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Review

Recent Developments in Myofibroblast Biology

Paradigms for Connective Tissue Remodeling

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The discovery of the myofibroblast has opened new perspectives for the comprehension of the biological mechanisms involved in wound healing and fibrotic diseases. In recent years, many advances have been made in understanding important aspects of myofibroblast basic biological characteristics. This review summarizes such advances in several fields, such as the following: i) force production by the myofibroblast and mechanisms of connective tissue remodeling; ii) factors controlling the expression of α-smooth muscle actin, the most used marker of myofibroblastic phenotype and, more important, involved in force generation by the myofibroblast; and iii) factors affecting genesis of the myofibroblast and its differentiation from precursor cells, in particular epigenetic factors, such as DNA methylation, microRNAs, and histone modification. We also review the origin and the specific features of the myofibroblast in diverse fibrotic lesions, such as systemic sclerosis; kidney, liver, and lung fibrosis; and the stromal reaction to certain epithelial tumors. Finally, we summarize the emerging strategies for influencing myofibroblast behavior *in vitro* and *in vivo*, with the ultimate goal of an effective therapeutic approach for myofibroblast-dependent diseases. ***(Am J Pathol 2012, 180: 1340–1355; DOI: 10.1016/j.ajpath.2012.02.004)***

Myofibroblasts regulate connective tissue remodeling by combining the extracellular matrix (ECM)–synthesizing features of fibroblasts with cytoskeletal characteristics of contractile smooth muscle cells. Since their first description in granulation tissue 40 years ago, remarkable progress has been made in understanding myofibroblast biological characteristics and their participation in physiological and pathological situations.[1] It is well established that myofibroblasts have multiple origins, contribute importantly to connective tissue remodeling by exerting traction forces and synthesizing ECM components, regress and disappear by apoptosis on wound epithelialization, and may persist in fibrotic situations and cause organ dysfunction.[1] Since our last review on the subject in this journal,[1] many new findings have emerged to advance our understanding of myofibroblast biological features, but many questions remain unanswered. Unresolved questions include the following: i) what is the progenitor or precursor cell for the myofibroblast, ii) is

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there a specific myofibroblast marker, iii) what regulates myofibroblast contractile activity, and iv) what is the basis for myofibroblast persistence in chronic or progressive fibrosis? Recent rapid progress in microRNA (miRNA) and epigenetics research[2] has furnished new tools and concepts for assessing molecular regulation of myofibroblast differentiation and perpetuation of the myofibroblast phenotype, as potentially mediated by epigenetic mechanisms. The ultimate aim is to suggest therapeutic strategies for influencing the widespread pathological situations that depend on this enigmatic cell.

Myofibroblast Basics

During normal tissue repair, such as skin wound healing, controlled and transient activation of myofibroblasts contributes to restoration of tissue integrity by forming a mechanically sound scar.[1] For example, scars stabilize the heart muscle after myocardial infarction and tendon, bone, and cartilage after fracture or rupture.[3] However, when myofibroblast activities become excessive and persist, beneficial tissue repair turns into the detrimental tissue deformities characteristic of organ fibrosis. In this review, we will discuss in more detail fibrosis of the skin, lungs, liver, and kidney. In addition to these organs, myofibroblasts play a substantial role promoting heart fibrosis and vascular remodeling, but because of space limitation, these topics are not covered herein, and we refer the reader to recent reviews of this rapidly growing body of literature.[4,5] Another fibrotic condition is the desmoplastic or stromal reaction to epithelial tumors, during which myofibroblasts contribute to the mediator and mechanical environment that promotes tumor progression.[6] We will discuss the similarities of the tumor-associated fibroblasts and myofibroblasts with those present in other fibrotic lesions.

Specific Aspects of Myofibroblast Contraction

High contractile activity of myofibroblasts is necessary for generating tissue contractures. In addition to the expression of α-smooth muscle actin (α-SMA; gene *ACTA*) in stress fibers, which promotes stronger force generation compared with other actin isoforms in fibroblastic cells, myofibroblasts appear to use specific modes of contraction.[3,7] In contrast to the reversible and comparably short-lived contraction of striated and smooth muscles, myofibroblast contractile activity, together with ECM synthesis and degradation, leads to connective tissue remodeling, followed by irreversible and long contractures in a process that can span weeks, months, or even years.[7] It is still unknown how myofibroblasts stabilize contractions that occur at the cellular or subcellular level to counteract the stress present in a tissue undergoing remodeling. Recent *in vitro* studies[8] indicate that myofibroblasts use a lockstep or ratchet mechanism of cyclic and incremental contractile events. This mechanism consists of strong (micronewtons) and far-ranging (tens of micrometers) contractions mediated by RhoA/Rho-associated kinase and weak (approximately 100 pN) and short-ranging (approximately 0.4 μm) cyclic contractions promoted by changes in intracellular calcium concentrations.[8] The model proposes that strong isometric contraction generates slack in the myofibroblast-associated fibrous and stressed collagen. Such tension-released fibrils are then straightened by the weak, but repeated, subcellular contractile events. By local ECM remodeling and/or deposition of new ECM, the shortened and repeatedly stressed collagen fibrils stabilize the status quo of the ECM, and a new myofibroblast contraction cycle can begin. It is intriguing that the level of stress (ie, the resistance of collagen fibers to pulling) may determine which mechanism of contraction will be engaged.

Characteristic Features of the Myofibroblast

Although there is considerable evidence to indicate the fibroblastic origin of myofibroblasts, other cell types, mostly from mesenchymal lineages, have been suggested as alternative or additional precursors.[1] Because different subsets of myofibroblast precursors are recruited in different organs, we will further address the question of the myofibroblast origin later. To target the fibrotic activity of myofibroblasts, irrespective of their provenance, it is important to define common denominators, a need that has stimulated the search for specific molecular markers. The most widely used molecular marker of the differentiated myofibroblast in research and clinical diagnostics is the *de novo* expression of α- SMA.[1] The convenience of a unique molecular marker has fostered the misconception that a myofibroblast must express α-SMA to be a myofibroblast. However, the most important defining feature of myofibroblasts is the *de novo* development of *in vivo* stress fibers and contractile force.[3,7] Several novel markers and modulators of the myofibroblast phenotype have been suggested. These include endosialin in tumor-associated fibroblasts,[9] P311 in hypertrophic scar myofibroblasts,[10] integrin $\alpha 11\beta 1$ in fibroblasts from various sources,[11] osteopontin in cardiac or dermal fibroblasts,[12] and periostin[13,14] (Table 1[15–77]). It remains to be confirmed whether these markers are tissue and/or condition specific or whether they are useful in identifying myofibroblasts in more general terms. To our knowledge, none of the other markers described over the years is unique to the myofibroblast.

Myofibroblast-Inducing Factors and Conditions

Mechanical resistance of the ECM, in conjunction with the action of profibrotic transforming growth factor $\beta 1$ (TGFβ1), is an amply documented primary stimulus for myofibroblast differentiation and persistence.[1] Myofibroblasts develop *in vitro* and *in vivo* their highly contractile cytoskeletal apparatus only above a certain ECM stiffness threshold.[3,62] Various fibrotic organs and tissues have recently exceeded this threshold at the micromechanical level.[78–80] Tissue stiffness increases as a consequence of ECM-remodeling activities of fibroblasts and myofibroblasts.[63] Contracting cells then generate the conditions that make them even more contractile in a

Table 1. Myofibroblast-Modulating Factors

Myofibroblast-inducing factors	Myofibroblast-suppressing factors
Transcription-Regulating Factors	
Smad3 and Smad2[15]	Smad7
MeCP2[16]	NF-κB[30]
KLF5[17]	KLF4[15,22,31]
HMGA2[18]	PPARγ[30,32]
SRF[19,20]	Nkx2.5[33]
RTEF-1[21]	YB-1[34]
Sp1 and Sp3[17,22]	
C/EBPβ[23]	
CSL[24]	
c-Myb[25]	
MRTF-A/MRTF-B[20,26–28]	
Fli-1[29]	
Epigenetic Regulators and miRNAs	
DNMT1[35]	Other DNMTs[16,42]
HDAC4[36,37]	miR-29[43–48]
(HDAC6 and HDAC8)[36]	
miR-192[38]	Let7[18]
miR-132[30]	miR-200a[49]
miR-21[39]	
miR-129[40]	
miR-200b/c[38]	
miR-216a[41]	
Growth Factors, Cytokines, and Others	
TGFβ1[50–52]	Interferon-γ[60]
P311[10]	CXCL10[61]
Wnt[51,53]	
Jagged1[51,54]	
FAK[52,55]	
NOX4[56–59]	
ECM, ECM-Modulating Proteins, and Physical Factors	
Stiff ECM[3,62–65]	Soft ECM[3,62,63]
ROS[66,67]	
LOX[68]	
LOXL2[69,70]	
Lysyl hydroxylase and PLOD2[71]	
Osteopontin[72]	
Periostin[13,14]	
CCN2 (CTGF)[51,73,74]	
ED-A fibronectin[1,75,76]	
Membrane-Bound and Surface-Expressed Proteins	
Endosialin (Tem1)[9]	
Integrin	
α11β1[11]	
α3β1[11,53]	
αvβ3[11,50]	
αvβ5[50]	
Notch1[24,51,54,77]	

The table summarizes recently identified proteins and factors that have regulated myofibroblast differentiation either directly or indirectly. For well-established myofibroblast modulators, such as TGFβ1, review articles are referenced.

C/EBP, CCAAT enhancer binding protein; CTGF, connective tissue growth factor; ED-A, extradomain A; FAK, focal adhesion kinase; Fli-1, friend leukemia integration; KLF, Kruppellike factor; PLOD, procollagen-lysine, 2-oxoglutarate 5-dioxygenase; ROS, reactive oxygen species; RTEF, R-transcription enhancer factor; Sp, specificity protein; SRF, serum response factor; YB-1, Y-box binding protein-1.

detrimental feed-forward loop. The chicken-and-egg question of how fibroblastic cells are initiated to become contractile in the soft ECM present after the injury may be answered by recent biomechanical studies. In an animal model of liver fibrosis, increased tissue stiffness precedes the activation of fibroblastic cells and the accumulation of collagen, thus suggesting that such early mechanical changes may be sufficient to trigger the contraction cascade.[68] Collagen cross-linking catalyzed by lysyl oxidase (LOX) enzymes is one possible factor responsible for early structural changes and tissue stiffening in these conditions.[68] Fibrosis-specific and stable collagen cross-links are further formed by lysyl hydroxylase and pro-collagen-lysine, 2-oxoglutarate 5-dioxygenase, which are responsible for pyridinoline and aldehyde-derived collagen cross-links in the fibrotic skin.[71] Furthermore, antibody-mediated inhibition of the enzyme LOX-like 2 (LOXL2) suppresses fibrosis and its progression in a variety of organ systems, including tumor desmoplasia.[69] However, it remains unclear whether this effect is due to reduced ECM stiffness. The potential role of initial tissue stiffening in the onset of fibrosis is particularly interesting given that cultured myofibroblasts can activate latent TGFβ1 from a sufficiently stiffened ECM by integrin-mediated contraction.[64,65] A purely mechanically driven mechanism of profibrotic growth factor activation has been experimentally proved in a cell-free system[81] and is supported by the recently revealed structure of latent TGFβ1.[82] These findings establish a direct link between the mechanical and chemical factors regulating myofibroblast differentiation and ultimately causing fibrosis.[50,83] Table 1 summarizes myofibroblast-inducing and myofibroblast-inhibiting factors.

Myofibroblast Differentiation and Regulation of ACTA *Gene Expression*

Because α-SMA expression is a common key element for detection of myofibroblast differentiation and a major player in contractile force production, most of the available data focus on the regulatory mechanisms underlying expression of this gene. Abundant information is available on transcriptional regulation of the *ACTA* gene, which indicates complex combinatorial mechanisms involving both stimulatory and inhibitory factors (Figure 1A and Table 1).

Activators of the α-SMA Promoter

Among the activators are serum response factor and the transcription enhancer factor-1 family member R-transcription enhancer factor, which bind to CC(A/T)6GG (CArG) and CATTCCT (MCAT) elements, respectively, in the upstream regulatory sequence of the α-SMA promoter.[19,21] In addition to the importance of Smad3 and its binding element,[15] another element found to be important in TGFβ1-induced differentiation is a proximal TGFβ control element, to which several factors [eg, Krüppel-like factor 5, specificity protein (Sp) 1, and Sp3] can bind to activate transcription.[17,21,22] Other upstream elements include the TGFβ hypersensitivity region and an Smad-binding element, which are activated by binding to Sp1/Sp3 and Smad3, respectively.[15,22] Studies in several different cell types have implicated additional transcription factors, such as CCAAT enhancer–binding protein β, CSL (from CBF1/RBP-J in mammals, Suppressor of Hairless [Su(H)] in Drosophila and Xenopus, and Lag-1 in Caenorhabditis elegans) (a down-

3stream target of Notch signaling), and c-Myb, in regulation of *ACTA* gene expression (Table 1).[23–25]

An important factor linking *ACTA* gene transcription to the level of mechanical stress and the state of the contractile cytoskeleton is myocardin-related transcription factor A (MRTF-A).[20,26,27] In a mechanism that involves the F-actin–organizing factor mDia1, MRTF-A docks to CArG elements and enhances the transcriptional activity of serum response factor.[20] MRTF-A and MRTF-B also mediate TGFβ1-induced myofibroblast differentiation and transcription of smooth muscle genes in fibroblasts.[28] Other mechanically regulated transcription of fibrosis-related genes have been recently reviewed.[20]

Repressors of the α-SMA Promoter

Several factors have down-regulated α-SMA expression and, thus, may be responsible for active suppression of myofibroblast differentiation (Table 1). Among these factors is Kruppellike factor 4, which can compete for binding to the TGFβ control element by its activators and interact with the Mad homology 2 (MH2) domain of Smad3 to suppress its binding to the *ACTA* promoter.[31] Additional repressors are Nkx2.5, peroxisome proliferator–activated receptor (PPAR)-γ, and Y-box binding protein-1.[32–34] In the case of Nkx2.5, reduction in its expression correlates with myofibroblast differentiation in lung fibroblasts. Thus, differentiation may be mediated by a derepression mechanism dependent on decreased expression of one or more of these repressors.

Smad-Independent Regulation of the α-SMA Promoter

In addition to Smad signaling, the multiple, complex signaling mechanisms implicated in regulation of myofibroblast differentiation may also involve mitogen-activated protein (MAP) kinases.[15] Depending, in part, on the precursor cell type, Wnt signaling pathways may also be involved.[53] Notch signaling is important in endothelial-to-mesenchymal transition and myofibroblast differentiation from lung fibroblasts.[24,54] This is likely mediated by CSL interacting with its binding element identified in the *ACTA* promoter. Induction of Jagged1, a ligand for Notch1, results in activation of this signaling pathway, with subsequent activation of *ACTA* gene transcription. Impaired Notch signaling *in vivo* results in reduced myofibroblast differentiation in an animal model.[54] Another important pathway in myofibroblast differentiation associated with the desmoplastic response in tumors is Hedgehog signaling, although the downstream target gene(s) have not been identified.[84] All these differing signaling pathways are likely to work in concert at one level or another in more complex scenarios to ultimately affect *ACTA* gene expression, both directly and indirectly, via other regulatory genes.

Myofibroblast Epigenetics: A Fibrotic Memory?

Various approaches have demonstrated differences in the protein, gene, and transcriptional profiles between myofibroblast precursors from different origins and their fibrotic counterparts.[85,86] Microarray studies have shown up-regulation of many genes, consistent with activation by TGFβ1, Wnt, and connective tissue growth factor (CCN2) signaling pathways, which are abnormally expressed in patients with fibrotic disorders.[85,87] These fibrotic signatures tend to disappear in explanted fibroblasts compared with whole tissue from the same patients.[88] A common observation in profiling studies is that specific signatures of myofibroblasts are retained over several passages *in vitro*, suggesting a myofibroblast memory that may be mediated by epigenetic modifications, with consequent preservation of the myofibroblast phenotype and its persistence. Epigenetic regulation of gene expression in a variety of fibrotic conditions has involved DNA methylation, modification of histones, and regulation of miRNAs targeting select genes (Figure 1, B and C).

Myofibroblast DNA Methylation

In the case of DNA methylation, modification at CpG islands represents an important mechanism for gene silencing, and this appears to be the case for the *ACTA* gene[16,89] (Figure 1B). Lung alveolar epithelial type II cells, which do not express α-SMA, exhibit high levels of methylation in the three CpG islands identified in the regulatory regions of this gene and in intronic regions. In contrast, lung fibroblasts exhibit significantly lower levels of DNA methylation in this gene, and its inhibition using 5-aza-2′-deoxycytidine activates gene transcription. DNA methylation is not limited to the *ACTA* gene in fibrotic conditions. In lung fibroblasts, hypermethylation of the promoter region of the *TYHY1* gene has been implicated in its silencing, correlating with fibrosis in the lung.[89,90] In scleroderma fibroblasts, ECM-inhibitory genes, such as Smad7 or Fli-1 are also hypermethylated and consequently down-regulated, suggesting that DNA methylation could cause derepression of ECM genes involved in fibrosis.[29]

Similarly induced specific deficiency of DNA methyl transferases (DNMTs), using small-interfering RNAs, causes activation of *ACTA* gene transcription in these fibroblasts[42] (Figure 1B). In the fibrotic kidney, DNMT1 is associated with persistent fibroblast activation and fibrogenesis via promotion of hypermethylation of *RASAL1*, a gene that encodes an inhibitor of the Ras oncogene.[35] *DNMT1*$^{+/-}$ heterozygous mice exhibit reduced renal fibrogenesis and kidney fibrosis. Conversely, induced DNMT overexpression in lung fibroblasts, using expression plasmids, results in repression of *ACTA* gene expression and, thus, of myofibroblast differentiation.[35] The use of these approaches to alter DNMT expression and/or inhibition of DNA methylation is likely to affect other genes as well, which may indirectly affect *ACTA* gene expression. This is the case in a study[30] of hepatic stellate cells (HSCs), in which inhibition of DNA methylation results in activation of PPARγ and NF-κB, which then repress *ACTA* gene expression. Direct and indirect effects of the methylated DNA-binding protein MeCP2 have also been observed. MeCP2 binds directly to the *ACTA* gene promoter and is es-

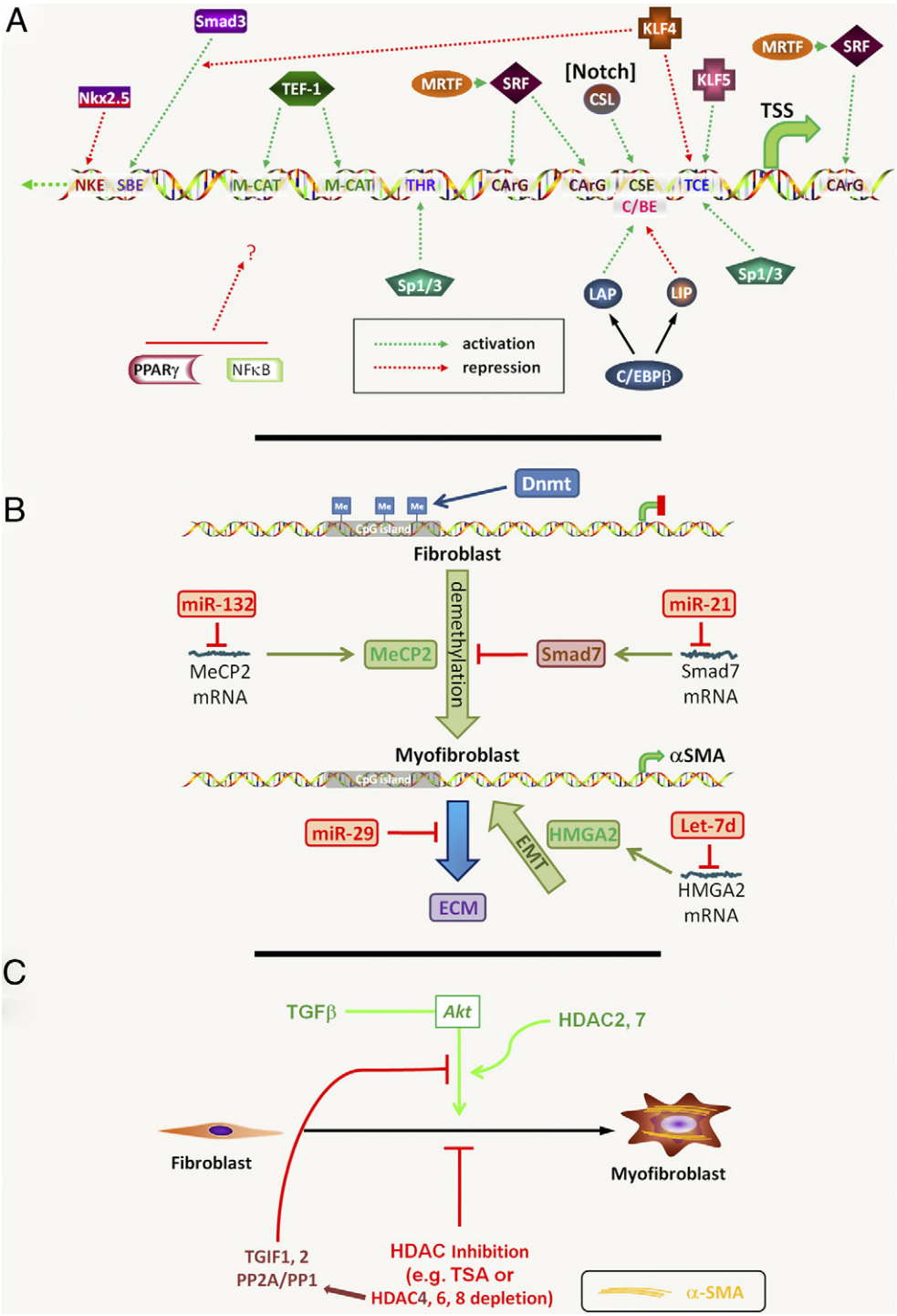

Figure 1. Myofibroblast epigenetics. **A:** Transcriptional regulation of the *ACTA* gene in myofibroblast differentiation. Activating or repressing transcription factors are shown directed at the relative locations of their cognate binding elements in the *ACTA* gene promoter. The interaction of Kruppellike factor (KLF) 4 with Smad3 results in decreased Smad3 binding to the Smad-binding element (SBE). In contrast, MRTF enhances serum response factor binding to its cognate element to activate differentiation. PPARγ and NF-κB are also shown, but the location/nature of their direct interaction with the promoter is uncertain (indicated with a question mark). The activation of Notch signaling activates its downstream factor CSL. CArG, CC(A/T)6GG; C/EBP, CCAAT enhancer binding protein; CSL, from CBF1/RBP-J in mammals, suppressor of hairless [Su(H)] in drosophila and xenopus, and Lag-1 in Caenorhabditis elegans; IM-CAT, binding element with sequence CATCCT; LAP, liver activator protein; LIP, liver inhibitory protein; NKE, Nkx2.5 binding element; SRF, serum response factor; TEF, transcription enhancer factor; THR, TGF-β1 hypersensitivity region; TSS, transcription start site. **B:** DNA methylation and miRNAs in myofibroblast differentiation. Methylated CpG islands are shown in the *ACTA* gene promoter in the fibroblast, which are maintained by DNMT. The differentiated myofibroblast shows reduced methylation with activation of *ACTA* gene expression. The methylated DNA-binding protein MeCP2 promotes myofibroblast differentiation; however, multiple target genes are regulated by this protein. The indicated miRNA species regulate their target mRNAs with downstream consequences on myofibroblast differentiation, as indicated. Although *ACTA* is used as a hallmark gene of the myofibroblast, other fibrosis-relevant genes have been regulated by hypermethylation as well (see *Myofibroblast DNA Methylation*). **C:** Histone deacetylation and myofibroblast differentiation. The indicated HDAC isoforms modulate differentiation via multiple mechanisms. In addition, inhibition/depletion of HDAC4 suppresses TGFβ activation of Akt via 5′-TG-3′-interacting factor 1,2 (TGIF1,2) and protein phosphatase 2A/1 (PP2A/PP1).

sential for *ACTA* gene expression.[16] Moreover, MeCP2 deficiency significantly reduces pulmonary fibrosis and myofibroblast differentiation in an animal model. The relative contribution of these direct versus indirect mechanisms may be decisive in determining the ultimate effect on myofibroblast differentiation.

Myofibroblasts, Fibrosis, and miRNAs

In addition to epigenetic modulation through DNA methylation, different miRNAs appear to contribute to the myofibroblast memory[43] (Figure 1B and Table 1). miR-29 expression is markedly reduced in skin biopsy specimens and explanted skin fibroblasts from patients with scleroderma, suggesting a post-translational epigenetic mechanism for increased ECM gene expression.[44] In fibrotic lung, low levels of miR-29 correlate with high expression levels of profibrotic genes, including previously unrecognized ECM and ECM remodeling genes. Knockdown or TGFβ1-mediated down-regulation of miR-29 in cultured lung fibroblast derepressed fibrosis-associated genes.[45] Down-regulation of miR-29 in cardiac fibroblasts *in vitro* and *in vivo* induces fibrosis-associated genes, whereas its overexpression has a fibrosis-suppressing effect.[46] A comparable fibrosis-suppressing role has been demonstrated for miR-200a in the kidney[49] and liver.[47]

Conversely, in an animal model of kidney fibrosis, up-regulation of miR-192 is reported on TGFβ1 signaling, and overexpression of an miR-192 analogue induces ECM production.[38] Similarly, miR-129,[40] miR-192, miR-200b/c,[38] and miR-216a[41] promote expression of fibrotic genes in mouse mesangial cells. Another miRNA involved in fibrosis is miR-132, which mediates inhibition of MeCP2 expression, reduces PPARγ expression, and consequently enhances *ACTA* gene expression in lung fibroblast.[30] Such an indirect mechanism has also been reported for additional miRNA species. Among these, Let7 and miR-21 affect lung myofibroblast differentiation via effects on HMGA2 and Smad7, respectively, although additional effects on other target genes have not been excluded.[18,39] Future miRNA profiling studies, such as the one recently started for bleomycin-induced lung fibrosis,[91] will hopefully provide a more complete picture of the miRNA species involved in the different aspects of fibrosis.

Myofibroblast Histone Modifications

Histone modification is involved in the perpetuation of fibrosis. The importance of histone modification in regulation of *ACTA* gene expression is generally indirect.[30] The histone deacetylases (HDACs) 4, 6, and 8 have been identified as the key HDACs involved in this mechanism[36] (Figure 1C and Table 1). Although HDAC4 possibly affects the activation of Akt in TGFβ1-induced differentiation,[37] the direct target genes of these modified histones have not been identified. Histone acetylation is usually associated with activation of target gene expression; thus, the mechanism of this inhibition on *ACTA* gene expression is likely due to activation of other genes that repress its expression. In addition to histone acetylation, methylation of histones is involved in myofibroblast development and fibrosis.[30] Considering the rapid progress made in understanding epigenetic processes, it will be years until the first potential targets are identified and tested for their anti-fibrotic potential.

Organ Fibrosis

Systemic Sclerosis

The Role of Fibrosis in Systemic Sclerosis

Systemic sclerosis or scleroderma is a chronic autoimmune and fibrotic disease of unknown etiology with no effective disease-modifying therapies and significant mortality. The disease is highly heterogeneous in its clinical manifestations. Widespread fibrosis affecting virtually every organ distinguishes scleroderma from organ-based fibrotic processes, such as pulmonary fibrosis, keloids, glomerulosclerosis, and hepatic fibrosis. The dermis, lung parenchyma, heart, gastrointestinal tract, tendons, and ligaments are prominently affected, with endocrine organs, such as the thyroid gland, occasionally affected.[51] Interestingly, neither the liver nor the central nervous system show significant fibrosis in scleroderma. Activated fibroblasts and myofibroblasts are the primary effector cells of fibrosis in scleroderma.[51]

The skin in scleroderma is characterized by replacement of the normal dermal architecture with collagen-rich connective tissue. Progressive dermal thickening and sclerosis obliterate eccrine and sebaceous glands, hair follicles, and small blood vessels. Analysis demonstrates excessive deposition of the main fibrillar collagens (types I and III) and types V and VII collagens, normally restricted to the dermal-epidermal junction.[92] The extradomain A fibronectin splice variant, which is critical for myofibroblast differentiation, shows increased deposition.[93] Other structural ECM molecules that are aberrantly expressed in scleroderma include fibronectin, proteoglycans, fibrillin, and elastin fibrils.[94] Fibrillin accumulation is of particular interest, because fibrillin-1 regulates the activation of ECM-bound latent TGFβ1, and mice with a fibrillin-1 mutation (tight skin mice) develop a sclerodermalike phenotype.[95] Microarray analysis of scleroderma skin reveals up-regulation of genes normally associated with bone and cartilage, including type XI collagen and cartilage oligomeric matrix protein (COMP). Integrins involved in cell-cell and cell-ECM adhesion and latent growth factor activation are also overexpressed in the lesional tissue.[86,87]

The Origin of Myofibroblasts in Scleroderma

Fibroblasts and myofibroblasts are the principal stromal cells responsible for the excessive ECM deposition and remodeling in scleroderma. Although *in situ* hybridization studies have identified an increased proportion of biosynthetically active fibroblasts in scleroderma dermis, *in vitro* clonal analysis of explanted skin fibroblasts shows substantial heterogeneity with respect to collagen synthesis, suggesting the existence of distinct subpopulations of dermal mesenchymal cells with different origins.

Cells positive for α-SMA are present in the skin with lesional scleroderma but are not detected in healthy skin.[93,96] Accumulation is prominent in areas with significant collagen deposition. Myofibroblastic cells are also observed in the esophagus and lung, even in the liver, despite the absence of fibrosis. Culture of bronchoalveolar lavage fluids from patients with scleroderma shows spontaneous outgrowth of α-SMA–positive cells with high production of collagen and fibronectin.[51] In contrast, myofibroblasts are not detected in bronchoalveolar lavage fluids from healthy individuals. The number of myofibroblasts in the lesional skin in scleroderma correlates with both the extent of local collagen deposition and the clinical assessment of skin stiffness.[97] Moreover, the myofibroblast score in the skin shows a decrease over 6 to 12 months in patients treated with cyclophosphamide, suggesting that myofibroblast levels in the skin may be used as biomarkers of changing skin involvement.

The origin of myofibroblasts in the fibrotic lesions has not been conclusively established. They may arise from the *in situ* activation of normally quiescent resident fibroblasts in response to extracellular triggers, such as TGFβ1, Wnt, Jagged/Notch, CCN2, endothelin-1, lysophosphatidic acid, and other signaling molecules, as well as hypoxia and mechanical stress due to increased ECM stiffness.[51] Each of these stimuli, which induce α-SMA expression and stress fiber formation in explanted healthy skin fibroblasts, has been aberrantly expressed or regulated in scleroderma. Alternatively, myofibroblasts in scleroderma lesional tissue may arise from other cell types, including vascular smooth muscle cells, pericytes,[93] and endothelial cells under the influence of TGFβ1[98] or epithelial cells in the skin or lungs.[99,100] Because both myofibroblasts and pericytes are associated with vascular remodeling and injury, which are prominent in patients with scleroderma, vascular smooth muscle cells and pericytes might provide the pathogenetic link between vascular injury and fibrosis characteristic of scleroderma. Although the significance of mesenchymal differentiation pathways in the context of scleroderma has not been conclusively demonstrated, and the mechanisms underlying such cellular plasticity *in vivo* remain mostly unknown, it is tempting to consider scleroderma therapies that are based on targeting these events.

Persistence of Scleroderma Fibroblasts

Historically, the study of scleroderma fibroblasts focuses primarily on the skin, because it is readily accessible for biopsy. Pioneering studies by Leroy[101] 30 years ago are the first to demonstrate the feasibility of studying explanted skin fibroblasts in scleroderma. These observations indicate that scleroderma-derived skin fibroblasts display a biosynthetically activated phenotype *in vitro* that is independent of extracellular signals. A cell-autonomous activated phenotype is maintained for multiple *in vitro* passages.[101] These studies have been subsequently reproduced and extended by other investigators.[102] In some studies, most explanted scleroderma fibroblasts are positive for α-SMA *in vitro*, with increased production of collagen and tissue inhibitor of metalloproteinase.[103] Moreover, myofibroblasts explanted from scleroderma skin are resistant to Fas-induced apoptosis.[96] Apoptosis resistance may be due to activation of the Akt prosurvival pathway and may account for the persistence of myofibroblasts in scleroderma skin.[104] Another potential mechanistic explanation accounting for the persistent α-SMA expression in scleroderma is provided by the demonstration that pharmacological inhibition of focal adhesion kinase phosphorylation significantly attenuates the myofibroblast phenotype of these cells.[55] Scleroderma fibroblasts show an elevated level of focal adhesion kinase phosphorylation, presumably reflecting their stimulation by autocrine TGFβ1. Consistent with the autocrine TGFβ1 hypothesis, explanted scleroderma fibroblasts show constitutive nuclear localization of phosphorylated Smad2/3, even in the absence of added TGFβ1.[105] These studies suggest that an autocrine TGFβ1-stimulatory loop induces focal adhesion kinase phosphorylation and apoptosis resistance, with consequent persistence of α-SMA–expressing myofibroblasts.[52]

In addition to enhanced ECM production, scleroderma fibroblasts in culture produce chemokines and growth factors and spontaneously generate reactive oxygen radicals, such as H_2O_2, through the NADPH oxidase (NOX) complex pathway, which is up-regulated in scleroderma skin.[66] In turn, intracellular production of reactive oxygen species is at least partially responsible for the constitutive up-regulation of collagen synthesis in these cells. The expression of surface receptors for relevant growth factors and chemokines, including TGFβ1, platelet-derived growth factor, and CCR2, are also elevated on scleroderma fibroblasts, suggesting an additional feed-forward amplification mechanism that might underlie the persistently activated phenotype.[106]

Renal Fibrosis

Renal interstitial fibrosis is a common pathological feature of chronic kidney disease (CKD), irrespective of etiology. The myofibroblast is a major player in the onset and evolution of renal fibrosis and has been implicated in pathogenesis.[107]

The Origin of Renal Myofibroblasts

Despite their pivotal role in disease progression, the source of renal myofibroblasts is still a matter of debate. Several progenitors have been proposed in addition to local fibroblasts, including circulating fibrocytes, local pericytes,[108,109] and resident epithelial cells, through epithelial-to-mesenchymal transition (EMT).[110] The possible contribution of EMT to fibrosis *in vivo* was recently challenged by a study[108] using a mouse model of renal interstitial fibrosis and genetic cell lineage tracing. However, under the same conditions of kidney obstruction, but using a different approach, EMT was observed by another group.[111] These conflicting data have been addressed elsewhere in several excellent critical articles.[112,113]

Tubuloepithelial-to-Mesenchymal Cross Talk Contributes to Kidney Myofibroblast Generation

It appears increasingly likely that loss of the normal, homeostatic microenvironment, due to alterations in the tubulointerstitium per se, triggers myofibroblast activation and causes progression of fibrosis.[114] This established concept is based on the histological evidence that proximal tubule cell (PTC) injury precedes interstitial fibrosis and that regions of active interstitial fibrosis predominantly exhibit a peritubular, rather than a perivascular, distribution. This notion is supported by *in vitro* studies[115] showing that cortical fibroblast proliferation is stimulated by paracrine signals generated by PTC in co-culture.

Moreover, in proteinuric nephropathies with progressive injury of the glomerular filtering barrier, abnormal uptake of ultrafiltered proteins by PTCs induces release of TGFβ1, which, in turn, promotes interstitial fibrogenesis.[116] Re-establishment *in vitro* of epithelial cell polarization and differentiation inhibits proliferation of mesenchymal stem cells (MSCs) from co-cultured adipose tissue fragments,[117] confirming that uninjured differentiated renal tubular cells in their normal configuration contribute to the maintenance of the homeostatic state of MSCs. A seminal work[73] evaluating the effects *in vitro* of aristolochic acid on several PTC lines reveals that damaged epithelial cells markedly up-regulate expression of profibrotic TGFβ1 and CCN2. This up-regulation is associated with a marked increase in the percentage of cells in the G_2/M phase of the cell cycle, which is confirmed *in vivo* using different acute kidney disease mouse models.[73] The contribution of PTCs is further supported in a tetracycline-inducible transgenic mouse model, in which conditional overexpression of TGFβ1, limited to renal tubules, induces widespread peritubular fibrosis and focal nephron degeneration.[114] During the course of this process, the remnants of one cell were removed by phagocytosis by neighboring cells with a mechanism akin to that seen in tubular atrophy.[118] Outside these degenerating tubules, a marked proliferation of resident fibroblasts, without contribution of EMT and only sparse macrophages, is associated with progressive deposition of ECM.

Notch signaling is involved in orchestrating kidney development in tubular epithelial cells and plays a role in tubulointerstitial development.[77] Interestingly, specific expression of cleaved Notch1 in tubular epithelial cells causes fibrosis in the surrounding interstitium, with a histological appearance that resembles the lesions seen in human CKD. Conversely, tubular-specific deletion of Notch signaling protects against kidney fibrosis.[77] The importance of an altered tubule-interstitial microenvironment to fibrosis is also supported by the clinical observation that the severity of acute kidney injury (AKI) with massive tubular epithelial cell death is a robust predictor of progression to CKD.[119] Even if mechanisms for AKI to CKD progression are unknown, it can be hypothesized that AKI progression toward CKD arises from incomplete repair of regenerating tubules. Another explanation is that persistently high profibrotic signaling activity in regenerating tubule epithelium produces paracrine activity that drives fibroblast proliferation and inflammation. Thus, in severe AKI and CKD, epithelial-mesenchymal cross talk alterations possibly lead to tubulointerstitial fibrosis and kidney failure.

It appears that an altered renal microenvironment is the main cause of myofibroblast differentiation in renal fibrosis, and cross talk between epithelial cells and fibroblasts is pivotal for maintenance of the local homeostatic microenvironment, a concept previously suggested for idiopathic pulmonary fibrosis[120,121] and for stromal reaction to carcinoma.[122] Therefore, interfering with tubular-mesenchymal cross talk emerges as a promising strategy for the development of new antifibrotic drugs, which are not solely directed to block myofibroblast activation.

Pulmonary Fibrosis

Structural Aspects of the Lung Relevant for Fibrosis

The adult human lung is a structurally complex organ system composed of >40 cell types. The upper conducting airway, composed of the trachea and a series of branching bronchi and bronchioles, terminates in the gas-exchanging alveoli of the lower respiratory tract. At each level, the epithelium-lined airways and endothelium-lined vasculature are integrated, both structurally and functionally, by an interconnected reticulum of mesenchymal cells and ECM extending from the upper airway down to the alveoli.[123] The lung is susceptible to various forms of short-/long-term injuries, both airborne and blood borne, that may culminate in fibrosis. In addition, fibrosis may involve the small conducting airways (eg, asthma and chronic obstructive pulmonary disease), vasculature (eg, pulmonary hypertension), or pleura (eg, pleural fibrosis). Some forms of fibrosis, such as acute lung injury or cryptogenic organizing pneumonia, are at least partially reversible, whereas others, in particular idiopathic pulmonary fibrosis, are progressive and usually fatal.[120,121] A central role for the myofibroblast in tissue remodeling and fibrosis involving these different tissue compartments and varied disease processes has been demonstrated.[124]

Origin of Lung Myofibroblasts

The concept that lung myofibroblasts may derive from multiple cellular sources, including bone marrow progenitors and the lung epithelium, has been previously reviewed.[1] Depending on the experimental model and tools used, conclusions on participation of myofibroblast progenitors to lung fibrosis sometimes appear contradictory. For example, recent studies using a surfactant protein C-CreER(T2) knock-in allele to follow the fate of type II alveolar cells *in vivo* indicated no contribution to myofibroblasts in the fibrotic reaction to an acute lung injury involving intratracheal instillation of bleomycin.[125] The resolving nature of bleomycin-induced lung fibrosis in mice contrasts with the progressive nature of the most recalcitrant forms of lung fibrosis in humans, in particular idiopathic pulmonary fibrosis. Whether chronicity of the injury may account for processes, such as EMT, remains

to be determined. Chronicity and irreversibility of the fibrotic process may also be linked to the epigenetic (dys) regulation[2,16,42,89] that controls the differentiation and fate of endogenous reparative cells.

Specific Features of Myofibroblasts in the Lung

The fate of myofibroblasts, regardless of their origin(s), in injured tissues may ultimately determine whether normal healing occurs or whether progression to end-stage fibrosis ensues.[121,126] There is likely to be significant heterogeneity in lung fibroblasts, including anti-fibrotic subpopulations, such as Thy-1–expressing fibroblasts and lipofibroblasts.[65,89,90] The mechanisms that produce an apoptosis-resistant myofibroblast phenotype have not been fully elucidated, although some of the same factors that mediate myofibroblast differentiation appear to promote myofibroblast survival.[90,127,128] A member of the NOX family of enzymes, NOX4 has been required for TGFβ1-induced myofibroblast differentiation, ECM production, and contractility of lung myofibroblasts.[56,57] NOX4 is up-regulated in lungs of human subjects with idiopathic pulmonary fibrosis, and genetic or pharmacological targeting of NOX4 attenuated lung fibrogenesis in two different murine models of lung injury.[56] It is unknown if the profibrotic effects of NOX4 are related solely to activation of myofibroblasts or if its expression in alveolar epithelial cells may also contribute to fibrogenesis.[58,59] It is also yet to be determined if targeting NOX4 modulates the apoptosis-resistant phenotype of myofibroblasts and whether this strategy may be effective in established fibrosis. However, the proof of concept for therapeutic targeting of prosurvival signaling with a protein kinase inhibitor in animal models of pulmonary fibrosis has been demonstrated.[129]

Another potential therapeutic strategy is to induce dedifferentiation of myofibroblasts as a mechanism for myofibroblast deactivation in progressive fibrotic disorders.[130] Cellular plasticity, specifically the ability of differentiated cells to dedifferentiate into more primitive multipotent or pluripotent progenitor cells, appears to be important in the remarkable regenerative potential observed in amphibians. The extent to which such mechanisms (or the lack thereof) explain the more limited regenerative capacity in mammals is unknown. It has also been proposed that lung fibrosis may represent a process of disordered redevelopment, in which there exists an aberrant recapitulation of developmental pathways.[131] Further studies on myofibroblast plasticity and its capacity for dedifferentiation may provide additional insights into tissue repair and fibrosis.

Liver Fibrosis

Characteristics of Liver Fibrosis

In experimental and clinical liver fibrosis, α-SMA–expressing myofibroblasts are the major source of fibrillar collagen and other ECM proteins. Although the impact of excessive and abnormal ECM deposition is well evaluated in the liver, little is known about the role of myofibroblast contraction. The contractive nature of liver fibrosis is suggested by the smaller size of the fibrotic liver (relative to the healthy liver) and its irregular or nodular surface due to underlying scar tissue. Collapse of the liver parenchyma results in a reduction of the distance between portal zones, which, together with ECM synthesis, is part of the fibrotic process. Distortion of liver architecture characterizing advanced fibrosis and shunting of the portal blood along porto-portal and porto-central fibrotic septa, away from hepatocytes, results in hepatic failure and portal hypertension. It is conceivable that these changes are mainly due to tissue remodeling and depend on contractile forces exerted by myofibroblasts in addition to ECM deposition.

Origins of Liver Myofibroblasts

Myofibroblasts are absent from normal liver. They are derived primarily from activated hepatic perisinusoidal cells (HSCs), at least in experimental liver injury.[132] However, other potential precursor cells are embedded in the portal tract stroma around portal structures, including vessels and biliary structures. For example, portal fibroblasts (PFs) are able to acquire a myofibroblastic phenotype,[133] and their contribution may be more important than generally assumed.[134] The contribution of liver MSCs to the myofibroblast population is as yet unclear. These mesenchymal progenitor cells are usually identified by the combined expression of different specific surface marker proteins and their capacity to differentiate into different mesenchymal cell lineages. The potential of MSCs to differentiate into myofibroblasts has gained increasing attention; however, it is unclear whether this fate is beneficial or detrimental for normal tissue repair and the envisaged medical use of MSCs for tissue regeneration purposes.[135] The association of MSCs with the well-known epithelial stem cells located in Hering's canals may constitute a niche, a concept that is being explored in the liver.[136] Several studies have demonstrated that, after injury, many myofibroblasts may originate from bone marrow,[137] but the contribution of these cells to collagen production is not known.[138] Lineage-tracing studies fail to demonstrate the generation of myofibroblasts from hepatic epithelial cells (hepatocytes and cholangiocytes) by EMT.[139] However, the controversy about the ability of hepatic epithelial cells to become myofibroblasts remains unsettled.[113] Thus, the possibility that MSCs, bone marrow–derived cells, and EMT may represent alternative sources of myofibroblasts cannot be excluded.

Evidence for the Reversibility of Myofibroblast Differentiation in Specific Liver Subpopulations

It is increasingly accepted that, depending on the underlying causative factor (eg, alcohol abuse, viral hepatitis, or biliary cholestasis), liver fibrosis may involve different fibrogenic cell subpopulations. To our knowledge, no reliable markers have been identified that allow unambiguous discrimination between HSC- and PF-derived

myofibroblasts. However, differences have been reported between these two fibrogenic cell populations, concerning the mechanisms underlying myofibroblastic differentiation, activation, and deactivation.[140] After isolation from healthy rat liver and culturing under the same conditions, both HSCs and PFs acquire a myofibroblast phenotype. HSC-derived myofibroblasts display rounded and spread morphological characteristics with an enlarged cytoplasm and, more important, a poor survival after two to three passages. Conversely, PF-derived myofibroblasts have more elongated morphological characteristics and proliferate over multiple passages. *In vivo*, during liver repair, HSC- and PF-derived myofibroblasts display different functions because of their specific locations and microenvironment. By using a model of cultured precision-cut liver slices, the behavior of the myofibroblast subpopulations during remodeling differs depending on the experimental model,[141] the pathological situation, and the disease cause.[142] HSC-derived myofibroblasts can lose α-SMA expression without undergoing cell death, whereas in similar conditions, PF-derived myofibroblasts die by apoptosis. HSC-derived myofibroblasts are involved in blood flow regulation and hepatocellular healing. The appearance of the myofibroblast phenotype in HSCs may lead to disordered vascular remodeling after liver injury, inducing ischemia and then fibrosis. PF-derived myofibroblasts are involved in scarring.[143]

Remodeling of the Fibrotic Injury and Potential Therapies

The traditional view of liver fibrosis as an irreversible process is obsolete, and the development of liver fibrosis is thought to be a dynamic and potentially bidirectional process. Because of the liver's extraordinary regeneration potential, myofibroblast and fibrosis reversion strategies may be more applicable than in other organs, such as the lung.[130] Spontaneous resolution of scarring is seen in animal models of liver fibrosis and in human trials in which the stimuli responsible for long-term or repeated hepatic damage are successfully removed. Key players in the process are myofibroblasts, matrix metalloproteinases, and matrix metalloproteinase inhibitors. However, it is still unclear whether advanced fibrotic or cirrhotic liver can revert back to normal architecture after the removal of profibrotic factors. It is conceivable that there is a point of no return, even for this highly regenerative organ. Important quantities of elastin and extensively cross-linked ECM components seem to be features of irreversibility.[144] Numerous preclinical studies[145] aimed at inhibiting myofibroblast activity and fibrosis progression in liver have been developed, but their translation into the clinic remains limited.

Stromal Reaction to Epithelial Tumors

Cancer cells do not exist independently but interact dynamically with local and distant host cells, best conceptualized as a gradually evolving microenvironment or even ecosystem.[6] Nonredundant changes in a single element may inevitably alter the organization of the whole system and may drive tumor initiation, invasion, and metastasis. Morphological evidence of host participation in the primary tumor, the premetastatic niche, and metastatic sites includes the following: i) desmoplasia consisting of carcinoma-associated fibroblasts (CAFs) and ECM; ii) inflammation and immune response represented by lymphocytes, macrophages, and dendritic cells; and iii) angiogenesis evidenced by newly formed blood vessels and lymph vessels. Herein, we focus on the prominent role of CAFs as host cells in the tumor microenvironment. Phenotypically, CAFs closely resemble myofibroblasts exhibiting different levels of specific marker expression. This raises the possibility that tumor-associated myofibroblasts fall into a diversity of functional subtypes, similar to what has been shown for inflammatory cells and macrophages.[122]

Origins of Myofibroblasts and CAF in Tumor Stroma

Current understanding of the origin and molecular events surrounding the genesis of myofibroblasts and CAFs in tumor stroma is still a matter of debate. CAFs are thought to arise from several mobilized cell types, such as tissue-resident MSCs or fibroblasts, as well as recruited bone marrow–derived MSCs and fibrocytes.[122] Alternatively, or in addition, CAFs may originate from multiple resident precursors, such as endothelial cells, myoepithelial cells, epithelial (cancer) cells, smooth muscle cells, adipocytes, and stellate cells. These findings suggest a high degree of plasticity and/or a diversity of origins for tumor myofibroblasts. This diversity may also explain the heterogeneous phenotype of myofibroblast populations observed within tumors.[122]

Specific Roles and Features of Myofibroblasts in the Tumor Environment

Traditionally, biomarker research has focused on molecular characteristics of the cancer cells, but increasing evidence suggests that myofibroblasts may refine the prognostic assessment of tumors and may provide predictive information about response to chemotherapy. Gene expression profiles of laser-capture microdissected tumor-associated host cells from primary breast tumors yield prognostic signatures strongly associated with a poor disease-specific survival. A wound-response signature, containing genes implicated in tissue remodeling, migration, angiogenesis, and inflammation, is established from 50 fibroblast cultures whose expression changed after exposure to 10% serum. Patients with breast cancer whose tumors express this wound-response signature have a markedly reduced overall survival and distant metastasis-free survival compared with those with tumors that do not express this signature.[80] Immunohistochemical analysis of breast tumors also reveals that many of the proteins encoded by genes identified in the previously mentioned signatures are expressed by myofibroblasts, indicating their striking

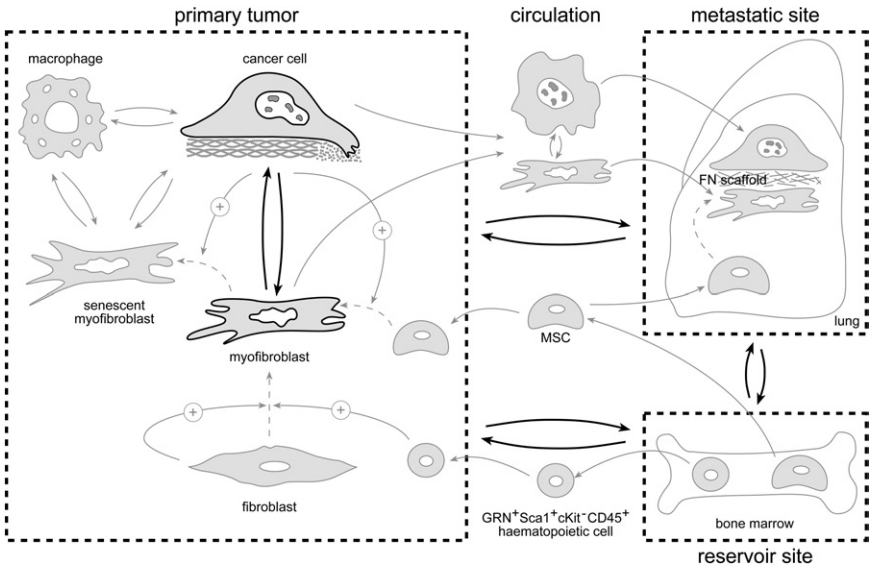

Figure 2. Tumor myofibroblasts. A schematic view highlights the cross signaling (**double arrows**) within and between local and distant tumor ecosystems. **Arrows** indicate displacement of cells between ecosystems; **dashed arrows**, conversion from one cell type into another; **positive arrows**, stimulation; and **bold arrows**, importance of the signaling.

prognostic value. Increased expression of platelet-derived growth factor receptor β, LOXL2, caveolin-1, and CD10 in CAFs has been correlated with poor clinical outcome in multiple cancer types, such as breast, colon, pancreas, and liver.[69,146–148] Increased co-expression of LOXL2 and α-SMA in colon and breast cancer suggests that myofibroblasts are the main producers of LOXL2. The ECM, including cross-linked collagen type I, has an influence on therapeutic response. Moreover, a tumor-associated host cell gene expression signature present in estrogen receptor–negative breast tumors predicts resistance to neo-adjuvant chemotherapy with 5-fluorouracil, epirubicin, and cyclophosphamide.[149]

In the primary tumor microenvironment, resident human mammary and prostate fibroblasts exhibit autocrine signaling loops mediated by TGFβ1 and chemokines and differentiated into myofibroblasts.[150] Epigenetic regulation by miRNAs and oxidative stress caused by reactive oxygen species in the primary tumor promote myofibroblast differentiation[67] and secretion of senescence-associated pro-inflammatory cytokines, such as IL-6 and IL-8.[151] When considering the tumor environment as an ecosystem, however, it is important to note that a tumor is not composed solely of cancer cells and myofibroblasts. The secretion of pro-inflammatory cytokines by myofibroblasts may influence the recruitment of inflammatory cells, which, in addition to their immunomodulatory activity, may also stimulate invasion of cancer cells. CAFs isolated from the initial hyperplastic stage in multistep skin tumorigenesis exhibit activated expression of the pro-inflammatory cytokine IL-6.[152] More important, chemical and physical insults encountered during therapeutic interventions may also cause stress-induced cellular senescence and reciprocal activation of pro-inflammatory, prosurvival, and pro-invasive pathways in the local ecosystem and at distant anatomical locations.[153]

Primary tumor–induced remodeling of distant ecosystems, which initiates metastatic niche formation and host

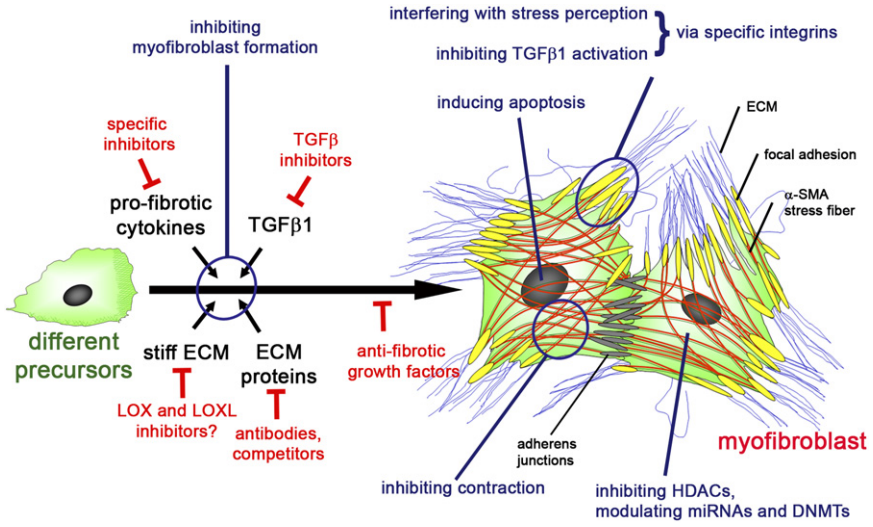

Figure 3. The myofibroblast in the center of attention. Anti-fibrotic therapies can be designed to interfere with the extracellular chemical and mechanical factors that lead to myofibroblast formation from a variety of different precursors. Alternatively, one might interfere with intracellular signaling pathways, transcription regulators, and epigenetic mechanisms that specifically modulate myofibroblast differentiation. Other potential anti-fibrotic targets are specific features of the differentiated myofibroblast, such as α-SMA in the contractile apparatus, specific integrins, and ECM proteins. Other strategies aim to induce myofibroblast regression and/or apoptosis (modified and reproduced with permission from the study by Hinz and Gabbiani[161]).

cell recruitment, suggests a crucial role for secretory products released into the circulation (Figure 2). The primary tumor signals to the bone marrow and stimulates the release of MSCs into the circulation, with recruitment by stromal cell–derived factor-1α chemokine gradients to the tumor site, where they are converted into myofibroblasts by TGFβ1.[154] Moreover, osteopontin released by an aggressive xenograft mobilizes granulin-secreting $ScaI^{+}cKit^{-}CD45^{+}$ bone marrow–derived hematopoietic cells into the circulation.[72] These granulin-secreting cells home to a second otherwise quiescent or indolent tumor in the same animal. Granulin accumulation converts resident fibroblasts into myofibroblasts and stimulates progression of an otherwise indolent tumor.[72] Secretory products from the primary tumor ecosystem recruit MSCs or hematopoietic cells from the bone marrow but also may promote myofibroblast differentiation in distant loci. Thus, TGFβ1, packaged in secreted nanovesicles termed *exosomes*, converts fibroblasts into myofibroblasts at distant organs and may prepare metastatic niches by focalized production of a fibronectin scaffold.[155,156] Alternatively, metastatic cancer cells can bring their own soil, including myofibroblasts, from the primary ecosystem to the distant ecosystem, as shown for the lung. Comigrating myofibroblasts provide prosurvival signals in the circulation and an early growth advantage in the ectopic lung site.[157]

Thus far, we have emphasized the invasion- and metastasis-promoting effects of myofibroblasts. However, transgenic mouse experiments suggest that suppressive signals derived from resident tissue fibroblasts can also control tumor initiation and progression.[80] For example, disruption of the TGFβ receptor type II (*TGFBR2*) gene in fibroblasts results in invasive squamous cell carcinoma of the forestomach.[158] In a model of prostate cancer, loss of p53 function in fibroblasts precedes inactivation of p53 in the epithelium.[159] Targeted genetic inactivation of the phosphatase and tensin homologue (PTEN), a tumor suppressor with lipid and protein phosphatase activity, in fibroblasts of mouse mammary glands accelerates the initiation and progression of ErbB2-driven mammary epithelial tumors characterized by massive accumulation of collagen type I and infiltration of F4/80-positive macrophages.[160] In conclusion, the complex molecular mechanisms that govern cancer cell–myofibroblast interactions in the primary tumor and in distant ecosystems justify the need for systems-level approaches to advance the field.

Conclusion and Perspectives

Based on the biological observations pinpointing the mechanisms of differentiation and activity of the myofibroblast, many attempts have been made to target them in anti-fibrotic therapies (Figure 3). However, no clinically effective compound has yet been identified, although several possibilities continue to be under investigation. Emerging and promising, but not yet clinically tested, strategies to counteract fibrosis include the use of miRNAs as potential therapeutic targets.[43] Another strategy is to interfere with collagen cross-linking and ECM stiffening by controlling the activities of LOX,[68] LOXL2,[70] and specific lysyl hydroxylases.[162]

The capacity of cytokines to inhibit α-SMA expression by fibroblastic cells suggests a different strategy. In the case of interferon-γ, locally applied cytokine is found to be effective for treatment of Dupuytren's nodules in one study,[60] but this result needs confirmation. Another more recent example for anti-fibrotic cytokines is CXCL10, which effectively treated lung fibrosis in an animal model.[61] The complementary approach is to block profibrotic cytokines, among which TGFβ1 is the most attractive target. However, administration of TGFβ1-inhibiting drugs and antibodies has shown little success in animal and human studies, indicating that targeting a single cytokine is unlikely to be successful.[52] More promising strategies appear to target TGFβ1 profibrotic cofactors, such as CCN2[74] or the ECM protein extradomain A fibronectin.[75,76] Alternatively, TGFβ1 can be counteracted at the level of its activation from latent stores; possible targets in the activation process are fibroblast/myofibroblast-associated integrins $\alpha v\beta 3$, $\alpha v\beta 5$, and $\alpha 8\beta 1$ integrin[50] and the epithelial integrin $\alpha v\beta 6$.[163] It is conceivable that inhibiting these integrins has alternative suppressing effects on myofibroblast development. Other integrins that have contributed to fibrosis and myofibroblast differentiation through different pathways include $\alpha 3\beta 1$, $\alpha 11\beta 1$, and $\alpha v\beta 3$, and all represent possible therapeutic targets.[11]

Other interesting approaches are based on the possibility of terminating myofibroblast persistence by inducing their apoptosis[164] or their reversion to nonfibrogenic cell phenotypes.[108] In the case of the former, the ability of nitric oxide to induce myofibroblast apoptosis[165] suggests a possible therapeutic approach, such as by the use of phosphodiesterase-5 inhibitors.[166] Finally, the experimental observation that the acetylated N-terminal peptide of α-SMA, Ac-EEED, inhibits both α-SMA and collagen type I expression, on intracellular delivery into myofibroblasts, makes this peptide a potential suppressor of fibrotic changes. This possibility has been successfully tested on experimental wound healing.[167] We believe that these different approaches will eventually open new therapeutic avenues.

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