FIBROSIS



# Tissue-resident mesenchymal stromal cells: Implications for tissue-specific antifibrotic therapies

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**Recent scientific findings support the notion that fibrosis is driven by tissue-specific cellular and molecular mechanisms. Analysis of seemingly equivalent mesenchymal stromal cell (MSC) populations residing in different organs revealed unique properties and lineage capabilities that vary from one anatomical location to another. We review recently characterized tissue-resident MSC populations with a prominent role in fibrosis and highlight therapeutically relevant molecular pathways regulating their activity in chronic disease.**

## INTRODUCTION

Due to the slow progression in the development of fibrosis therapeutics, most degenerative fibrosing disorders remain diseases with high morbidity and mortality. This is in part due to our incipient understanding of the biology of the disease-causing mesenchymal stromal cells (MSCs) and the mechanisms that regulate their activity in vivo. Here, we define adult MSCs as cells derived from the embryonic mesenchyme that reside in the tissue stroma and the perivascular niche and contribute to the generation of extracellular matrix (ECM) and/or connective tissue cells in tissue homeostasis, injury, and chronic disease. As discussed below, this definition comprises multiple MSC types, including primitive perivascular stem cell–like progenitors—called mesenchymal stem cells by others—as well as further committed connective tissue precursors and fibroblasts.

The notion that fibrosis in multiple tissues is driven by virtually identical MSC populations and a common set of signals is rapidly becoming obsolete. The anatomical distribution and organization of resident MSC populations in different organs are established during development and are driven by unique combinations of local signals, resulting in durable tissue identity. The view that both embryonic origin and local signals define MSC identity is supported by fate tracing experiments in mice identifying waves of cells from different embryonic origins which sequentially populate the same anatomical locations at different times during development. Those waves comprise mesenchymal progenitor cells (MPCs) from at least two different sources, namely, the neuroectoderm and the primitive streak (via the mesoderm), which contribute to the generation of connective tissues throughout the body (*1*, *2*). Despite phenotypic similarities, derivatives from those progenitor cell populations present in adult tissues have unique epigenetic properties and retain lineage-specific capabilities (*3*–*5*).

Further evidence supporting the unique anatomical identity of fibrogenic stromal cells comes from a recent systematic study of phenotypically similar perivascular MSC populations residing in multiple adult human tissues. Because those cells share the common marker signature $CD146^+$ $CD45^-$ $CD34^-$ and were previously found to generate multiple connective tissues in vitro, it was assumed that they constituted one ubiquitous population of adult mesenchymal progenitor/stem cells (*6*). Recent analysis of transcriptional profiles and connective tissue lineage capabilities in vivo, however, indicated that although $CD146^+$ $CD45^-$ $CD34^-$ cells residing in skeletal tissues, including bone and skeletal muscle (SM), can generate osteoblasts or chondroblasts in vivo, cells residing in nonskeletal tissues do not differentiate into those lineages (*7*). In addition, whole transcriptome analysis identified essential differences in gene programs, including expression of membrane receptors and signaling molecules (*7*). From that analysis, expression of signal transducer and activator of transcription 2 (STAT2), transforming growth factor–β receptor 2 (TGFβ2), fibroblast growth factor-18 (FGF-18), and retinoic acid receptor-α (RARA) was found to be significantly higher in bone marrow (BM) MSCs compared to MSCs from other tissues, whereas expression of insulin-like growth factor 2 (IGF-2), jagged 1 (JAG-1), bone morphogenic protein 2 (BMP-2), FGF-13, and angiopoietin-like 1 (ANGPTL-1) was predominant in MSCs residing in the SM, and TGFBR3, platelet-derived growth factor receptor-α (PDGFR-α), WNT1-inducible signaling protein 1 (WISP-1), interleukin-7 (IL-7), osteoglycin (OGN), IGF-1, and suppressor of cytokine signaling 5 (SOCS-5) characterized MSCs in the periosteum (*7*). These findings constitute evidence that, in vivo, MSCs have a unique identity associated with the tissue in which they reside, and also bring into question the validity of commonly used in vitro differentiation assays, because they may not reflect the in vivo capabilities of those cells.

It is not feasible to study MSCs in vivo in human tissues. However, the use of genetic labeling techniques allows us to visualize their counterparts in mouse organs. Using those techniques, the behavior of MSCs can now be traced and studied in fibrotic disease, with the cautionary note that often mice models can only partially recapitulate human disease. Fate tracing of genetically labeled MSCs has led to the identification and characterization of several tissue-resident MSC populations in vivo (Table 1). From these studies, it has become clear that the pool of MSCs residing in a tissue actually comprises multiple cell populations with varying lineage capabilities (degrees of multipotency). Resident MSC pools may include perivascular MPCs capable of giving rise to specific connective tissue cell types (Fig. 1), as well as interstitial fibroblasts that contribute to the basement membrane surrounding parenchymal structures. Although the relationship between MPCs and pericytes remains unclear (*8*), MPCs have pericyte features that define them anatomically as mural cells, partially or completely embedded in the capillary basement membrane. Those cells make contact with the abluminal surface of the endothelium in the microvascular bed of all adult tissues, particularly at

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**Table 1. In vivo lineage-traced fibrogenic MSC populations shown to participate in tissue fibrosis.** MSC, mesenchymal stromal cell; Lrat, lecithin-retinol acyltransferase; EN-1, engrailed 1.

| Organ/tissue | Lineage tracing marker | MSC populations labeled | Adult mesenchymal tissue contribution in vivo | References |
|---|---|---|---|---|
| Lung | FoxD1 | Pericyte-like MPC | Myofibroblast | (*59*) |
| | NG2 | Pericyte | Pericyte | (*60*) |
| | Adrp | Lipofibroblast | Myofibroblast | (*61*) |
| | PDGFRα | Fibroblast | Fibroblast | (*60*) |
| Skeletal muscle | PDGFRα | Pericyte-like MPC | Myofibroblast, adipocyte | (*20*, *25*) |
| | ADAM12 | Pericyte-like MPC | Myofibroblast | (*23*) |
| Liver | Lrat | Pericyte-like MPC | Myofibroblast | (*45*) |
| Kidney | FoxD1 | Pericyte-like MPC, pericytes, pertibular fibroblasts | Myofibroblast, pericyte | (*33*) |
| | Gli1 | Pericyte-like MPC | Myofibroblast, pericyte | (*34*) |
| Bone marrow | LepR | Pericyte-like MPC | Myofibroblast, osteocyte, chondrocyte, adipocyte | (*10*–*12*) |
| | Gli1 | Pericyte-like MPC, endosteal MPCs | Myofibroblast | (*18*) |
| Skin | ADAM12 | Pericyte-like MPC | Myofibroblast | (*23*) |
| | EN-1 | Fibroblast | Myofibroblast | (*68*) |
| | Dlk1 | Fibroblast/MPC | Myofibroblast, adipocyte | (*69*) |

branch points of capillaries (*6*). The resulting anatomical distribution pattern within organs allows MSCs to rapidly detect local cues within tissues, including damage-associated signals. As we will discuss in the following sections, the organization and composition of resident MSC pools are unique to every tissue. In certain organs such as the lung and the kidney, interstitial fibroblast populations are prominent, and perivascular progenitor cells constitute a smaller fraction of the stroma, whereas in other tissues such as the liver and the SM, the opposite is true. These differences will result in tissue-specific fibrogenic processes and are relevant to the design of antifibrosis therapies.

## TISSUE-SPECIFIC RESIDENT MSC POPULATIONS EMERGE AS TARGETS OF FIBROSIS THERAPIES

### BM LepR$^+$ MSCs in myelofibrosis

MSCs expressing leptin receptor (LepR) with fibrogenic, adipogenic, and osteogenic potential have been identified in human and murine BM (*9*, *10*). Genetic labeling studies indicate that LepR$^+$ MSCs reside along the BM vasculature, in areas adjacent to the endosteum (Table 1 and Fig. 2) (*11*). In addition to exhibiting multipotency, the association with the vasculature defines LepR$^+$ MSCs as a population of BM-resident MPCs (*12*). LepR$^+$ MSCs effectively generate most of the osteocytes in adult mice and therefore play a central role in bone turnover and homeostasis (*11*). LepR$^+$ MSCs also participate in bone regeneration. After long-bone damage, LepR$^+$ MSCs proliferate and generate a pool of osteoblasts, becoming the major source of newly formed bone during the repair process (*11*).

In the context of degenerative damage, LepR$^+$ MSCs detach from the vasculature, proliferate, and differentiate into the fibrogenic and adipogenic lineages. Fate tracing experiments have indicated that BM irradiation induces the differentiation of LepR$^+$ MSCs into adipocytes and myofibroblasts (Fig. 2) (*10*, *11*). Ectopic adipocyte formation is a hallmark of myelodegenerative BM disease. In the clinic, BM adipogenesis impairs hematopoietic activity and characterizes the aplasia observed in the marrow of patients with primary myelofibrosis (MF) or patients receiving radiotherapy or chemotherapy (*13*).

The LepR receptor signals via janus kinase (JAK)/STAT (*14*), a major pathway driving MF (*15*). Inhibition of JAK1/JAK2 signaling by ruxolitinib (Jakafi) was shown to be effective in a controlled randomized, double-blind, placebo-controlled trial involving patients with intermediate- or high-risk MF (*16*). In 2011, ruxolitinib (Jakafi) was approved by the U.S. Food and Drug Administration (FDA) for the treatment of intermediate- or high-risk MF. In addition to JAK/STAT, a role for PDGFRα signaling has been reported in the activation of LepR$^+$ MSCs for the initiation of MF (*10*), indicating that this receptor constitutes a potential target for the treatment of early phases of MF. Although caution is warranted when comparing experimental and clinical data, the results obtained in LepR lineage tracing experiments might help interpret the outcomes from previous clinical attempts to inhibit PDGFRα in MF patients using imatinib mesylate, in which the treatment was found to be effective in early disease phases but ineffective in patients at more advanced disease stages (*17*). Further in vivo studies may confirm the efficacy of PDGFRα inhibitors as a therapeutic alternative to ruxolitinib for the treatment of MF in its early stages (Fig. 2).

In addition to LepR$^+$ MSCs, a population of fibrogenic MSCs expressing glioma-associated oncogene homolog 1 (Gli1) identified in the human and murine BM plays an important role in BM fibrosis (Fig. 2) (*18*). Gli1$^+$ stromal cells profusely populate the BM of patients with MF, and their density correlates with the fibrosis grade as determined by pathological scoring (*18*). Analysis of BM Gli1$^+$ MSCs in transgenic mice carrying a reporter transgene that allows for cell fate tracing showed that this cell population comprises both a small subset of pericyte-like MSCs residing along the BM sinusoids and

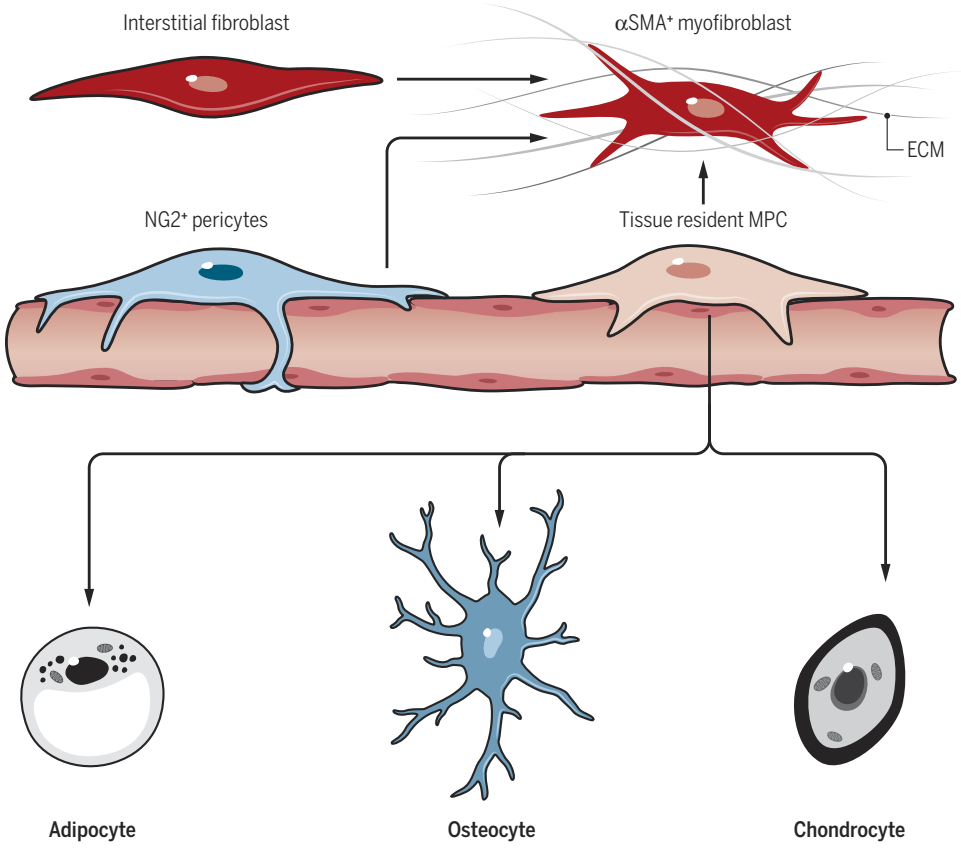


**Fig. 1. Perivascular MPCs with varying lineage capacities generate distinct connective tissues and specialized mesenchymal cells, depending on the tissue of residence.** Mesenchymal progenitor cells (MPCs), neural/NG2$^+$ (glial antigen 2) pericytes, and fibroblasts participate in tissue-specific fibrosis processes through the generation of αSMA$^+$ (α–smooth muscle actin) myofibroblasts that drive excessive production of extracellular matrix (ECM). The arrows indicate cell fate.

arterioles, and a larger subpopulation of MSCs that lie along the endosteum (*18*). Neither subpopulation expressed LepR, indicating that Gli1$^+$ stromal cells constitute an MSC population distinct from LepR$^+$ MSCs. Murine Gli1$^+$ MSCs behave like their human counterpart, proliferating and differentiating into SMA$^+$ myofibroblasts in a murine model of MF (Fig. 2) (*18*). GANT61, a Hedgehog/Smoothened inhibitor, impairs the differentiation of the progenitor cells and induces apoptosis in differentiated cells, in both murine and human Gli1$^+$ MSCs (*18*). These findings point to GLI transcription factors as promising therapeutic targets for both early and advanced stages of MF. Therefore, the combination of clinical and experimental tools to target human- and murine-specific BM MSC populations is opening an exciting path for the discovery of novel therapeutic targets for BM fibrosis.

## SM fibro/adipogenic progenitors in chronic muscle disease

The characterization of MSC populations in the SM has led to the identification of molecular mechanisms with therapeutic potential for chronic muscle disease. In this tissue, CD31$^-$ CD45$^-$ Sca1$^+$ PDGFRα$^+$ bipotent MSCs reside attached to the abluminal side of capillaries in the interstitial spaces between the myofibers in both human and mouse SM (*19*–*21*). Also identified in the heart (*22*) and referred to as fibro/adipogenic progenitors (FAPs), these cells have the ability to differentiate into fibroblasts and adipocytes both in vivo and in vitro (Table 1) (*19*–*21*). FAPs constitute the main MPC population in the SM capable of fibro/adipogenesis, but also ectopic osteogenesis in vivo (*19*, *23*, *24*).

SM damage triggers the activation and proliferation of FAPs (*17*). Those cells infiltrate the area of damage and synthesize ECM components during a stage of transient fibrogenesis (*19*, *20*, *25*). The number of FAPs declines during the resolution phase because the cells undergo programmed cell death (*19*, *20*, *25*). Chronic SM damage, on the other hand, leads to the perpetuation of FAPs, as observed in degenerative muscle disease. FAPs are the main collagen type I synthesizing muscle resident cell population in the SMs of dystrophic MDX mice, a model of Duchenne muscular dystrophy (Fig. 2) (*20*). Both in SM regeneration and chronic SM damage conditions, such as muscular dystrophy, the activity of FAPs is largely controlled by TGFβ receptors and PDGFRα (*25*–*27*). Inhibition of PDGFRα and TGFβ signaling results in reduced FAP number and a reduction in collagen deposition (*25*–*27*). In recent years, tyrosine kinase inhibitors (TKIs) have been found to effectively reduce SM fibrosis both in experimental acute damage and models of muscular dystrophy. Imatinib mesylate and nilotinib inhibit FAP proliferation and survival, reducing fibrosis in the muscles of dystrophic MDX mice (*26*, *28*). Nilotinib was shown to reduce FAP survival by blocking TGFβ signaling, leaving FAPs exposed to inflammatory proapoptotic signals such as tumor necrosis factor–α in an experimental model of acute SM damage, as well as in MDX mice (*25*). Another TKI, crenolanib, which inhibits both PDGFRα and PDGFRβ signaling, has been recently found to effectively reduce fibrosis and improve muscle force in MDX mice (*27*). Although more clinical data are needed to ascertain their effectiveness, TKIs have become a promising therapeutic option for the treatment of fibrosis in muscle diseases, including muscular dystrophy.

FAPs support myogenesis via the secretion of myoblast differentiation factors. In muscular dystrophy, the promyogenic role of FAPs is age-dependent and regulated by histone deacetylases (HDACs), which control the expression of promyogenic paracrine factors (*29*). HDAC inhibitors block FAP adipogenic differentiation while enhancing their ability to support myogenic differentiation of adjacent myoblasts through up-regulation of the soluble factor follistatin (*29*). This effect was restricted to young MDX mice, whereas FAPs from old mice were refractory to treatment with HDAC inhibitors (*29*). In keeping with such experimental findings, results from a phase 2 open-label clinical trial in boys with Duchenne muscular dystrophy aged 7 to 11 showed that the HDAC inhibitor givinostat significantly slowed disease progression after 12 months of treatment, noted by increased muscle fibers size, decreased necrosis, and reduced total fibrosis and fatty infiltration in the interstitium (*30*). Givinostat constitutes yet another example of how analysis of MSC

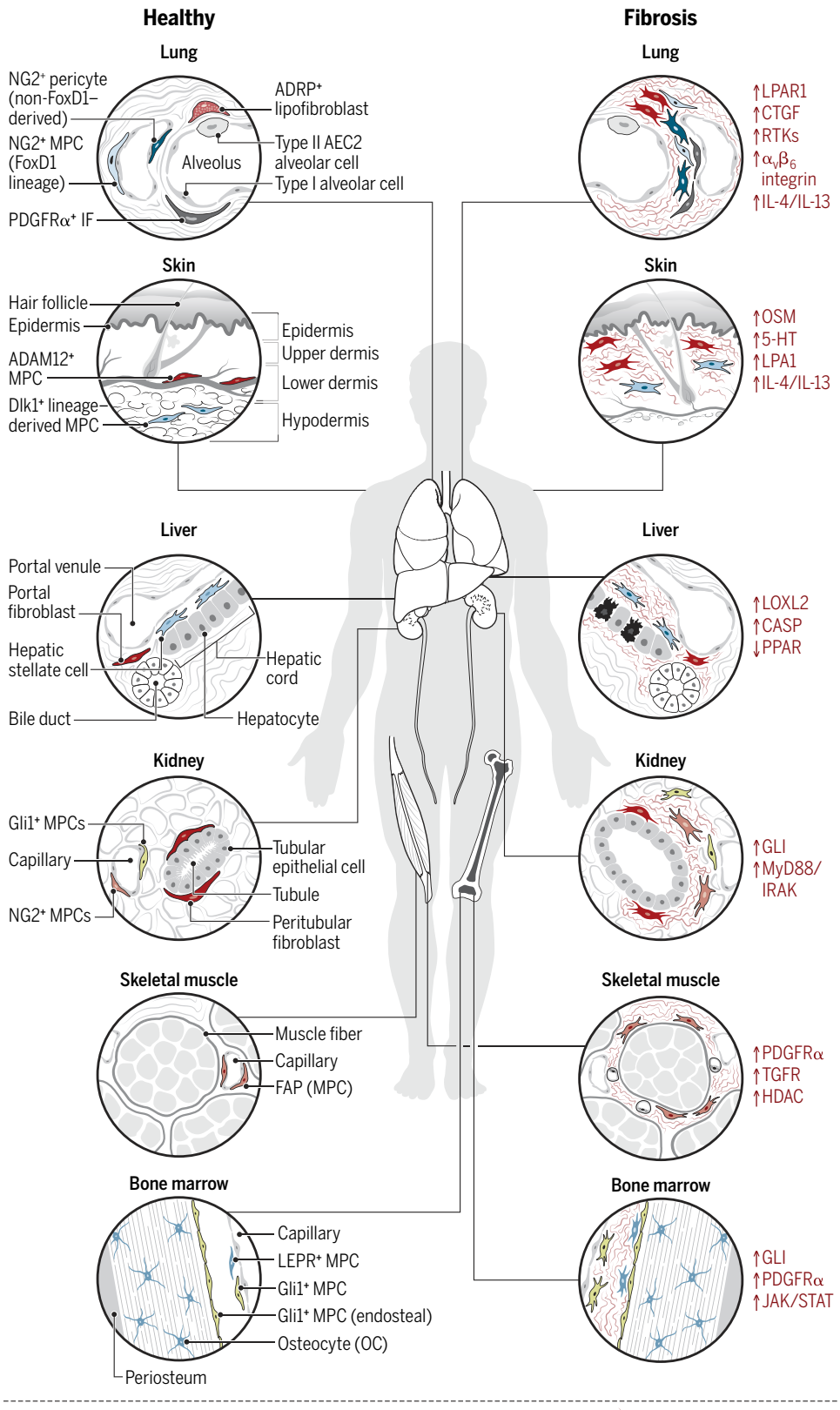


**Fig. 2. Tissue-specific mechanisms of fibrosis driven by mesenchymal stromal cells with unique anatomical identities.** A combination of perivascular MPCs, pericytes, and fibroblasts residing in the alveolar niche participate in idiopathic pulmonary fibrosis. Dlk1$^+$ (delta-like notch ligand 1) lineage–derived dermal MPCs and ADAM12$^+$ (disintegrin and metallopeptidase domain 12) perivascular MPCs drive cutaneous fibrosis. In the liver, hepatic stellate cells (HSCs) and, to a lesser extent, portal fibroblasts drive hepatic fibrosis. Peritubular fibroblasts, perivascular Gli1$^+$ (glioma-associated oncogene homolog 1) MPCs and NG2$^+$ pericytes contribute to renal fibrosis in tubulointerstitial disease. Perivascular FAPs (fibro/adipogenic progenitors) are the main fibrogenic cell population driving skeletal muscle fibrosis in muscular dystrophy. LepR$^+$ (leptin receptor) and Gli1$^+$ MPCs generate myofibroblasts in bone marrow myelofibrosis. The latest therapeutically relevant molecular pathways are indicated in red. Progenitor cells and their fibrogenic derivatives are color-coded. IF, interstitial fibroblast; RTKs, receptor tyrosine kinases; PDGFRα, platelet-derived growth factor receptor α; LPAR1, lysophosphatidic acid receptor 1; $\alpha_v\beta_6$, alpha v beta 6; PPAR, peroxisome proliferator–activated receptor; IRAK, interleukin-1 receptor–associated kinase; TGFR, transforming growth factor receptor; HDAC, histone deacetylase; JAK, janus activate kinase; STAT, signal transducer and activator of transcription; OSM, oncostatin M; 5-HT, 5-hydroxytryptamine; LOXL2, lysyl oxidase-like 2; CASP, caspases; FoxD1, forkhead box D1.

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behavior in vivo can lead to the discovery of new promising therapeutic tools.

### MSCs in kidney tubulointerstitial fibrosis

Lessons learned from recent clinical study failures are paving the way for new therapeutic approaches to block MSCs in kidney tubulointerstitial fibrosis. In the kidney, quiescent peritubular fibroblasts and pericytes with characteristics of MPCs capable of generating fibroblasts de novo contribute to fibrogenesis in response to injury (*31*–*33*). In addition, recent reports have shown that Gli1, a member of the Hedgehog signaling pathway, labels a population of tissue-resident perivascular MSCs in the kidney (Table 1) (*34*). Gli1$^+$ MSCs express the MPC markers CD29, Sca1, CD44, and CD105 and are capable of trilineage differentiation in vitro (*34*). Gli1$^+$ MPCs contribute to vascular homeostasis. Gli1$^+$ MPCs associated with the adventitia supply the lining cells of larger vessels, whereas Gli1$^+$ MPCs associated with the microvasculature appear to supply the lining cells of microvessels. Vascular support by Gli1$^+$ MPCs involves the generation of further committed neural/glial antigen 2 (NG2)$^+$ mural fibrogenic MPCs (*34*), which stabilize capillaries and contribute to microvasculature homeostasis in the healthy kidney (*35*, *36*).

Both acute and chronic kidney injuries drive the rapid activation of interstitial fibroblasts and their differentiation into the myofibroblast phenotype (*37*, *38*). Injury signals also activate Hedgehog Gli signaling, including up-regulation of Gli1 and Gli2, a mechanism found in fibrosing human and mouse kidneys (*39*), specifically in Gli1$^+$ MPCs and NG2$^+$ pericyte–like MPCs (*31*, *34*). Upon activation, both cell types detach from the vasculature and proliferate to generate new fibroblasts and myofibroblasts (*34*, *36*). Recent experimental data indicate that Gli1$^+$ MPCs generate NG2$^+$ pericytes that, in turn, generate α–smooth muscle actin (αSMA)–expressing PDGFRβ$^+$ fibroblasts and myofibroblasts de novo (Fig. 2) (*34*). Fibroblasts and myofibroblasts generated by both MPCs and activated preexisting interstitial fibroblasts are at the end of the fibrogenic lineage and constitute a major source of collagen, fibronectin, and fibrillin found in the ECM. Lineage tracing studies showed that the Gli1 progeny covers injured areas rich in collagen type I deposition, indicating that those cells actively synthesize collagen and participate in ECM deposition during transient fibrogenesis (Fig. 2) (*34*). Genetic ablation of Gli1$^+$ cells in the context of injury led to a 50% reduction of the fibroblast/myofibroblast content in injured kidneys, indicating that Gli1$^+$ cells are major contributors to fibrosis but also that preexisting interstitial fibroblasts contribute considerably to the pool of myofibroblasts (*34*).

Clinical trial failures in fibrotic kidney disease have brought into question the long-standing hypothesis that TGFβ is the central regulator of fibrosis across multiple organs and diseases. A phase 2 trial of pan-TGFβ blockade in diabetic nephropathy using a blocking antibody resulted in no impact on any end points in the disease, despite progressive fibrosis being strongly implicated in the disease pathogenesis (clinicaltrials.gov NCT01113801). A phase 2 study of TGFβ blockade in focal and segmental glomerulosclerosis has not been taken forward (clinicaltrials.gov NCT01665391). On the other hand, novel pathways, including the previously mentioned Hedgehog signaling pathway, are emerging as promising therapeutic avenues for the treatment of kidney fibrosis. GANT61, a compound that inhibits GLI activity in the nucleus, specifically blocks the proliferation of Gli1$^+$ MPCs and myofibroblasts in vitro (*39*). In vivo treatments with two separate compounds that reduce GLI protein expression—darinaparsin, an antineoplastic drug approved by the FDA, and GANT67—resulted in reduced collagen deposition and αSMA expression in an experimental model of kidney fibrosis (*39*). Neither molecule inhibited tubular epithelial cell proliferation, highlighting the specificity of the Hedgehog pathway to the mesenchymal compartment of the kidney.

Kidney-resident MSCs can contribute to nephron damage through the secretion of inflammatory cytokines. Both in the mouse kidney and in human cell cultures, fibrogenic NG2$^+$/PDGFRβ$^+$ MPCs sense acute damage-associated molecular signals via Toll-like receptor (TLR) signaling and respond by producing inflammatory cytokines and chemokines including IL-6, IL-1, and monocyte chemoattractant protein 1 (MCP1) (*40*, *41*). Sustained IL-6 and MCP1 contribute to parenchymal injury (*42*) and drive the recruitment of circulating immune cells, respectively, in a process that results in further inflammation. In addition, TLR signaling in kidney-resident MSCs also drives differentiation toward the matrix-producing contractile myofibroblast. These results highlight a potential critical signaling node that controls both fibrogenic as well as innate immune properties of kidney MSCs. Inflammatory signals converge on the multiprotein signaling complex called "myddosome," a signaling node formed by myeloid differentiation primary response 88 (MYD88), IL-1 receptor–associated kinase 4 (IRAK4), and IRAK1 (*40*). This complex integrates signals from multiple TLRs, IL-1, IL-18, and IL-33 to control downstream nuclear factor κ light chain enhancer of activated B cells (NFκB) and stress-activated kinase signaling pathways (*43*). Unlike the deleterious effects on cell survival observed when distal parts of NFκB signaling cascade are inhibited, blockade of the more proximal portions of the pathway appears safe. Recent advances in structure-based drug design have enabled the development of highly specific inhibitors of IRAK4, a kinase recently shown to directly transduce signals from the myddosome present in fibroblasts and damage-activated NG2$^+$ pericytes (*40*). IRAK4 inhibition appears to be a novel and potentially safe approach to directly target pathological kidney fibroblasts.

### Hepatic stellate cells in liver function and fibrosis

Hepatic fibrosis is the result of liver damage, which can be caused by a variety of etiological agents including viral infection, alcoholic liver disease, and nonalcoholic fatty liver disease (*44*). Analysis of the damage-associated fibrogenesis in the liver indicates that multiple fibrogenic MSC populations reside in this tissue. Lecithin-retinol acyltransferase (Lrat) expression identifies PDGFRβ$^+$ hepatic stellate cells (HSCs), a pericyte-like liver-specific MPC population residing along the sinusoids that represents ~10% of resident liver cells and is common to both human and mouse liver (Table 1) (*45*). In addition to HSCs, the liver contains CD146$^+$ pericytes with characteristics of MPCs (*46*), and portal fibroblasts (PFs) also known as biliary fibroblasts found in the mesenchyme surrounding the bile ducts (*47*). PFs play an important structural role, providing stability to ducts and the vasculature through the synthesis of basement membrane and microfibrillar components of the periductal ECM (*47*).

Injury to hepatocytes and cholangiocytes activates HSCs and PFs, respectively (*44*, *45*). Both cell types generate myofibroblasts: HSCs through transdifferentiation and PFs through direct fibrogenic differentiation (Fig. 2) (*48*). Although the contribution of HSCs to liver fibrosis is more prominent than that of PFs, the two cell populations have distinct dynamics of activation and differentiation that vary throughout the time elapsed after injury (*49*). Furthermore, the degree of activation seems to depend on the type of injury (*49*). Fate tracing studies in rodents indicated that HSCs generate 82 to 96% of

myofibroblasts in models of toxic, cholestatic, and fatty liver disease (*45*), whereas temporal analysis of both cell populations indicated that early myofibroblasts in cholestatic liver injury arise predominantly from PFs, whose contribution is prominent at the onset of injury and declines steadily thereafter (*44*, *45*). Similar to other tissues with regenerative capacity such as SM, liver fibrosis is a reversible process that can be at least partially abrogated after injury ceases (*50*). During regeneration, ECM clearance occurs as the number of myofibroblasts decreases. The latter is the result of three processes: activation of p75NTR leading to HSC apoptosis (*51*); senescence-activated killing by natural killer (NK) cells via NKG2D receptor, which can be induced by IL-30 treatment (*52*, *53*); and reversion through down-regulation of fibrogenic gene expression to a phenotype similar to that of quiescent HSCs (*54*). These three restorative processes offer interesting therapeutic avenues that remain to be explored.

Despite the fact that multiple mechanisms of liver fibrosis and resolution have been described, there are currently no therapies approved that target liver HSCs in fibrosis. One of the most promising therapies currently being tested clinically, cenicriviroc (CVC), is an oral antagonist targeting both C-C chemokine receptor 2 (CCR2) and CCR5 (*55*). CVC has both anti-inflammatory and antifibrotic effects, reducing monocyte/macrophage recruitment and tissue infiltration, as well as HSC activation (*56*). The latter mechanism is supported by recent data showing that CCL5 secreted by macrophages exposed to hepatitis C virus activates the expression of inflammasome and fibrosis markers in HSCs (*57*). Three clinical trials are currently underway to determine whether CVC is effective and safe in the treatment of nonalcoholic steatohepatitis (NASH) (CENTAUR, NCT02217475), to assess the long-term safety of continued treatment with CVC in participants who participated in the CENTAUR study (NCT03059446), and to confirm the efficacy and safety of CVC for the treatment of liver fibrosis in adult subjects with NASH (NCT03028740). In addition to inflammatory signaling, epigenetic targets are emerging as promising candidates with antifibrotic effects. Inhibition of the bromodomain and extraterminal protein family member BRD4 by the small-molecule JQ1 has been shown to significantly block HSC activation and proliferation, limiting the fibrotic response in carbon tetrachloride–induced fibrosis in a mouse model (*58*). Collectively, those findings point to the importance of inflammatory mechanisms and HSC epigenetics as future avenues toward liver fibrosis therapeutics.

### Perialveolar MSCs in pulmonary fibrosis

Pulmonary fibrosis is a heterogeneous type of interstitial lung disease associated with high morbidity and mortality. Although the causes are not completely understood, pulmonary fibrosis can be initiated when epithelial injury triggers a fibrogenic process that, similar to one in the liver, can be resolved upon interruption of the etiological agent. Unlike other tissues in which the major fibrogenic progenitor MSC populations have been identified, the lung stroma contains a complex milieu of tissue-resident MSCs. Independent fate tracing studies have partially clarified the fibrogenic process in this organ, albeit without completely elucidating the origin of all fibroblasts and myofibroblasts (*59*, *60*). These studies have identified multiple pericyte-like populations closely associated with the pulmonary microvasculature, as well as different types of perialveolar fibroblasts (Fig. 2) (*59*, *60*). Within the population of pericyte-like MSCs, a population of PDGFRβ$^+$/NG2$^+$ MPCs derived from embryonic forkhead box D1 (FoxD1)$^+$ mesenchymal progenitors gives rise to an estimated 45% of the αSMA$^+$ myofibroblasts observed in the mouse bleomycin lung injury model (Table 1) (*59*). Notably, not all pericyte-like cells in the lung are fibrogenic progenitors, as indicated by lineage tracing of PDGFRβ$^+$/NG2$^+$ pericyte-like mural cells, which proliferate in response to damage but are reported not to give rise to myofibroblasts (Table 1) (*60*). In addition, collagen I–expressing perialveolar NG2$^-$/desmin$^+$/PDGFRα$^+$ fibroblasts, which proliferate upon alveolar damage, contribute to interstitial fibrosis (Table 1) (*59*, *60*). In addition to those lung MSC populations, recent data have highlighted a role for lipofibroblasts, lipid droplet–containing fibroblasts located adjacent to alveolar epithelial cells type 2 (AEC2), in lung fibrosis has been reported (*61*). The lipofibroblast population overlaps with the population of PDGFRα$^+$ fibroblasts, and it is possible that one subpopulation gives rise to the other, although more evidence will be needed to test that hypothesis. After injury, lipofibroblasts traced by the expression of Adrp—also known as perilipin 2—undergo a lipogenic-to-myogenic phenotype switch driven by FGF-10 signaling to generate myofibroblasts (*61*). Lipofibroblast conversion can be reverted by peroxisome proliferator–activated receptor γ activation in the context of repair during fibrosis resolution, when lipofibroblast-derived myofibroblasts revert their phenotype by means of a myogenic-to-lipogenic switch (*61*). Lineage tracing analysis of AEC2 in the bleomycin model indicated that those cells do not generate myofibroblasts through epithelial-to-mesenchymal transition (*60*), as was previously believed.

Similar MSC populations have also been identified in the lungs of patients with idiopathic pulmonary fibrosis (IPF) (*60*), the most common clinical form of lung fibrosis. IPF begins in the alveolar and distal bronchiolar regions of the lung because injured alveolar and distal bronchiolar epithelium activates its associated mesenchyme. Fibrotic lesions predominantly affect the peripheral regions of the lung. In more advanced disease stages, gross destruction of the lung architecture results in a "honeycombed" tissue appearance. Two therapies have been approved for IPF by the FDA, namely, pirfenidone (Esbriet, Genentech) and nintedanib (Ofev, Vargatef, Boehringer Ingelheim). Both pirfenidone and nintedanib reduce proliferation as well as αSMA and fibronectin expression in lung fibroblasts from IPF patients in vitro (*62*), indicating that MSCs are the primary target for both molecules. Nintedanib is a small molecule that inhibits vascular endothelial growth factor receptor (VEGFR), PDGFR, and FGF receptor signaling. Although no precise molecular mechanism or target has been identified for pirfenidone, clinical results from a phase 3 trial (ASCEND study) showed reduced IPF progression, improved exercise tolerance, and increased progression-free survival in patients with IPF (*63*).

Lysophosphatidic acid (LPA) receptor 1 (LPAR1), a G protein–coupled receptor restricted to fibroprogenitor cells and their progeny, has become an attractive target for fibrosis (*64*). Activated by the bioactive lipid LPA released by many cells in response to injury, LPAR1 stimulates activation and differentiation of MSCs to fibroblasts and activates profibrotic cell signaling pathways including protein kinase B, extracellular signal–regulated kinase (ERK), and yes-associated protein/transcriptional coactivated with PDZ-binding motif signaling, which has recently been implicated in persistence of fibroblasts in a pathological state (*65*, *66*). An inhibitor of LPAR1, Bristol-Myers Squibb's BMS-986020 (formerly Amira AM-152), has been shown to be effective in preclinical models of degenerative diseases and is currently in phase 2 clinical trials for the treatment of IPF (*67*). Other molecules currently tested in clinical trials include biological agents blocking connective tissue growth factor (CTGF) (Pamrelumab, FibroGen), alpha v beta 6 ($\alpha_v\beta_6$) integrin (BG00011, Biogen), IL-4/IL-13

(SAR156597, Sanofi), and IL-13 (Lebrikizumab, Roche), all of which target activated fibroblasts derived from the resident MSC populations.

### Lower dermis as a source of fibrogenic MSCs in dermal fibrosis

Dermal fibrosis is a hallmark of several cutaneous disorders. Recently, populations of skin MSCs residing in distinct layers of the dermis that contribute to skin connective tissue have been identified. A single embryonic lineage has been defined in mice by the expression of engrailed 1 (EN-1), a homeobox protein that is no longer expressed in adulthood (*68*). Although at early embryonic stages EN-1–derived cells represent less than 1% of total dermal fibroblasts, this fibroblast pool expands throughout development and early postnatal stages to comprise 75.3% of total dermal fibroblasts at postnatal day 30 (*68*). In the adult mouse skin, EN-1–derived fibroblasts express several fibroblast markers, including *Pdgfrα*, Vimentin (*Vim*), prolyl-4 hydroxylase β (*P4hb*), collagen 1a1 (*Col-1a1*), *Col-3a1*, and fibrillin 1 (*Fbn1*) (*66*) and the surface markers CD34, LY-51, CD54, CD61, and CD26. EN-1–derived fibroblasts produce the majority of the deposited connective tissue within the underlying dermis, the stroma surrounding hair follicles, and dermal papillae in homeostasis (*68*). Further analysis of CD26, Sca1, and delta-like notch ligand-1 (Dlk1) expression led to the identification of three main fibroblast subpopulations. Papillary dermal $CD26^+$ $Sca1^-$ fibroblasts contribute to upper dermis connective tissue, whereas $Dlk^+$ $Sca1^+$ and $Dlk^-$ $Sca1^+$ MSCs include reticular fibroblasts, preadipocytes, and adipocytes residing in the hypodermis (Table 1) (*69*). $Dlk1^+$ MSCs become activated rapidly and generate the initial wave of myofibroblasts that produce most of the ECM during the fibrogenic response associated with wound healing (Table 1) (*69*). Upper dermis MSCs, on the other hand, generate fibroblasts that lie underneath the repaired epidermis, late in the healing process (*69*). $Dlk^+$ MSCs of the hypodermis therefore appear to be the major ECM-producing population with fibrotic activity in the dermis.

Expression of a disintegrin and metallopeptidase domain 12 (ADAM12) labels a population of perivascular MSCs identified in the mouse dermis (*23*). $ADAM12^+$ MSCs are unipotent fibrogenic progenitors, which, similar to $Dlk1^+$ MSCs, become activated promptly after skin injury and proliferate to generate pools of $\alpha SMA^+$ myofibroblasts (Table 1 and Fig. 2) (*23*). Activation of ADAM12 occurs in multiple types of fibrosis (*23*), including diffuse cutaneous systemic sclerosis (SSc) (*70*). The splice variant ADAM12-L is a target of miR-29b, a microRNA targeting ECM genes in several fibrotic diseases. A phase 1 clinical study to test the tolerability of a synthetic miR-29b microRNA mimetic in healthy patients is underway (miRagen), and the trial will pave the way to test the effect of the molecule in scleroderma/SSc, a disease of autoimmune origin. Fibrogenic reticular and perivascular MSCs of the dermis are activated through the 5-hydroxytryptamine receptor 2B (5-HT2B) receptor by serotonin released by platelets (*71*). Targeting platelet activity was first regarded as a potential therapeutic avenue to reduce scleroderma-associated fibrosis, and new findings support a role for clopidogrel, a drug blocking the activation of platelets in SSc. However, clopidogrel has been found to worsen endothelial function without reducing serotonin levels in 13 patients with SSc (*72*). Similar to lung fibrosis, the LPA signaling pathway activates skin fibrogenic MSCs and plays a major role in scleroderma (*73*). In a small-scale phase 2 clinical trial, treatment of SSc patients with a well-tolerated LPAR antagonist molecule (SAR100842, Sanofi) resulted in significantly reduced key skin fibrotic biomarkers and improved modified Rodnan skin scores. In addition, after a successful phase 1 clinical trial completed in 2015, an efficacy and tolerability trial is planned for the LPAR1 inhibitor currently undergoing phase 2 clinical trials for IPF (Bristol-Myers Squibb's BMS-986020, formerly Amira AM-152).

Oncostatin M, a cytokine from the IL-6 family, activates JAK/STAT, ERK1/2, and p38 mitogen-activated protein kinase (MAPK) signaling in dermal fibroblasts to stimulate ECM production (*74*). A study is currently ongoing to test the safety, tolerability, pharmacokinetics, and pharmacodynamics of repeated subcutaneous doses of a humanized monoclonal antibody that blocks oncostatin M (GSK2330811, GlaxoSmithKline). Development of scleroderma is also associated with increased local (dermal) and systemic production of IL-4 (*75*). IL-4 and IL-13 bind types I and II IL-4Rα expressed in dermal fibroblasts, activating JAK-2 and p38 MAPK to up-regulate the expression of TGFβ, tenascin, and tissue inhibitor of metalloproteinase 2 (*75*). A study to test the effectiveness and safety of subcutaneous delivery of a tetravalent bispecific antibody against IL-4/IL-13 (SAR156597) is currently recruiting participants. In sum, a deeper understanding of the molecular pathways driving fibrogenic dermal MSC activity in cutaneous fibrosis can accelerate the development of more efficacious therapies for the treatment of cutaneous fibrosis.

## CONCLUDING REMARKS

MSCs present unique properties depending on their environment and tissue of residence. Several lines of evidence further support the notion that TGFβ and other molecules, previously considered to play a well-defined role in fibrosis across multiple organs, are actually less prominent than other tissue-specific mechanisms. The combination of transgenic mouse models, instrumental for both the study of mechanisms of fibrosis in vivo and preclinical testing of antifibrotic compounds, allows the evaluation of emerging antifibrotic therapies with higher accuracy. Limitations arising from differences between mice and human biology and from the etiology of the fibrogenic process in those experimental models versus human diseases, however, preclude the direct extrapolation of experimental data and require careful interpretation of results obtained with those models.

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