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Fibrosis in systemic sclerosis: Emerging concepts and implications for targeted therapy

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Abstract

Systemic sclerosis (SSc) is a complex and incompletely understood disease associated with fibrosis in multiple organs. Recent findings identify transforming growth factor-ß (TGF-ß), Wnt ligands, toll-like receptor-mediated signaling, hypoxia, type I interferon, type 2 immune responses and mechanical stress as extracellular cues that modulate fibroblast function and differentiation, and as potential targets for therapy. Moreover, fibrillin-1 has a major role in storing and regulating the bioavailability of TGF-ß and other cytokines, and fibrillin-1 mutations are implicated in a congenital form of scleroderma called stiff skin syndrome. Fibrosis is due not only to the activation of tissue-resident fibroblasts and their transdifferentiation into myofibroblasts, but also the differentiation of bone marrow-derived fibrocytes, and transition of endothelial and epithelial cells, pericytes and adipocytes into activated mesenchymal cells. These responses are modulated by signaling mediators and microRNAs that amplify or inhibit TGF-ß and Wnt signaling. Gain-of-function and loss-of-function abnormalities of these mediators may account for the characteristic activated phenotype of SSc fibroblasts. The nuclear orphan receptor PPAR-γ plays a particularly important role in limiting the duration and intensity of fibroblast activation and differentiation, and impaired PPAR-γ expression or function in SSc may underlie the uncontrolled progression of fibrosis.

Identifying the perturbations in signaling pathways, mediators and differentiation programs that are responsible for SSc tissue damage allows their selective targeting. This in turn opens the door for therapies utilizing novel compounds, or drug repurposing by innovative uses of already-approved drugs. In view of the heterogeneous clinical presentation and unpredictable course of SSc, as well as its complex pathogenesis, only robust clinical trials incorporating the judicious application of biomarkers will be able to clarify the clinical utility of these innovative approaches.

Keywords

Fibrosis; Systemic sclerosis; Fibroblast; TGF-ß; Wnt; Collagen

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1. Introduction

Systemic sclerosis (SSc) is a progressive autoimmune disease of unknown cause associated with substantial mortality. To date there are no disease-modifying therapies, and immunomodulatory agents that are highly effective in other rheumatic diseases have shown disappointing results in SSc. The explanation may lie in the complex nature of SSc, characterized by a constellation of vascular injury, inflammation and fibrosis. It is not clear which of these perturbations is primary. Every SSc patient manifests some degree of vascular injury, immune activation and tissue fibrosis, but the relative contributions of these distinct processes to individual disease phenotype and natural history varies greatly from patient to patient. This variability accounts for the strikingly heterogeneous clinical picture of SSc. The three seemingly disparate yet interrelated pathophysiologic processes account for the clinical manifestations of SSc. Evidence of vascular injury and endothelial damage are detected at initial evaluation in a majority of patients. Over time, progressive vascular damage and obliteration of small and medium sized arteries cause tissue ischemia and its myriad complications. Vascular injury also plays a role in activation of the innate and adaptive immune systems, and contribute directly and indirectly to tissue fibrosis. The interrelationship of the three cardinal processes in the pathogenesis of SSc is illustrated in Fig. 1. In this review we focus on recent exciting developments in understanding fibrosis, the final common pathway in SSc that accounts for much of its mortality.

2. Fibrosis

2.1. Skin fibrosis

Skin fibrosis is prominent and widespread in diffuse cutaneous SSc (dcSSc), whereas in limited cutaneous SSc (lcSSc) vascular complications rather than fibrosis tend to predominate. Fibrotic skin is characterized by thick dermis and obliteration of appendages such as hair follicles, sweat glands and cutaneous blood vessels. Initially fibrosis is most prominent in the reticular dermis, but with progression, the subjacent adipose layer also becomes affected. Biochemical analysis of affected skin demonstrates increased accumulation of the main fibrillar collagens (Type I and Type III), and appearance of Type VII collagen. Moreover, lesional skin has abundant fibrillin and elastin fibrils, and elevated levels of collagen-modifying enzymes such as lysyl hydroxylase 2 (PLOD2) responsible for increased formation of aldehyde-derived collagen cross-links.

The early lesion is accompanied by perivascular inflammatory cell infiltrates, composed largely of T lymphocytes and monocytes. The number of alpha smooth muscle actin-positive myofibroblasts is increased. Eventually the skin becomes atrophic. The epidermis is thin, rete pegs are effaced, and small blood vessels are virtually absent. Vascular rarefaction results in tissue hypoxia and induction of the hypoxia-inducible factor HIF-1 with increased local production of vascular endothelial growth factor (VEGF) and other angiogenic factors. Evidence of tissue hypoxia can even be found in clinically uninvolved apparently “normal” skin of patients with SSc. Hypoxia itself serves as a potent stimulus for fibroblast activation, epithelial–mesenchymal transition (EMT) and progression of fibrosis.

Genome-wide expression profiling using microarrays has provided powerful new insight into the nature of skin fibrosis. These studies reveal strikingly altered patterns of gene expression in skin from SSc patients compared to healthy controls. Prominent perturbations were seen in genes associated with transforming growth factor-ß and Wnt signaling, extracellular matrix, innate immunity and hypoxia. The number of genes differentially expressed in skin biopsy microarrays greatly exceeds the number in explanted dermal fibroblasts, suggesting partial “extinction” of the activated phenotype with ex vivo propagation. Microarray studies also highlight the tremendous patient-to-patient variability in the molecular fingerprint of scleroderma.

2.2. Lung fibrosis

Clinically significant lung fibrosis develops in over 20% of SSc patients. Compared to the skin, the cellular and molecular events that underlie lung fibrosis, and the role of alveolar epithelial cell injury and transdifferentiation, remain obscure. In early lung disease, patchy infiltration of the alveolar walls with lymphocytes, plasma cells, macrophages and eosinophils may be seen, and bronchoalveolar lavage fluid contains Th2-polarized leukocytes, and increased levels of growth factors and chemokines. The hallmark histological lesion is nonspecific interstitial pneumonitis or NSIP, characterized by moderate interstitial inflammation and fairly uniform distribution of fibrosis. Less common in SSc is a histological picture of usual interstitial pneumonia (UIP) characterized by scattered fibroblastic foci and patchy fibrosis, and associated with a worse prognosis. Progressive thickening of the alveolar septae ultimately results in air space obliteration and honeycombing.

2.3. Fibrosis of other organs

Fibrosis can occur in virtually all organs in SSc. The esophagus is virtually always affected, with fibrosis in the lamina propria, submucosa and muscular layers. In the kidneys vascular lesions predominate, and glomerulonephritis is rare. Cardiac involvement is associated with interstitial and perivascular fibrosis, which may be clinically silent or associated with diastolic dysfunction.

3. Fibroblast activation in SSc

Over three decades ago, Carwile LeRoy demonstrated that lesional skin fibroblasts explanted from patients with SSc synthesize increased amounts of collagen in vitro compared to healthy control fibroblasts [1,2]. Additional features that phenotypically define SSc fibroblasts include increased synthesis of extracellular matrix; constitutive production of cytokines and chemokines; altered expression of cell surface integrins and receptors for transforming growth factor-ß (TGF-ß), platelet-derived growth factor (PDGF) and the CC chemokine MCP-1; and myofibroblast transdifferentiation [3]. These phenotypic alterations partially persist for a limited number of ex vivo passages. However, it remains unanswered whether they represent cell-autonomous perturbations of intracellular signaling molecules and pathways (Table 1), or reflect paracrine/autocrine fibroblast activation triggered by extracellular cues [4–6].

4. Cell types and cell fate switching in fibrosis

It is not surprising that a multiplicity of cell types are activated or deregulated in SSc, and play a role in tissue damage. Effector cells prominent at various stages of the disease include endothelial cells and vascular pericytes, cellular components of both the adaptive and innate arms of the immune system (Th2 cells and B lymphocytes, dendritic cells, NK cells, monocytes, alternatively-activated macrophages, mast cells and eosinophils) and possibly plasmacytoid dendritic cells. In the tissue, proximal effector cells directly responsible for fibrosis include bone marrow-derived mesenchymal progenitors such as fibrocytes and monocytes, and most importantly, myofibroblasts. The transition of differentiated non-fibroblastic cell lineages (such as epithelial or endothelial cells or adipocytes) into fibroblasts and myofibroblasts in the context of deregulated fibrogenesis in SSc is an area of substantial research interest. Recent studies enhance our understanding of the intracellular signal transduction pathways that enable mesenchymal effector cells to drive fibrosis and other manifestations of tissue damage. Factors promoting the transition of various precursor and mature cell types toward an activated fibroblastic phenotype include TGF-ß, Wnt and hypoxia, whereas PPAR-γ appears to play an important inhibitory role in these pathways and promotes the maintenance of cellular quiescence.

5. Persistent fibrotic responses: innate immune recognition signaling via toll-like receptors

Recent studies indicate a potential important role for innate immune signaling via toll-like receptors (TLRs) in fibrosis in SSc. A critical aspect of normal host defence against invading microorganisms is the ability to recognize pathogen-associated molecular patterns. Pattern recognition receptors (PRRs) are the critical cellular sensors for exogenous danger. The family of PRRs includes, in addition to TLRs, lectin receptors, scavenger receptors CD36 and MARCO and complement receptors [7]. The TLRs represent a family of 11 receptors expressed on macrophages, neutrophils, B cells and dendritic cells, as well as non-immune cells such as epithelial cells, keratinocytes and myocytes. The expression and function of TLRs on fibroblasts is poorly understood. TLRs allow the innate immune system to sense and rapidly respond to microbial and viral pathogens. Each of the distinct TLRs shares one of two adapter molecules termed MyD88 and TRIF (specific for TLR3 and TLR4). Some TLRs are at the cell surface (TLR2 and TLR4) while others are intracellular, located within endosomes (TLR3, TLR7, and TLR9). The endosomal TLRs recognize viral RNA and bacterial DNA. Upon interaction with a TLR ligand, the adaptor proteins MyD88 and IRAKs are recruited to the TLR cytoplasmic domain, resulting in phosphorylation of IRAK1/4 and activation of downstream TRAF6 and the MAP kinase TAK1. IRF5 is recruited to the TRAF6/TAK1 complex, resulting in activation of IKK with subsequent degradation of IkB and nuclear translocation of NF-kB, as well as activation of AP-1, and induction of type I IFN and pro-inflammatory cytokines.

An increasingly strong case can now be made for the involvement of TLR-mediated innate immune signaling in fibrosis. For instance, TLR4 activation by high-dose LPS plays critical role in liver fibrosis, with sensitization to TGF-ß as the underlying mechanism [8]. Moreover, TLR4 activation also induced the expression of the profibrotic transcription

factors Egr-1 and Egr-2. How might innate immune signaling be implicated in the pathogenesis of fibrosis? It seems likely that fibroblast TLRs are activated by endogenous TLR ligands that are generated in situ as a consequence of tissue injury, autoimmunity and oxidative stress. It is well recognized that TLRs can recognize damage-associated molecular patterns (DAMPs), and aberrant TLR-mediated self-recognition is implicated in chronic inflammation, arthritis and autoimmunity [9]. Injury—via hypoxia or oxygen radical-induced cleavage or modification of normal tissue components—generates DAMPs. Endogenous TLR ligands implicated in SSc belong to three broad categories: matrix-derived molecules such as hyaluronan and its small molecular weight degradation products, alternatively spliced extra domain A of fibronectin (Fn^{EDA}) and biglycan; cellular stress proteins such as HMGB1 and Hsp60; and nucleic acids and immune complexes released from damaged or necrotic cells. We have demonstrated elevated expression of both TLR3 and TLR4 in lesional skin and lung biopsies from SSc patients, accompanied by substantial increase in HA staining. Similar changes were noted in mice with bleomycin-induced scleroderma (Bhattacharyya S, Melichian D, Varga J; unpublished). Remarkably, incubation of normal fibroblasts with the synthetic TLR3 ligand poly(I:C), a mimick for viral RNA, causes dramatic induction of IL-6 and other inflammatory cytokines, as well as type I IFN. These observations suggest that activated fibroblasts exposed to endogenous TLR ligands during tissue injury, switch to an activated phenotype, exacerbating the uncontrolled fibrotic process. In this way, fibroblast TLR signaling initiated by endogenous TLR ligands might be a key factor for converting a self-limited tissue repair with full regeneration into an aberrant and intractable fibrotic scar (Fig. 2).

6. Soluble factors and extracellular cues regulating fibroblast activation and differentiation

Transforming growth factor-ß is the pre-eminent cue for initiating connective tissue remodeling during both normal wound healing and pathological fibrosis. The expression of receptors for TGF-ß as well as surface integrins that activate latent TGF-ß are elevated on SSc fibroblasts, suggesting that the SSc phenotype reflects autocrine TGF-ß stimulation of sensitized cells [10]. While recent studies provide support for the “autocrine TGF-ß hypothesis” [11–14], pharmacological blockade of Type I TGF-ß receptor only incompletely “normalized” SSc fibroblasts [15]. Multiple cytokines, growth factors and chemokines, as well as autoantibodies, hypoxia, and mechanical strain serve as additional extracellular cues for fibroblast activation in SSc (Table 2). Genome-wide analysis comparing skin biopsies from patients with SSc and healthy controls has revealed some 2000 genes that distinguish SSc from healthy controls [16,17].

6.1. Type I Interferon signaling in SSc

Type I interferon (IFN) is a potent immunomodulatory signal that is strongly implicated in the pathogenesis of systemic lupus erythematosus. Increased expression of IFN-regulated genes in peripheral blood leukocytes correlates with disease activity and severity in lupus. It is thought that interaction of antinuclear autoantibodies with circulating plasmacytoid dendritic cells results in the induction of IFN. Increased expression of type I IFN-regulated genes in peripheral blood leukocytes and monocytes has also been reported in SSc [18–20].

Moreover, IFN expression is elevated in the lesional skin [21]. A strong “IFN signature” in SSc appears to be associated with the presence of anti-topo I autoantibodies, and may reflect stimulation of plasmacytoid dendritic cells by nucleic acid-containing immunocomplexes via toll-like receptors such as TLR3. A potential role of IFN in the pathogenesis of SSc is supported by genetic association of alleles coding for genes involved in IFN signaling, such as IRF5 [22]. Remarkably interferons are generally thought to act as potent inhibitors of collagen synthesis and other fibrotic responses. It remains unclear at this time whether elevated IFN signaling in SSc contributes to tissue fibrosis directly or via promoting microvascular injury and apoptosis, or instead represents an attempt to limit injury and attenuate fibrosis. This issue might be resolved by results from clinical trials using agents that block IFN signaling.

6.2. Transforming growth factor-ß (TGF-ß)

Multiple lines of evidence implicate TGF-ß as the master regulator of both physiologic fibrogenesis and pathological fibrosis [23]. TGF-ß is a member of a large cytokine family that also includes BMP and activin. TGF-ß is secreted in a latent form, and is sequestered by LTBP and fibrillin-1 in the extracellular matrix as a biologically inactive molecule. Upon injury, matrix-bound TGF-ß is activated and engages its surface receptors. Latent TGF-ß activation is mediated by thrombospondins and via integrins present on epithelial cells and also on fibroblasts (to be discussed in the later part). The three TGF-ß isoforms recognize a cell surface TGF-ß receptor complex of two serine–threonine kinases that in turn activate downstream signaling cascades. The best studied of these are the Smads, which upon their phosphorylation by the activated Type I TGF-ß receptor, translocate into the nucleus where they act as sequence-specific DNA-binding transcription factors. We have shown that in both normal and SSc fibroblasts TGF-ß induces Smad2/3 activation and heteromeric complex formation. The stimulation of collagen gene transcription by TGF-ß is mediated in part via binding of the activated Smad complex to its cognate SBE site located in the proximal COL1A2 promoter [24,25]. Specificity and potency of the transcriptional response is dictated in part through association with other transcription factors such as Sp1, and by recruitment of the p300 coactivator that acts as a histone acetyltransferase [26]. By inducing locus-specific chromatin relaxation, p300 facilitates Smad2/3 access to the collagen promoter, and potentiates transcriptional stimulation. Although it is present in fibroblasts in limiting amounts, p300 is nevertheless an absolute requirement for TGF-ß induced fibrotic responses [27]. Multiple abnormalities in the Smad signaling pathways have been identified in SSc fibroblasts [28]. These include elevated expression or phosphorylation of Smad2/3, constitutive (ie. ligand-independent) association of p300 with Smad2/3 [29], elevated levels of p300 (Ghosh AK, Bhattacharyya S. Ms in preparation) and defective function of Smad7, the endogenous inhibitor of Smad signaling [30]. Selective pharmacological inhibition of Smad2/3 activation abrogated TGF-ß-induced fibrotic responses in vitro and in vivo [10]. Multiple small molecules that block Type I TGF-ß receptor are in preclinical development for the treatment of fibrosis.

6.3. Profibrotic stimulation by TGF-ß: novel concepts

6.3.1. c-Abelson (c-Abl) tyrosine kinase—Physiologic TGF-ß responses can be mediated via Smad-independent pathways. It was recently demonstrated that in normal lung

and skin fibroblasts, TGF-ß induces Smad-independent activation of c-Abl, a Src family non-receptor tyrosine kinase implicated in chronic myelogenous leukemia (CML) [31,32]. Furthermore, endogenous c-Abl was required for the profibrotic responses induced by TGF-ß in vitro. Therefore, c-Abl is an important previously unrecognized component of the fibroblast response to TGF-ß. Imatinib mesylate is a small molecule inhibitor of c-Abl kinase that is successfully used for the treatment of CML and gastrointestinal stromal tumors (GIST). In vitro, imatinib blocked the stimulation of collagen synthesis, fibro-blast proliferation and morphologic alterations elicited by TGF-ß. Moreover, imatinib suppressed elevated basal collagen gene expression in SSc fibroblasts. In vivo studies in mice showed that imatinib attenuated the severity of lung fibrosis induced by bleomycin [31]. These studies implicate c-Abl is an important component of the profibrotic TGF-ß responses. Results from on-going multicenter clinical trials of imatinib in idiopathic pulmonary fibrosis and SSc will reveal whether inhibiting aberrant c-Abl activity in vivo is associated with a clinically meaningful anti-fibrotic effect.

6.4. Early growth response genes: the Egr family

Egr-1 is the prototypical member of a family of DNA-binding protein zinc finger transcription factors. The expression of Egr-1 is induced at sites of injury by cytokines, lipids and mechanical injury, and implicated in cell proliferation, differentiation and survival. Closely related family members include Egr-2, Egr-3 and Egr-4 that are also DNA-binding transcription factors, as well as NAB-2 which is itself induced by Egr-1 and antagonizes its function. TGF-ß was shown also to induce Egr-1 expression in normal dermal fibroblasts [33]. The response was rapid and transient, occurred via an ERK1/2-dependent and Smad-independent mechanism, and involved transcriptional stimulation. Fibroblasts lacking Egr-1 showed loss of collagen stimulation in response to TGF-ß, identifying Egr-1 as a novel downstream mediator of profibrotic TGF-ß responses. A GC-rich Egr-1 binding element was mapped within the proximal promoter of the human COL1A2 gene, and binding of Egr-1 to this element was required for full TGF-ß stimulation of COL1A2 transcription. Additional observations highlight the potential significance of Egr-1 as a novel mediator of the fibrotic process. For example, Egr-1 is known to induce the production of TGF-ß and TGF-ß receptors, and also stimulates the coactivator p300, thereby greatly amplifying TGF-ß-induced cellular responses [34]. The levels of Egr-1 are elevated in lesional SSc skin and in the bleomycin-induced mouse model of scleroderma. Significantly, although mice lacking Egr-1 are viable and appear normal, they show markedly attenuated fibrotic response to bleomycin in vivo [35]. Furthermore, transgenic mice that over-express activated TGF-ß or IL-13 on an Egr-1-deficient genetic background fail to develop lung fibrosis [36]. Together, these findings implicate a novel role for Egr-1 in fibrosis in SSc. The role of other Egr family members in mediating TGF-ß responses and fibrosis has not yet been evaluated.

6.5. Wnt-ß-catenin signaling in SSc: aberrant activation of a developmental program

The Wnts constitutes a family of 19 secreted signaling glycoproteins with key roles in embryonic development and organogenesis. Reactivation of Wnt signaling in adults is generally associated with disease, including cancer and fibrosis. Wnt signaling occurs primarily via the canonical ß-catenin pathway initiated by binding of Wnt ligand to Frizzled

(FZD) cell surface receptors, resulting in stabilization and accumulation of cytosolic ß-catenin. In the absence of ligand, cellular ß-catenin is phosphorylated by glycogen synthetase kinase 3-ß (GSK3-ß), leading to its ubiquitination and proteasomal degradation [37]. In the presence of ligand, the FZD receptor becomes activated, leading to inhibition of GSK-3ß and blockade of ß-catenin degradation. ß-catenin is translocated into the nucleus where is regulates target gene transcription via interaction with DNA-binding factors such as TCF/LEF. Expression of a large number of genes is regulated by canonical Wnt signaling in a cell type-specific manner [38]. Moreover, the Wnt pathway engages in cross-talk with TGF-ß signaling.

Wnt signaling is aberrantly activated in fibrosis, suggesting the reactivation of developmental programs as a potential mechanism underlying various forms of fibrosis [39]. Unbiased genome-wide profiling using microarray screens reveals elevated expression of Wnt ligands including Wnt2 and Wnt5a, in patients with pulmonary fibrosis [40] and SSc [16,41]. Other studies have described nuclear localization of activated ß-catenin or increased phosphorylation of GSK-3ß in fibrotic lungs, indicating increased canonical Wnt signaling [42,43]. By immunohistochemistry, we have found evidence of constitutive Wnt-ß-catenin activation in the lungs of patients with SSc-associated pulmonary fibrosis (Lam A et al., Ms. in preparation). The source of Wnt ligand in fibrosis and SSc, and the triggers for aberrant Wnt signaling, are unknown. In light of the ability of canonical Wnt pathway to stimulate fibroblast activation and induce adipocyte and mesenchymal progenitor cell differentiation in vitro, and the association of transgenic Wnt expression with the development of scleroderma-like skin fibrosis in the mouse (Wei J, Macdougald O, Varga J, unpublished), aberrant Wnt signaling, perhaps reflecting reactivation of developmental programs in response to tissue injury, is likely to be an important factor in the pathogenesis of SSc, and an interesting potential target for therapy.

6.6. Integrin signaling in SSc

Fibroblast function is strongly influenced by interaction with the surrounding extracellular matrix mediated via cell surface integrins [44]. Moreover, integrins are intimately involved in localizing growth factors and regulating their activity. Recent studies have addressed the role of integrin signaling in the pathogenesis of SSc. The αvβ5 and αvβ3 integrins are significantly elevated in SSc fibroblasts and can mediate the activation of latent TGF-ß sequestered in the extracellular matrix [45,46]. The functional significance of the elevated integrin expression was further elucidated using cultured fibroblasts. These studies showed that integrin receptors directly contribute to the phenotypic alterations of SSc fibroblasts, including elevated collagen synthesis and conversion to myofibroblasts. A recent report focused on the stiff skin syndrome, a rare congenital condition associated with striking scleroderma-like changes in the skin [47]. Individuals with stiff skin syndrome were found to have mutations in the fibrillin-1 gene that disrupts αvβ3 integrin function, and therefore the ability of fibroblasts to interact with the surrounding matrix. Moreover, skin biopsies from these patients show evidence of activated TGF-ß signaling in the dermis with elevated levels of phospho-Smad2.

6.7. Hypoxia

Vascular rarefaction resulting from the double insults of vascular injury and excessive matrix accumulation in SSc results in tissue hypoxia. Hypoxia acting primarily through HIF-1, serves as a potent stimulus for the synthesis of extracellular matrix molecules including collagen and fibronectin [48]. Moreover hypoxia stimulates the secretion of TGF-ß and CTGF. We and others have shown that hypoxia can also drive the transition of fully differentiated epithelial cells into myofibroblasts, a process called epithelial–mesenchymal transition (EMT) that is mediated via autocrine TGF-ß as well as HIF-1, and can be blocked by liagands of peroxisome proliferator-activated receptor gamma (to be discussed in the later part) [49,50]. Thus, tissue hypoxia that occurs as a consequence of vascular damage and capillary rarefaction directly contributes to fibrosis and maintenance of a vicious cycle. A recent paper provides compelling experimental support for the role of hypoxia in exacerbating and perpetuating the progression of fibrosis [51]. In this study, mice with myeloid-cell-specific VEGF knockout showed markedly increased tissue hypoxia and activation of Wnt-ß-catenin signaling upon bleomycin-induced lung injury, culminating in striking exacerbation of experimental fibrosis.

6.8. Bioactive lipids

A variety of bioactive lipids have emerged as potent modulators of fibroblast function, and have been shown to positively or negatively regulate extracellular matrix biosynthesis and accumulation. While certain prostanoids inhibit fibrotic responses and tissue remodeling through a variety of mechanisms [52], prostaglandin F ($PGF_{2\alpha}$) was recently shown to be elevated in the lungs of patients with pulmonary fibrosis, and be capable to stimulating collagen production and fibroblast proliferation [53]. Moreover, mice with targeted deletion of the PGF receptor were protected from bleomycin-induced pulmonary fibrosis, demonstrating that an important role for this prostanoid in fibrosis. The expression and function of PGF and its receptor in SSc has not been evaluated to date. Lysophosphatidic acid (LPA) is generated via the hydrolysis of membrane phospholipids. LPA exerts multiple biological activities via signaling through G-protein coupled transmembrane receptors. Recently LPA was shown to induce fibroblast chemotaxis and CTGF production [54]. The levels of LPA are elevated in the lungs of patients with pulmonary fibrosis. Moreover, mice with targeted deletion of LPA1 are protected from bleomycin-induced lung fibrosis. A recent study indicates that LPA induces avß6-mediated TGF-ß activation in epithelial cells, contributing to sustained autocrine TGF-ß signaling [55]. In light of these findings, LPA might be a potential target for therapy for fibrosis of the lungs, kidneys and liver. The availability of small molecules that block LPA1-mediated signaling makes this approach particularly appealing.

7. Peroxisome proliferator-activated receptor-γ: negative regulation of fibroblast activation and differentiation

Peroxisome proliferator-activated receptor gamma (PPAR-γ) directly modulates TGF-ß signaling and mesenchymal cell plasticity, and is increasingly associated with regulation of matrix remodeling and fibrosis. Originally identified in adipocytes, PPAR-γ is a nuclear hormone receptor and ligand-inducible transcription factor with fundamental roles in

adipogenesis and lipid metabolism. In the absence of a recognized true physiologic ligand, PPAR-γ is considered to be an orphan receptor. Multiple lipid moieties and prostanoids such as 15d-prostaglandin J_2 (15d-PGJ_2) can act as endogenous PPAR-γ agonists. Ligand activation of cellular PPAR-γ results in its nuclear accumulation and binding to conserved DNA elements (PPRE) in target gene promoters. Homozygous deletion of the PPAR-γ gene results in embryonic lethality in mice due to defective placental development. The thiazolidinedione class of insulin-sensitizing drugs such as rosiglitazone or pioglitazone are potent agonists of PPAR-γ, indicating that a crucial role of PPAR-γ in the regulation of glucose homeostasis. Moreover, it is becoming evident that in addition, PPAR-γ is involved in vascular biology, cell proliferation and immune responses, and abnormal PPAR-γ function is implicated in lipodystrophy, atherosclerosis, pulmonary hypertension, cancer and inflammatory diseases. Allelic polymorphisms of the PPAR-γ gene are associated with type 2 diabetes, obesity, cardiovascular diseases and asthma. Thus the PPAR-γ pathway represents an appealing therapeutic target in a variety of diseases.

Recent studies have revealed an entirely novel function for PPAR-γ in connective tissue homeostasis and matrix remodeling as a cell-intrinsic anti-fibrotic pathway [56]. It was shown in normal fibroblasts that activation of fibroblasts with either natural (15d-PGJ_2) or synthetic (rosiglitazone) PPAR-γ ligands resulted in a virtual abrogation of TGF-ß-induced collagen production and Smad3-dependent transcriptional responses [57]. Subsequent studies exploring the mechanism underlying the anti-TGF-ß activity of PPAR-γ showed that PPAR-γ blocked p300 recruitment due to competition for limiting amounts of this indispensable Smad3 coactivator (squelching), resulting in inhibition of Smad-dependent transcriptional responses [58,59]. Moreover, PPAR-γ blocks the activation of Egr-1 [60], a TGF-ß-inducible transcription factor required for stimulation of collagen synthesis (as previously discussed). Epithelial to mesenchymal transition (EMT) is a cellular differentiation pathway regulated by TGF-ß that is considered to be important in embryonic development. Inappropriate EMT is implicated in cancer and the pathogenesis of organ fibrosis. Incubation of TGF-ß-stimulated alveolar epithelial cells with PPAR-γ ligands prevented EMT and the associated decline in E-cadherin levels [61]. Preadipocytes are multipotent mesenchymal progenitor cells that can be induced to differentiate into both adipocytes and fibroblasts. The principal mediator of adipogenic differentiation is PPAR-γ which is permissive for preadipocyte differentiation into mature adipocytes. Evidence suggests that tissue injury can lead to loss of cellular PPAR-γ , which is associated with transdifferentiation of quiescent preadipocytes and so-called lipofibroblasts into activated myofibroblasts via the process of adipocytemesenchymal transition (AMT). Therefore, PPAR-γ plays a fundamental role in regulating mesenchymal cell lineage fate determination and can shift progenitor cell differentiation along fibrogenic or non-fibrogenic pathways.

8. Impaired PPAR-γ in fibrosis and SSc: role in pathogenesis

Fibroblast-specific gene targeting of PPAR-γ resulted in markedly exaggerated skin fibrosis when mice were injected with bleomycin [62]. Similarly, PPAR-γ deletion in follicular stem cells in mice causes localized fibrosis that resembles scarring alopecia [63]. On the other hand, we found that treatment with rosiglitazone attenuated bleomycin-induced dermal fibrosis in mice, suggesting a critical physiologic role for PPAR-γ in preventing excessive

fibrotic responses. Consistent with this notion are recent findings from a full-thickness incisional model of normal wound healing in mice [64]. This study demonstrated that resolution of wound healing was associated with local up-regulation of PPAR-γ expression, suggesting that the shift from PGE to 15d-PGJ_2-induced PPAR-γ signaling might be the crucial physiologic switch that initiates the resolution phase of tissue repair. These studies suggest that a key role of PPAR-γ is to serve as an endogenous anti-fibrotic. Indeed, there is abundant evidence indicating that reduced PPAR-γ expression or function is associated with spontaneous or inducible fibrosis in vivo. Studies have shown a decline of PPAR-γ tissue expression concomitant with the onset of fibrosis in experimental models of lung, liver and kidney fibrosis [65–67]. The process of aging itself is associated with declining PPAR-γ expression [68].

We have found that PPAR-γ expression and activity are impaired in patients with dcSSc. Furthermore, PPAR-γ expression shows an inverse relationship with enhanced TGF-ß signaling in lesional tissue (Wei J, Varga J; Ms. in review). Although the cause underlying the PPAR-γ deficit in SSc and other fibrosing conditions is not yet known, it is noteworthy that multiple factors implicated in the pathogenesis of fibrosis potently inhibit PPAR-γ expression. The list of factors inhibiting PPAR-γ expression includes TGF-ß, along with members of the Wnt family (Wnt3a and Wnt10b), IL-13, hypoxia, LPA, CTGF and leptin. Therefore, aberrant expression or activity of these profibrotic mediators may be responsible for suppressing PPAR-γ function, which in turn contributes to the progression of fibrosis. Genome-wide studies show an association of dcSSc with the PPAR-γ locus in European populations. Together, these studies findings implicate altered PPAR-γ expression and function in SSc, and raise the possibility that activating PPAR-γ signaling using synthetic agonists, or enhancing defective PPAR-γ tissue expression, may be effective novel approaches to treatment of fibrosis.

9. Conclusion

The intractable problem of fibrosis is finally beginning to yield its secrets. Unbiased genome-wide expression analysis using DNA microarrays, population-based genetic association studies and transgenic animal models are contributing to a substantially enhanced understanding of the cellular and molecular basis of fibrosis in SSc. Cell-intrinsic alterations in SSc fibroblasts contribute to their activated phenotype. These include aberrant expression of TGF-ß receptors, activation of downstream Smads and Smad-independent pathways, c-Abl, integrins and Egr-1; and a functional deficiency of endogenous repressors of fibroblast differentiation and collagen production such as PPAR-γ and microRNAs. The roles of hypoxia, perturbed Wnt, TGF-ß and lipid signaling, progenitor cell differentiation and cellular transitions in the development of fibrosis are becoming clearly defined. These emerging cellular and molecular targets provide myriad novel opportunities for therapeutic interventions, as well as for the discovery and validation of pathogenesis-based biomarkers for clinical studies. In the coming years we anticipate very rapid research progress toward finding effective treatments for SSc.

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Take-home messages

- SSc is highly heterogeneous in its clinical and autoimmune manifestations; individualized therapy required
- SSc is distinct from other rheumatic diseases; the unique combination of fibrosis and vascular disease is prominent
- because its manifestations and clinical course of SSc are highly heterogeneous, there is an urgent need for the discovery and validation of biomarkers to help classify patients, identify high risk groups, predict drug responders
- There are no effective therapies for reversing or even controlling fibrosis; however, multiple cellular and molecular targets are emerging from basic research
- The efficacy of therapeutically targeting these molecules and pathways will be determined by carefully designed randomized prospective trials and careful assessment of clinical responses and changes in relevant biomarkers

BCG Vaccination: A Role for Vitamin D?

BCG vaccination is administered in infancy in most countries with the aim of providing protection against tuberculosis. There is increasing interest in the role of vitamin D in immunity to tuberculosis. Lalor MK, et al. (PLoS One 2011;6:16709) determined if there was an association between circulating 25(OH)D concentrations and BCG vaccination status and cytokine responses following BCG vaccination in infants. Blood samples were collected from UK infants who were vaccinated with BCG at 3 (n=47) and 12 (n=37) months post BCG vaccination. These two time-points are denoted as time-point 1 and time-point 2. Two blood samples were also collected from age-matched unvaccinated infants (n=32 and 28 respectively), as a control group. Plasma vitamin D concentrations (25(OH)D) were measured by radio-immunoassay. The cytokine IFNγ was measured in supernatants from diluted whole blood stimulated with M.Tuberculosis (M.tb) PPD for 6 days. 58% of infants had some level of hypovitaminosis (25(OH)Db30ng/ml) at time-point 1, and this increased to 97% 9 months later. BCG vaccinated infants were almost 6 times (CI: 1.8-18.6) more likely to have sufficient vitamin D concentrations than unvaccinated infants at time-point 1, and the association remained strong after controlling for season of blood collection, ethnic group and sex. Among vaccinees, there was also a strong inverse association between IFNγ response to M.T. PPD and vitamin D concentration, with infants with higher vitamin D concentrations having lower IFNγ responses. Vitamin D may play an immuno-regulatory role following BCG vaccination. The increased vitamin D concentrations in BCG vaccinated infants could have important implications: vitamin D may play a role in immunity induced by BCG vaccination and may contribute to non-specific effects observed following BCG vaccination.

Anti-class A scavenger receptor autoantibodies from systemic lupus erythematosus patients impair phagocytic clearance of apoptotic cells by macrophages in vitro

Inadequate clearance of apoptotic cells by macrophages is one of the reasons for the breakdown of self-tolerance. Class A scavenger receptors, macrophage receptor with collagenous structure (MARCO) and scavenger receptor A (SR-A), which are expressed on macrophages, play important roles in the uptake of apoptotic cells. A previous study reported the presence of the anti-MARCO antibody in lupus-prone mice and systemic lupus erythematosus (SLE) patients. In this regard, Chen XW, et al. (Arthritis Res Ther 2011;13:R9) aimed to investigate the prevalence of anti-class A scavenger receptor antibodies in patients with various autoimmune diseases, in particular SLE, and the functional implication of those autoantibodies in the phagocytic clearance of apoptotic cells by macrophages. Purified recombinant scavenger receptor cysteine-rich (SRCR) polypeptide (ligand-binding domain of MARCO) and recombinant SR-Awere used as antigens. By using enzyme-linked immunosorbent assay, the anti-SRCR and anti-SR-A antibodies were detected in the sera of untreated patients with SLE (n=65), rheumatoid arthritis (n=65), primary Sjogren syndrome (n=25), and healthy blood donors (n=85). The effect of IgG purified from SLE patients or healthy controls on the phagocytosis of apoptotic cells by macrophages was measured by the flow cytometry assay. Anti-SRCR antibodies were present in patients with SLE (18.5%) and rheumatoid arthritis (3.1%), but not in those with primary Sjogren syndrome. Anti-SR-A antibodies were present in patients with SLE (33.8%), rheumatoid arthritis (13.8%), and primary Sjogren syndrome (12.0%). IgG from SLE patients positive for anti-SRCR or anti-SRA antibodies showed a higher inhibition rate on binding of apoptotic cells to macrophages than IgG from healthy controls (both Pb0.05). IgG from SLE patients positive for both anti-SRCR and anti-SR-A antibodies showed a significantly higher inhibition rate on ingestion of apoptotic by macrophages than IgG from healthy controls (Pb0.05). Authors concluded that autoantibodies to class A scavenger receptors might contribute to the breakdown of self-tolerance by impairing the clearance of apoptotic debris and play a role in the pathogenesis of autoimmune disease, especially in SLE.

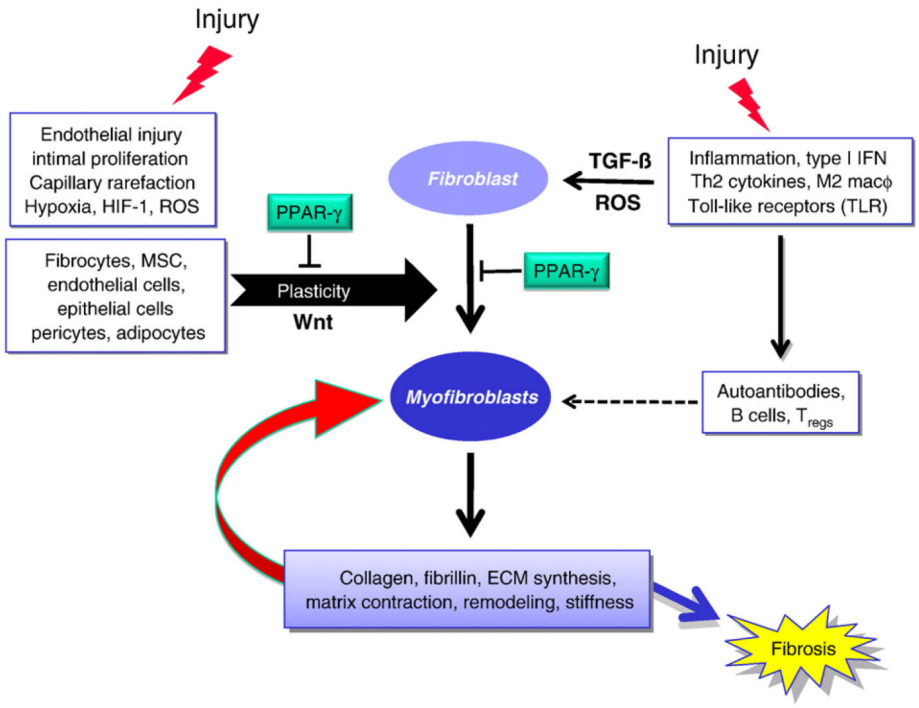

Fig. 1.

Schematic overview of the cellular and molecular pathways underlying fibrosis in systemic sclerosis. Injury due to virus, autoantibodies, ischemia–reperfusion, toxins causes inflammation, including T cell and macriphage activation and the generation of autoantibodies. Cytokines and growth factors such as TGF-ß and Wnt10b, and reactive oxygen species geenaretyd at the injury cause fibroblast activation and differentiation into myofibroblasts that produce excess amounts of collagen, contract and remodel the connective tissue, and are resistant to elimination by apoptosis. Injury also directly induces transition of pericytes, epithelial and endothelial cells into myofibroblasts, expanding the tissue pool of matrix–synthesizing activated fibroblasts.

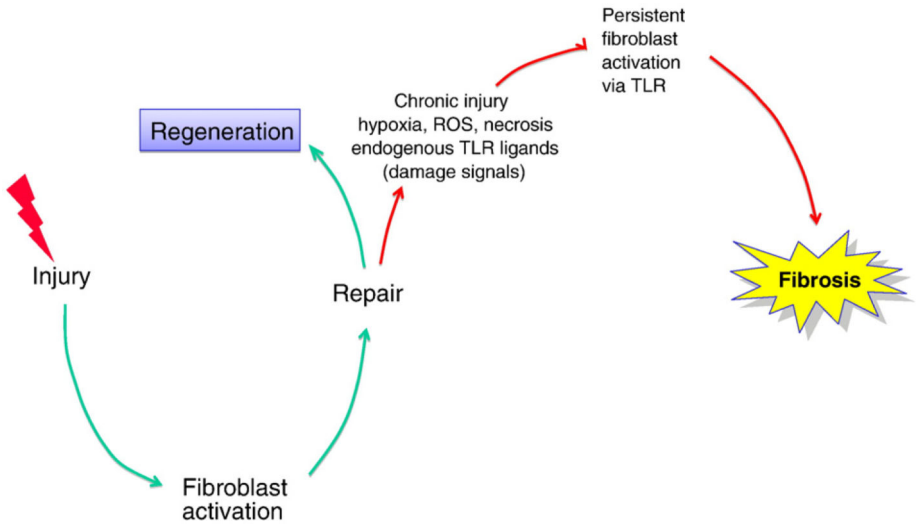

Fig. 2.

Tissue damage and innate immune signaling transform an orderly self-limited repair into a disorderly sustained fibrogenic process. Following injury, fibroblasts undergo a regulated activation. Once repair has been accomplished, tissue regeneration is complete. When recurrent or sustained injury leads to damage, matrix molecules are fragmented, resident cells die and toll like receptors are up–regulated. This enables activation of toll like receptor–mediated innate immune recognition signaling and sustained fibroblast activation culminating in excessive fibrogenesis.

Table 1

Intracellular signal mediators showing aberrant expression in SSc.

Molecule	Increased expression/activity	Decreased expression/activity
Egr-1, Egr-2	⇑	
Sp1	⇑	
p300/CBP	⇑	
Fli-1		⇓
Smad7		⇓
Ski/Sno		⇓
Nab2		⇓
PTEN		⇓
PPAR-gamma microRNA		⇓

Table 2

Extrinsic mediators of fibroblast activity potentially implicated in SSc.

Cytokines
TGF-ß
IL-4
IL-13
IL-17
IL-33
Growth factors, peptides and bioactive lipids
Wnt family (Wnt3a, Wnt10b, others)
CTGF (matricellular protein)
PDGF
IGFBP-5
Endothelin-1
Adenosine
Lysophosphatidic acid (LPA)
Prostaglandin F
Chemokines
CXCL12
MCP-1
Autoantibodies
Antibody to Topo I
Anti-fibroblast antibody
Anti-PDGF antibody