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Endothelial to mesenchymal transition (EndoMT) in the pathogenesis of Systemic Sclerosis-associated pulmonary fibrosis and pulmonary arterial hypertension. Myth or reality?

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Endothelial to mesenchymal transition (EndoMT) in the pathogenesis of Systemic Sclerosis-associated pulmonary fibrosis and pulmonary arterial hypertension. Myth or reality?

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ABSTRACT

Systemic Sclerosis (SSc) is a systemic autoimmune disease characterized by progressive fibrosis of skin and multiple internal organs and severe functional and structural microvascular alterations. SSc is considered to be the prototypic systemic fibrotic disorder. Despite currently available therapeutic approaches SSc has a high mortality rate owing to the development of SSc-associated interstitial lung disease (ILD) and pulmonary arterial hypertension (PAH), complications that have emerged as the most frequent causes of disability and mortality in SSc. The pathogenesis of the fibrotic process in SSc is complex and despite extensive investigation the exact mechanisms have remained elusive. Myofibroblasts are the cells ultimately responsible for tissue fibrosis and fibroproliferative vasculopathy in SSc. Tissue myofibroblasts in SSc originate from several sources including expansion of quiescent tissue fibroblasts and tissue accumulation of CD34+ fibrocytes. Besides these sources, myofibroblasts in SSc may result from the phenotypic conversion of endothelial cells into activated myofibroblasts, a process known as endothelial to mesenchymal transition (EndoMT). Recently, it has been postulated that EndoMT may play a role in the development of SSc-associated ILD and PAH. However, although several studies have described the occurrence of EndoMT in experimentally induced cardiac, renal, and pulmonary fibrosis and in several human disorders, the contribution of EndoMT to SSc-associated ILD and PAH has not been generally accepted. Here, the experimental evidence supporting the concept that EndoMT plays a role in the pathogenesis of SSc-associated ILD and PAH will be reviewed.

Keywords: Systemic Sclerosis, Fibrosis, Interstitial Lung Disease, Pulmonary Fibrosis, Pulmonary Arterial Hypertension, EndoMT, Endothelial cell, Myofibroblast.

INTRODUCTION

Systemic Sclerosis (SSc) is a systemic autoimmune disease of unknown etiology characterized by progressive fibrosis of skin and multiple internal organs, and severe functional and structural fibroproliferative alterations in the microvasculature [1-3]. Although numerous studies have examined the pathogenesis of SSc the exact mechanisms involved are not well understood and have remained elusive [4-7]. However, it has been recognized that the most serious clinical manifestations of the disease and its high mortality are the result of SSc-associated-interstitial lung disease (ILD) or pulmonary arterial hypertension (PAH). SSc-associated ILD results from exaggerated and often progressive accumulation of fibrous collagens and other extracellular matrix molecules in the lung parenchyma, whereas, SSc-associated PAH is caused by functional alterations and structural fibroproliferative vasculopathy affecting the small and medium sized pulmonary arterioles [8-12].

The cells ultimately responsible for the severe fibrotic process affecting the lung parenchyma and for the fibroproliferative alterations in the small and middle size pulmonary arterioles in SSc are the activated myofibroblasts [13-18]. Myofibroblasts are a unique population of mesenchymal cells displaying a marked profibrotic cellular phenotype characterized by the increased production of fibrillar type I and type III collagens, initiation of expression of α-smooth muscle actin (α-SMA), and reduction in the expression of genes encoding ECM-degradative enzymes [19-23]. Myofibroblasts also acquire a motile behavior and pronounced contractile properties, features that allow them to migrate to unaffected tissues and to induce a progressive increase in tissue stiffness, a recently recognized potent profibrotic stimulus [24,25]. Given the crucial role of myofibroblasts in the pathogenesis of organ-specific and systemic fibrotic disorders there has been wide-ranging interest in the precise identification of their origin [26-28]. Extensive research studies have shown that myofibroblasts originate from diverse sources including expansion and activation of quiescent resident tissue fibroblasts [29],

migration and tissue accumulation of bone marrow-derived circulating CD34+ fibrocytes [30-32], or from epithelial cells or perivascular cells (pericytes) that have undergone a phenotypic transition into mesenchymal cells [33-35]. More recent studies, however, have demonstrated that another source of activated myofibroblasts are endothelial cells (EC) that have acquired a mesenchymal phenotype through a process known as endothelial to mesenchymal transition (EndoMT). During EndoMT EC lose their specific EC markers such as CD31/PECAM-1, von Willebrand Factor (vWF), and VE-cadherin, and initiate the expression of mesenchymal cell products including α–SMA, vimentin, and type I collagen [36,37]. Despite the demonstration of the occurrence of EndoMT in numerous animal models of experimentally-induced cardiac, pulmonary, and renal fibrosis [37-43], and several studies suggesting a role for EndoMT in the pathogenesis of various human disorders [37,44-50], the possibility that EndoMT participates in the development or progression of the fibrotic process or the fibroproliferative vasculopathy in SSc has not been generally accepted.

Here, we will review the available experimental evidence that supports a role for EndoMT in the pathogenesis of SSc-associated ILD and PAH. First, we will briefly review the evidence that certain pathways that have been shown to participate in SSc-pathogenesis also play a role in EndoMT as depicted in **Figure 1**. Secondly, we will discuss the occurrence of EndoMT in experimental models of pulmonary fibrosis and pulmonary vascular disease. Finally, we will review the available evidence that supports a role of EndoMT in various human disorders and will discuss studies supporting the notion that the phenotypic change from EC to activated myofibroblasts participates in the development of SSc-associated ILD and PAH.

1. ROLE OF PATHWAYS INVOLVED IN SSC PATHOGENESIS IN THE MOLECULAR MECHANISMS OF ENDOMT.

1.1 The TGF-β pathway. Given the crucial role that TGF-β plays in the development of tissue fibrosis and in the pathogenesis of numerous fibrotic diseases including SSc [51-54]

several studies have investigated the role of this extended family of profibrotic growth factors in the generation of myofibroblasts through EndoMT [55-58]. The detailed molecular events and the intracellular cascades activated by TGF-β that result in the phenotypic change of EC into mesenchymal cells have not been entirely elucidated. However, several studies have shown that both Smad-dependent and Smad-independent pathways and numerous transcriptional regulators such as Snail1, Snail2 (or Slug), Twist, and some members of Zeb family of proteins are involved [55-59]. We recently examined the intracellular transduction pathways mediating TGF-β-induced EndoMT in cultured murine lung EC. We found that TGF-β induction of EndoMT was mediated by the c-Abl kinase and by protein kinase c-δ and was associated with a strong upregulation in the expression of Snail-1 [60].

1.2. Caveolin-1 (CAV1). CAV1, the main protein component of caveolae plays an important role in the internalization, trafficking and degradation of TGF-β receptors and, therefore, is involved in the regulation of TGF-β signaling and TGF-β-mediated fibrotic responses [61,62]. Extensive studies in *Cav1* knock out mice (*Cav1*-/-) showed that these mice exhibited a remarkable phenotype characterized by extensive fibrotic tissue accumulation in skin and lungs [63]. Following these observations several groups examined whether CAV1 may play a role in human fibrotic disorders and described that CAV1 protein and gene expression were markedly decreased in affected tissues from patients with SSc and SSc-associated ILD as well as in lung tissues from patients with idiopathic pulmonary fibrosis [63-65]. Restoration of CAV1 functional domains by supplementation with a caveolin scaffolding domain peptide or by adenoviral-mediated expression of CAV1 corrected the profibrotic phenotype of SSc and IPF cells *in vitro* [63-65] and *in vivo* in animal models of pulmonary fibrosis and PAH, including the monocrotaline-induced model of PAH and the bleomycin-induced pulmonary fibrosis in mice [64-66]. These studies, however, did not examine whether CAV1 was involved in EndoMT. To address this point, we examined the role of CAV1 in EndoMT employing immunopurified EC

isolated from lungs of *Cav1*-/-mice [67]. The results demonstrated the spontaneous occurrence of EndoMT in *Cav1*-/- pulmonary EC as evidenced by the constitutive expression of α-SMA, the high levels of production of type I collagen, and the high expression of the transcriptional repressors *Snai1* and *Snai2*, molecules previously shown to be upregulated in TGF-β1-induced EndoMT. These observations demonstrated that EndoMT occurred spontaneously in *Cav1*-/- mice *in vivo* and suggested that CAV1 deficiency, a molecular alteration that is a characteristic feature of SSc cells, may participate in the development of the progressive tissue fibrosis and proliferative vasculopathy in the disease through the establishment of EndoMT.

1.3. The role of endothelin-1 (ET-1) in EndoMT. Besides its crucial role in the development of primary and secondary PAH, a growing body of evidence has implicated ET-1 as a participant in organ fibrosis and numerous studies have considered it as an important trigger of the fibrotic process in SSc [68-70]. Although ET-1 is a major vasoactive peptide with multiple effects on EC it is not known whether it is capable of inducing EndoMT. Recent studies have examined whether ET-1 may also participate in the development of tissue fibrosis by inducing EndoMT. In one study, Widyantoro, et al. [71] showed that EC-derived ET-1 promotes cardiac fibrosis and heart failure in diabetic hearts through stimulation of EndoMT. In recent studies from our group employing murine lung EC it was found that although ET-1 was not capable of inducing EndoMT by itself it potentiated TGF-β-induced EndoMT. In subsequent studies we showed that cultured CD31+ human EC cells also were induced to undergo EndoMT *in vitro* when treated with ET-1 in the presence of TGF-β (Wermuth and Jimenez, unpublished observations). In these studies we also demonstrated that ET-1 exerted potent synergistic stimulation on TGF-β effects on EndoMT. A similar study employing immunopurified CD31 dermal EC from SSc patients and from normal individuals showed that TGF-β and ET-1 induced EndoMT in normal and SSc-EC and that these effects involved the Smad pathway and were blocked by the ET-1 receptor antagonist, macitentan [72]. The results of the studies with human

EC demonstrate that ET-1 is capable of generating either by itself or in combination with TGF-β activated tissue myofibroblasts through EndoMT. These studies also provide a novel mechanism for ET-1 participation in the development of tissue fibrotic reactions.

1.4. Role of Notch and Hedgehog signaling pathways. The morphogens Notch and Hedgehog play crucial roles during embryonic development [73-76]. Although in the adult these pathways are tightly regulated, under some circumstances their aberrant activation may occur leading to serious pathological consequences, including fibrotic diseases. Indeed, there are numerous recent publications implicating alterations in Notch pathways in the pathogenesis of SSc and other fibrotic diseases [77-79].

Although not extensively studied, it has recently become apparent that Notch pathways may also participate in the regulation of EndoMT. The role of Notch signaling in EndoMT was first described by Noseda, et al. [80] and it was suggested that the Notch pathway may be crucial for heart valve and cardiac cushion development and/or vascular smooth muscle differentiation [81,82]. In human microvascular EC, Notch and TGF-β synergistically stimulate Snail expression and upregulate a subset of genes by recruiting Smad3 to Smad binding sites [81-83]. However, while there is limited, although significant information, concerning the interaction of Notch and TGF-β signaling in EndoMT, the potential role of Notch in SSc-associated ILD and fibroproliferative vasculopathy has not been studied.

Another important pathway that has been shown to participate in the pathogenesis of various fibrotic disorders is the Sonic Hedgehog (SHh) pathway [84-88]. Recently, an extensive study [89] showed increased SHh expression in SSc affected tissues and demonstrated that TGF-β increased its expression. Characterization of the cells displaying increased SHh expression employing immunofluorescence for SHh epitopes showed intense EC staining in affected SSc skin. Furthermore, SHh was capable of strong stimulation of fibroblast to

myofibroblast transition with a potency similar to that of TGF-β [89]. However, the possibility that SHh may be involved in EndoMT has not been examined.

1.5. **Wnt pathway activation.** Wnts comprise a multigene family of secreted glycoproteins that play crucial roles during embryogenesis signaling through canonical and non-canonical pathways [90,91]. Following binding of Wnt ligands to their specific cell surface receptor and co-receptors the activation of glycogen synthetase kinase (GSK)-3ß is inhibited resulting in the accumulation of unphosphorylated β-catenin in the cytoplasm, followed by its translocation into nucleus, and the activation of target genes. Secreted Frizzled-related proteins (SFRP), Wnt inhibitory factors and other Wnt inhibitors such as Dkk-1 have been shown to negatively modulate Wnt responses [90,91]. Recent studies showed that the Wnt/β-catenin pathway is involved in the activation of numerous profibrotic steps in SSc pathogenesis [92-95]. Indeed, increased Wnt activation has been found in skin biopsies from SSc patients and it was further shown that Wnt3a induced myofibroblast differentiation via Smad-dependent autocrine TGF-β signaling promoting pathologic fibrogenesis [93]. A crucial role of β-catenin as a mediation of the profibrotic effects of the Wnt pathway was also demonstrated [94]. It was also shown that the Wnt pathway antagonists Dkk-1 and SFRP1 were epigenetically downregulated in SSc cells [95]. Similar findings were also obtained in SSc-associated lung fibrosis with the nuclear accumulation of β-catenin in activated fibroblasts present in fibroblastic foci in the lungs of patients with SSc-associated ILD [96]. Despite these observations very few studies have examined the role of Wnt pathway activation on the generation of activated myofibroblasts through EndoMT. One recent study demonstrated that induction of canonical Wnt signaling resulted in EndoMT pathway activation in cultured EC and in myocardial EC following experimentally-induced myocardial infarction [97]. In contrast, two studies showed that the Wnt inhibitor Dkk-1 enhanced EndoMT in aortic EC [98] and in human renal glomerular EC cultured in a high glucose medium [99] results that indicate that further study is needed to conclusively

determine the precise role of Wnt and of its inhibition on EndoMT-induced myofibroblast generation.

1.6. Involvement of hypoxia in EndoMT. The transcription factor HIF-1α is the key regulatory molecule responsible for the induction of a vast array of cellular and molecular responses to hypoxia and has been implicated in various pathologic conditions [100-102]. Hypoxia-induced dysregulation of HIF-1α expression and activity has been implicated in the pathogenesis of various fibrotic disorders including kidney and cardiac fibrosis [103,104]. The mechanisms involved in HIF-1α-induced fibrosis are very complex and include activation of a vast array of profibrotic genes as shown in global gene expression changes in hypoxic hepatic stellate cells [105], stimulation of expression of growth factors such as TGF-β and VEGF, and induction of epithelial to mesenchymal transition [106-108]. However, the possibility that hypoxia and HIF-1α may mediate some physiologic or pathologic responses through induction of EndoMT has just begun to be explored. Indeed, two very recent studies showed that one important downstream effect of HIF1-α is the induction of EndoMT in human coronary EC and that this effect may ultimately lead to development of cardiac fibrosis [109] and that HIF-1α mediated EndoMT during the development of radiation-induced pulmonary fibrosis [110].

2. DEMONSTRATION OF ENDOMT IN ANIMAL MODELS OF PULMONARY FIBROSIS.

Although in the past EndoMT was believed to be a rare phenomenon confined to certain stages of human embryonic development [36] numerous studies have described its occurrence in various experimental models of fibrosis including cardiac, renal, and pulmonary fibrosis [37-43]. One of the first studies to evaluate whether EC could represent a source of myofibroblasts involved in the development of pulmonary fibrosis examined bleomycin-induced lung fibrosis in double-transgenic mice with stable LacZ expression in EC [111]. In this setting any cells originated from the EC lineage will be LacZ labeled. Morphological evaluation of lungs from the

transgenic mice following endotracheal injection of bleomycin showed that the areas of fibrotic involvement contained large numbers of LacZ-positive fibroblasts indicative of their endothelial origin. To directly demonstrate the presence of EC–derived fibroblasts, lung fibroblasts from either saline-injected control mice or from bleomycin treated mice were isolated and cultured. LacZ detection revealed that approximately 16% of lung fibroblasts in the cultures from bleomycin-treated mice were derived from EC. Immunocytochemical staining for type I collagen and α-SMA showed that some cells from the bleomycin-treated mice expressed LacZ, type I collagen, and α-SMA, demonstrating their EC origin. These findings conclusively showed that lung EC could give rise to a substantial number of myofibroblasts through EndoMT in the bleomycin-induced lung fibrosis model. Furthermore, the study demonstrated that the phenotypic change was a permanently acquired trait. Another study examined the occurrence of EndoMT in experimentally induced PAH. In this study PAH was induced in mice following exposure to hypoxia and treatment with the antiangiogenic compound SU5416, a potent VEGF receptor inhibitor [112]. The results showed that approximately 6% of pulmonary arterioles displayed colocalization of vWF and α-SMA indicative of the occurrence of EndoMT *in vivo* in this animal model [112].

Despite the extensive experimental evidence supporting a role of EndoMT in the pathogenesis of tissue fibrosis there have been some reports raising controversy as to whether EndoMT was indeed a source of activated myofibroblasts contributing to the development of fibrotic reactions *in vivo* [113,114]. However, a recent study using detailed endothelial lineage tracing in transgenic mice with experimentally-induced renal fibrosis showed that from 10 to 20% of fibroblasts in the fibrotic kidneys arise from endothelial cells via EndoMT [42] *in vivo*, and another study in experimentally induced cardiac fibrosis employing rigorous genetic cell lineage tracing also showed that a population of cardiac interstitial fibroblasts were of EC origin [115].

3. DEMONSTRATION OF ENDOMT IN NON-PULMONARY HUMAN FIBROTIC DISEASES.

Given the intense interest raised by the study of EndoMT in various animal models of tissue fibrosis numerous studies have examined the occurrence of EndoMT in human pathologic conditions [37,42]. Results from the rapidly growing literature about the possible contribution of EndoMT to human disorders has provided strong support to the concept that EndoMT may also play a role in the pathogenesis of SSc-associated ILD and PAH. Following the pioneering description of Zeisberg et al. of the important role of EndoMT in the development of cardiac fibrosis [38] numerous reports appeared describing the occurrence of EndoMT in various human pathologic conditions [37,42]. For example, Bertram and collaborators demonstrated that EndoMT played a significant role in the development of chronic kidney disease in diabetes observations that were expanded to include other causes of chronic renal failure [41]. In more recent studies, the possibility that EndoMT of portal vein endothelium via TGF-β/Smad activation may also be involved in portal venopathy was examined [48]. The results showed enhanced expression of phosphorylated Smad2 (pSmad2) in venous endothelium of smaller portal veins in idiopathic portal hypertension, which was associated with colocalization of EC and mesenchymal cell (myofibroblast) protein markers. The authors concluded that the conversion of portal vein EC into cells expressing a myofibroblastic phenotype may be responsible for exaggerated periportal-venous deposition of collagen and other fibrous tissue proteins, and may represent the ultimate mechanism responsible for portal venous obliteration in idiopathic portal hypertension. Other studies that described the occurrence of EndoMT in human disorders include the demonstration of EndoMT in the process of neointima formation in human vein graft tissues [47], the participation of EndoMT in the severe heterotopic ossification occurring in the course of fibrodysplasia ossificans progressiva

[49], and a prominent role in the development of intestinal fibrosis [45] and radiation induced rectal fibrosis [50].

4. EVIDENCE SUPPORTING THE ROLE OF ENDOMT IN SSC-ASSOCIATED ILD PULMONARY FIBROSIS AND PAH.

4.1. Demonstration of EndoMT in primary or in SSc-associated PAH. Arciniegas et al. were among the first investigators to suggest a role of EndoMT in the pathogenesis of chronic PAH [116]. Following this novel suggestion, two more recent studies implicated EndoMT in the pathogenesis of PAH. The first study examined Primary PAH [117] and the second studied PAH secondary to SSc [112]. In the first study Ranchoux and co-workers [117] applied transmission electron microscopy, and correlative light and electron microscopy, providing unequivocal ultrastructural-level evidence of ongoing dynamic EndoMT in lung tissue samples from patients with primary PAH. Indeed, they demonstrated that typical EC identified by the presence of Weibel-Palade bodies acquired expression of the myofibroblast-specific marker α-SMA, as well as, displayed invaginations into the neointima of the abnormal pulmonary arterioles. In the second study, Good et al. [112], assessed EndoMT in the pulmonary arterioles in lung tissues from patients with SSc-associated-PAH. Assessment of the cellular phenotype in intimal and plexiform lesions from PAH lungs showed the unambiguous expression of endothelial (CD31, CD34, VE-cadherin) and mesenchymal (α-SMA) markers. A quantitative assessment of the co-expression of vWF and α-SMA indicated that up to 4% of pulmonary arterioles in the lungs of patients with SSc-associated PAH displayed co-expression of EC and mesenchymal cell markers. Furthermore, the protein and mRNA expression patterns confirmed the notion of a key role of EndoMT in SSc-associated PAH pathology. The novel observations described in these two studies provide conclusive evidence for the occurrence of EndoMT in small and medium size arterioles of lung tissues from patients with both primary PAH and SSc-associated PAH as discussed recently [118,119].

4.2. Demonstration of EndoMT in lung tissues from patients with SSc-associated ILD. We recently performed a study to examine the possibility that Endo-MT is involved in the fibrotic process of SSc-associated ILD [120]. In this study lung tissues from six patients with SSc and pulmonary fibrosis and 2 normal lung controls were examined by histopathology, immunohistochemistry, and confocal laser microscopy for the simultaneous expression of markers of EC (CD31 and vWF) and myofibroblasts (α-SMA or type I collagen). Immunohistology studies showed expression of the EC marker CD31/PECAM in mesenchymal cells embedded within the neointima of small pulmonary arteries as well as in the parenchymal fibrotic areas in the six SSc lung specimens as illustrated in **Figure 2**. These observations demonstrated for the first time the presence of cells carrying EC molecular markers removed from the vessel endothelium and embedded within the fibrotic lung parenchyma in all SSc-associated ILD samples examined. Co-expression of CD31 or vWF with the mesenchymal markers, collagen type I or α-SMA was demonstrated employing confocal laser microscopy in numerous EC lining the small and medium sized pulmonary arteries as illustrated for small arterioles in **Figure 3**. These findings were not present in the small or medium sized arteries of the normal lung tissues. The results demonstrated that EC co-expressing EC-specific and myofibroblastic cell markers are present in the endothelium of small pulmonary arteries from patients with SSc-associated pulmonary fibrosis and suggest that mesenchymal cells of endothelial origin are likely to be responsible for the production and accumulation of subendothelial fibrotic tissue in the affected vessels that in turn results in their luminal obliteration.

These observations were confirmed by an extensive assessment of the differences in gene expression patterns between microvascular EC isolated from normal lungs compared to microvascular EC isolated from lungs from patients with SSc-ILD. The gene expression assessment of immunopurified CD31+/CD102+ EC obtained from lung tissues from two patients

with SSc-associated ILD compared to the average gene expression of immunopurified CD31+/CD102+ EC from two control lungs is shown in **Figure 4**. The results demonstrated a very strong expression of COL1A1 and COL3A1 in the CD31+/CD102+ purified EC from lungs from SSc patients and these values were up to 21 times and 26 times higher, respectively, than the expression of the same collagen genes in CD31+/CD102+ EC purified from the normal control lungs. The expression of FN1 and ACTA2 (α-SMA), other profibrotic genes such as TGFB1 and CTGF, and that of several EndoMT-related genes such as SNAI2 and TWIST was also substantially increased in the CD31+/CD102+ EC from the lungs of SSc patients. Thus, we believe that the results of the extensive study performed in lung tissues from patients with SSc-associated ILD provide conclusive evidence for the occurrence of EndoMT during the fibrotic process affecting the lungs in SSc.

CONCLUDING REMARKS.

The results of the various studies reviewed here including evidence from experimental animal models of tissue fibrosis and from several studies with tissues from patients with SSc-associated ILD and PAH certainly indicate that the participation of EndoMT in these processes should no longer be considered a myth but it is, indeed, a reality. Furthermore, the results of these studies also suggest that greater understanding of the molecular mechanisms involved in EndoMT and its pharmacological modulation may represent a novel therapeutic approach for devastating effects and the high mortality of the SSc-associated tissue fibrosis and fibroproliferative vasculopathy complications that currently do not have effective therapies.

REFERENCES

1. Varga J, Abraham D. (2007). Systemic sclerosis: a prototypic multisystem fibrotic disorder. J Clin. Invest. 117,557-67.

2. Gabrielli A, Avvedimento EV, Krieg T. (2009). Scleroderma. N. Engl. J. Med. 360, 1989-2003.

3. Matucci-Cerinic M, Kahaleh B, Wigley FM. (2013). Systemic Sclerosis (scleroderma, SSc) is a vascular disease. Arthritis Rheum. 65, 1953-62.

4. Jimenez SA, Derk CT. (2004). Following the molecular pathways toward an understanding of the pathogenesis of Systemic Sclerosis. Ann. Int. Med. 140,37-50.

5. Katsumoto TR, Whitfield ML, Connolly MK. (2011). The pathogenesis of systemic sclerosis. Annu. Rev. Pathol. 6, 509-37.

6. Pattanaik D, Brown M, Postlethwaite BC, et al. (2015). Pathogenesis of Systemic Sclerosis. Front. Immunol. 6,272.

7. Stern EP, Denton CP. (2015). The pathogenesis of systemic sclerosis. Rheum. Dis. Clin. North. Am. 41, 367-82.

8. Veraldi KL, Hsu E, Feghali-Bostwick CA. (2010). Pathogenesis of pulmonary fibrosis in systemic sclerosis: lessons from interstitial lung disease. Curr. Rheumatol. Rep. 12,19-25.

9. Herzog EL, Mathur A, Tager AM, et al. (2014). Review: interstitial lung disease associated with systemic sclerosis and idiopathic pulmonary fibrosis: how similar and distinct? Arthritis Rheumatol. 66, 1967-78.

10. Wells AU, Margaritopoulos GA, Antoniou KM, et al. (2014). Interstitial lung disease in systemic sclerosis. Semin. Respir. Crit. Care. Med. 35, 213-21.

11. Tan A, Denton CP, Mikhailidis DP, et al. (2011). Recent advances in the diagnosis and treatment of interstitial lung disease in systemic sclerosis (scleroderma): a review. Clin. Exp. Rheumatol. 29(2 Suppl 65), S66-74.

12. Solomon JJ, Olson AL, Fischer A, et al. (2013). Scleroderma lung disease. Eur. Respir. Rev. 22, 6-19.

13. Krieg T, Abraham D, Lafyatis R. (2007). Fibrosis in connective tissue disease: the role of the myofibroblast and fibroblast-epithelial cell interactions. Arthritis Res. Ther. 9 Suppl 2, S4.

14. Abraham DJ, Eckes B, Rajkumar V, et al. (2007). New developments in fibroblast and myofibroblast biology: implications for fibrosis and scleroderma. Curr. Rheumatol. Rep. 9, 136-43.

15. Beon M, Harley RA, Wessels A, et al. (2004). Myofibroblast induction and microvascular alteration in scleroderma lung fibrosis. Clin. Exp. Rheumatol. 22, 733-42.

16. Watsky MA, Weber KT, Sun Y, et al. (2010). New insights into the mechanism of fibroblast to myofibroblast transformation and associated pathologies. Int. Rev. Cell. Mol. Biol. 282, 165-92.

17. Gilbane AJ, Denton CP, Holmes AM. (2013). Scleroderma pathogenesis: a pivotal role for fibroblasts as effector cells. Arthritis Res. Ther. 15, 215.

18. Kendall RT, Feghali-Bostwick CA. (2014). Fibroblasts in fibrosis: novel roles and mediators. Front. Pharmacol. 5,123.

19. Gabbiani G. (1981). The myofibroblast: a key cell for wound healing and fibrocontractive diseases. Prog. Clin. Biol. Res. 54, 183-94.

20. Kirk TZ, Mark ME, Chua CC, et al. (1995). Myofibroblasts from scleroderma skin synthesize elevated levels of collagen and tissue inhibitor of metalloproteinase (TIMP-1) with two forms of TIMP-1. J. Biol. Chem. 270, 3423-8.

21. Hinz B, Phan, SH, Thannickal VJ, et al. (2012). Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. Am. J. Pathol. 180, 1340-55.

22. Hu B, Phan SH. (2013). Myofibroblasts. Curr. Opin. Rheumatol. 25, 71-7.

23. Kis K, Liu X, Hagood JS. (2011). Myofibroblast differentiation and survival in fibrotic disease. Expert Rev. Mol. Med. 23, 13:e27.

24. Wells RG, Discher DE. (2008). Matrix elasticity, cytoskeletal tension, and TGF-beta: the insoluble and soluble meet. Sci. Signal. 1(10):pe13. doi: 10.1126/stke. 110pe13.

25. Hinz B. (2009). Tissue stiffness, latent TGF-beta1 activation, and mechanical signal transduction: implications for the pathogenesis and treatment of fibrosis. Curr. Rheumatol. 11, 120-6.

26. Hinz B, Phan SH, Thannickal VJ, et al. (2007). The myofibroblast: one function, multiple origins. Am. J. Pathol. 170, 1807-16.

27. McAnulty RJ. (2007). Fibroblasts and myofibroblasts: their source, function and role in disease. Int. J. Biochem. Cell. Biol. 39, 666-71.

28. Falke LL, Gholizadeh S, Goldschmeding R, et al. (2015). Diverse origins of the myofibroblast-implications for kidney fibrosis. Nat. Rev. Nephrol. 11, 233-44.

29. Poslethwaite AE, Shigemitsu H, Kanagat S. (2004). Cellular origins of fibroblasts: possible implications for organ fibrosis in systemic sclerosis. Curr. Opin. Rheumatol. 16, 733-8.

30. Strieter RM, Keeley EC, Hughes MA, et al. (2009). The role of circulating mesenchymal progenitor cells (fibrocytes) in the pathogenesis of pulmonary fibrosis. J. Leukoc. Biol. 86, 1111-8.

31. Herzog EL, Bucala R. (2010). Fibrocytes in health and disease. Exp. Hematol. 38, 548-56.

32. Bellini A, Mattoli S. (2007). The role of the fibrocytes, a bone marrow-derived mesenchymal progenitor, in reactive and reparative fibroses. Lab. lnvest. 87, 858-70.

33. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. (2010). Fate tracing reveals the pericyte and not epithelial origin on myofibroblasts in kidney fibrosis. Am. J. Pathol. 176, 85-97.

34. Lamouille S, Xu J, Derynck R. (2014). Molecular mechanisms of epithelial-mesenchymal transition. Nat. Rev. Mol. Cell. Biol. 15, 178-96.

35. Kramann R, Schneider RK, DiRocco DP, et al. (2015). Perivascular Gli1+ progenitors are key contributors to injury-induced organ fibrosis. Cell Stem Cell. 16, 51-66.

36. Arciniegas E, Neves CY, Carrillo LM, et al. (2005). Endothelial-mesenchymal transition occurs during embryonic pulmonary artery development. Endothelium. 12, 193-200.

37. Piera-Velazquez S, Li Z, Jimenez SA. (2011). Role of Endothelial-Mesenchymal Transition (EndoMT) in the Pathogenesis of Fibrotic Disorders. Am. J. Pathol. 179,1074-84.

38. Zeisberg EM, Taranavski O, Zeisberg M, et al. (2007). Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat. Med. 13, 952-61.

39. Goumans MJ, van Zonneveld AJ, ten Dijke P. (2008). Transforming growth factor β-induced endothelial-to-mesenchymal transition: A switch to cardiac fibrosis? Trends. Cardiovasc. Med. 18, 293-8.

40. Zeisberg EM, Potenta SE, Sugimoto H, et al. (2008). Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. J. Am. Soc. Nephrol. 19, 2282-7.

41. Li J, Qu X, and Bertman JF. (2010). Review: Endothelial-Myofibroblast Transition, a new player in diabetic renal fibrosis. Nephrology (Carlton). 15, 507-12.

42. LeBleu VS, Taduri G, O'Connell J, et al. (2012). Origin and function of myofibroblasts in kidney fibrosis. Nat. Med. 19, 1047-53.

43. Zeisberg M, Kalluri R. (2013). Cellular mechanisms of tissue fibrosis. 1. Common and organ-specific mechanisms associated with tissue fibrosis. Am. J. Cell. Physiol. 304, C216-25.

44. Lin F, Wang N, Zhang TC. (2012). The role of endothelial-mesenchymal transition in development and pathological process. IUBMB. Life. 64, 717-23.

45. Rieder F, Kessler SP, West GA, et al. (2011). Inflammation-induced endothelial-to-mesenchymal transition: a novel mechanism of interstitial fibrosis. Am. J. Pathol. 179, 2660-73.

46. Yoshimatsu Y, Watabe T. (2011). Roles of TGF-β signals in endothelial-mesenchymal transition during cardiac fibrosis. Int. J. Inflam. 724080.

47. Cooley BC, Nevado J, Mellad J, et al. (2014). TGF-β signaling mediates endothelial-to mesenchymal transition (EndMT) during vein graft remodeling. Sci. Transl. Med. 6, 27 ra 34.

48. Kitao A, Sato Y, Sawada-Kitamura S. et al. (2009). Endothelial to mesenchymal transition via transforming growth factor-beta1/Smad activation is associated with portal venous stenosis in idiopathic portal hypertension. Am. J. Pathol. 175, 616-26.

49. Ramirez DM, Ramirez MR, Reginato AM, et al. (2014). Molecular and cellular mechanisms of heterotopic ossification. Histol. Histopathol. 29, 1281-5.

50. Mintet E, Rannou E, Buard V, et al. (2015). Indentification of endothelial-to-mesenchymal transition as a potential participant in radiation proctitis. Am. J. Pathol. Jul 14. pii: S0002-9440(15)00329-6. doi: 10.1016/l.ajpath.2015.04.028. [Epub ahead of print].

51. Denton CP, Abraham DJ. (2001). Transforming growth factor-beta and connective tissue growth factor: key cytokines in scleroderma pathogenesis. Curr. Opin. Rheumatol. 13, 505-11.

52. Verrecchia F, Mauviel A, Farge D. (2006). Transforming growth factor-beta signaling through the Smad proteins: role in systemic sclerosis. Autoimmun. Rev. 5, 563-9.

53. Varga J, Whitfield ML. (2009). Transforming growth factor-beta in systemic sclerosis (scleroderma). Front. Biosci. (School Ed). 1, 226-35.

54. Lafyatis R. (2014). Transforming growth factor β—at the centre of systemic sclerosis. Nat. Rev. Rheumatol. 10, 706-19.

55. Goumans MJ, Liu Z, ten Dijke P. (2009). TGF-beta signaling in vascular biology and dysfunction. Cell Res. 19, 116-27.

56. Medici D, Potenta S, Kalluri R. (2011). Transforming growth factor-β2 promotes Snail-mediated endothelial mesenchymal transition through convergence of Smad-dependent and Smad-independent signaling. Biochem. J. 433, 515-20.

57. van Meeteren LA, ten Dijke P. (2012). Regulation of endothelial cell plasticity by TGF-β. Cell Tissue. Res. 347, 177-86.

58. Piera-Velazquez S, Jimenez SA. (2012). Molecular mechanisms of endothelial to mesenchymal cell transition (EndoMT) in experimentally induced fibrotic diseases. Fibrogenesis. Tissue. Repair. 5, Suppl 1:S7.

59. Vandewalle C, Van Roy F, Berx G. (2009). The role of the ZEB family of transcription factors in development and disease. Cell. Mol. Life. Sci. 66, 773-87.

60. Li Z, Jimenez SA. (2011). Protein kinase Cδ and c-Abl kinase are required for transforming growth factor β induction of endothelial-mesenchymal transition in vitro. Arthritis Rheum. 63, 2473-83.

61. Razani B, Zhang XL, Bitzer M, et al. (2001). Caveolin-1 regulates transforming growth factor (TGF)-beta/SMAD signaling through an interaction with the TGF-beta type I receptor. J. Biol. Chem. 276, 6727-38.

62. Del Galdo F, Lisanti MP, Jimenez SA. (2008). Caveolin-1, transforming growth factor-beta receptor internalization, and the pathogenesis of systemic sclerosis. Curr. Opin. Rheumatol. 20, 713-19.

63. Del Galdo F, Sotgia F, de Almeida CJ, et al. (2008). Decreased expression of caveolin 1 in patients with systemic sclerosis: crucial role in the pathogenesis of tissue fibrosis. Arthritis Rheum. 58, 2854-65.

64. Tourkina E, Richard M, Gööz P, et al. (2008). Antifibrotic properties of caveolin-1 scaffolding domain in vitro and in vivo. Am. J. Physiol. Lung Cell. Mol. Physiol. 294, L843-861.

65. Wang XM, Zhang Y, Kim HP, et al. (2006). Caveolin-1: a critical regulator of lung fibrosis in idiopathic pulmonary fibrosis. J. Exp. Med. 203,2895-906.

66. Jasmin JF, Mercier I, Dupuis J, et al. (2006). Short-term administration of a cell-permeable caveolin-1 peptide prevents the development of monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy. Circulation. 114, 912-20.

67. Li Z, Wermuth PJ, Benn BS, et al. (2013). Caveolin-1 deficiency induces spontaneous endothelial-to-mesenchymal transition in murine pulmonary endothelial cells in vitro. Am. J. Pathol. 182, 325-31.

68. Abraham DJ, Vancheeswaran R, Dashwood MR, et al. (1997). Increased levels of endothelin-1 and differential endothelin type A and B receptor expression in scleroderma-associated fibrotic lung disease. Am. J. Pathol. 151, 831-41.

69. Xu S, Denton CP, Holmes A, et al. (1998). Endothelins: effect on matrix biosynthesis and proliferation in normal and scleroderma fibroblasts. J. Cardiovasc. Pharmacol. 31, S360-3.

70. Leask A. (20110. The role of endothelin-1 signaling in the fibrosis observed in systemic sclerosis. Pharmacol. Res. 63, 502-3.

71. Widyantoro B, Emoto N, Nakayama K, et al. (2010). Endothelial cell-derived endothelin-1 promotes cardiac fibrosis in diabetic hearts through stimulation of endothelial-to-mesenchymal transition. Circulation. 121, 2407-18.

72. Cipriani P, Di Benedetto P, Ruscitti P, et al. (2015). The endothelial-mesenchymal transition in systemic sclerosis is induced by endothelin-1 and transforming growth factor-β and may be blocked by macitentan, a dual endothelin-1 receptor antagonist. J. Rheumatol. Aug 15. pii: jrheum.150088. [Epub ahead of print].

73. Louvi A, Artavanis-Tsakonas S. (2012). Notch and disease: a growing field. Semin. Cell. Dev. Biol. 23, 473-80.

74. Penton AL, Leonard LD. (2012). Notch signaling in human development and disease. Spinner NB. Semin. Cell. Dev. Biol. 23, 450-7.

75. Chari NS, McDonnell TJ. (2007). The sonic hedgehog signaling network in development and neoplasia. Adv. Anat. Pathol. 14, 344-52.

76. Briscoe J, Thérond PP. (2013). The mechanisms of Hedgehog signalling and its roles in development and disease. Nat. Rev. Mol. Cell. Biol. 14, 416-29.

77. Horn A, Palumbo K, Cordazzo C, et al. (2012). Hedgehog signaling controls fibroblast activation and tissue fibrosis in systemic sclerosis. Arthritis. Rheum. 64, 2724-33.

78. Beyer C, Distler JH. (2013). Morphogen pathways in systemic sclerosis. Curr. Rheumatol. Rep. 15, 299.

79. Beyer C, Dees C, Distler JH. (2013). Morphogen pathways as molecular targets for the treatment of fibrosis in systemic sclerosis. Arch. Dermatol. Res. 305, 1-8.

80. Noseda M, McLean G, Niessen K, et al. (2004). Notch activation results in phenotypic and functional changes consistent with endothelial-to-mesenchymal transformation. Circ. Res. 94, 910-7.

81. Niessen K, Fu Y, Chang L, et al. (2008). Slug is a direct Notch target required for initiation of cardiac cushion cellularization. J. Cell. Biol. 182, 315-25.

82. Chang AC, Fu Y, Garside VC. et al. (2011). Notch initiates the endothelial-to-mesenchymal transition in the atrioventricular canal through autocrine activation of soluble guanylyl cyclase. Dev. Cell. 21, 288-300.

83. Fu Y, Chang A, Chang L, et al. (2009). Differential regulation of transforming growth factor beta signaling pathways by Notch in human endothelial cells. J. Biol. Chem. 284, 19452-62.

84. Ding H, Zhou D, Hao S, et al. (2012). Sonic Hedgehog Signaling Mediates Epithelial-Mesenchymal Communication and Promotes Renal Fibrosis. J. Am. Soc. Nephrol. 23, 801-13.

85. Syn WK, Jung Y, Omenetti A, et al. (2009). Hedgehog-mediated epithelial-to-mesenchymal transition and fibrogenic repair in nonalcoholic fatty liver disease. Gastroenterology. 137, 1478-88.

86. Fabian SL, Penchev RR, Jacques BS, et al. (2012). Hedgehog-Gli Pathway Activation during Kidney Fibrosis. Am. J. Pathol. 180, 2935-51.

87. Jung IH, Jung DE, Park YN, et al. (2011). Aberrant Hedgehog ligands induce progressive pancreatic fibrosis by paracrine activation of myofibroblasts and ductular cells in transgenic zebrafish. PLoS. One. 6:e27941. Epub 2011 Dec 2.

88. Bolaños AL, Milla CM, Lira JC, et al. (2012). Role of Sonic Hedgehog in idiopathic pulmonary fibrosis. Am. J. Physiol. Cell. Mol. Physiol. 303, L978-90.

89. Horn A, Palumbo K, Cordazzo C, et al. (2012). Hedgehog signaling controls fibroblast activation and tissue fibrosis in systemic sclerosis. Arthritis Rheum. 64, 2724-33.

90. Clevers H, Nusse R. (2012). Wnt/β-catenin signaling and disease. Cell. 149, 1192-205.

91. Niehrs C. (2012). The complex world of WNT receptor signaling. Nat. Rev. Mol. Cell. Biol. 13, 767-79.

92. Lafyatis R. (2012). Connective tissue disease: SSc-fibrosis takes flight with Wingless inhibition. Nat. Rev. Rheumatol. 8, 441-2.

93. Wei J, Fang F, Lam AP, et al. (2012). Wnt/β-catenin signaling is hyperactivated in systemic sclerosis and induces Smad-dependent fibrotic responses in mesenchymal cells. Arthritis Rheum. 64, 2734-45.

94. Beyer C, Schramm A, Akhmetshina A, et al. (2012). B-catenin is a central mediator of pro-fibrotic Wnt signaling in systemic sclerosis. Ann. Rheum. Dis. 71, 761-7.

95. Dees C, Schlottmann I, Funke R, et al. (2014). The Wnt antagonists DKK1 and SFRP1 are downregulated by promoter hypermethylation in systemic sclerosis. Ann. Rheum. Dis. 73, 1232-9.

96. Lam AP, Flozak AS, Russell S, et al. (2011). Nuclear β-catenin is increased in systemic sclerosis pulmonary fibrosis and promotes lung fibroblast migration and proliferation. Am. J. Respir. Cell. Mol. Biol. 45, 915-22.

97. Aisagbonhi O, Rai M, Ryzhov S, et al. (2011). Experimental myocardial infarction triggers canonical Wnt signaling and endothelial-to-mesenchymal transition. Dis. Model. Mech. 4, 469-83.

98. Cheng SL, Shao JS, Behrmann A, et al. (2013). Dkk1 and MSX2-Wnt7b signaling reciprocally regulate the endothelial-mesenchymal transition in aortic endothelial cells. Arterioscler. Thromb. Vasc. Biol. 33, 1679-89.

99. Li L, Chen L, Zang J, et al. (2015). C3a and C5a receptor antagonists ameliorate endothelial-myofibroblast transition via the Wnt/β-catenin signaling pathway in diabetic kidney disease. Metabolism. 64, 597-610.

100. Semenza GL. (2012). Hypoxia-inducible factors in physiology and medicine. Cell. 148, 399-408.

101. Lokmic Z, Musyoka J, Hewitson TD, et al. (2012). Hypoxia and hypoxia signaling in tissue repair and fibrosis. Int. Rev. Cell. Mol. Biol. 296, 139-85.

102. Hasse VH. (2009). Pathophysiological consequences if HIF activation; HIF as a modulator of fibrosis. Ann. N. Y. Acad. Sci. 1177, 57-65.

103. Higgins DF, Kimura K, Iwano M, et al. (2008). Hypoxia-inducible factor signaling in the development of tissue fibrosis. Cell Cycle. 7, 1128-32.

104. Ruthenborg RJ, Ban JJ, Wazir A, et al. (2014). Regulation of wound healing and fibrosis by hypoxia and hypoxia-inducible factor-1. Mol. Cells. 37, 637-43.

105. Coople BL, Bai S, Burgoon LD, et al. (2011). Hypoxia-inducible factor-1α regulates the expression of genes in hypoxia hepatic stellate cells important for collagen deposition and angiogenesis. Liver Int. 31, 230-44.

106. Sun S. Ning X, Zhang Y, et al. (2009). Hypoxia-inducible fact -1alpha induces Twist expression in tubular epithelial cells subjected to hypoxia, leading to epithelial-to-mesenchymal transition. Kidney Int. 75,1278-87.

107. Xu X, Tan X, Tampe B, et al. (2015). Snail is a direct target of hypoxia-inducible factor 1α (HIF1α) in hypoxia-induced endothelial to mesenchymal transition of human coronary endothelial cells. J. Biol. Chem. 290, 16653-64.

108. Higgins DF, Kimura K, Bernhardt WM, et al. (2007). Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. J. Clin. Invest. 117, 3810-20.

109. Xu X, Tan X, Tampe B, et al. (2015). Snail is a direct target of Hypoxia-inducible Factor 1α 9Hipoxia-induced Endothelial to Mesenchymal Transition of human coronary endothelial cells. J. Biol. Chem. 290, 16653-64.

110. Choi SH, Hong ZY, Nam JK, et al. (2015). A Hypoxia-induced vascular endothelial-to-mesenchymal transition in development of radiation-induced pulmonary fibrosis. Clin. Cancer Res. 21, 3716-26.

111. Hashimoto N, Phan SH, Imaizumi K, et al. (2010). Endothelial-mesenchymal transition in bleomycin-induced pulmonary fibrosis. Am. J. Respir. Cell. Mol. Biol. 43, 161-72.

112. Good RB, Gilbane AJ, Trinder SL, et al. (2015). Endothelial to Mesenchymal Transition Contributes to Endothelial Dysfunction in Pulmonary Arterial Hypertension. Am. J. Pathol. 185, 1850-8.

113. Moore-Morris T, Guimarães-Camboa N, Banerjee I, et al. (2014). Resident fibroblast lineages mediate pressure overload-induced cardiac fibrosis. J. Clin. Invest. 124, 2921-34.

114. Moore-Morris T, Guimarães-Camboa N, Yutzey KE, et al. (2015), Cardiac fibroblasts: from development to heart failure. J. Mol. Med. (Berl). 93, 823-30.

115. Ali SR, Ranjbarvaziri S, Talkhabi M, et al. (2014). Developmental heterogeneity of cardiac fibroblasts does not predict pathological proliferation and activation, Circ. Res. 115, 625-35.

116. Arciniegas E, Frid MG, Douglas IS, et al. (2007). Perspectives on endothelial-to-mesenchymal transition: potential contribution to vascular remodeling in chronic pulmonary hypertension. Am. J. Physiol. Lung Cell. Mol. Physiol. 293, L1-8.

117. Ranchoux B, Antigny F, Rucker-Martin C, et al. (2015). Endothelial-to-mesenchymal transition in pulmonary hypertension. Circulation. pii:CIRCULATIONAHA.114.008750. [Epub ahead of print].

118. Xiong J. (2015). To be EndoMT or not to be, that is the question in pulmonary hypertension. Protein Cell. 6, 547-50.

119. Jimenez SA. (2013). Role of endothelial to mesenchymal transition in the pathogenesis of the vascular alterations in systemic sclerosis. ISRN. Rheumatol. Sep 23, 2013: 835948. doi: 10.1155/2013/835948.

120. Mendoza F, Piera-Velazquez S, Farber J, et al. (2015). Endothelial cells expressing endothelial and mesenchymal cell gene products in Systemic Sclerosis-associated interstitial lung disease lung tissues. Arthritis Rheum. In Press.

Figure Legends.

Figure 1. Signaling pathways involved in SSc pathogenesis that may also play a role in EndoMT.

The diagram shows the TGF-β, ET-1, NOTCH, and Wnt pathways, as well as, other putative pathways such as cellular stress and hypoxia that may participate in the EndoMT process and may be involved in SSc pathogenesis. One central pathway is initiated following TGF-β-binding and subsequent activation of the ALK-1-mediated Smad-independent TGF-β pathway. Activation of this pathway causes phosphorylation of GSK-3β mediated by PKC-δ and the c-Abl non-receptor kinase. Phosphorylation of GSK-3β at serine 9 (ser9) causes its inhibition which then allows Snail-1 to enter the nucleus. Nuclear accumulation of Snail-1 results in marked stimulation of Snail-1 expression which then leads to acquisition of the myofibroblast phenotype with stimulation of α-SMA expression. The inhibition of GSK-3β ser9 phosphorylation by specific inhibition of PKC-δ or c-Abl activity allows GSK-3β to phosphorylate Snail-1 targeting it for proteosomal degradation and thus, effectively abolishes the acquisition of the myofibroblastic phenotype and the fibrotic response. ET-1 effects appear to be mediated by a synergistic stimulation of TGF-β-induced EndoMT involving the canonical Smad pathways although the possibility of a direct stimulation of EndoMT by ET-1 has also been suggested. Other pathways such as those involving Wnt, NOTCH, hypoxia and cellular stress responses may also participate although the molecular events have not been fully elucidated. Modified from Ref. 119.

Figure 2. **Histopathology and immunohistology of SSc-associated ILD lung tissues.**

A. A small artery in the lung of a patient with SSc-associated ILD shows severe narrowing of the vessel lumen with accumulation of elongated mesenchymal cells and large amounts of fibrous tissue in the subendothelial intimal space. **B**. Immunohistochemical staining of the same tissue

for the endothelial cell specific antibody marker CD31. Note the presence of CD31 positive cells in the subendothelial space besides their expected endothelial location. **C**. Lung tissue from another patient showing two cells bearing the EC-specific CD31 marker embedded within the neointimal tissue removed from the endothelium, and a CD31-positive cell cluster within the fibrotic lung parenchyma. **D-F**. CD31 immunohistological staining of lung tissues from three additional SSc patients showing similar findings. (Reproduced from Ref. 120 with permission).

Figure 3. **Confocal microscopy staining for vWF and α-SMA of a small arteriole in the lung of a patient with SSc-associated ILD.**

Staining for vWF is shown in green; staining for α-SMA is shown in red, and co-expression of vWF and α-SMA is shown in yellow in the merged image. All EC (vWF-stained) present within the endothelium and subendothelial tissue express the mesenchymal cell marker α-SMA. (Reproduced from Ref. 120 with permission).

Figure 4. Quantitative PCR assessment and Western blot analysis of expression levels of selected genes and proteins in CD31+/CD102+ lung EC from SSc-associated ILD. Quantitative PCR of two different preparations of CD31+/CD102+ EC from lungs of two SSc patients or from normal lungs analyzed in duplicate. Shown are transcript measurements for interstitial collagen genes (COL1 and COL3), fibronectin 1 (FN1), α-SMA (SMA), EC-specific genes (COL4A1, VE-cadherin, vWF and VEGF), profibrotic genes (TGF-β1 and CTGF), and EndoMT-related transcription factors (SNAI2, and TWIST1). Fold change in CD31+/CD102+ EC from each of the SSc lungs (SSc1 and SSc2) compared to the average levels of the CD31+/CD102+ EC from the two normal lungs. (Reproduced from Ref. 120 with permission).