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Molecular Pathogenesis of Skin Fibrosis: Insight from Animal Models

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Abstract

Skin fibrosis occurs in a variety of human diseases, most notably systemic sclerosis (SSc). The end stage of scleroderma in human skin consists of excess collagen deposition in the dermis with loss of adnexal structures and associated adipose tissue. The initiating factors for this process and the early stages are believed to occur through vascular injury and immune dysfunction with a dysregulated inflammatory response. However, because of the insidious onset of the disease, this stage is rarely observed in humans and remains poorly understood. Animal models have provided a means to examine these early stages and to isolate and understand the effect of perturbations in signaling pathways, chemokines, and cytokines. This article summarizes recent progress in the understanding of the molecular pathogenesis of skin fibrosis in SSc from different animal models, both its initiation and its maintenance phases.

Keywords

Scleroderma; Systemic sclerosis; CREST; Fibrosis; Animal models; Mouse models

Introduction

Scleroderma, or systemic sclerosis (SSc), is a systemic connective tissue disease. Most commonly affected organs include the lungs, skin, heart, kidney, and gastrointestinal tract. The initiating event and early changes of this disease remain unknown in part because of the slow onset, resulting in patients being seen at a fairly advanced stage of the disease upon diagnosis. Early stages are believed to consist of vascular injury and immune dysfunction with patients presenting with fibrotic changes at later stages. Scleroderma, however, has a wide diversity of presentations and several broad categories of disease are recognized. The broadest subcategorization of scleroderma is between systemic and localized scleroderma. Systemic scleroderma is then subdivided into diffuse cutaneous and limited cutaneous forms. Diffuse cutaneous involves skin thickening of the trunk, proximal and distal extremities, and face, and most commonly involves multiple internal organ systems, although single organ involvement has occasionally been documented. Limited cutaneous SSc, although also involving skin thickening, is usually restricted to the face, neck, and areas distal to the elbows and knees. This form also includes the CREST (calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias) syndrome as a distinct subset, although patients frequently do not exhibit all of these characteristics.

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In contrast, localized scleroderma tends to lack sclerodactyly, Raynaud phenomenon, and internal organ involvement. Localized scleroderma can be further subdivided into localized morphea, generalized morphea, and linear scleroderma. Localized morphea is usually limited to a contiguous region but may affect underlying joints if it extends over these. This can then be further divided into subtypes depending on the depth of involvement: classic morphea primarily involves the dermis; subcutaneous morphea involves the fat; morphea profunda extends to the subcutaneous fat and fascia; and pansclerotic morphea has sclerosis extending to the bone. There are also clinically distinct subtypes of localized morphea, such as atrophoderma of Pasini and Pierini, in which cliff-like borders extend to an area of atrophy. Generalized morphea involves skin thickening without visceral involvement, although muscle atrophy may be present. Linear morphea tends to extend linearly. Lesions may follow lines of Blaschko on the arms or legs or occur parasagittally on the frontal scalp. This subtype is more common in children. There also exist overlap forms in which morphea coexists with lesions of lichen sclerosus et atrophicus or SSc coexists with features of another connective tissue disease such as systemic lupus erythematosus, myositis, or rheumatoid arthritis. In addition, a number of scleroderma-like diseases may present similarly and be initially misdiagnosed as scleroderma, including eosinophilic fasciitis, eosinophilia myalgia syndrome, and other sclerodermatous conditions related to a toxic exposure, including nephrogenic systemic fibrosis from gadolinium contrast media in patients with severe renal disease. Forming an accurate animal model of skin fibrosis is complicated by this diversity of clinical entities and even in the presentations of the prototypical skin fibrosis disorder itself, scleroderma. Indeed, scleroderma should be understood in terms of a spectrum of clinical conditions under the umbrella of dermal fibrosis. In addition, even when examining only one subtype of sclerodermatous disease, animal models often fail to reproduce all of the clinical and histopathologic features of the subtype. As such, one-for-one mapping of pathogenetic events from animal model to human disease is often not possible. However, animal models provide the opportunity to examine the potential early stages of disease, which are often missed in vivo and allow examination of particular molecules and pathways and the effect of their dysregulation, either through therapeutic intervention or genetic models such as knockout experiments. We will discuss the insights provided by studies into the early molecular and cellular changes that may evoke the initial fibrotic response and those that contribute to its maintenance.

Animal Models

The following sections discuss different animal models of fibrosis, including their generation, similarities to SSc, their weaknesses as a model, and insights into the molecular pathogenesis of skin fibrosis provided by such models. The components of pathogenesis present in animal models of fibrosis are summarized in Table 1 and key contributing factors are summarized in Table 2.

Tsk1

Although the homozygous tight skin (Tsk) murine Tsk/Tsk genotypes die in utero within 8 days, Tsk/+ mice survive, developing tight skin, especially intracapsularly, firmly bound to underlying structures. The Tsk/+ genotype, therefore, has been used as an animal model to understand the phenotypic skin changes of SSc, even though it lacks key pathologic features, such as an early inflammatory infiltrate.

It has been shown that the Tsk murine model is a spontaneous autosomal dominant mutation consisting of a large in-frame intragenic duplication of the fibrillin 1 gene (*FBN1*) from exons 17 to 40 [1]. RNA extracts prepared from Tsk/+ mice show transcripts for both a normal 11 kb *FBN1* and a larger 14 kb version. In addition, both normal 350 kD *FBN1* and a 450 kD variant have been shown to be secreted by Tsk/+ dermal fibroblasts. Although a definitive

mechanism for fibrogenesis by this altered fibrillin molecule has not been found, two possible mechanisms can easily be postulated. First, it is known that fibrillin-1 is a component of connective tissue microfibrils and as such is important in correct elastic fiber assembly. Any alteration in fibrillin-1 could therefore also secondarily alter this production. Second, fibrillin can partially control the bioavailability of the profibrotic molecule transforming growth factor β (TGF-β) via interaction with the latent TGF-β binding proteins 1 and 4. Indeed, it is interesting to note that the altered fibrillin binds more TGF-β than normal fibrillin. This may support the hypothesis that similarities in the duplicated regions of the fibrillin molecule to TGF-β binding protein domains are responsible for altering the TGF-β profibrotic cascade.

In addition to upregulation of the foregoing microfibrillar proteins gene expression, analysis of 6-week old Tsk/+ mice using GeneChip arrays also demonstrated upregulation in collagen, bone morphogenic protein (BMP), secreted frizzle-related protein (SRFP4), connective tissue growth factor (CTGF), and Wnt signaling proteins, including Wnt3a, which have been implicated in the regulation of cell fate and integration of signals from other pathways such as TGF-β, fibroblast growth factor (FGF), and BMP [2••]. Application of Wnt3a to cultured fibroblasts produced increased levels of Fbn-1 and collagen, and elevated levels of SRFP4 were also noted in tissue from SSc patients.

There is also evidence of fibroblast activation that persists into culture. Dermal fibroblast cultures from Tsk/+ mice show increased collagen deposition associated with elevated steady state levels of collagen types 1 and 3 messenger RNA (mRNA) [3]. In addition, probes to type 1 and 3 collagen show upregulation in Tsk fibroblasts in comparison to normal controls.

Although autoantibodies are a normal feature of SSc, it remains unclear if autoimmunity is a part of the pathogenesis of fibrosis initiation or maintenance in Tsk mice. In support of a role for immune activation, transplantation of Tsk bone marrow into normal mice resulted in tissue fibrosis and increased collagen gene expression. In addition, Tsk/+ mice with no functioning interleukin-4 (IL-4) or Stat6 genes [4], as well as mice with a targeted mutation in the IL-4 receptor α chain [5], did not develop dermal fibrosis, suggesting that IL-4 is at least in part responsible for regulating fibrosis in Tsk mice. CD19 knockout mice also showed less dermal fibrosis [6]. However, conflicting evidence for immune regulation stems from observations that murine strains lacking α/β receptor T cells, B cells, or lacking all T and B cells, such as the severe combined immune deficiency (SCID) or recombination activating gene (Rag) knockout mouse still developed tight skin when combined with the Tsk mutation.

Although the Tsk/+ mouse may superficially resemble clinical findings of SSc, as a model for SSc it does have some weaknesses. For example, histologic analysis shows that there is no fibrosis in the deep dermis, in contrast to typical SSc, but instead skin tethering to the subjacent muscle occurs. In addition, although the Tsk/+ mouse does show pulmonary changes, these are of an emphysema-like pattern with increased alveolar spaces, rather than the pulmonary fibrosis seen in SSc. Tsk/+ mice do not exhibit a vasculopathy, in contrast to SSc. Despite these weaknesses, the Tsk/+ mouse model has provided insight into the possible role of Wnt dysregulation, or TGF-β in skin fibrosis, the interaction with the immune system, and the ability to develop extensive skin fibrosis from a single mutation.

Tsk2

The tight skin 2 mutation (Tsk2) is a chemically (ethylnitrosourea) induced autosomal dominant mutation in mouse chromosome 1. Although this produces similar phenotypic skin changes to Tsk1, the histologic changes are closer to that of human SSc with dermal fibrosis and adipose tissue loss. In addition, there are positive antinuclear antibodies in 88% of Tsk2/+ mice compared with 10% of controls, and, as in SSc, anticentromere, antitopoisomerase-I,

and anti-RNA polymerase II autoantibodies [7]. Vessels appear thickened in Tsk2 but still lack the frank vasculopathy of SSc.

Elevated steady state levels of collagen type 1 α1 (COL1A1) mRNA exist with transcriptional activation of COL1A1and collagen type 3 α1 (COL3A1) mediated by Sp1 and nuclear factor (NF)-1 demonstrated in cultured Tsk2 fibroblasts [8]. Increased collagen gene expression was also supported by use of a collagen transgene expressing green fluorescent protein that showed an age-dependent increase in green fluorescent protein (GFP)–expressing fibroblasts when compared with wild-type littermates [9].

Mutant Fibrillin Transgene

A number of transgenic mice with mutant fibrillin genes have been produced with a variety of resulting phenotypic pictures. Transgenic mice demonstrating persistent cutaneous thickening and positive anti-topoisomerase antibodies were produced by the use of a plasmid vector, plasmid-bearing mutated Fbn-1 gene, carrying the Tsk Fbn1 sequence [10]. However, injection of the same plasmid to newborn mice resulted in only transient skin thickening. Other mutant fibrillin transgene experiments have produced mice that show anomalies in TGF-β signaling but no skin thickening. Perhaps the most successful model of this kind was a conditional mutant-fibrillin transgene model. In embryonic fibroblast cultures, fibrillin fibers had a distorted structure with increased collagen deposition despite a lack of change in COL1A1 mRNA or procollagen production. Instead, elevated levels of microfibril-associated glycoprotein 2 that stimulates elastic fiber assembly were detected, and has also been shown to be present in the tissue of SSc patients.

Conditional Constitutively Active TGF-β Receptor 1 Transgenic Mouse

For this model, a knockin murine strain with a constitutively active mutant of the activin receptor-like kinase 5 TGF-β type I receptor (TβR1ca) inserted in a transcription stop cassette, flanked by two loxP sites, was targeted to the ROSA26 locus promoter. The receptor was therefore fused to a ubiquitously expressed promoter that could only be activated by a Cre-mediated rearrangement of the LoxP sites. This murine strain was then bred with a transgenic mouse with a tamoxifen inducible Cre (CreER) driven by the COL1A2 promoter. Injection of tamoxifen for five consecutive days to the offspring of this pairing produced TβR1 activation in fibroblasts [11••] and offspring recapitulated many of the clinical skin features of SSc, such as progressive dermal fibrosis, epidermal thinning, loss of adnexal and subcutaneous adipose structures, as well as extracutaneous findings such as intramural collagen accumulation in small pulmonary vessels. Analysis of these fibroblasts showed ligand-independent Smad2/3 phosphorylation and nuclear accumulation, as well as elevated gene expression of downstream targets of TGF-β, including CTGF, tissue inhibitor of metalloproteinase-1, type 1 collagen, fibronectin, plasminogen activator inhibitor-1, and secreted protein, acidic and rich in cysteines (SPARC). P38 and mitogen-activated protein (MAP) kinase pathway activity was also detected. This model therefore demonstrated production of an SSc phenotype, even without initial inflammation, purely by TGF-β signal dysregulation.

Type 2 TGF-β Receptor Mutant Transgenic Mouse

A different model involving fibroblast-restricted expression of a kinase-dead type 2TGF-β receptor (TβRIIδk) also resulted in fibrosis [12]. This result proved surprising given that TβRIIδk in cultured fibroblasts was reported to be a dominant negative inhibitor of TGF-β signaling. Further analysis showed upregulation of plasminogen activator inhibitor-1, connective tissue growth factor, Smad3, Smad4, and Smad7, and that Smad2/3 phosphorylation was increased in transgenic fibroblasts. Although difficult to understand in the context of prior experiments, this model provided dramatic evidence of the ability of even small perturbations in TGF-β signaling to result in the formation of a fibrotic phenotype.

Relaxin-Null Mouse

Studies have shown that the hormone peptide relaxin is effective in blocking TGF-β activity in either normal or SSc skin fibroblasts, reducing collagen production and fibrosis. Although relaxin-null mice develop spontaneous skin and lung fibrosis, which could be attenuated by administration of relaxin [13], and in vitro studies showed higher collagen production in relaxin-null fibroblasts than wild type, none of the vascular or immunologic features of SSc were evident.

Bleomycin-Induced Fibrosis

Following the observation of pulmonary fibrosis in cancer patients treated with bleomycin, this pharmaceutical has been used to produce fibrosis in murine models. Unlike models of lung fibrosis in which fibrosis is produced via a one-time intratracheal injection, skin fibrosis is produced by repeated subcutaneous injections for at least 3 weeks [14]. This latter technique produces a small peri-injection area of induration with histologic changes showing epidermal hypertrophy, dermal fibrosis, consisting of accumulation of myofibroblasts, collagen and dense extracellular matrix material, and adipose atrophy. This effect varies by murine strain, with C3H/He and B10.A giving the most pronounced histologic changes [15]. Biopsy of lesional skin shows upregulated TGF-β production, phosphorylated Smad2/3 [16], coactivators of the TGF-β/Smad pathway, such as histone acetyltransferase p300 [17], and CTGF [18•]. In contrast Smad3-null mice treated with bleomycin showed significantly less fibrosis supporting the importance of this pathway [19]. However, TGF-β stimulated collagen production is also upregulated via Smad-independent signal transduction with chronic upregulation of the early growth response factor (Egr)-1 in bleomycin-treated mice, a factor normally rapidly but only transiently elevated in injury or hypoxia [20]. Both pathways, Smad-dependent and Smad-independent stimulation of TGF-β, including downregulation of Egr-1 and hepatocyte growth factor (HGF), are inhibited by ligation by peroxisome proliferator-activated receptor (PPAR)-γ, a ubiquitous nuclear hormone receptor [21], and treatment of C3H mice with 15-deoxyprostaglandin J_2 (PGJ_2), an endogenous ligand of PPAR-γ, or rosiglitazone, a pharmacologic ligand [22•], that showed attenuation of bleomycin-induced fibrosis. Part of the antifibrotic effect may also be explained by the downregulation of HGF, a factor secreted by mesenchymal cells but acting on epithelium via the c-Met receptor coupled by Gab1 and Grb2 to downstream tyrosine kinases, thought to play an antifibrotic role in acute injury repair and shown to downregulate collagen synthesis in normal and SSc fibroblasts in vitro [23]. Indeed intramuscular injection of cDNA coding for HGF gave partial protection against skin fibrosis in bleomycin-treated mice [24].

Similar to Egr-1, adenosine levels rise rapidly in normal tissue but transiently in injury or hypoxia, reflecting both its anti-inflammatory role [25] and its role in wound healing in which it stimulates fibroblast collagen production via the A_{2A} cell surface receptor [26] and downstream MAP kinase/extracellular regulated kinase (MEK1/ERK) signal transduction pathway [27]. Attenuation of bleomycin-induced fibrosis is seen in A_{2A} receptor knockout mice [27]. Mice deficient for the adenosine-catabolizing enzyme adenosine deaminase (ADA) with subsequent adenosine accumulation in blood and tissues exhibit augmented dermal fibrosis with enhanced expression of IL-13 and CTGF, as occurs in bleomycin-treated mice [28].

Chemokines have also shown to be important. Monocyte chemoattractant protein (MCP)-1 also known as chemokine ligand (CCL)-2 and its receptor (CCR)-2 are known to be upregulated in SSc patients. In addition MCP-1 was shown to stimulate collagen production in cultured fibroblasts. For this reason, the effect of bleomycin on MCP-1 knockout mice was examined, and showed a greatly reduced early inflammatory infiltrate, less fibrosis, and more regular collagen [29]. In contrast, wild-type mice had smaller-sized irregular collagen, with

unregulated expression of MCP-1 and CCR-2 on early infiltrating monocytes and late-stage fibroblasts.

Activated adhesive signaling is noted in fibroblasts from SSc patients and believed to be a key step in fibrosis formation. This is supported by observations showing that the deletion of β1 integrin resulted in resistance to bleomycin-induced fibrosis in mouse models [30•].

Fibrosis has also been associated with a T-helper (Th)-2 immune response, in part due to downregulation of the antifibrotic Th1 cytokine interferon (IFN)-γ. In comparison to wild-type littermate mice, mice deficient for T-bet, a regulator essential for initiating Th1, showed increased and more rapid fibrosis with an exaggerated immune response to bleomycin [31]. Other factors identified in the increased fibrosis of T-bet null mice were extensive eosinophil degranulation and constitutively elevated IL-13 [32]. As in scleroderma, autoimmune antibodies have been detected in murine sera and transfer of CD4(+) T cells from bleomycin-treated BALB/c mice induced the same pathologic changes and antibody production in untreated-BALB/c nude mice, supporting the immunologic basis of disease in this model [33].

Fli1−/− Mouse

Friend leukemia integration-1 (Fli1), a member of the Ets family of transcription factors, represses transcription of collagen genes via an Sp1-dependent pathway [34]. Indeed, embryonic fibroblasts from Fli1−/− mice showed significantly increased collagen type I mRNA and protein levels and examination of cultured SSc fibroblasts and in SSc skin in vivo shows downregulation of Fli1 expression levels [35]. This downregulation of Fli1 is also associated with increased CTGF and decreased MMP1 [36] and may be caused by Fli1 degradation when TGF-β initiates a PCAF-dependent acetylation of Fli1 [37••].

Sclerodermatous Graft-Versus-Host Disease

The sclerodermatous graft-versus-host mouse model is produced by transplantation of immunologically incompatible spleen and bone marrow cells. Female Balb/c mice are irradiated and receive donor cells from male B10.D2 mice, allowing tracking of donor cells via Y specific sequences. These murine strains differ at minor histocompatibility loci setting up a sequence of immune activation and subsequent fibrosis. First, elevated levels of mRNA for Th1 and Th2 cytokines, TGF-β1, MCP-1, macrophage inflammatory protein-1α and RANTES (regulated on activation, normal T Expressed, and secreted) are detected, and tissue is infiltrated by donor T cells and monocytes/macrophages [38]. This is then followed by skin and lung fibrosis, which develops at 14–21 days posttransplantation, accompanied by elevated levels of type I collagen RNA synthesis. Interestingly this cascade was blocked by anti-TGF-β antibodies, indicating the key role of TGF-β in the fibrotic process. In addition, a recent study identified the key role of downregulation of TNF-α in producing the sclerodermatous phenotype [39•].

A similar model, avoiding the need for irradiation has also been produced using the Rag 2 mouse [40]. This mouse is immune deficient secondary to the loss of ability to rearrange immunoglobulin and T cell receptor genes and the donor cells can therefore be directly injected. This resulted in a similar skin phenotype between weeks 3 and 5, as well as producing fibrosis and vascular endothelial changes in the kidney but did not reproduce the lung pathology.

MRL/lpr IFN-γ Receptor Deficient Mouse

The basic MPR/lpr mouse model produces autoimmune antibodies and develops arthritis, vasculitis, cutaneous lesions, and lethal glomerulonephritis. Versions with deficiency of IFN-γ or its receptor do not show the glomerulonephritis but instead have been shown to have a

vasculopathy characterized by intimal thickening and accumulation of collagen [41]. Despite this observation, and the known antifibrotic properties of IFN-γ, the role of IFN-γ in fibrosis in this model is not clear. Although accumulation of thick collagen bundles in the kidneys, liver, lungs, and salivary glands in IFN-γ receptor deficient mice was in contrast to the MPR/lpr/γ R+/+ mice, which had normal collagen bundles in these organs, both lines showed fibrosis in skin lesions. It may be that the IFN-γ mutation, by protecting against glomerulonephritis in this animal model, provides longer life expectancy allowing time for the fibrosis to develop in different organs, and that skin is fibrotic in both as it is the first organ affected. Thus, an alternative, as yet unexplained mechanism of the fibrosis in this model is likely also at work.

UCD-200 Chicken

One to 2 weeks after hatching, University of California at Davis line 200 (UCD-200)/206 chickens develop microvascular occlusions, followed by prominent perivascular lymphocytic infiltration involving skin and viscera. This usually first manifests as erythema, edema, and necrosis of the comb, analogous to Raynaud phenomenon in SSc. There is also pronounced subsequent skin fibrosis and thickening. This sequence of vasculopathy, inflammation, and subsequent fibrosis has been suggested as a good model of human scleroderma, despite being much more fulminant, encompassing as it does the vasculopathic and inflammatory stages noted to be absent in models such as Tsk1 and Tsk2, and thus giving further insight into the earliest pathophysiologic changes of SSc. As in human SSc, these animals also develop autoantibodies, including antinuclear antibodies, antiendothelial cell antibodies (AECA), anticardiolipin antibodies, and rheumatoid factors. Indeed, some authors have suggested that endothelial cells are the primary target in scleroderma pathogenesis targeted by AECA-dependent cellular cytotoxicity via Fas/Fas ligand interaction [42], and that AECA induces microvascular alterations via upregulation of endothelial adhesion molecules such as E-selectin, P-selectin, the Ig-supergene family members intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 [43]. Although AECA has been noted in human SSc, it only appears to occur in localized morphea and in the very early stages of SSc. This, therefore, poses the following question: if this is indeed the initiating factor, why does the disease not always become systemic and what maintains the ongoing fibrosis? In the UCD-200 chicken, the subsequent inflammatory phase is composed of T cell receptor (TCR) γ/δ+/CD3+/major histocompatibility complex (MHC) class II T cells in the stratumpapillare and TCR α/β+/CD3+/CD4+/MHC class II + T cells in the deeper dermis [44]. Finally, skin thickening is caused by excessive accumulation of collagen types I, III, and VI although no mutation of the collagen genes is noted on restriction fragment length polymorphism studies [45].

Conclusions

The experiments to date with animal models of skin fibrosis point to a number of potentially important factors in the pathogenesis of this disease state and continue to identify new pathways, and thus new potential therapeutic targets for the modulation of fibrosis. Although no currently available model exactly mimics the development of human fibrosing diseases such as SSc or nephrogenic systemic fibrosis, this is in part because of the wide clinical spectrum of diseases such as SSc. They do, however, allow us to attempt to see the early stages of fibrosis, a stage frequently missed in the evaluation of human disease because of the slow, almost asymptomatic initial onset, as well as allowing us to target specific genes or proteins to evaluate potential therapies as well as hypotheses of pathogenesis.

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Table 1

Components of pathogenesis in animal models of fibrosis

Model	Vasculopathy	Inflammation	Autoimmunity
Tsk-1	−	−	+
Tsk-2	−	±	+
Mutant fibrillin transgene	−	−	+
Conditional TGF-βRI	+	+	−
TGF-βRII DN	−	+	−
Relaxin null	−	−	−
Bleomycin-induced	−	+	−
Fli1−/−	+	−	−
GVHD (B10.D2 versus Balb/C)	+	+	−
GVHD (B10.D2 versus Rag-2−/−)	+	+	+
MRL/lpr/IFN-γR−/−	−	+	+
UCD-200 chicken	+	+	+

Fli1 friend leukemia integration-1; *GVHD* graft-versus-host disease; *IFN* interferon; *Rag* recombination activating gene; *TGF-βRI* transforming growth factor β type I receptor; *TGF-βRII DN* TGF-β type II receptor dominant-negative; *Tsk* homozygous tight skin; *UCD-200* University of California at Davis line 200

Table 2

Key components believed to be important in fibrogenesis in the skin of different animal models

Model	Mechanism
Tsk-1	Large in frame intragenic duplication of the fibrillin-1 gene that may either alter elastic fiber assembly or alter TGF-β bioavailability.
Tsk-2	Autosomal dominant mutation in mouse chromosome 1; mechanism unknown.
Mutant fibrillin transgene	Fibrillin fibers have a distorted structure and microfibril-associated glycoprotein 2 is elevated.
Conditional TGF-βRI	Constitutively active TGF-β receptor in fibroblasts.
TGF-βRII	Small perturbation in TGF-β may lead to fibrosis, perhaps surprising given TGF-βRII cultured fibroblasts has been shown to be a dominant negative inhibitor of TGF-β signaling
Relaxin-null mouse	Relaxin blocks TGF-β activity.
Bleomycin-induced	Production of reactive oxygen species. Upregulated TGF-β via Smad-dependent and independent pathways. Activation of adenosine 2A receptors.
Fli1−/−	Fli1 represses transcription of collagen genes via Ets-1 and Sp1-dependent pathway
GVHD (B10.D2 versus Balb/C)	Chronic GVHD caused by differences at minor histocompatibility loci.
GVHD (B10.D2 versus Rag-2−/−)	Same as above.
MRL/lpr/IFN-γR−/−	IFN-γ is antifibrotic, however, the underlying mechanism may be different as the IFN-γ mutation provides longer life expectancy due to protecting against glomerulonephritis.
UCD-200 Chicken	Anti-endothelial cell antibodies may target endothelial cells via Fas/Fas ligand interaction, and induce microvascular alterations via up-regulation of endothelial adhesion molecules.

Fli1 friend leukemia integration-1; *GVHD* graft-versus-host disease; *IFN* interferon; *TGF-βRI* transforming growth factor β type I receptor; *TGF-βRII DN* TGF-β type II receptor dominant-negative; *Tsk* homozygous tight skin; *UCD-200* Univeristy of California at Davis line 200