HHS Public Access

Author manuscript

Curr Opin Rheumatol. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

Curr Opin Rheumatol. 2015 November ; 27(6): 555–562. doi:10.1097/BOR.0000000000000217.

Origin of Fibrosing Cells in Systemic Sclerosis

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Abstract

Purpose of review—Systemic sclerosis (SSc), an autoimmune disease of unknown origin, is characterized by progressive fibrosis that can affect all organs of the body. To date, there are no effective therapies for the disease. This paucity of treatment options is primarily due to limited understanding of the processes that initiate and promote fibrosis in general and a lack of animal models that specifically emulate the chronic nature of systemic sclerosis. Most models capitulate acute injury-induced fibrosis in specific organs. Regardless of the model however, a major outstanding question in the field is the cellular origin of fibrosing cells.

Recent findings—A multitude of origins have been proposed in a variety of tissues, including resident tissue stroma, fibrocytes, pericytes, adipocytes, epithelial cells, and endothelial cells. Developmentally derived fibroblast lineages have recently been elucidated with fibrosing potential in injury models. Increasing data supports the pericyte as a fibrosing cell origin in diverse fibrosis models and adipocytes have recently been proposed. Fibrocytes, epithelial cells, and endothelial cells have been examined, though data does not as strongly support these possible origins.

Summary—In this review, we discuss recent evidence arguing in favor of and against proposed origins of fibrosing cells in diverse models of fibrosis. We highlight outstanding controversies and propose how future research may elucidate how fibrosing cells arise and what processes can be targeted in order to treat systemic sclerosis.

Keywords

fibrosis; myofibroblast; fibrosing cell; lineage tracing; systemic sclerosis

Introduction

Fibrotic tissue is characterized by excessive deposition of extracellular matrix proteins including collagen and fibronectin, as well as increased numbers of fibroblasts expressing the contractile protein alpha smooth muscle actin (αSMA) within the interstitial spaces of tissues. Both of these aberrant processes increase tissue stiffness. Uncorrected, these pathologic processes ultimately result in organ failure. In all forms of fibrosis, including

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Conflicts of Interest
None.

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systemic sclerosis (SSc), the major cell type considered to deposit excess collagen and smooth muscle actin is called the myofibroblast (1). This term was given by Gabbini and colleagues to describe fibroblast-like cells present in granulation tissue that harbor extensive filamentous structures in their cytoplasm and exhibit deformed nuclei, evidencing cell contraction (2,3). The microfilametous contractile apparatus in these cells was later shown to contain myosin and αSMA, which subsequently became the primary marker for the cell type. (4). In addition to fibrosis, myofibroblasts are predominantly responsible for depositing collagenous scar tissue during wound healing, and their presence has been noted in association with epithelial tumors (5). During wound healing, myofibroblasts ultimately undergo apoptosis resulting in scar resolution (5). In fibrosis however, myofibroblasts persist, leading to progressive and chronic scar deposition. As myofibroblast presence is characteristic of all forms of organ fibrosis, the criteria for a cell to be deemed fibrotic is the loss of its initial features and the acquisition of a myofibroblast phenotype. We will therefore primarily examine lineage-tracing studies that follow the fate of cells from various origins, outlined below, in the context of diverse organ fibrosis models.

Many studies on the origin of fibrotic cells have examined whether myofibroblasts arise from circulation or from resident cells within the afflicted tissue. Fibrocytes are hematopoietic cells that express markers in common with leukocytes, including CD45, CD11, CD18, and CD13 as well as some fibroblast markers including Vimentin and Collagen type 1 (6). They also express CD34, a marker expressed by bone marrow progenitor cells, capillary endothelium, and fibroblasts (7). Parabiosis and hematopoietic cell transplantation experiments indicate that resident stromal cells are the primary source of fibrosing cells and that fibrocytes support fibrosis progression. We highlight below the major evidence for the role of fibrocytes, and possible resident effector cells, including developmentally derived tissue fibroblasts, pericytes, adipocytes, epithelial cells, and endothelial cells (Table 1).

Fibrocytes

Fibrocytes have been considered a source of fibrosing cells, as they express *Collagen 1* and display elevated numbers in acute wounds and fibrotic tissues (6). Fibrocytes have been detected in approximately half of patients with systemic sclerosis (SSc)-associated interstitial lung disease and their presence was positively correlated with disease severity (36). Intravenous delivery of CD45+ Col1+ fibrocytes into mice with TGFα-induced lung fibrosis increased disease severity while delivery of CD45− Col1− fibroblasts did not (37). Additionally, fibrocytes have been shown to be elevated in patients with idiopathic pulmonary fibrosis and are mostly absent from healthy lungs (36,38).

Most studies on fibrocytes have examined their role in bleomycin-induced lung fibrosis. In this model, it was recently observed that resident tissue fibroblasts actively secrete a factor that blocks fibrocyte differentiation from monocytes, the neuronal guidance protein Slit2 (39). Injection of Slit2 led to a reduction of fibrocyte number in fibrotic lesions with a reduction of lesional collagen and overall fibrosis severity (39). A previously discovered, liver-derived factor called serum amyloidP (SAP) also inhibits fibrocyte differentiation *in vitro* (40) and reduces fibrosis severity when injected *in vivo* (41-43). However, it was later

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suggested that the therapeutic effect may be attributed more to a reduction of activated M2 macrophage numbers in fibrotic lesions than the effect on fibrocytes specifically (42,43).

Despite the correlation between fibrocyte presence and fibrosis severity, a recent genetic ablation study, in which the *Col1a1* gene was deleted from cells expressing the pan-hematopoietic marker Vav1, suggested that fibrocytes are not major source of Collagen 1 in bleomycin-induced lung fibrosis (11). Though the authors observed that fibrocytes synthesized Collagen 1 at the transcript level, they found that CD45+ Col1+ fibrocytes produced far less Collagen 1 protein than CD45− Col1+ fibroblasts. Furthermore, the detection of intracellular Collagen 1 in fibrocytes using *Vav1*Cre; *Col1a1* floxed mice indicated that the majority the intracellular Collagen 1 harbored by fibrocytes might be taken up from other cells rather than synthesized *de novo*. Additionally, fibrocytes were observed to be mostly negative for αSMA co-localization (less than 10%) in the TGFα-induced pulmonary fibrosis model (37). In a recent kidney fibrosis study, it was noted that although approximately 50% of αSMA+ myofibroblasts were bone-marrow derived, ablation of circulatory fibroblasts had no impact on fibrosis severity (16). Other bone marrow-transplantation studies have shown that fibrosing cells derive from local sources and not from circulation (8,12,16,20,21). Collectively, these findings indicate that despite plausible importance of fibrocytes in mediating disease severity, fibrocytes are not a major fibrosing cell origin. It will be important in the future to elucidate the precise function of fibrocytes in the progression of fibrosis, specifically, how they facilitate increased deposition of extracellular matrix by myofibroblasts from other sources.

Developmentally Derived Tissue Fibroblasts

A possible source of fibrosing cells is the resident fibroblasts that make up the connective tissue of organs during homeostatic conditions. In culture, fibroblasts are known to acquire myofibroblast markers in response to mechanical stimuli or treatment with signaling molecules such as TGFβ1 or Wnt ligand (1). However, recent studies have illuminated high heterogeneity within resident tissue fibroblast populations. Genetic lineage tracing wherein mice expressed Cre recombinase driven by the *Dlk1* or *Lrig1* promoters revealed that dermal fibroblasts arise developmentally from two separate lineages (8). Dlk1-labeled stromal cells made up the majority of the lower dermis, while Lrig1- labeled cells constituted the upper dermis. During wound healing, Dlk1-traced fibroblasts infiltrated the wound bed and co-stained for α-smooth muscle actin, indicating myofibroblast transition, while Lrig1+ fibroblasts were observed within the wounded area during later stages of repair. Bone marrow transplantation revealed that there was no contribution of bone-marrow derived cells to the wound fibroblasts, indicating that in dermal wound healing, 100% of the wound myofibroblasts derive from resident cells (8,25).

Subsequently, two additional lineages of resident dermal fibroblasts have been identified, denoted by expression of *Engrailed-1* in the dermis and *Wnt1* in the oral dermis (9). These lineages were examined for their function in wound healing, radiation induced fibrosis, and melanoma. Ablation of *Engrailed-1*-expressing fibroblasts using the diphtheria toxin system resulted in diminished collagen deposition in wounded skin and reduced expansion of fibroblasts associated with melanoma. Whether developmentally derived fibroblast lineages

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are distinct in adult tissue, and which of these lineages contribute to diverse fibrosis models will be important to define.

Perivascular Stem Cells (includes mesenchymal stem cells and pericytes)

Pericytes and mesenchymal stem cells are non-endothelial (CD31−), nonhematopoietic (CD45−) cells that reside in tight association with organ vasculature and have been implicated in fibrosis. Mesenchymal stem cells (MSCs) are defined by their ability to differentiate into many lineages *in vitro*, though there is much controversy over the specific markers they express (44). Evidence suggests that these cells can be directed towards different fates in healthy versus pathologic conditions (24,45). Following skeletal muscle injury, for example, myogenic MSCs are shifted away from the myogenic fate and towards adipocyte or fibroblast fates (46-50).

Pericytes reside directly adjacent to endothelial cells on the outer walls of blood vessels, and markers typically used to identify them include NG2, PDGFRβ, CD135, Nestin, Desmin, and FoxD1 (Table1). Additionally, pericytes have been noted to share some myofibroblasts markers, including αSMA and Thy-1 (51). Pericytes also demonstrate multi-lineage differentiation capacity *in vitro* and are therefore considered to be somewhat synonymous with mesenchymal stem cells (44). Though their function is not well understood, they seem to be required for vascular integrity, as their ablation leads to dilated vessels and hemorrhage (52-55). They are also increasingly considered as important forerunners of fibrosing cells in acute tissue injury and fibrosis. Activated perivascular cells expressing *PDGFRβ* have been observed in the dermis in association with the vasculature in skin biopsies from patients with early stage systemic scleroderma and autoimmune Raynoud's syndrome, but not in healthy controls, primary Raynoud's, or late stage scleroderma (51).

Perivascular cells are found in the perivascular space of every examined organ and are implicated in diverse fibrosis models. In injured skeletal muscle and ear dermis, perivascular cells lineage-traced from the *ADAM12* promoter were found to proliferate and produce both αSMA and Collagen 1 (12). As with resident fibroblasts during skin wound healing, these activated cells diminished as the injury resolved. Ablation of these cells led to a substantial decrease in fibrotic tissue formation (12). Similarly, following spinal cord injury, pericytes were shown to disassociate from vasculature, proliferate, and express pro-fibrotic proteins αSMA and Fibronectin (13,14). They were also required for injury resolution when traced from the *Glast1* promoter (13) or *Nestin* promoter (14).

The functional role of pericytes has been intensely examined in kidney fibrosis. Lineage tracing studies from several promoters, including *Cspg4* (*NG2*), *PDGFRβ, Col1a1*, and *FoxD1* have demonstrated the ability of pericytes to expand and differentiate into myofibroblasts in response to UUO-induced kidney fibrosis (10,16,17,19,21). Additionally, *Foxd1*-expressing pericytes follow this scheme in the context of bleomycin-induced lung injury (18). In white adipose tissue fibrosis, induced by overactive PDGFRα signaling, pericytes lineage-traced from the *Nestin* promoter were shown to proliferate and acquire myofibroblast characteristics (15). Deletion of α_v*-integrin* from *PDGFRβ*-expressing pericytes in the lung and kidney attenuated fibrosis (19) suggesting a functional role for

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these cells as contributors to fibrosis. However, ablation of NG2 or PDGFRβ-labeled cells did not ameliorate fibrosis in the kidney (16).

A recent study described a class of *Gli1+*, *PDGFR*β-expressing perivascular mesenchymal progenitor cells at the origin of fibrosis in diverse organs (20). Using genetic lineage tracing wherein tdTomato was expressed from the *Gli1* promoter, the authors showed that *Gli1*-expressing perivascular cells are present in low numbers in the heart, kidney, liver, and lung and that they expand in fibrotic conditions. Concomitantly, ablation of *Gli1*-expressing cells ameliorated fibrosis. Another study, wherein pericytes were examined in different organ models of fibrosis, including lung, kidney, heart, spinal cord, and brain, suggested a functional distinction between Type 1 pericytes (defined as Nestin−, NG2+) which accumulated with fibrosis progression, and Type 2 pericytes (Nestin+, NG2+), which did not expand (14).

Collectively, these studies indicate that perivascular cells make a substantial contribution to the formation of fibrotic tissue. Despite this, most studies indicate that they do not contribute 100% to the myofibroblast pool, implying that there are still other cellular origins. Additionally, it is still unclear to what extent these cells functionally contribute to fibrosis severity. In the future, it will be important to more fully understand the role of these cells under homeostatic conditions, to determine the extent of their contribution to fibrosis, and to elucidate the mechanisms that orient them towards the myofibroblast fate in pathological contexts.

Adipocytes

Adipocytes have not been predominantly considered as an important source of fibrosing cells in SSc, though there has been increasing evidence that they contribute to fibrosis. Strikingly, development of dermal fibrosis in SSc patients is accompanied by loss of subcutaneous adipocytes (57), and this phenotype is well recapitulated in animal models of dermal fibrosis (58-68). Loss of adipose tissue and its replacement with fibrous tissue has been observed in other pathologies as well, including cancer cachexia, epithelial tumors, and obesity (69-72). In the liver, trans-differentiation of lipid containing hepatic stellate cells into myofibroblasts is the primary driver of fibrosis (22,27,73,74). Therefore, a novel yet attractive model is that adipocytes or related lipid-containing cells may be an origin of fibrosis.

Using genetic lineage tracing analysis wherein the *Adiponectin* promoter drove Cre-mediated expression of tdTomato, labeling mature adipocytes, Marangoni and colleagues recently observed that induction of fibrosis caused *Adiponectin*-expressing cells to trans-differentiate into a cell type resembling myofibroblasts, migrate into the dermis, and express αSMA (26). They also observed that dermal adipocytes were attenuated prior to increased collagen deposition and dermal thickness, and that a reduction of adipogenic gene expression preceded the increase in fibrotic gene expression. Interestingly, several therapeutic strategies shown to ameliorate fibrosis in mice, such as treatment with TGFβ neutralizing antibody or the PPARγ agonist rosiglitazone, positively facilitate adipogenesis (59,65,75-79). Future studies will elucidate whether adipocytes are essential to the development of fibrosis and whether this mechanism will apply to fibrosis in other organs.

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Epithelial Cells

Epithelial to mesenchymal transition (EMT) has been considered as a possible source of myofibroblasts in fibrosis, though this model has become less popular in light of most recent lineage tracing studies. In the fibrosis context, the model was largely based on *in vitro* studies showing that epithelial cells can express myofibroblast markers such as FSP1 and αSMA in response to TGFβ stimulation (23,28,80-83). EMT is also known to support metastasis in epithelial cancers and is a key component of vertebrate development (82). However, lineage-tracing studies examining EMT in fibrosis models have not strongly supported the contribution of epithelium to fibrotic cells. In liver fibrosis models, lineage-tracing via albumin-Cre in hepatic epithelial cells yielded controversial results (23,28), and a subsequent study where hepatic epithelial cells were traced with promoter *Alfp* found no contribution of these cells to the myofibroblast pool (29). In UUO-induced kidney fibrosis, lineage-tracing of *γGT*-expressing tubular epithelial cells suggested co-localization of labeled cells with myofibroblasts markers FSP1 and Hsp47 (30), though later studies lineage tracing tubular epithelial cells via promoters *Six2* and *HoxB7* did not find any trace-labeled interstitial myofibroblasts (17). Another UUO-induced kidney fibrosis study tracing tubular epithelial cells with *γGT*-Cre found that only approximately 5% of myofibroblasts originated from these cells (16). In bleomycin-induced lung fibrosis, alveolar epithelial cells traced with the *Sftpc* or *Scgbla1* promoters proliferated in fibrotic conditions, however they did not stain positive for myofibroblasts markers S100A4, Vimentin, or αSMA and therefore were not shown to contribute to the fibrosis (31).

Endothelial Cells

There is not strong evidence supporting a large contribution of endothelial cells to tissue fibrosis. In bleomycin-induced pulmonary fibrosis, cells marked by the pan-endothelial marker Tie2 were isolated and shown to upregulate myofibroblast genes *αSMA* and *Col1a1* compared to cells from saline-treated mice (79). Additionally, this group showed that endothelial cell lines could be induced to acquire a myofibroblast phenotype when treated with either TGFβ or activated Ras, while losing expression of endothelial markers CD31, VE-cadherin, and CD34 (79). In a cardiac fibrosis model, lineage tracing of another endothelial marker Tie1 showed co-labeling of Tie1 with myofibroblast marker Fsp1, concomitant with similar loss of endothelial markers in labeled cells (35). In the kidney, lineage tracing, in which YFP was expressed under control of either the *Tie2* or *Cdh5* promoter, also showed a contribution of endothelial cells to the fibrotic cellular pool (16,34).

Despite some arguments in favor of endothelial contributions in several forms of fibrosis, these studies are confounded by other evidence that Tie2 is expressed in not only endothelial cells but also other non-endothelial cell types, including non-endothelial mesenchymal stem cells (32). Additionally, CD34 is not entirely specific for endothelium, as it also labels bone marrow stem cells, nonendothelial mesenchymal stem cells, subsets of dendritic cells, and dermal fibroblasts (7,8). Additional lineage tracing studies using more specific Cre drivers, such as CD31 or VEcadherin, as well as the use of more specific endothelial cell markers, will be needed to determine if endothelial to mesenchymal transition actually contributes to fibrosis.

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Conclusion

Identifying the origin of fibrosing cells has been a daunting task, due to the diversity of cell types proposed, numerous organ fibrosis models, and to the specificity and variable efficiency of labeling cell subsets in lineage-tracing studies. While myofibroblasts producing αSMA and collagen are regarded as the principal effector cells, it should be considered that cells expressing either of these markers might contribute to increased extracellular matrix deposition and tension within fibrotic tissues.

Perivascular stem cells are increasingly being acknowledged for their capability to produce myofibroblasts in diverse models of injury and fibrosis. A future challenge will be to more fully identify markers to characterize these cells and to better understand their functions. However, evidence in favor of contributions of diverse cell types, from endothelial cells to adipocytes to resident mesenchymal cells is being actively generated. It remains to be seen how interactions between fibrosing cells and other cell types affected by the process leads to disease progression. Currently, there are not effective therapies for the treatment of fibrosis, despite an abundance of molecular targets (84). In the future, the development of new therapeutics able to specifically target the cells responsible for the deposition of extraneous collagen and expression of alpha smooth muscle actin will be needed to halt fibrosis progression while leaving bystanding cells intact, in the hopes that researchers can identify ways to regenerate tissue for a full recovery from fibrosis.

Acknowledgments

None.

Financial support and sponsorship

SE is supported by the National Science Foundation Graduate Research Fellowship Program and NIH Training Grant (T32 GM007223). VH is supported by the NIH (AR060295) and the Connecticut Department of Public Health (12SCBYALE01)

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Key Points

- Recently defined lineages of resident tissue fibroblasts have the capacity to become myofibroblasts during injury and tumor formation, though it remains to be seen how they contribute to chronic fibrosis.
- Lineage tracing studies indicate pericytes as a myofibroblast origin in diverse fibrosis models.
- Adipocytes have recently been proposed as an origin of myofibroblasts in dermal fibrosis, reminiscent of hepatic stellate cell activation in liver fibrosis.
- Studies examining the contribution of epithelial and/ or endothelial cells to fibrosis suggest that these are not a primary source of fibrosing cells.

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Table 1

Murine genetic tools for analysis of fibrotic cell types.

Cell Type	Driver	Use	Organ	Reference
Dermal fibroblast	*Dik1*	• Lineage tracing	• Dorsal dermis	(8)
	Lrig1	• Lineage tracing	• Dorsal dermis	(8)
	Blimp1	• Lineage tracing	• Dorsal dermis	(8)
	PDGFRα	• Genetic reporter	• Dorsal dermis	(8)
	Engrailed -1	• Lineage tracing • Ablation	• Dorsal dermis	(9)
Oral fibroblast	*Wnt1*	• Lineage tracing • Ablation	• Oral dermis	(9)
Interstitial fibroblast	*Myelin protein zero (P0)*	• Lineage tracing • Genetic reporter	• Kidney	(10)
Fibrocyte	*Vav1*	• Conditional knock out of *Col1a1*	• Hematopoietic cells	(11)
Pericyte	*ADAM12*	• Lineage tracing • Ablation • Genetic Reporter	• Ear dermis • Skeletal muscle	(12)
	Glast1	• Lineage tracing • Blocking cell proliferation	• Spinal cord	(13)
	Nestin	• Lineage tracing • Genetic reporter	• Spinal cord • Brain • Lung • Kidney • Heart • White adipose tissue	(14,15)
	NG2 (Cspg4)	• Genetic reporter • Ablation	• Spinal cord • Brain • Lung • Kidney • Heart	(14,16)
	αSMA (Acta2)	• Lineage tracing • Genetic reporter • Conditional knock out of *TGFβR2*	• Kidney	(16)
	FoxD1	• Lineage tracing • Genetic reporter	• Kidney • Lung	(17,18)
	PDGFRβ	• Genetic reporter • Ablation • Conditional knock out of *αv integrin*	• Kidney • Lung	(16,19)
	Gli1	• Lineage tracing • Ablation	• Heart • Kidney • Liver • Lung	(20)
	Col1a1	• Lineage tracing • Genetic reporter	• Kidney • Liver • Dermis • Skeletal muscle	(21,22,23,24,25)
Adipocyte	*Adiponectin*	• Lineage tracing	• Dorsal dermis	(26)
Hepatic stellate cell	*Lrat*	• Genetic reporter	• Liver	(27)
Epithelial cell	*Albumin*	• Lineage tracing	• Liver	(23,28)
	Alfp	• Lineage tracing	• Liver	(29)
	γGT	• Lineage tracing	• Kidney	(16,30)
	FSP-1	• Genetic reporter	• Kidney	(30)

Cell Type	Driver	Use	Organ	Reference
	Six2	• Lineage tracing	• Kidney	(17)
	HoxB7	• Lineage tracing	• Kidney	(17)
	Sftpc	• Lineage tracing	• Lung	(31)
	Scgbla1	• Lineage tracing	• Lung	(31)
Endothelial cell	*VECadherin (Cdh5)*	• Lineage tracing	• Kidney • Skeletal muscle	(16,32)
	Tie2	• Lineage tracing	• Kidney • Lung • Skeletal muscle	(32,33,34)
	Tie1	• Lineage tracing	• Heart	(35)

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