# The Vessels Contribute to Fibrosis in Systemic Sclerosis

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**ABSTRACT:** Microvascular damage, clinically expressed by Raynaud's phenomenon, is generally the first symptom of the disease and the injured vascular cells, both endothelial and perivascular, may transdifferentiate to myofibroblasts, thus leading to collagen deposition in the tissue and consequent fibrosis. Systemic sclerosis (SSc, scleroderma) is a complex disease characterized by autoimmunity, vasculopathy, and fibrosis. It has been shown that microvascular damage may be the first symptom of SSc. Injured endothelial cells and pericytes may transdifferentiate into myofibroblasts, the cells responsible for fibrosis and collagen deposition in the tissue. Based on these factors, the process of myofibroblast generation may link two pivotal events of SSc: microvascular damage and fibrosis. Understanding the development, differentiation, and function of myofibroblasts is therefore crucial to individuate early pathogenetic events and develop new therapeutic target for SSc, a condition in which no disease-modifying agents are available. The aim of this review was to discuss the possible origins of myofibroblasts in SSc, highlighting the process of endothelial mesenchymal transition and pericytes to myofibroblast transition and to show how these events may contribute to pathogenesis of the disease.

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**KEY WORDS:** endothelial cells, fibrosis, pericytes, mesenchymal stem cells, systemic sclerosis

Systemic sclerosis (SSc, scleroderma) is a rare and complex, multi-system connective tissue disorder affecting the skin and internal organs. It is characterized by widespread microangiopathy, fibrosis, and autoimmunity [1-4]. SSc usually affects young women and leads to severe disability [5-8] such as reduced mouth opening, loss of hand function, pain, and psychological consequences [8].

Clinically, SSc is a very heterogeneous disease, classified by the pattern and extent of skin involvement. In limited cutaneous SSc (lcSSc), fibrosis is mainly present in hands, arms, and face. In diffuse cutaneous SSc (dcSSc), the fibrosis affects the trunk, extremities, and internal organs [1,2,9,10] such as lungs [11], gastrointestinal tract [12], kidneys [13], heart [14], and central and peripheral nervous system [15].

**Microvascular injury may be the first symptom of systemic sclerosis**

In lcSSc, the course of fibrosis is typically benign and does not cause significant disability, although these patients often present more vascular features. In dcSSc, skin and internal organ fibrosis tends to be accelerated in the first 5 years of disease duration. In the latter subset of patients, there is radiographic evidence of lung fibrosis in more than 90% and it becomes clinically significant in 25% of patients [16,17]. Compared to skin fibrosis, lung fibrosis in SSc is typically progressive. In addition, the two subsets of the disease are characterized by different autoantibody patterns, identifying the risk of specific complication [1,18,19]. In fact, it has been shown that antinuclear antibodies (ANAs) may help to predict the clinical development of SSc. The presence of autoantibodies for endothelin or angiotensin II may be associated with more progressive forms of SSc [20]. Furthermore, the autoantibodies for platelet-derived growth factor receptor may play a role in fibrotic alterations, activating fibroblast intracellular signaling [21].

In this context, accumulating evidence indicates that the primary target for both initiating and propagating SSc is blood vessels [3,4]. Signs of dysfunctional vascular thermoregulation, clinically presenting as Raynaud's phenomenon, occur at disease onset in almost every patient. The capillary network of SSc patients shows a reduced density and an irregular chaotic architecture. These changes result in a decreased capillary blood flow, leading to tissue hypoxia, which is the major stimulus for angiogenesis that is necessary to compensate for the lack of oxygen [22].

Despite tissue hypoxia in SSc patients, the compensatory angiogenic mechanisms are impaired, leading to progressive vessel degeneration. It is well known that the vascular damage precedes and contributes to the onset of fibrotic alterations during SSc. In fact, endothelial cells (ECs) and pericytes, after injuries, could transdifferentiate toward myofibroblast, which releases increased amounts of collagen type I, III, VI, and VII; fibronectin; and glycosaminoglycans [23], resulting in the disruption of the affected tissue's architecture [16].

Accordingly, the understanding of the pathogenic link between vascular damage and myofibroblast generation in SSc may help to assess the early mechanisms responsible of fibrosis and to individuate new therapeutic targets. In fact, at present, no disease-modifying agents are available for SSc, partially due to the lack of understanding of its pathophysiology [24]. In this review, we aimed to discuss the possible

origin of myofibroblasts in SSc, how these cells may derive from both ECs and perivascular cells activated after vascular injuries, and how they may contribute to pathogenesis of the disease.

### INTERPLAY BETWEEN ENDOTHELIAL CELLS AND PERIVASCULAR CELLS OF SYSTEMIC SCLEROSIS PATIENTS

The ECs are the basic layer of blood vessels. During physiological angiogenesis, they may proliferate and migrate toward the perfused tissues. During this migration, ECs interact with surrounding perivascular cells and extracellular matrix (ECM), establishing among them an interplay, which supports the new blood flow. In fact, the cross-talk between ECs and pericytes is crucial in regulating vasculogenesis and angiogenesis and its disruption leads to a variety of congenital and acquired diseases [25,26]. Furthermore, the cross-talk between ECs and pericytes is mediated by the release of several growth factors, modulating the cell–cell contact and the interaction with ECM [3,27]. An impaired expression and production of these molecules may be responsible for the impaired angiogenesis observed in SSc patients. SSc is a disease with prominent vascular damage, starting with ECs and leading to an impaired ECs cross-talk with the surrounding perivascular cells.

**Both endothelial cells and pericytes may transdifferentiate into myofibroblast cells, the cells responsible for collagen deposition in the tissue that leads to fibrosis**

Conversely, in the presence of ECs derived from SSc patients (SSc-ECs), MSCs derived from both healthy HC and SSc patients were unable to support the tube formation. The mechanism responsible for this altered tube formation may be dysregulated SSc-EC production of growth factors, such as vascular endothelial growth factor (VEGF), platelet derived growth factor-BB (PDGF-BB), and transforming growth factor beta (TGFβ) [4,16,29], which are also profibrotic mediators. In fact, VEGF, which is elevated in SSc-ECs before and after co-culture with MSCs, is a mediator of vasculopathy in SSc [22,23]. In addition, VEGF is connected with fibrosis, suggesting a possible link between EC damage and profibrotic alteration. In fact, recent studies have shown that transgenic mice, which over express VEGF, spontaneously develop skin fibrosis and have exacerbated fibrotic responses [30].

PDGF-BB is a mitogen for mesenchymal cells and may promote skin fibrosis [31] as is TGFβ, which is produced by injured SSc-ECs and plays a profibrotic role on perivascular cells. Together, these molecules, which are released by injured ECs, may promote a profibrotic phenotypic switch on SSc perivascular cells. Different works substantiated the concept of disease inherent abnormalities in differentiation capacity and biologic functions of SSc-perivascular MSCs, which displayed an increased expression of fibrotic molecules, resulting in a more contractile profibrotic phenotype and low proliferative capacity [32]. According to this concept, the vascular damage may be responsible for a phenotypic switch of perivascular cells toward profibrotic myofibroblasts; thus, linking the vascular damage to some fibrotic events underling the development and progression of SSc.

**The molecules controlling the interplay between endothelial cells and pericytes may play a key role in triggering myofibroblasts generation**

### ENDOTHELIAL MESENCHYMAL TRANSITION

After microvascular damage, both ECs and pericytes may transdifferentiate into myofibroblasts. SSc-ECs, under the synergistic effects of TGFβ and ET-1, which are upregulated in SSc, may product collagen and express αSMA. This situation may result in their transdifferentiate toward myofibroblasts and contribute to fibroblast accumulation.

It has been shown that in certain environments, ECs may acquire a mesenchymal phenotype by a process of endothelial-to-mesenchymal transition (EnMT). This process, which shares several molecular signalling pathways with epithelial-to-mesenchymal transition (EMT), may be induced in vitro by TGFβ [33]. During EnMT, resident ECs delaminate from the polarized cell layer and invade the underlying tissue. This EC mesenchymal phenotype is characterized by the loss of cell–cell junctions and EC markers, such as Von Willebrand factor, CD31, and vascular endothelial–cadherin (VE–cadherin), as well as the acquisition of invasive properties, associated with the gain of mesenchymal markers, such αSMA, smooth muscle 22, and collagen [34]. According to this concept, it has been proposed that the endothelial damage in SSc skin occurs due to a progressive loss of VE-cadherin, a strictly endothelial-specific adhesion molecule, located at the junctions between ECs [34]. Many recent studies have shown that myofibroblasts involved in tissue fibrosis may derive from ECs through a process known as EnMT [30], supporting the idea that dysregulation of ECs function within the vascular wall may play an important role in vascular remodeling associated with the fibroproliferative vasculopathy observed in SSc.

### PERICYTES TO MYOFIBROBLAST TRANSITION

Perivascular cells (mural cells) were first histologically described as cells closely associated to the endothelial layer of blood vessel and are found in all organs throughout the body [35]. In the past, they were thought to only support the blood vessels; however, it is now clear that these cells have a variety of functions. The contractile properties of perivascular cells allow the regulation of blood supply by changing the vessel diameter in response to vasoactive substances [35]. Furthermore, several studies provided evidence that, during adult life, resident pericytes represent the local sources of stem cells. In fact, pericytes

may differentiate toward a variety of mesenchymal populations such as osteoblasts, chondrocytes, adipocytes, and fibroblasts [28]. Adult multipotent MSCs are members of the pericytes family and reside in a specialized physical location known as perivascular niche [3,4,35]. In this niche, the fate of MSCs is controlled by the crosstalk with ECs [35]. This interaction maintains MSCs in their quiescent state or, alternatively, provides stimuli leading to their differentiation [37].

Different authors have shown that MSCs express pericyte markers and cooperate with ECs to form a vascular network, thus suggesting that these cells may derive from the bone marrow perivascular sites and probably act as pericytes [28]. In a previous article [36], we showed that MSCs isolated from SSc patients are a good model to study pericytes, expressing the same markers and supporting ECs during tube formation. Of note, it has been shown that SSc–MSCs showed a profibrotic phenotype, thus contributing to disease progression [4]. Microvascular pericytes act as a crucial cellular interface between vessels and surrounding tissue signals, and given their anatomic location, they control the vessel development, stabilization, and integrity [37].

**During systemic sclerosis, the two processes, which link vasculopathy to fibrosis, may be the EnMT and pericytes-to-myofibroblast transition**

Recently, the use of in vivo experimental models in which cells producing collagen have been tracked has helped to identify the progenitors in perivascular cells of myofibroblasts cells. These studies showed that perivascular cells and resident fibroblasts are probably the main sources of myofibroblasts in animal models of chronic kidney fibrotic diseases [38]. In fact, it has been shown that perivascular fetal cells, which contribute to the generation of perivascular cells in the adult skeletal muscle, express the isoform 12 of a disintegrin and metalloprotease (*ADAM12*) gene. This gene is downregulated during adult life, but after injury, it is reactivated, aiming to restore the vascular integrity. Under chronic stimuli, this compensatory mechanism may lead to an inappropriate fibrotic outcome, characterized by the detachment and migration of ADAM12+ perivascular cells lineage to the tissue, and their transdifferentiation toward activated myofibroblast. Thus, perivascular progenitor cells with profibrotic fate and function, may be identified by the expression of *ADAM12* and the genetic ablation of ADAM12+ cells may limit the generation of profibrotic cells [38]. In a previous article, we provided evidence of activated *ADAM12* expression in SSc–MSC, suggesting their commitment toward a profibrotic activity. Furthermore, we showed that *ADAM12* was constitutively activated in SSc–MSCs and could play an active role in regulating TGFβ signaling. It is possible that after EC damage, activated ADAM12+ perivascular cells may contribute in the fibrotic process. They are the irreversible end stage of SSc [39].

It has been shown that in microvascular lesions of SSc patients with active Raynaud's phenomenon, pericytes overexpressed the PDGF-receptor beta and the high-molecular weight melanoma-associated antigen, promoting their proliferation and the increase of the vascular wall thickness [40].

## CONCLUSIONS

We reviewed the link between vasculopathy and fibrosis in SSc. We discussed that both ECs and perivascular cells after injury, may produce several molecules, which may promote their differentiation in myofibroblasts, the cells responsible for collagen production and tissue fibrosis. The origin of myofibroblasts is still a matter of debate; however, a growing body of evidence highlights that diverse cell types may contribute to generate these fibrosing cells. It remains to be clarified how interactions between fibrosing cells and other affected cell types may lead to disease progression. At present, no curative treatment is available for SSc, and only symptomatic treatments are used to alleviate the disability affecting these patients.

Although recently many efforts have been made to individuate the earlier therapeutic target to prevent or arrest the fibrosis, there are still no effective therapies for the treatment of fibrosis. Emerging data further support a key role for the molecules regulating the cross-talk between ECs and perivascular cells. These molecules may ultimately provide new therapeutic strategies for SSc by having a direct antifibrotic effect and preventing the earlier mechanism leading to myofibroblasts accumulation in fibrotic tissue.

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**Capsule**

## Targeted tumor immunotherapy

Although immunotherapy has transformed the cancer therapeutics landscape, a number of problems remain to be solved, from improving efficacy to limiting side effects. **Wang** et al. engineered nanoparticles that could be activated specifically in tumors. They conjugated antibodies against programmed death ligand 1 (PDL1) with matrix metalloproteinase protein 2 (MMP-2)-sensitive nanoparticles carrying a photosensitizer. MMP-2 is highly expressed in tumors, and delivery of the nanoparticle to tumors elicited release of the antibody from the nanoparticle. When used in conjunction with localized near-infrared radiation that activated the photosensitizer to produce reactive oxygen species, the nanoparticles outperformed systemic anti-PDL1 in limiting growth and metastasis of murine tumors.

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