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Transforming growth factor-beta in systemic sclerosis (scleroderma)

John Varga[1], Michael L. Whitfield[2]

[1]Feinberg School of Medicine, Northwestern University, Chicago IL, [2]Dartmouth Medical School, Hanover, NH

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1. ABSTRACT

Deregulated transforming growth factor-beta (TGF-beta) activity and responses play prominent roles in the pathogenesis of systemic sclerosis (SSc), a chronic and progressive connective tissue disease characterized by fibrosis of the skin and internal organs. Systemic sclerosis has highly heterogeneous clinical manifestations, and patients can be classified into multiple subgroups on the basis of distinct molecular signatures defined by transcriptional profiling of gene expression in target organs. Current research to uncover how TGF-beta regulates fibroblast function opens the door for the discovery of targeted therapies. Anti-fibrotic treatments that selectively block TGF-ß expression, biological activity or intracellular signaling in SSc are currently under development.

2. INTRODUCTION: SYSTEMIC SCLEROSIS (SCLERODERMA) IN A NUTSHELL

Recent studies firmly implicate transforming growth factor-ß (TGF-ß) in the pathogenesis of systemic sclerosis (SSc), and TGF-ß is emerging as a molecular target of choice for therapy. Systemic sclerosis has a complex and poorly-understood pathogenesis, with multiple genes influencing disease susceptibility and clinical course. Like other connective tissue diseases, SSc occurs predominantly in women with onset in young-to middle age. It has strikingly heterogeneous clinical manifestations, generally follows a chronic and progressive course and is associated with substantial morbidity and mortality. Death is often due to pulmonary, renal, cardiac and gastrointestinal complications. There are currently no effective disease-modifying treatments, and

Table 1. Definition of systemic sclerosis

Key pathophysiologic processes	Clinical correlates
Immune activation and autoimmunity	Tissue infiltration with activated T cells, macrophages; type 2 polarized immune responses; specific autoantibodies (anti-Topoisomerase I; anticentromere; anti-RNA polymerase III)
Vascular injury and obliteration	Raynaud phenomenon; mucocutaneous telangiectasia; obliterative arteriopathy of small and medium-sized blood; ischemic finger ulcers, gastric vascular ectasia ("watermelon stomach"), malignant hypertension and acute renal failure; capillary rarefication
Tissue fibrosis	Tight skin; interstitial lung disease; skin sclerosis; heart failure; gastroesophageal hypomotility; muscle atrophy; tendon rubs and joint contractures

immunosuppressive therapy has been generally ineffective (Table 1). Based on the extent and pattern of skin involvement, most patients can be subclassified into one of two subsets: diffuse cutaneous SSc and limited cutaneous SSc (Figure 1). These two subsets of SSc are associated with distinct clinical features and long-term outcome, and non-overlapping autoantibody profiles. Localized forms of scleroderma are not associated with internal organ complications, and carry a benign prognosis, and will not be further considered here.

The clinical and pathological characteristics of SSc are summarized in Table 2. Virtually all patients develop Raynaud phenomenon (episodic vascospasms in the digits) at some point during their disease. Other common vascular manifestations include ischemic digital ulcers, pulmonary arterial hypertension, gastric vascular ectasia, mucocutaneous telangiectasia, and renal arterial involvement with acute renal failure. Circulating autoantibodies can be detected in >90% of patients, and unique anti-centromere and anti-topo-I antibodies are found in 40%, distinguishing SSc from other autoimmune conditions such as systemic lupus erythematosus. A recent report described the presence of a serum autoantibody to the platelet-derived growth factor (PDGF) receptor that was capable of inducing activation of fnormal fibroblasts (1). Furthermore, the highly SSc-specific Topo-I autoantibody induces production of interferon-alpha (IFN-α) in plasmacytoid dendritic cells *ex vivo*, which may account for the "interferon signature" detected by gene expression analysis of peripheral blood leukocytes in SSc patients (2). Elevated IFN-α contributes to persistent T and B cell activation and autoantibody production, and may also play a role in loss of V-cadherin in small blood vessels, suppression of vasculogenesis and resultant capillary rarefaction characteristic of SSc (3,4).

Organ fibrosis, autoimmunity and progressive vascular loss are invariable features in all patients, but vary in their severity, with some patients having extensive vascular damage but little organ fibrosis, and others with severe fibrosis but clinically insignificant vascular disease. Autoimmune manifestations (circulating autoantibodies, tissue-infiltrating self-reactive T and B cells), and vascular injury (Raynaud phenomenon, mucocutaneous telangiectasia) can be detected in many patients before the onset of fibrosis. The fibrotic process is generally progressive, and ultimately accounts for much of the mortality of SSc. Clinical manifestations include tight skin, joint contractures, muscle wasting, gastrointestinal hypomotility, interstitial lung disease and cardiomyopathy.

3. FIBROSIS IN SSC

Although inflammation, autoimmunity and vascular injury feature prominently in early SSc, fibrosis is the characteristic hallmark of established disease (5). Its extent varies greatly among individual patients, and is a powerful predictor of survival. The key cellular effector of fibrosis is the activated fibroblast. Ordinarily quiescent tissue fibroblasts are primed to respond to extracellular signals by modulating the production of collagens and other extracellular matrix proteins, growth factors and cytokines, and enhancing their migratory, adhesive and contractile phenotypes. Early studies indicated that SSc fibroblasts were autonomously activated (6). The "scleroderma phenotype" has been attributed, in part, to constitutive autocrine stimulation by endogenously produced TGF-ß (7). It remains a matter of debate whether SSc fibroblasts are intrinsically abnormal, or simply reflect activation within the inflammatory and profibrotic microenvironment. Recent DNA microarray studies of gene expression, discussed in detail below, shed light on the pathogenesis of fibrosis in SSc and the molecular heterogeneity of this process. The results reveal prominent activation of the TGF-ß, as well as the Wnt-ß-catenin signaling pathways in the skin. In contrast, only modest and inconsistent activation of these pathways was found when explanted skin fibroblasts were analyzed under *in vitro* culture conditions (vide infra).

4. INSIGHTS FROM GENETIC STUDIES

Recent studies provide evidence that SSc occurs in genetically susceptible individuals upon exposure to as-yet unidentified environmental triggers (8). Although familial SSc is rare, first-degree relatives of patients are at increased risk for SSc (9,10). Twin studies reveal low disease concordance rates but high concordance for antinuclear autoantibodies, suggesting that genetic factors may influence susceptibility, but are insufficient for the development of SSc (11,12). Multiple candidate gene single nucleotide polymorphisms (SNPs) are associated with SSc or with specific disease manifestations, or particular SSc-specific autoantibodies. However, the functional consequences of these genetic polymorphisms, and their precise roles in pathogenesis, remain unclear.

5. GENE EXPRESSION PROFILING IN SSC

Gene expression profiling in SSc has revealed substantial deregulation of gene expression, and unique valuable insight into the molecular processes in end target tissues (13-17) (Figure 2). The number of genes with altered expression in SSc, which is in the thousands,

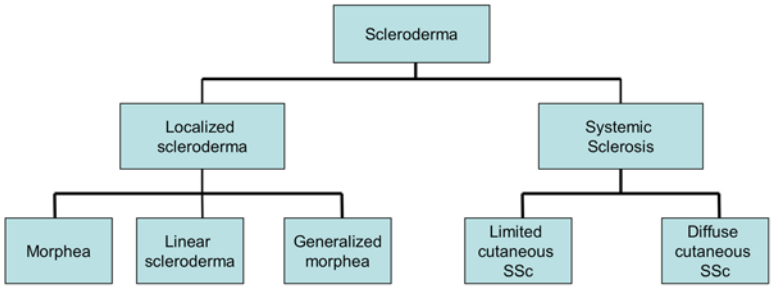

Figure 1. Classification of scleroderma. The two principal subclasses are systemic sclerosis (SSc), which can occur in diffuse and limited forms, and is invariably associated with internal organ complications; and localized scleroderma, which can occur in thee different forms, is more common in children, and generally carries a benign prognosis..

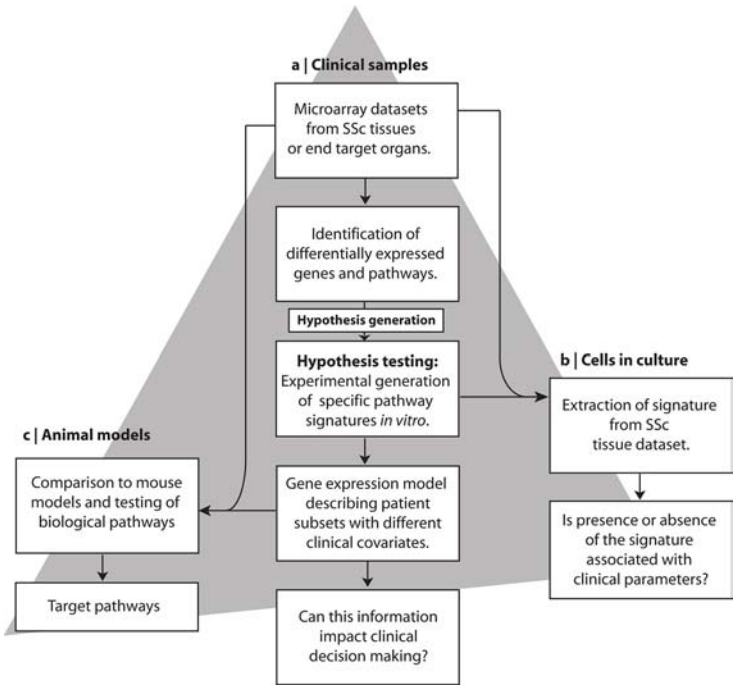

Figure 2. Integrative strategy for the genome-wide analysis of systemic sclerosis and associated animal models of disease. Analysis of the gene expression in clinical samples such as skin or lung biopsies, or peripheral blood cells can lead to the identification of deregulated pathways, infiltrating cells, sub-setting of patients, and hypotheses regarding the underlying pathogenesis (a). Potential deregulated pathways can be interrogated in cells in culture and the resulting mechanism-derived signatures analyzed for association with clinical parameters in clinical samples (b). Alternatively, the pathways can be analyzed using mouse models of disease (c), which provide a more complex tissue environment and can provide a quantitative comparison to the gene signatures in patient tissues.

reflects deregulation of multiple biological pathways. Recent studies indicate that patients with SSc can be grouped into distinct classes with unique gene expression signatures (14), similar to what has been found in cancer (18-20).

5.1. Insights from gene expression in the Skin

Three studies of gene expression in SSc skin biopsies have been reported to date. Two studies compared gene expression in patients with dSSc and healthy controls (13,15). Both studies found deregulation of thousands of genes encompassing many different pathways. A third study analyzing the heterogeneity of gene expression identified distinct subsets in SSc skin (14).

Whitfield *et al.* found that more than 2,776 genes were changed at least two-fold in patients with dSSc (13). Lesional (forearm) and non-lesional (lower back) skin from these patients showed nearly identical gene expression that was distinct from that found in healthy controls. Differentially expressed genes were found to be associated with B lymphocytes, microvascular endothelial cells, epithelial cells, fibroblasts and smooth muscle cells. Surprisingly, there were no significant differences in gene

expression differences between explanted SSc fibroblasts and normal skin fibroblasts studied in culture, suggesting the aberrant gene expression in SSc reflect the tissue environment. A second study analyzed gene expression in the skin and explanted fibroblasts from 9 patients with SSc and 9 healthy controls. 1,800 genes were identified that distinguished SSc from normal skin (15). Deregulation of the TGF-βand WNT signaling pathways, ECM production and expression of CCN family members were again prominent. In contrast to skin, differences in gene expression in explanted fibroblasts from SSc and controls were highly variable.

5.2. Studies in fibroblasts

A number of studies examined gene expression in explanted dermal fibroblasts (12,1 3, 21,22). One study analyzed the fibroblasts from sets of identical twins (12). Although fewer than 5% of twin pairs with SSc were concordant for the disease, most were concordant for anti-nuclear antibodies (11). Explanted fibroblasts from lesional and non-lesional skin showed similar gene expression, which was distinct from normal controls and unaffected dizygotic twins, recapitulating the lesional/non-lesional similarity of Whitfield *et al* (13) in fibroblasts cultures. Surprisingly, the unaffected monozygotic twins showed a disease-specific gene expression profile similar to that found in the fibroblasts of the affected twins. These results suggest that the disease specific gene expression profiles may be established very early in the disease, raising the intriguing hypothesis that a test could be developed to identify individuals at risk for developing SSc.

5.3. Peripheral blood cells and interferon

Gene expression studies of peripheral blood mononuclear cells (PBMCs) and isolated monocytes and CD4+ T-cells have revealed an "interferon signature" similar to that seen in systemic lupus erythematosus, dermatomyositis, and rheumatoid arthritis (16,17,23-26). In SSc the interferon signature has been observed in unfractionated whole-blood (16,25) and in isolated monocytes and CD4+ T-cells (17). Recent studies indicate that anti-Topo-I antibodies can stimulate the production of IFN-α in plasmacytoid dendritic cells (3).

5.4. Quantification of heterogeneity at the molecular level

Gene expression can be used to quantify the heterogeneity in SSc. Skin biopsies were analyzed from different groups of patients with scleroderma along with healthy controls (14). Lesional and non-lesional skin biopsy pairs again showed nearly identical, disease-specific patterns of gene expression. Analysis of the genes most consistently expressed within an individual and likely indicative of systemic disease, but most diverse across all individuals in the study, revealed the existence of multiple subgroups, including three subgroups with dSSc and two with lSSc. One subgroup of dSSc and lSSc patients formed an overlapping molecular subset characterized by an inflammatory profile.

Distinct gene expression signatures characterized each group and the gene expression driven groupings were found to be statistically robust. The intrinsic subsets could each be mapped to distinct clinical covariates such as modified Rodnan skin score, Raynaud severity, digital ulcers, ILD, and GI involvement (14). Analysis for differences in disease duration (onset of first non-Raynaud symptom) showed a trend in the intrinsic subsets, with patients with the earliest disease divided across two groups and patients with later stage disease in a second group. Preliminary analysis of gene expression in a second patient cohort has confirmed the intrinsic subgroups found in dSSc (S.A. Pendergrass, R. Lemaire, R. Lafyatis, and M.L.Whitfield, *unpublished*).

5.5. Heterogeneity in SSc skin at the pathway level

The recognition of these subsets with distinct gene expression profiles in SSc suggests that each group may have different pathways that are deregulated. The use of sets of genes compiled from the literature can be error-prone due to the cell type and context specificity of gene expression (27-29). One way to specifically analyze signatures is to use experimentally-derived gene sets created by treating cells *in vitro* (Figure 2). This provides an experimentally derived set of regulated genes in the cell type of interest. The negative of this approach is that one is looking at single cell type in culture in the absence of the more complex milieu of other cell types and circulating factors that pertains *in vivo*.

A second approach is to identify a signature in genetically-engineered mice with tissue or cell-type specific conditional over-expression of a specific modifier. The advantage to this approach is that one is working in a whole tissue with a complex mixture of cell types and other possible confounding factors, therefore providing a more realistic view of what the signature may look like in the end target organ. The negative is that the complex tissue will also report the infiltration of different cell types, rather than simply the activation of genes, complicating the interpretation of the results.

5.6. TGF-ß-regulated gene expression

Regulation of gene expression by TGF-ß has been analyzed in dermal fibroblasts (30), fetal lung fibroblasts (31), keratinocytes (32) and adult lung fibroblasts (33). In lung fibroblasts from patients with idiopathic pulmonary fibrosis (IPF) and SSc-associated pulmonary fibrosis, 129 transcripts were found to be regulated by TGF-ß (33). There were no differences between the fibroblasts derived from patients with IPF or SSc-associated pulmonary fibrosis. Since lung fibrosis appears to be largely mediated by fibroblasts (34) and TGF-ß has been implicated in driving the fibrotic process in the lung (35,36), the lack of significant differences between the two types of fibroblasts suggests the cellular environment is driving the scleroderma phenotype.

In recent studies we examined TGF-ß-responsive gene expression in SSc (J.L. Sargent, M.K. Connolly, J. Varga, H.Y. Chang, and M.L. Whitfield, *unpublished*). First, using fibroblasts explanted from healthy controls and patients with dSSc, we identified 894 genes as TGF-ß-responsive *in vitro*, and then analyzed this TGF-ß signature

Table 2. Mediators of fibrosis elevated in systemic sclerosis

Mediator	Cellular source
TGF-ß	inflammatory cells, platelets, fibroblasts, macrophages
Wnt ligands	epithelial cells
PDGF	platelets, macrophages, fibroblasts, endothelial cells
CTGF/CCN2	fibroblasts
Insulin-like growth factors	fibroblasts
IL-4, IL-13	Th2 lymphocytes, mast cells
IL-6	macrophages, B cells, T cells, fibroblasts
Chemokines (MCP-1, MCP-3)	neutrophils, epithelial cells, endothelial cells, fibroblasts
Fibroblast growth factor	fibroblasts
Endothelin-1	Endothelial cells
Autoantibodies (anti-PDGFR)	B lymphocytes

Table 3. Fibrogenic TGF-ß activities relevant for the pathogenesis of SSc

Stimulates synthesis of collagens, fibronectin, proteoglycans, elastin, TIMP's; inhibits matrix metalloproteinases
Stimulates fibroblast proliferation, chemotaxis, contraction, adhesion
Induces production of connective tissue growth factor and endothelin-1
Stimulates expression of membrane receptors for TGF-ß, PDGF
Enhances matrix stiffness
Promotes fibroblast-myofibroblast differentiation, monocyte-fibrocyte differentiation; epithelial-mesenchymal transition
Inhibits fibroblast apoptosis
Suppresses intracellular levels of caveolin-1

in SSc skin using a large microarray dataset (14). We find high expression of the TGF-ß responsive gene signature in a subset of dSSc patients and low expression in another subset, demonstrating that the heterogeneity captured in the intrinsic subsets (14) extends to the pathway level.

6. **TGF-ß in SSc: insights from animal models**

A number of putative animal models of SSc have been characterized, although none recapitulate all of the cardinal features of human SSc (37). The cancer drug bleomycin induces scleroderma and fibrosis of the lung and kidneys in inbred strains of mice. The development of fibrosis in this disease model is governed by the relative balance of type 1 and type 2 cytokines, as is also the case in human SSc (38,39). As highlighted by loss-of-function experiments, fibrosis this model is critically dependent on Smad-mediated TGF-ß signaling (40,41). The bleomycin model is now used extensively to investigate the pathogenesis of fibrosis, and screen potential anti-fibrotic interventions (42). Another scleroderma model involves the transplantation of bone marrow or spleen cells into lethally-irradiated HLA mismatched recipient mice. This results in chronic graft-versus-host disease with features of SSc, including dermal fibrosis and strong TGF-ß dependence (43). More recently, novel strains of transgenic mice have been created by genetic engineering, including a mouse strain with expression of a constitutively active TGF-ß type 1 receptor ($T\beta R1^{ca}$) mutant restricted to fibroblasts (44). Activation of Cre recombinase by tamoxifen injection in these mice results in excision of a LoxP-flanked STOP codon upstream of the $T\beta R1^{ca}$ transgene, leading to fibroblast-restricted $T\beta R1^{ca}$ expression and ligand-independent TßR1 signaling, with consequent development ofdermal and pulmonary fibrosis and obliterative vasculopathy in the absence of inflammation. This transgenic mouse strain should prove uniquely valuable for evaluating novel anti-fibrotic therapies and the specific contributions of their anti-inflammatory versus anti-fibrotic activities. The central role of TGF-ß in fibrosis is further supported by studies with mice with null mutation of Smad3, which renders the mice resistant to bleomycin-induced pulmonary fibrosis and scleroderma (41,45). Adipocyte-restricted transgenic expression of Wnt10b, a member of the Wnt family, in the mouse leads to extensive scleroderma-like changes in the skin, suggesting a potential important role of Wnt signaling in the pathogenesis of scleroderma (46).

7. MEDIATORS OF FIBROSIS IMPLICATED IN SSC

Inflammatory leukocytes and macrophages, platelets, endothelial cells and fibroblasts are all capable of generating TGF-ß, as well as a host of other chemokines, cytokines and growth factors that are potentially important in the pathogenesis of SSc (Table 3). Pharmacological inhibition profibrotic mediators, and enhancing the expression or activity of anti-fibrotic mediators, represent complementary therapeutic strategies for the prevention and/or control of fibrosis in SSc.

7.1. Pro-fibrotic factors: TGF-ß in SSc

In SSc, tissue fibroblasts are exposed to TGF-ß secreted from activated platelets, macrophages and lymphocytes accumulating within the cellular microenvironment. Significantly, by virtue of enhanced expression of integrins $\alpha v\beta 5$ and $\alpha v\beta 6$, which can activate latent TGF-ß (47), SSc fibroblasts release TGF-ß sequestered within the extracellular matrix (48). In fibroblasts and other mesenchymal cells, TGF-ß stimulates the synthesis of fibrillar collagens and elicits a broad profibrotic program summarized in Table 4. These TGF-ß responses are mediated primarily via canonical Smad signaling, with some role for Smad-independent pathways involving the MAP kinases ERK1/2 and FAK1 and transcription factors such as Egr-1 (49). The ubiquitous histone acetyltransferase p300/CBP is recruited by DNA-bound Smad3 to target gene promoters, and functions as an indispensable transcriptional coactivator for TGF-ß/Smad-mediated fibrotic responses (50).

Because of its evident importance in the progressive fibrotic pathology of SSc, novel therapies

Table 4. Anti-TGF-ß strategies for pharmacological inhibition of fibrosis in SSc

Approach to inhibiting TGF-ß	Comment
Existing drugs (approved for other indications)	
Tranilast	blocks TGF-ß production; extensive in clinical use for allergic diseases in Japan
Angiotensin II receptor blockers (losartan)	in clinical use for hypertension; decreased TGF-ß production
Statins	in clinical use as cholesterol-lowering drugs; antagonize stimulation of collagen,
Thiazolidenediones (rosiglitazone)	in clinical use as insulin-sensitizing drugs; antagonize stimulation of collagen
Imatinib mesylate (Gleevec)	in clinical use in chronic myelogenous leukemia; antagonizes collagen stimulation
Novel therapies (investigational)	
Block ligand production or activity	Isotype-specific neutralizing antibodies Soluble TßR1-3 receptors Antibodies to αvß6 integrin Natural TGF-ß binding proteins (eg. Decorin) Nucleic acid-based (antisense, ribozyme, siRNA)
Block activation of TGF-ß receptors	Orally active small molecule TßR kinase inhibitors
Block Smad function	Physiologic endogenous inhibitor Smad7
Block coactivator recruitment and function	Aptamers (Trx-SARA)

targeting the expression or function of TGF-ß are currently being developed. As summarized in Table 5, several approaches to block TGF-ß are currently under investigation. These include small molecule kinase inhibitors such as SB431542 and SD208 that block the activity of the type 1 TGF-ß type I receptor (TßR1), soluble TGF-ß receptors/decoys, and isotype-specific or pan-neutralizing antibodies to the TGF-ßs (51). Recent studies implicate the tyrosine kinase c-Abl in mediating certain TGF-ß responses in fibroblasts, including stimulation of collagen synthesis and myofibroblast transdifferentation (52). Imatinib mesylate (Gleevec), a small molecule kinase inhibitor widely used in the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia, blocks fibrotic responses elicited by TGF-ß, as well as by PDGF (52). Imatinib has been shown to prevent bleomycin-induced lung fibrosis (52) and scleroderma (53) in the mouse; whether these significant anti-fibrotic consequences of pharmacological c-Abl blockade are due to the direct antagonism of TGF-ß signaling by imatinib, or other biological activities such as inhibition of PDGF signaling, remain to be clarified. Neutralizing antibodies to TGF-ß or to αvß6 integrins, have also been shown to prevent experimentally induced fibrosis in animal models (47), and have emerging clinical utility in the treatment SSc.

7.2. Other pro-fibrotic factors implicated in scleroderma

Connective tissue growth factor (CTGF), also known as CCN2, is a TGF-ß-inducible matricellular protein that mediates some of the fibrotic responses elicited by TGF-ß. Levels of CTGF are elevated in SSc skin (54), and in mouse models of scleroderma (41). Insulin-like growth factors and their binding proteins (IGFBPs) have recently emerged as novel pro-fibrotic mediators *in vitro* and *in vivo* (55). Levels of IGFBP-3 and IGFBP-5 are significantly increased in patients with SSc (56). Both IGF-II and IGFBP-5 can elicit fibrotic responses *in vitro*, but the precise mechanisms underlying these profibrotic effects are still under investigation.

7.3. Anti-fibrotic factors

Interferon-γ is a Th1 cytokine with potent anti-fibrotic effects that can antagonize multiple activities of TGF-ß. It has been proposed that SSc is associated with a skewed balance of Th1 versus Th2 cytokines, with a predominance of Th2 cytokines such as IL-4, and a reduction in interferon-γ (57). Hepatocyte growth factor (HGF) has potent anti-fibrotic activity and was shown to ameliorate tissue fibrosis in several organs, most notably the skin (58) and the kidney (59). Small molecule HGF mimetics for anti-fibrotic therapy are currently under development.

Peroxisome proliferator-activated receptor-γ (PPAR-γ), a widely expressed nuclear receptor with essential roles in adipogenesis and insulin homeostasis, can blocs TGF-ß-induced stimulation of collagen synthesis and other fibrotic responses *in vitro* (60,61). The mechanism for the anti-TGF-ß activities of ligands of PPAR-γ are not fully understood, and may involve disruption of Smad-mediated transcription. *In vitro*, ligand agonists of PPAR-γ such as rosiglitazone or pioflitazone ameliorate experimental fibrosis (62) and bleomycin-induced scleroderma (63). Because TGF-ß, as well as Wnt ligands, are potent inhibitors of PPAR-γ expression and function, a reciprocally-antagonistic relationship exists between TGF-ß and PPAR-γ, whereby each of these cellular signaling pathway blocks the activity of the other, and may have implications for governing the propensity for fibrosis following injury (Wei J, Varga J; unpublished). Significantly, recent observations indicated that skin and lung fibrosis in SSc is associated with reduced PPAR-γ in these tissues, suggesting that PPAR-γ may play a physiologic role as an endogenous anti-fibrotic to restrict the duration or magnitude of fibroblast activation and limit repair processes, and prevent pathological fibrosis. Orally active thiazolidinedione agonist ligands of PPAR-γ, well-tolerated and widely used as an insulin sensitizers for the treatment of type 2 diabetes, may be effective for the prevention and reversal of TGF-ß-mediated fibrosis.

8. SUMMARY AND PERSPECTIVES

The pathogenesis of SSc involves a dynamic interplay among immunologic/inflammatory events, vascular injury and damage, and fibroblast activation and matrix deposition, presumably triggered by environmental exposure in a genetically-susceptible host. Robust experimental strategies such as transgenic mouse modeling,

genomewide analysis and DNA microarray analysis of gene expression are beginning to yield an increasingly coherent picture of the pathogenesis. Current data confirm the paramount role of TGF-ß in the development of fibrosis, and suggests that different subsets of SSc patients could have differential responses to targeted therapies. While interventions targeting the inflammatory phase of SSc show limited efficacy, investigational therapeutic approaches focusing on fibrosis, such as blockade of TGF-ß, are more promising. However, the effectiveness of anti-fibrotic interventions in ameliorating fibrosis and meaningfully altering the course of the disease in SSc remain to be determined.

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Send correspondence to: John Varga, Northwestern University Feinberg School of Medicine, McGaw 2300, 240 East Huron Street, Chicago IL 60611-2909, Tel: 312-503-0377, Fax: 312-503-0994, E-mail: j-varga@northwestern.edu

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