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Invited Review

The contribution of mesenchymal transitions to the pathogenesis of systemic sclerosis

Irene Rosa*, Eloisa Romano*, Bianca Saveria Fioretto, Mirko Manetti

Abstract

Systemic sclerosis (SSc) is a multifaceted connective tissue disease characterized by widespread vasculopathy and autoimmune reactions that evolve into progressive interstitial, perivascular, and vessel wall fibrosis that affects the skin and multiple internal organs. Such an uncontrolled fibrotic process gradually disrupts the physiologic architecture of the affected tissues and frequently leads to significant organ dysfunction, thus representing a major cause of death in SSc patients. The main fibrosis orchestrators in SSc are represented by chronically activated myofibroblasts, a peculiar population of mesenchymal cells combining the extracellular matrix-synthesizing features of fibroblasts with cytoskeletal characteristics of contractile smooth muscle cells. Multiple lines of evidence support the notion that profibrotic myofibroblasts may derive not only from the activation of tissue resident fibroblasts but also from a variety of additional cell types, including pericytes, epithelial cells, vascular endothelial cells and preadipocytes/adipocytes. Here we overview an emerging picture that espouses that several cell transitional processes may be novel essential contributors to the pool of profibrotic myofibroblasts in SSc, potentially representing new suitable targets for therapeutic purposes. An in-depth dissection of the multiple origins of myofibroblasts and the underlying molecular mechanisms may be crucial in the process of deciphering the cellular bases of fibrosis persistence and refractoriness to the treatment and, therefore, may help in developing more effective and personalized therapeutic opportunities for SSc patients.

Keywords: Systemic sclerosis, scleroderma, fibrosis, myofibroblasts, mesenchymal transitions

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Introduction

Systemic sclerosis (SSc, scleroderma) is a connective tissue disorder characterized by multisystem clinical manifestations and a variable and unpredictable course. Despite the considerable improvement in clinical outcomes, primarily due to better management of complications, current treatments are mainly organ-based and do not result in a definite cure (1, 2). The precise mechanisms implicated in SSc pathogenesis are not fully understood, but it is well-established that the disease development and clinical manifestations are a result of three primary and distinct processes, namely: i) activation of both innate and adaptive immunity with production of a variety of autoantibodies, ii) widespread vasculopathy, and iii) progressive fibrosis of the skin and multiple internal organs such as lungs, gastrointestinal tract, and heart (1-3). The hallmark of SSc is interstitial, perivascular, and vessel wall fibrosis due to the excessive deposition of extracellular matrix (ECM) proteins (i.e., collagen and fibronectin) that gradually disrupts the physiologic architecture of the affected tissues and frequently leads to significant organ dysfunction, thus representing a major cause of death in SSc patients (3). According to the extent of skin fibrosis, clinicians commonly classify SSc patients into two well-recognized subsets: the limited cutaneous subset (lcSSc) and the diffuse cutaneous subset (dcSSc) (4). Although in both subsets the fibrotic process begins at the distal ends of the extremities and then progresses centripetally, patients with lcSSc do not show skin fibrosis beyond the elbows and knees, while skin fibrosis in dcSSc may also affect the arms, legs, and trunk (4). The progression of the fibrotic process is frequently rapid in patients with dcSSc, whereas the disease course is protracted in those with lcSSc.

In the last few decades, numerous histologic and molecular investigations of the skin, lungs, heart, and other organs highlighted that the main orchestrators of the severe fibrotic process in SSc are chronically activated myofibroblasts, a peculiar population of mesenchymal cells combining the ECM-synthesizing features of fibroblasts with cytoskeletal characteristics of contractile smooth muscle cells (5, 6). Indeed, myofibroblasts display a marked profibrotic cellular phenotype, consisting of an increased production of fibrillar type I and III collagens and other ECM macromolecules. They are also characterized by a reduction in the

expression of genes encoding ECM-degrading enzymes, and α-smooth muscle actin (α-SMA) expression and assembly into stress fibers, which endows them with the aforementioned contractile properties (7). Owing to their contractile force, myofibroblasts can induce substantial changes in SSc-affected skin, contributing to a progressive increase in dermal tissue stiffness, which has been recently recognized as a potent profibrotic trigger (8, 9). Myofibroblasts can also be observed in the esophagus and gastrointestinal wall of SSc patients with severe internal organ fibrosis, where they may contribute to gastro-esophageal reflux disease and gut dysmotility (10), and in the pulmonary interstitial space and bronchoalveolar lavage fluid, where they negatively affect lung functions (11, 12). In addition, other organs such as the myocardium and kidneys can also be affected by myofibroblast-driven fibrosis (13).

Given the universally accepted central role of myofibroblasts in the pathogenesis of organ fibrosis in SSc and many other fibrotic disorders (14), the identification of their possible cellular origins has increasingly become a central research topic in the most recent years. Indeed, the accumulation and persistence of fibrogenic myofibroblasts during disease development and progression can be attributed either to a reduced susceptibility of these cells to proapoptotic stimuli or to their increased formation through trans-differentiation/transition processes of different cell types (3, 5, 14, 15). In particular, extensive investigations have revealed that pathogenic myofibroblasts may be derived not only from proliferating tissue resident fibroblasts, but also from different sources, including the recruitment of bone marrow-derived circulating fibroblast precursors (also referred to as circulating fibrocytes), activation of perivascular pericytes, transformation of preadipocytes/adipocytes, and trans-differentiation of epithelial cells or vascular endothelial cells (3, 5, 14) (Figure 1). It is important to note that, the multiple pathways implicated in SSc pathogenesis, such as canonical and non-canonical transforming growth factor- β (TGF-β) and Wnt/β-catenin signaling, appear to regulate the transition process of the aforementioned cell types toward an activated myofibroblastic phenotype (3). Taken together, since activated myofibroblasts can undoubtedly be considered the critical effector cells driving the severity of the SSc fibrotic phenotype, an in-depth unraveling of their multiple origins may be crucial for deciphering the cellular and molecular mechanisms underlying fibrosis persistence and refractoriness to therapies and, therefore, to identify novel strategies effective in reversing this process. In this context, here we will provide an overview of recent insights supporting the notion that a variety of cell transitional processes may be novel essential contributors to the pool of profibrotic myofibroblasts, thus representing emerging mediators of SSc pathophysiology and opening new therapeutic perspectives.

Main Points

- In systemic sclerosis, progressive multiorgan fibrosis is orchestrated by profibrotic myofibroblasts originating not only from tissue resident fibroblast activation, but also from a variety of cell transition processes.
- Endothelial-to-mesenchymal transition may be pathogenetically crucial in systemic sclerosis by contributing to both tissue fibrosis and fibroproliferative or destructive vasculopathy.
- Deciphering the multiple origins of profibrotic myofibroblasts may be the key to unravel the cellular and molecular bases of fibrosis persistence and refractoriness to treatment and develop more effective and personalized therapeutic opportunities for systemic sclerosis patients.

Pericytes and bone marrow-derived mesenchymal stem cells as myofibroblast precursors

Pericytes are perivascular cells located at intervals along the outer walls of blood capillaries, pre-capillary arterioles, and post-capillary venules, residing in intimate contact with the underlying endothelium and extending processes both along and around microvessels. Due to their close relationship with endothelial cells, pericytes play a pivotal role in the morphogenesis, remodeling, and maintenance of the vascular network system (16), as demonstrated by the evidence that their ablation leads to vessel dilation and hemorrhage (17). Pericytes are identifiable by the expression of numerous markers, such as platelet-derived growth factor receptor-β (PDGFR-β), chondroitin sulfate proteoglycan 4 (also known as nerve/glial antigen 2 or NG2), CD135, nestin, desmin, and the transcription factor FoxD1 (18, 19). Pericytes demonstrate a capacity for *in vitro* multi-lineage differentiation into different mesodermal lineages, including bone, fat, cartilage, and skeletal muscle and can be considered a type of mesenchymal stem cell (MSC) (20). Several studies have focused on pericytes as a possible source of activated myofibroblasts. Indeed, they already express α-SMA and can differentiate into myofibroblasts when starting to express proteins, such as type I collagen and ED-A fibronectin splice variant, as demonstrated in the fibrotic skin of SSc patients with the diffuse cutaneous subset (21). By using a fate-mapping approach in skin- and muscle-wounding experiments, pericytes derived from the ADAM12(+) lineage were found to migrate into the perivascular tissue and differentiate into myofibroblasts, producing both α-SMA and type I collagen and accumulating

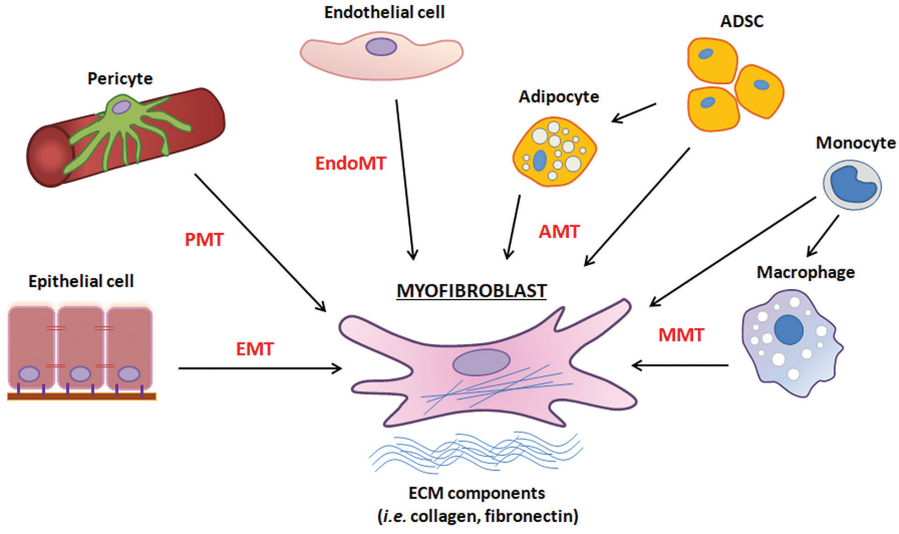

Figure 1. Different cell trans-differentiation processes may be responsible for accumulation of profibrotic myofibroblasts in the tissues of patients with systemic sclerosis. Myofibroblasts can originate not only from tissue resident fibroblasts but also from pericytes, mature adipocytes/adipose-derived stem cells (ADSC), monocytes/macrophages, epithelial cells, and vascular endothelial cells.

AMT: adipocyte-to-myofibroblast transition; ECM: extracellular matrix; EMT: epithelial-to-mesenchymal transition; EndoMT: endothelial-to-mesenchymal transition; MMT: macrophage-to-myofibroblast transition; PMT: pericyte-to-myofibroblast transition.

in skin scars (22). Similarly, after spinal cord injury, pericytes were shown to dissociate from the vasculature and migrate into the interstitial space, where they proliferate and express profibrotic proteins (23, 24). In a recent study, lung pericytes isolated from patients with idiopathic pulmonary fibrosis exhibited an increased propensity to migrate and invade the surrounding ECM, features that may contribute to the function of these cells in lung fibrosis (25). Furthermore, the pericyte-to-myofibroblast transition has been observed in lung, liver, kidney, and myocardial fibrosis (19, 26, 27), indicating that this process is shared by many fibrotic disorders. Pericytes were also found to contribute to the appearance of myofibroblasts in different experimental models of scleroderma (28, 29). *In vitro,* mouse lung pericytes treated with TGF-β have been shown to trans-differentiate into myofibroblastic cells, as testified by the increased expression of type I collagen, connective tissue growth factor, and α-SMA (30). In addition, an immunophenotypical analysis of myofibroblasts and perivascular mesenchymal cells in the bleomycin-induced rat skin scleroderma model revealed that pericytes may represent possible myofibroblast progenitors in experimental sclerotic lesions (31).

As already discussed, pericytes exhibit a phenotype and differentiation ability that is strikingly similar to that of multipotent MSC. In particular, several studies have shown that pericytes and bone marrow-derived MSC (BM-MSC) share similar markers, including PDGFR-β, α-SMA, NG2, and desmin (17). Moreover, *in vitro* BM-MSC are able to assist endothelial cells in forming and maintaining a vascular network, distributing themselves around the tubes (17). On these bases, recent investigations employing BM-MSC from SSc patients as pericyte surrogates provided evidence that these cells display a myofibroblast-like phenotype and may be involved in myofibroblast accumulation in the fibrotic skin of SSc patients (32, 33). In support of these findings, a prolonged exposure of SSc BM-MSC to different profibrotic factors mimicking the SSc-microenvironment was reported to increase the propensity of these cells to differentiate toward myofibroblasts (34).

Epithelial-to-mesenchymal transition

Epithelial-to-mesenchymal transition (EMT) is a biological process by which epithelial cells trans-differentiate into cells with a mesenchymal phenotype, thus providing an additional source of fibroblasts/myofibroblasts in both wound healing and pathological fibrosis (35, 36). Epithelial cells are tightly adherent to the basal membrane and to each other, forming one or more layers that act as a barrier, the main functions of which are to prevent permeability and contribute to defining the structure of tissues and organs (35, 36). Indeed, the important features of epithelial cells are the lack of motility, apical-basal polarity, and cell-cell adhesion, which are established through the arrangement of tight junctions, adherent junctions, desmosomes, and gap junctions (35, 36). The EMT process follows a complex program that takes multiple steps, starting with the loss of cell junctions and apical-basal polarity and a significant rearrangement of the cytoskeleton, ultimately resulting in changes in the cell shape (i.e., from a cuboidal to a spindle form) (36). Newly formed mesenchymal cells acquire the ability to degrade the underlying basement membrane and to migrate away from the epithelial layer in which they originated, thus invading the surrounding connective tissue (35, 36). Immunophenotypically, epithelial cells undergoing EMT lose their specific cell marker E-cadherin and gain mesenchymal/myofibroblast markers such as α-SMA, vimentin, and type I collagen (37). The most significant growth factor that is able to trigger EMT appears to be TGF-β, whose signaling cascade induces the expression of transcription factors such as Snail1 and Snail2 (also referred to as Slug), Twist, and zinc-finger E-box-binding, all having important roles in the induction of the EMT process (35, 36). EMT, which is particularly important during embryologic development, wound healing, and tissue regeneration, has also recently been implicated in a variety of pathological conditions, including cancer and renal, liver, pulmonary, and skin fibrosis (36). As far as SSc is concerned, the expression of Snail1 and Twist1 was observed in the eccrine glands of a small cohort of patients with the diffuse cutaneous subset (38). In another study, the lesional SSc epidermis has been reported to exhibit an increased amount of TGF-β signaling and Snail1-encoding gene (*SNAI1*) expression (39). However, since SSc keratinocytes did not appear to lose the epithelial E-cadherin marker, the aforementioned changes were suggested to be consistent with a "partially evoked" EMT process, i.e., SSc epidermal cells may acquire some mesenchymal features and may contribute to dermal fibrosis without fully transforming into a myofibroblast population (39). In SSc, EMT may also be triggered by the lacking activity of the transcription factor Friend leukemia integration factor-1 (Fli1), as suggested by the experimental evidence that mice with keratinocyte Fli1 deficiency spontaneously develop autoimmunity and SSc-like dermal fibrosis with epithelial activation (40, 41). Besides the EMT process, SSc keratinocytes were found to exhibit increased expression of NF-κB-regulated cytokines and chemokines. They were also found to promote fibroblast activation and transition to myofibroblasts in a TGF-β-independent manner (42).

Endothelial-to-mesenchymal transition

Endothelial-to-mesenchymal transition (EndoMT) refers to a trans-differentiation process during which endothelial cells undergo a phenotypical change characterized by the down-regulation of their specific markers, such as CD31/platelet-endothelial cell adhesion molecule-1 (PECAM-1), von Willebrand factor (vWF), vascular endothelial (VE)-cadherin, and the parallel acquisition of mesenchymal cell/myofibroblast products, including α-SMA, S100A4/fibroblast-specific protein-1 (FSP1), and type I collagen (43, 44). Such an immunophenotypical switch is mainly driven by the stabilization and nuclear translocation of the transcriptional regulator Snail1, a crucial trigger of the gene expression program underlying EndoMT (43, 44). During this mesenchymal transition, endothelial cells gain a spindle-shaped/fibroblast-like morphology, disaggregate, and lose polarity, thereby showing migratory capacity, invasiveness, and enhanced resistance to apoptosis (43, 44). EndoMT appears to share several molecular patterns with EMT and may be induced by different cytokines and growth factors, including TGF-β, interleukin-1β, tumor necrosis factor-α, endothelin-1 (ET-1), Notch, and Wnt, and by other putative pathways such as oxidative stress and hypoxia (43). In recent years, EndoMT has emerged as an important player in the pathogenesis of tissue fibrosis and fibroproliferative vasculopathy in various diseases, including cardiac and intestinal fibrosis, diabetic nephropathy, portal hypertension, and pulmonary arterial hypertension (PAH) (43, 44). Increasing experimental evidence supports the prominent role of EndoMT in SSc as well (7, 43-45). In particular, it has been proposed that the SSc-related EndoMT process may have differential pathogenetic roles depending on the type of affected vessels (45). In arterioles and small arteries, EndoMT may lead to an accumulation of profibrotic myofibroblasts in the vessel intima and media, thereby contributing to vessel remodeling (i.e., "fibroproliferative vasculopathy" characterized by fibrosis and thickening of the vessel wall with occlusive vascular disease) and clinically manifesting as digital ulcers and gangrene of the extremities or SSc-associated PAH, scleroderma renal crisis, and myocardial blood vessel anomalies (45) (Figure 2). When affecting capillary vessels, EndoMT may instead result in an increase in the number of perivascular myofibroblasts that are responsible for tissue fibrosis and a parallel loss of endothelial cells (i.e., "destructive vasculopathy" characterized by microvessel rarefaction)

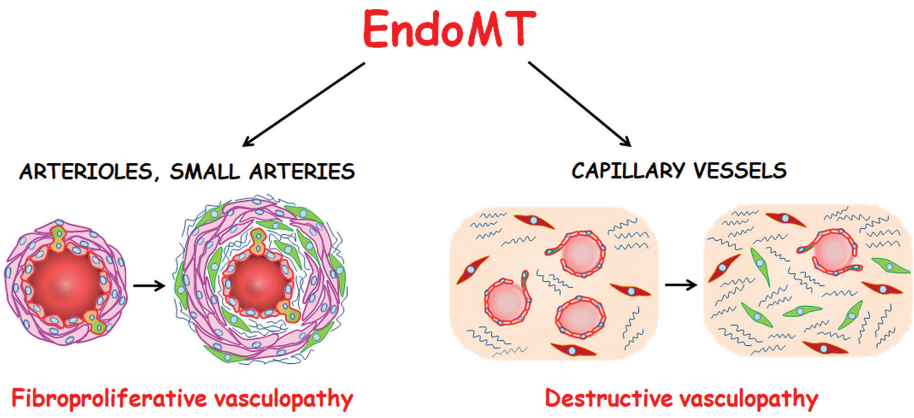

Figure 2. Endothelial-to-mesenchymal transition (EndoMT) may contribute to different pathogenetic aspects of systemic sclerosis depending on the type of affected vasculature. In arterioles and small arteries, EndoMT may be crucial for the development of fibroproliferative vasculopathy through the accumulation of profibrotic myofibroblasts in the vessel intima and media, resulting in progressive thickening of the vessel wall and occlusion of the vascular lumen. In capillary vessels, EndoMT may instead generate myofibroblasts that migrate into the perivascular space with consequent tissue fibrosis and microvessel rarefaction (destructive vasculopathy).

with disturbed angiogenic responses as testified by nailfold videocapillaroscopy abnormalities (45) (Figure 2). A considerable body of evidence supports the implication of EndoMT in the development of SSc-associated interstitial lung disease (ILD), PAH, and dermal fibrosis (7, 40, 43, 45). For instance, the co-expression of endothelial and mesenchymal/fibroblastic cell markers (vWF and α-SMA, respectively) has been reported in the pulmonary arterioles of patients with SSc-associated-PAH and in an experimental murine model of hypoxia-induced PAH (46, 47). Similar findings have been reported in the lung tissue of patients suffering from SSc-associated ILD (47, 48). Moreover, using a transgenic mouse model, a recent study demonstrated that an endothelial cell-specific and constitutive activation of TGF-β downstream signaling pathway induces EndoMT in lung vessels, resembling the fibrotic alterations characteristic of SSc (49). In addition, abnormal fibrillin-1 expression and chronic oxidative stress were found to mediate EndoMT in the tight-skin mouse model of SSc (50). Several lines of evidence also support the notion that a deficiency of the transcription factor Fli1, which plays a pivotal role in the maintenance of endothelial cell homeostasis, may be implicated in the multifactorial pathogenetic mechanisms of SSc mainly through the induction of the EndoMT process (51, 52). Indeed, Fli1 expression is markedly downregulated in SSc dermal microvascular endothelial cells (MVEC), and the endothelial lineage-specific Fli1 deficiency in mice recapitulates the histopathological and functional abnormalities characteristic of SSc vasculopathy, including a downregulation of endothelial VE-cadherin and PECAM-1, an impaired development of the vessel basement membrane, and a decrease in the pericyte coverage of dermal capillaries (7, 53). Strikingly, Fli1 haploinsufficiency in the bleomycin-induced skin fibrosis model was also reported to induce a profibrotic phenotype in dermal MVEC, further corroborating Fli1 as an important factor underlying EndoMT (51). Endothelial cells in the intermediate stages of EndoMT (i.e., co-expressing α-SMA with either CD31 or VE-cadherin) have been detected in dermal microvessels from the affected skin of both SSc patients and bleomycin-induced and urokinase-type plasminogen activator receptor (uPAR)-deficient mouse models of SSc (7). Furthermore, dermal MVEC explanted from the clinically involved skin of SSc patients (SSc-MVEC) exhibited a spindle-shaped morphology, co-expressed both endothelial and myofibroblast markers, and were able to contract at variance with healthy skin donor-derived MVEC (H-MVEC) (7). Interestingly, H-MVEC were shown to acquire a myofibroblast-like morphology and a contractile phenotype when exposed to sera from SSc patients (7). Mechanistically, the matrix metalloproteinase-12-dependent cleavage of uPAR, a process altering the uPAR-mediated regulation of integrin functions and playing a pivotal role both in the impaired angiogenic performance of SSc-MVEC and in fibroblast-to-myofibroblast differentiation, was found to be implicated in the pro-EndoMT effects exerted by SSc serum (7). Similarly, EndoMT could be induced in healthy pulmonary artery endothelial cells by inflammatory cytokines, as demonstrated by a profound reorganization of the actin cytoskeleton and the development of a mesenchymal/myofibroblast morphology (46). In another study, MVEC isolated from SSc-unaffected skin were found to undergo EndoMT when co-cultured with fibroblasts from SSc-affected skin and treated parallelly with TGF-β and ET-1 (54). In this *in vitro* model system, both bosentan and macitentan, two different ET-1 receptor antagonists, were effective in inhibiting the ET-1/TGF-β-mediated EndoMT (54). Consistent with these findings, macitentan was found to block both ET-1- and TGF-β-induced EndoMT in skin MVEC isolated from healthy donors and SSc patients (55). The ET-1/TGF-β-mediated EndoMT process has also been confirmed in skin and lungs *in vivo* in an animal model of TGF-β-induced tissue fibrosis (56).

As mentioned above, other several important regulatory pathways such as Wnt/β-catenin, Notch, and caveolin-1 signaling may also participate in the regulation of EndoMT (40, 43, 44). Although the effective participation of Wnt/β-catenin and Notch signaling in the SSc-related EndoMT process has yet to be demonstrated, the evidence that these pathways are activated in SSc-affected skin suggests that they may play a role in the overall disease pathogenesis and may thus represent potential targets for disease modifying therapeutic approaches (40). Similarly, despite numerous studies supporting the role of caveolin-1 in the pathogenesis of SSc (43, 57), to date, the possibility of its direct participation in the regulation of SSc-related EndoMT has not been explored in detail. Finally, microRNA (miRNA), a class of small endogenous noncoding RNA that regulate post-transcriptional gene expression, have been recently reported to play important roles in SSc pathogenesis and in the EndoMT process (40, 44). Nevertheless, specific studies will be necessary to definitively prove that, in SSc, miRNA might be directly implicated in the EndoMT process (40, 44).

Besides fully differentiated endothelial cells, it has been suggested that bone marrow-derived circulating endothelial progenitor cells (EPC) might also trans-differentiate toward a myofibroblast-like phenotype in SSc (58). Several studies have shown that the EPC detected in the peripheral blood of SSc patients have an impaired function (58). In particular, the occurrence of mesenchymal trans-differentiation has been demonstrated in the early circulating EPC from SSc patients (59). Thus, it has been proposed that SSc EPC may be defective in vasculogenesis because they preferentially undergo EndoMT, representing an additional source of profibrotic myofibroblast-like cells in the disease process (59).

A schematic representation of the main molecular mechanisms and signaling pathways that have been suggested to regulate the EndoMT process in SSc is shown in Figure 3.

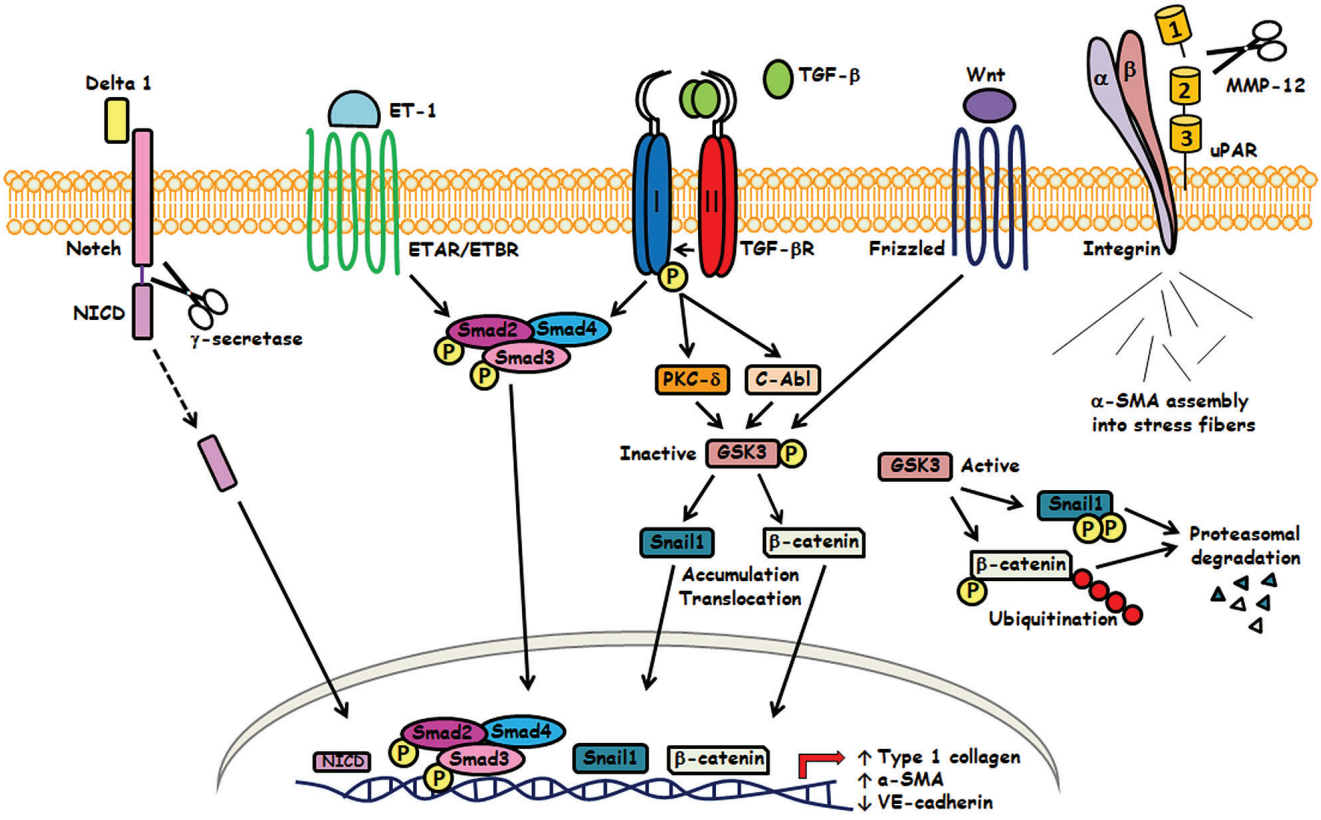

Figure 3. Schematic representation of the putative molecular mechanisms and signaling pathways that may regulate the endothelial-to-mesenchymal transition (EndoMT) process in systemic sclerosis. One central pathway is initiated following TGF-β-binding and subsequent activation of both Smad-dependent and Smad-independent TGF-β pathways. Activation of the Smad-independent pathway results in GSK 3β phosphorylation/inhibition by the PKC-δ and c-Abl non-receptor kinases, and consequent stabilization and nuclear translocation of the transcriptional regulator Snail1. Upon accumulation in the nucleus, Snail 1 triggers the acquisition of the myofibroblast phenotype through the upregulation of mesenchymal markers and downregulation of endothelial markers. The Wnt/Frizzled pathway may also lead to GSK 3β phosphorylation, followed by β-catenin stabilization and nuclear translocation. ET-1 may participate in the EndoMT process through a synergistic stimulation of the Smad-dependent TGF-β pathway. Activation of the Delta/Notch pathway with cleaved intracellular domain of Notch (NICD) translocation to the nucleus may also contribute to EndoMT induction. The cleavage of uPAR by MMP-12 results in an integrin-dependent increase in adhesion of cells to the extracellular matrix and generation of the cell tension, which is required for the assembly of α-SMA into stress fibers and the acquisition of a myofibroblast-like phenotype.

Adipocyte-to-myofibroblast transition

Besides the aforementioned mesenchymal plasticity-equipped cells, recent research has reported that both intradermal adipocytic progenitors, also referred to as preadipocytes or adipose-derived stem cells (ADSC), and mature white adipocytes may trans-differentiate into contractile myofibroblasts through a process termed adipocyte-to-myofibroblast transition (AMT), a conversion characterized by downregulation of adipocytic markers and acquisition of mesenchymal products (60-62). Loss of adipose tissue and its replacement with fibrotic tissue have been observed in numerous pathologies, including epithelial tumors and liver fibrosis (17). As far as SSc is concerned, the development of skin fibrosis is accompanied by a loss of subcutaneous adipose tissue over the course of the disease, and this feature is well-recapitulated in various animal models (17). By using a genetic lineage tracing analysis in the bleomycin-induced mouse model of SSc, it has been recently reported that the induction of fibrosis causes adiponectin-expressing progenitor cells, normally confined to the intradermal adipose tissue compartment, to lose their adipocytic markers, acquire α-SMA expression and migrate into the dermis, thus reprogramming into myofibroblasts (60). Of note, the loss of intradermal adipose tissue preceded the onset of dermal fibrosis, with a reduction in adipogenic gene expression closely followed by an increase in fibrotic genes (60).

AMT can be driven by different molecules/pathways, such as TGF-β (60), found in inflammatory zone 1 (FIZZ1) and possibly Wnt/β-catenin (5). In particular, *in vitro* analyses on human healthy ADSC indicated that profibrotic TGF-β induces the loss of adipocytic markers and reciprocal acquisition of mesenchymal/myofibroblast markers (60, 63). Moreover, FIZZ1 has been reported to suppress adipogenesis and stimulate myofibroblast differentiation in murine 3T3-L1 preadipocytes via Notch signaling (5). In addition, mice lacking FIZZ1 preserve more adipose tissue and develop less fibrosis in response to bleomycin-induced skin injury (5). As far as Wnt is concerned, a hyperactivation of canonical Wnt/β-catenin signaling has been reported in SSc skin biopsies (64). Moreover, Wnt-3a was shown to inhibit the adipogenesis of healthy human subcutaneous adipocytes by repressing the adipogenic master regulator peroxisome proliferator-activated receptor-γ (PPAR-γ) and promoting the differentiation of these cells into myofibroblasts (64). Indeed, PPAR-γ is considered the principal mediator of adipogenic differentiation and its loss is known to be associated with the trans-differentiation of quiescent ADSC into activated myofibroblasts (65). Therefore, PPAR-γ can direct progenitor cell differentiation toward fi-

brogenic or non-fibrogenic pathways, playing a pivotal role in mesenchymal cell lineage fate determination (65). Interestingly, several therapeutic strategies, such as treatment with TGF-β neutralizing antibodies or the PPAR-γ agonist rosiglitazone, have been shown to ameliorate fibrosis in mice potentiating adipogenesis (66). Finally, *in vitro* studies demonstrated that early passage SSc-ADSC harvested from the adipose-derived stromal vascular fraction display a profibrotic and anti-adipogenic phenotype characterized by high levels of the myofibroblast marker α-SMA and low expression of both caveolin-1 and the adipogenic marker fatty acid binding protein 4 (FABP4) (63). Collectively, all these findings suggest a substantial contribution of the adipocytic cell lineage in the development of cutaneous fibrosis.

Conclusion and future directions

Extensive and progressive tissue fibrosis represents the primary pathogenic process responsible for the severity of the clinical manifestations of SSc, largely determining the disease clinical course, response to therapy, prognosis, and overall mortality. Although several therapies have been proposed for the treatment of SSc-associated fibrosis in small case series or pilot studies, owing to the extreme disease heterogeneity, their efficacy and safety have yet to be fully validated in larger cohorts of patients (1, 2, 4). An in-depth deciphering of the multifactorial disease pathogenesis is also essential to set new targeted treatment strategies. Even if important advances have been made in the last decades toward understanding the molecular mechanisms underlying SSc-associated fibrosis, several pieces are still missing, which halts the complete unraveling of the differential origins of the key profibrotic effector cell, i.e., the myofibroblast. Indeed, we have recently witnessed a profound reevaluation of the classic view, which considered that SSc myofibroblasts were derived exclusively from an expansion/activation of resident tissue fibroblasts, because it has now been well-established that these profibrotic orchestrators may also originate from the trans-differentiation of epithelial, endothelial, and adipocytic cells (3, 5, 14). Among the different transitions, to date, most investigations have focused on the role of EndoMT, a process that in SSc may be pathogenetically crucial and represent a unique therapeutic target because of its contribution to both progressive tissue fibrosis and fibroproliferative and destructive vasculopathies (7, 40, 44-48). Additionally, although there is some evidence supporting the beneficial effects of autologous adipose-derived stromal vascular fraction transplantation in SSc, further experimental work is necessary to clarify whether, in the presence of a specific pathological environment, ADSC might preferentially undergo AMT and behave as an unwanted source of profibrotic myofibroblasts (67). It is also worth considering that additional cell trans-differentiation processes toward the myofibroblast phenotype have been uncovered, though they currently remain almost unexplored in SSc. For instance, it has been reported that α-SMA expression can be induced in lymphatic endothelial cells and *in vitro* by TGF-β stimulation and *in vivo* during wound repair, providing proof of the existence of a lymphatic-EndoMT process (68). In this regard the evidence that activated profibrotic fibroblasts express the lymphatic endothelial marker podoplanin in SSc skin suggests the potential implication of lymphatic-EndoMT in SSc-related dermal fibrosis development (69). Moreover, tissue macrophages have been shown to directly differentiate into myofibroblasts through a process termed macrophage-to-myofibroblast transition in interstitial renal fibrosis (70) and SSc circulating CD14+ monocytes displayed the potential to transform into myofibroblasts (71), which leads to the speculation that cells of the monocyte/macrophage lineage might contribute in enriching the myofibroblast pool in SSc fibrotic tissues. Finally, it is reasonable to hypothesize that the contribution of each of the aforementioned mesenchymal transitions to the generation of profibrotic myofibroblast populations may vary among different SSc subsets. Therefore, by unveiling the preferential associations of these cell trans-differentiations with specific disease phenotypes and by dissecting the underlying molecular pathways, there is realistic hope to take a step toward the development of more effective and personalized therapies for SSc patients.

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