling pathways, downstream transcriptional, and epigenetic regulation.

TGFβ signaling

The stimulation of myofibroblast differentiation by TGFβ is well documented and mediated by Smads and relevant Ras/ERK/MAPK kinases in conjunction with other transcription factors, such as Sp1/Sp3, TEF-1, and KLF4 [1■,2,33]. Additionally, recent studies [34,35] indicate that TGFβ also induces NADPH oxidase 4 (Nox4), a source for reactive oxygen species, thus providing a link between oxidative stress and myofibroblast differentiation. Moreover, expression of Nox4 induces Smad2/3 phosphorylation that promotes myofibroblast differentiation [34,35]. Elevated expression of Nox4 is reported in hyperplastic alveolar type II cells and fibroblasts in the lungs of patients with IPF [34,36], thus suggesting a potential role in pathogenesis. This possibility is supported by animal model studies [37■■,38] showing deficient fibrosis in Nox4 knockout mice or by treatment with Nox inhibitors. Another recent study [39] confirms the importance of MyoD in TGFβ-induced myofibroblast differentiation and concludes that differentiation is reversible. However, other studies [40,41] suggest that disappearance of myofibroblasts in successful wound healing occurs via apoptosis rather than a process of dedifferentiation. Interestingly, bFGF or FGF-2 is found to inhibit myofibroblast differentiation in the latter study and is likely mediated by enhanced expression of Nkx2.5, a repressor of α-*SMA* gene expression [42]. Another modulator of TGFβ signaling is Cx43, which is found to mediate the activation of the α-*SMA* gene by TGFβ [43] by competing with Smads for binding to microtubules [44]. Finally, another soluble agonist capable of inducing myofibroblast differentiation is lysophosphatidic acid [45], which activates a chloride channel and depends

on autocrine TGFβ to induce differentiation [46]. The importance of lysophosphatidic acid in fibrosis [47] may be mediated in part through this ability to promote myofibroblast differentiation.

Wnt signaling

The importance of Wnt signaling in fibrosis [48,49■] suggests its potential importance in myofibroblast differentiation. Moreover, its importance in EMT [50,51] suggests another way in which this signaling pathway can participate in genesis of the myofibroblast. Indeed, several recent studies indicate that Wnt signaling is important in induction of myofibroblast differentiation [5] and in part by being activated by TGFβ [52]. However, Wnt3a is also reported to enhance TGFβ expression and signaling [53], suggesting a potential positive feedback loop on its effect on myofibroblast differentiation.

Notch signaling

Four members of Notch signaling have been identified in mammalian cells [54]. All of them except for Notch4 are capable of regulating myofibroblast differentiation [10,55–58]. Notch1 and Notch3 are known to stimulate α-*SMA* gene expression in lung fibroblasts [10] and hepatic stellate cells [55], whereas Notch2 inhibits TGFβ-induced α-*SMA* and collagen I gene expression through downregulation of Notch3 in myoblasts [57]. However, in 10T1/2 fibroblasts, Notch3 represses expression of smooth muscle target genes including α-*SMA* by inhibition of the activation of Smad3 and p38 mitogen-activated protein kinase [58]. In contrast, in alveolar epithelial cells, Notch1 induces the phosphorylation of Smad3 and activates α-*SMA* gene transcription in a SRF-binding site [$CC(A/T)_6GG$, termed CArG box]-dependent and TGFβ control element-dependent manner [59]. Other experiments also suggest that Notch1 suppresses fibroblast proliferation that depends on Wnt11-dependent WISP-1 expression [60]. The importance of Notch signaling in fibrosis [61] including in scleroderma may be due to the activating effects of this signaling pathway on myofibroblast differentiation, including that via EMT and endothelial–mesenchymal transition.

Hedgehog signaling

Hedgehog signaling is primarily known for its critical function in development and cell differentiation as well as in cancer [62–66]. Sonic hedgehog (Shh) is the most widely expressed and recently shown to be implicated in fibrotic disorders [62]. It is highly induced in epithelial cells at sites of fibrotic disease [67]. Activation of hedgehog induces, whereas its inhibition with either siRNA or inhibitors suppresses, myofibroblast differentiation markers of gene expression including α-*SMA*, desmin, fibronectin, and collagen I expression [68]. Additionally Shh can mediate EMT in liver fibrosis [69]. *In vivo*, Gli1-deficient mice exhibit reduced interstitial fibrosis in kidneys after obstructive injury [68]. Suppressing the Shh signal with inhibitor against either Shh or its downstream mediator Smo prevented myofibroblast differentiation, reduced extracellularmatrix expression, and mitigated fibrotic lesions [68,70■■,71■■].

EPIGENETIC REGULATION

The epigenetic regulation of gene expression includes DNA methylation, histone modification and their interaction with DNA, as well as small interfering RNA-mediated gene regulation [2,3,72]. All these factors are found to be involved in the regulation of myofibroblast differentiation.

DNA methylation

DNA methylation is commonly associated with repression of the affected genes and is catalyzed by DNA methyl transferases (DNMTS) [72]. There is mounting evidence to suggest its importance in the regulation of myofibroblast differentiation [1■,6]. A recent study [73■] reveals widespread differences in global DNA methylation patterns between lung tissue from IPF patients when compared with those from controls [73■]. Interestingly these altered patterns of DNA methylation in IPF lung show some similarities to the changes observed in lung cancer samples. Although no significant alterations in overall global DNA methylation are observed, differentially methylated CpG islands and RNA expression of their affected genes have been identified between IPF and control lungs [73■]. However, global hypomethylation of genomic DNA is observed in cancer-associated myofibroblasts and in early-stage liver fibrosis [74,75]. For the α-*SMA* gene, differential DNA methylation has been identified between fibroblasts and lung alveolar epithelial type II cells [6]. Although the α-*SMA* gene promoter region is highly methylated in both cell types, the first intronic region is only highly methylated in the epithelial cells, which do not express this gene. Moreover induced overexpression or underexpression of DNMTS suppresses or activates α-*SMA* gene expression, respectively, consistently with inhibition of myofibroblast differentiation by DNA methylation. This is also supported by in-vitro evidence that DNA hypermethylation of the α-*SMA* promoter abolished its activity [6]. However, DNA methylation will also affect expression of genes other than α-*SMA*, which may also affect myofibroblast differentiation indirectly. For example, in hepatic stellate cells, inhibition of DNA methylation leads to activation of Peroxisome Proliferator-Activated Receptor γ (PPARγ) [76], a repressor for α-*SMA* gene expression [77,78], resulting in inhibition of myofibroblast differentiation. The specific mechanism by which DNA methylation affects α-*SMA* gene expression is not clear; however, it does enhance binding of the trans-acting factor MeCP2 to the methylated α-*SMA* DNA fragments [79■]. Although methylation of the α-*SMA* gene increases binding of MeCP2 and inhibits myofibroblast differentiation, paradoxically MeCP2 is found to be essential for fibrosis and enhances myofibroblast differentiation. This may indicate that additional effects of MeCP2 on other target genes also significantly influence myofibroblast differentiation, perhaps via repression of PPARγ expression [78]. Another relevant gene target subject to regulation by DNA methylation is Thy-1 [80] whose expression and interaction with αVβ5 integrin disrupt contraction-dependent TGFβ activation and myofibroblast differentiation [81,82].

Histone modification and their interaction with DNA

The importance of histone acetylation in regulating myofibroblast differentiation is initially suggested by evidence that trichostatin A, a histone deacetylase (HDAC) inhibitor, is an inhibitor of TGFβ1-induced α-*SMA* and type I collagen expression, but has since been

confirmed in multiple studies of fibrosis in other organ systems [83]. For example, knockdown of HDAC4 inhibits TGFβ-induced α-*SMA* expression through phosphorylation of Akt [84]. Another HDAC inhibitor, spiruchostatin A, is also found to be effective in suppressing TGFβ-induced human lung myofibroblast differentiation [85]. It is noteworthy that HDAC inhibition also activates Thy-1 expression, in part by reducing DNA methylation status of this gene with expected consequences on myofibroblast differentiation [86■]. Thus, it is likely that future studies will yield further insights into these complex interactions between these two modes of epigenetic regulation.

Regulation by small interfering RNAs

Small interfering RNAs are small noncoding RNAs (approximately 22 nucleotides) that lead to silencing of genetic information through posttranscriptional degradation of messenger RNA and/or translational inhibition of protein expression [87]. These are primarily microRNAs, many of which were found recently to regulate myofibroblast differentiation and fibrosis [88]. Despite their broad range of targets, their overall effect on myofibroblast differentiation has begun to be identified. For example, miR-21, which targets Smad7 [89] and programmed cell death 4 [90], enhances myofibroblast differentiation and lung fibrosis. On the contrary, miR-146a by targeting SMAD4 [91■], miR-132 by targeting MeCP2 [78], andmir-155 by inhibiting ERK1/2 phosphorylation [92] have a suppressive effect on myofibroblast differentiation. Other microRNAs such as miR-29 also may play a role in myofibroblast differentiation and fibrosis, but their relevant target genes remain unclear. There is some evidence that miR-29 targets collagen types I and IV mRNAs [93], but appears to enhance collagen gene transcription by targeting DNMTs and consequent inhibiting DNA methylation [94]. Further studies are necessary to resolve these apparently conflicting effects of miR-29 on a key phenotypic property of the myofibroblast.

SIGNIFICANCE OF MYOFIBROBLAST DIFFERENTIATION

Myofibroblast differentiation represents a key event during wound healing, tissue repair, as well as chronic fibrosis [1■,2,3]. The high contractile force generated by myofibroblasts is beneficial for physiological tissue remodeling but detrimental for tissue function when it becomes excessive such as in hypertrophic scars, in virtually all fibrotic diseases, and during stromal reaction to tumors [3]. The myofibroblast are shown to be the major extracellular matrix producing cells in fibrotic diseases in a variety of organs [1■,8]. However, despite evidence suggesting that suppression of myofibroblast differentiation correlates with reduced fibrosis [1■,2,3], direct proof is lacking that this is due specifically to the suppression of de-novo genesis of the myofibroblast. More direct evidence was obtained recently in a study [95■■] using mesenchymal cell/fibroblast-specific conditional CCAAT/ Enhancer Binding Protein β (C/EBPβ) knock out mice. These mice had reduced myofibroblasts and pulmonary fibrosis but an intact inflammatory/immune cell response when endotracheally injected with bleomycin [95■■]. Thus, despite the broad spectrum of C/EBPβ target genes in multiple cell types, its selective depletion in fibroblasts results in diminished myofibroblast differentiation and fibrosis.

CONCLUSION

The focus of recent studies is on critical mechanisms underlying genesis of myofibroblasts (summarized in Fig. 1). These studies elucidate the importance of the major signaling pathways, including TGFβ, Wnt, Notch, and hedgehog pathways along with their downstream transcription factor targets that mediate their effects on gene expression. Additionally, mounting evidence for epigenetic regulatory mechanisms has been identified in the control of myofibroblast differentiation. Future studies should reveal more of the complexities underlying these mechanisms and how they interact to ultimately regulate myofibroblast differentiation and fate.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 151).

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KEY POINTS

- Increasing evidence for local stromal origin of myofibroblasts.
- Importance of the hedgehog, Notch, and Wnt signaling pathways highlighted.
- NOX4 mediates myofibroblast differentiation.
- Epigenetic mechanisms regulate myofibroblast differentiation.

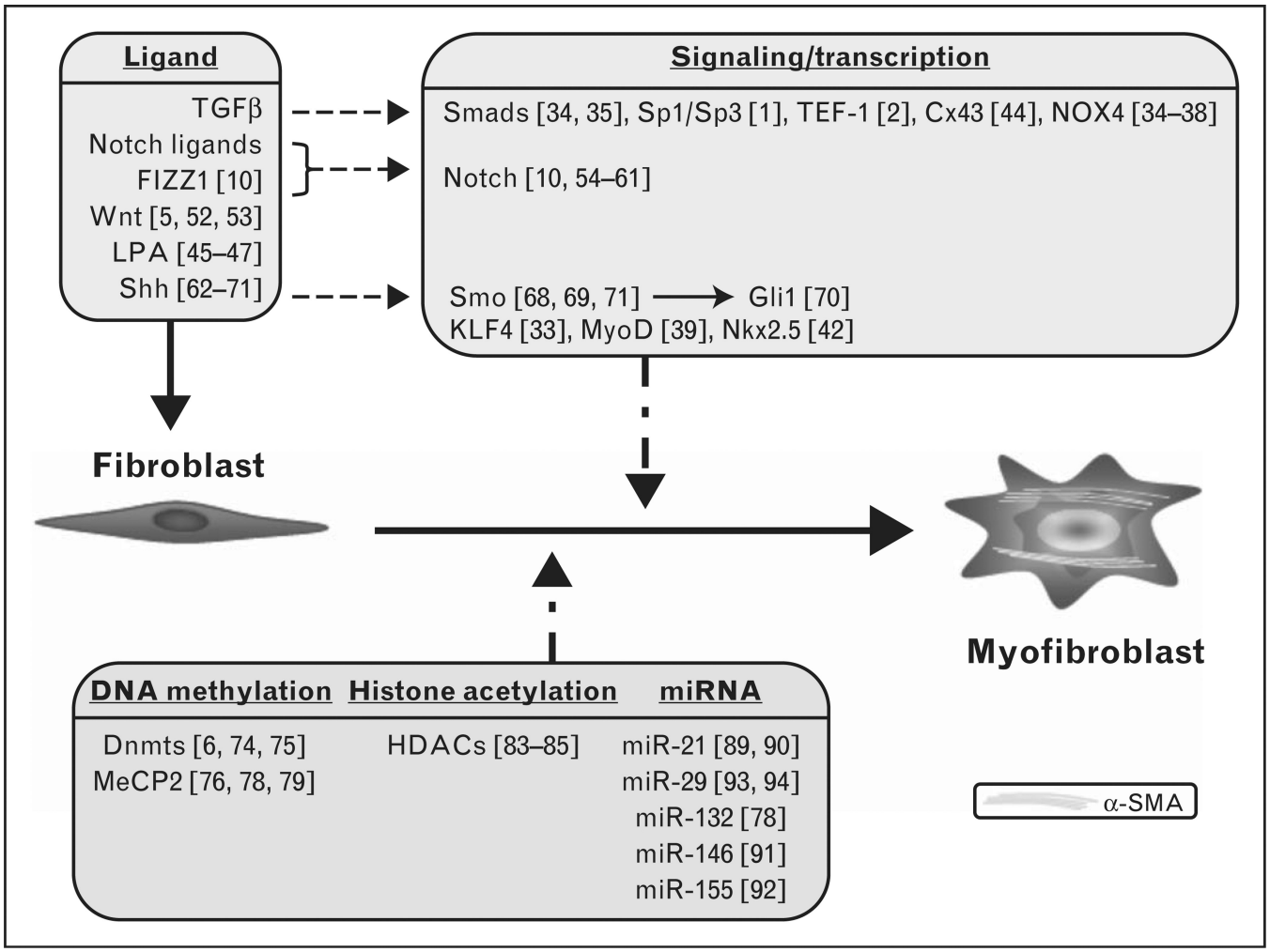

FIGURE 1.

Regulation of myofibroblast differentiation. Recently reported diverse ligands, signaling pathways, transcription, and epigenetic factors are summarized in this cartoon. The numbers within the square brackets refer to the relevant references. The respective factors are primarily reviewed from the standpoint of α-SMA as the target myofibroblast marker gene, but are also relevant to other genes associated with myofibroblast differentiation and function as described in the text. The fibroblast is indicated as the myofibroblast progenitor cell, but many of these factors play similar roles in differentiation from other progenitor cell types as discussed in the relevant sections. DNMTS, DNA methyl transferases; HDAC, histone deacetylase; LPA, lysophosphatidic acid; SMA, smooth muscle actin.