Animal models of systemic sclerosis: insights into systemic sclerosis pathogenesis and potential therapeutic approaches

Paul J. Christner and Sergio A. Jimenez

Purpose of review

Animal models have been extremely valuable in contributing to a better understanding of the pathogenesis of systemic sclerosis. Discussed here are recent studies that have examined the molecular pathways and potential therapeutic approaches for systemic sclerosis using animal models.

Recent findings

Reported evidence further indicates that the immune system plays a role in modulating the fibrosis observed in the tight skin-1/+ mouse model for systemic sclerosis. CD19, interleukin-6, and interleukin-4 are involved. The injection of spleen cells into immune-compromised mice resulted in fibrotic, vascular, and immunologic alterations quite similar to those of systemic sclerosis. Transforming growth factor-β and its signaling pathway (JAK kinase and STAT-6, Smad2/3, and Smad7) appear to play a central role in the development of fibrosis as well as monocyte chemoattractant protein-1, CCR-2, platelet-derived growth factor C, and excessive apoptosis. Viruses were shown to be possible cofactors. The therapeutic agents hepatocyte growth factor and halofuginone were shown to prevent fibrosis in animal models of systemic sclerosis.

Summary

The transforming growth factor-β signaling pathway is a common mechanism of tissue fibrosis in animal models of systemic sclerosis, although numerous additional molecules modulate this pathway or have a direct effect on fibrosis.

Keywords

systemic sclerosis, animal models, Tsk (tight skin) mouse, bleomycin-induced scleroderma, transforming growth factor β

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Division of Rheumatology, Department of Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

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Correspondence to Sergio A. Jimenez, Thomas Jefferson University, Division of Rheumatology, 233 South 10th Street, Room 509 BLSB, Philadelphia, PA 19107, USA
Tel: 215 503 5042; fax: 215 923 4649; e-mail: sergio.jimenez@jefferson.edu

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Abbreviations

BALF	bronchoalveolar lavage fluid
ECM	extracellular matrix
HGF	hepatocyte growth factor
IL	interleukin
MAGP-2	microfibril-associated glycoprotein 2
MCP	monocyte chemoattractant protein
MMP	matrix metalloproteinase
PAI	plasminogen activator inhibitor
PDGF	platelet-derived growth factor
SSc	systemic sclerosis
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinase
Tsk	tight skin

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Introduction

Animal models of systemic sclerosis (SSc) have provided valuable insights into the causative mechanisms and the pathogenesis of SSc and have furnished the means to potentially test useful therapeutic interventions [1–3]. Among the numerous animal models for SSc, the most extensively studied are murine because of the large number of inbred mouse strains available and the detailed genetic and molecular information available for this species. Although the animal models described to date do not reproduce precisely all the clinical and pathologic alterations of the human disease, several of them show some of the typical abnormalities of this disorder, and prudent interpretation of the results obtained from their study has provided substantial and valuable information about the pathogenesis of SSc. Here we review recent studies using animal models of SSc, emphasizing their contribution to understanding the pathogenesis of the human disease and potential approaches for its treatment.

Insights into pathogenesis and molecular pathways of systemic sclerosis

Numerous recent studies have examined molecular pathways that may be of relevance to the pathogenesis of SSc.

Studies with Tsk1 mice

Saito *et al.* [4] reported that the level of the cytokine CD19 can influence skin fibrosis and autoimmunity in the tight skin (Tsk)1/+ mouse. Tsk1/+ mice were hyperresponsive to CD19 transmembrane signals and had a decreased expression of IgM expression, enhanced serum Ig levels, and spontaneous autoantibody production. The reason for this aberrant immune response appeared

to be the constitutive increase in tyrosine phosphorylation of CD19 in B cells from the Tsk1/+ mouse. In addition, CD19-mediated $[Ca^{2+}]_i$ responses, Vav phosphorylation and Lyn kinase activity were increased. Furthermore, Tsk1/+ mice deficient in CD19 had significantly less skin fibrosis and showed a much higher expression of surface IgM on their B cells, did not develop the autoantibodies characteristic of Tsk1/+ animals, and had reduced interleukin (IL)-6 production. The authors concluded that chronic B cell activation resulting from augmented CD19 signaling could lead to skin sclerosis and autoantibody production through a mechanism of IL-6 overproduction.

In another study, Kodera *et al.* [5] reported that the IL-4 gene was crucial for the expression of the Tsk1 phenotype. Tsk1/Tsk1 mice are not viable, and they die *in utero* at approximately the same time that expression of the mutated fibrillin 1 gene is initiated. These authors reported that elimination of either one copy or both copies of the IL-4 gene allowed survival of the homozygous Tsk1/Tsk1 mouse. Histopathology indicated that these mice did not show any cutaneous hyperplasia but still displayed pulmonary emphysema. *In vitro* experiments indicated that IL-4 regulated the levels of transforming growth factor (TGF)-β, and the authors postulated that the downregulation of TGF-β levels in these IL-4–deficient Tsk mice prevented the development of cutaneous fibrosis. The important conclusion of these studies is that IL-4 is crucial in the development of the Tsk phenotype and is involved in the mortality of Tsk/Tsk embryos. In a follow-up study, McGaha *et al.* [6] reported that IL-4 induced a substantial increase in collagen synthesis in both Tsk1/+ and normal mouse dermal fibroblasts, but the effect was greater in Tsk1/+ cells. They further showed that the IL-4 signaling cascade was altered in Tsk1/+ fibroblasts compared with control specimens. In Tsk1/+ cells, the phosphorylation of JAK kinases, which in turn phosphorylate the IL-4 receptor, was constitutive, whereas in cells from normal mice, IL-4 was required for JAK phosphorylation. Another signal transduction molecule, STAT-6, was also involved because IL-4 induced higher levels of phosphorylated STAT-6 in Tsk1/+ cells than in control cells. Transfection studies with a portion of the α2(I) collagen gene promoter showed that IL-4 and STAT-6 could upregulate its transcriptional activity and that AP-1 and Sp-1 transcription factors were involved in this effect. The authors concluded that type I collagen gene expression is enhanced by IL-4 either directly or through TGF-β–mediated mechanisms.

Another study characterized the tissue expression of elastic fiber–related proteins in Tsk1/+ mouse skin. Lemaire *et al.* [7] showed that Tsk mutant fibrillin 1 increases extracellular matrix (ECM) incorporation of microfibril-associated glycoprotein 2 (MAGP-2) and type I collagen. The authors analyzed the effect of the Fbn1 mutation present in the Tsk1/+ mouse on the structure and composition of the ECM by transfecting the mutated Fbn1 gene under the control of a tetracycline-dependent promoter into a mouse embryonic cell line. It was found that when the mutated Fbn1 was expressed in the mouse embryonic cell line, the sharply defined fibrillin network became smudged and blurred when examined by immunofluorescence. The fibrillin fibers produced in the presence of the mutated Fbn1 were also broader and less intensely stained. However, in contrast with previous studies, Col1a1 mRNA was equally expressed in the presence or absence of expression of the mutated Fbn1. This was true even when TGF-β1 was added to the cultures. However, expression of the mutated Fbn1 had a significant effect on the deposition and incorporation of type I collagen molecules into the ECM. There was also an increase in the MAGP-2 fibrillar structures within the ECM caused by the expression of the mutated Fbn1. The authors also reported an increase in MAGP-2 deposits in lesional SSc skin and concluded that alterations in the microfibril structure or deposition may contribute to cutaneous fibrosis in SSc.

Studies with the bleomycin-induced model of cutaneous fibrosis

Murota *et al.* [8] examined the effects of disruption of the TNF-α receptor p55 on collagen turnover in skin fibroblasts from mice injected with bleomycin subcutaneously. The treatment caused mild sclerosis after 14 days in normal mice, whereas it resulted in severe sclerosis after only 3 days in TNF-α receptor $p55^{-/-}$ mutants. The mutant mice showed thickened and homogeneous collagen bundles and dermal inflammatory infiltrates, whereas at the same time-points the control mice had severe inflammatory infiltrates but no cutaneous sclerosis or fibrosis. After 3 days of bleomycin injections, the skin in the mutant mice was significantly thicker than that in the control mice, and the collagen content at the site of injection was almost threefold higher. At sites distant from the injection site, there was no increase in dermal thickness, and the expression levels of IL-4 and platelet-derived growth factor (PDGF) were also not different. By contrast, TGF-β mRNA was reduced and TNF-α mRNA was induced in a time-dependent and dose-dependent manner in the mutant and control mice as a result of the bleomycin injections. Most interestingly, the expression level of Col1a1 mRNA was not altered in the mutant mice compared with control mice. However, bleomycin induced an increased expression of matrix metalloproteinase (MMP)-1 mRNA in the control mice, whereas MMP-1 expression was significantly less in the mutants. MMP-2, MMP-9, and gelatinase were equal in both sets of mice. These same results were confirmed when mouse embryonic fibroblasts were treated with bleomycin *in vitro*. The authors concluded that TNF-α receptor p55 is an essential component of the signaling

pathway for MMP-1 expression leading to the degradation of collagen.

In another study, Takagawa *et al.* [9] examined the effects of repeated injections of bleomycin into the skin of mice, which induced inflammation and fibrosis by 1 week. They further characterized the role of cellular Smad3 expression in the skin using specific antibodies. Mice injected with bleomycin had abundant Smad3 throughout the dermis, epidermis, hair follicles, and sebaceous glands. This effect was absent in control mice injected with phosphate-buffered saline. At 3 days and 1 week, Smad3 was detected mainly in infiltrating macrophages; the inflammation began to resolve in 2 to 3 weeks, and Smad3 expression was confined mainly to the nuclei of fibroblasts. The authors also examined Smad 2 and Smad3 phosphorylation as an indicator of the activation level of the TGF-β/Smad pathway. They showed that 70% of the lesional fibroblasts in mice injected with bleomycin were positive for phospho-Smad2/3 and that it was detectable even after 3 weeks of bleomycin injection, primarily in fibroblast nuclei. By contrast, phospho-Smad2/3 was undetectable in control mice. The authors concluded that activation of the Smad signaling pathway is ongoing in resident lesional fibroblasts even after the inflammation has resolved. In experiments with dermal fibroblasts, it was shown that bleomycin has no direct effect on the phosphorylation of Smad2/3 but that its effect is mediated by TGF-β. To understand the mechanism by which the resident lesional fibroblasts continued to produce phospho-Smad2/3 in the absence of inflammation, the authors postulated that there might be a defect in Smad7 that downregulates TGF-β activation of the Smad signaling pathway. *In vivo* expression of Smad7 in the dermis of TGF-β–injected mice was demonstrated after 24 hours. By contrast, in the bleomycin-injected mice Smad7 was induced in the infiltrating mononuclear cells but not in the fibroblasts. These results indicated that the ratio of phospho-Smad2/3 to Smad7-positive fibroblasts was significantly altered in bleomycin-injected mouse dermis and that this alteration was responsible for the cutaneous fibrosis that develops in these mice.

In a related study, Yamamoto and Nishioka [10] examined the role of monocyte chemoattractant protein-1 (MCP-1) and its receptor, CCR-2, in the pathogenesis of bleomycin-induced scleroderma. The authors first examined the expression of MCP-1 and CCR-2 in the lesional skin of mice injected subcutaneously with bleomycin. They found that MCP-1 was weakly detectable on scattered mononuclear cells in control mice. In contrast, MCP-1 and CCR-2 were detectable on infiltrating mononuclear cells in the dermis of bleomycin-injected mice, and the number of positive cells peaked between 2 and 3 weeks during treatment. Positive fibroblasts were also detected in the sclerotic dermis at later stages. The authors concluded that there was concurrent upregulation of MCP-1 and CCR-2 in mononuclear cells at early inflammatory stages and in fibroblasts at a later sclerotic stage in the skin of bleomycin-injected mice. The authors next investigated the effect of antibodies against MCP-1 on the course of dermal fibrosis. Administration of the antibody every other day decreased the bleomycin-induced dermal fibrosis and reduced the number of infiltrating mononuclear cells compared with control animals injected with bleomycin but receiving no antibody. The link between MCP-1 and increased expression of Col1a1 mRNA was examined in cultured fibroblasts. The addition of MCP-1 to the fibroblast cultures increased collagen mRNA levels by more than fourfold. In addition, decorin mRNA levels were also increased. These mRNA increases were MCP-1 dose dependent. However, the levels of fibronectin and biglycan mRNA were not significantly altered. The authors concluded that MCP-1 may induce fibrosis by directly upregulating Col1a1 mRNA in fibroblasts as well as by exerting an indirect effect through cytokines released from immunocytes recruited into lesional skin.

Another study by Yamamoto and Nishioka [11] examined the possible role of apoptosis in the pathogenesis of bleomycin-induced scleroderma. These authors showed that murine skin treated with bleomycin showed strong apoptotic signals indicative of cell death in the infiltrating mononuclear cells, hair follicles, and sebaceous glands in the dermis. The signals were present as early as 1 week and increased markedly after 3 weeks of bleomycin treatment. The molecular mechanism of the induced apoptosis involved Fas and FasL. Fas was detected in the cell membrane of some mononuclear cells after 1 week of bleomycin treatment and increased to a maximum after 2 weeks of treatment. Fas was also expressed constitutively in fibroblasts from 1 to 4 weeks of bleomycin treatment. FasL was detectable after 1 week of treatment in inflammatory cells and in fibroblastic cells at 3 to 4 weeks. Reverse transcription polymerase chain reaction showed that Fas mRNA was clearly detectable throughout all layers of skin during 1 to 4 weeks of bleomycin treatment, whereas FasL mRNA expression was upregulated and peaked at 7.5 times the control values at 3 weeks after the beginning of bleomycin treatment. The authors next investigated the role of caspase-3, a downstream regulator of Fas/FasL. Immunohistochemistry showed that caspase-3 expression was detected in epidermis, hair follicles, and sebaceous glands but not in cellular infiltrates in control mice. In bleomycin-treated skin, caspase-3 was detected in both the nucleus and the cytoplasm of infiltrating mononuclear cells and a small portion of the fibroblasts. Reverse transcription polymerase chain reaction showed that both caspase-1 and caspase-3 mRNA expression was upregulated in lesional skin and peaked at 3 weeks of bleomycin treatment. The upregulation of caspase-3 fol-

lowed the same time course as FasL upregulation. An anti-FasL antibody partially prevented the development of dermal fibrosis after bleomycin treatment, and collagen content was reduced 50% compared with skin from controls treated with bleomycin and normal IgG. The number of apoptotic mononuclear cells in the sclerotic skin was also decreased. The authors conclude that there is a relation between the Fas/FasL system and caspase-3 activation that mediates apoptosis and that the continuous and extensive expression of FasL may participate in the development of cutaneous fibrosis/sclerosis by inducing excessive apoptosis or by modulating inflammatory mediators.

Zhuo *et al.* [12] examined the modulation of PDGF-C and PDGF-D in bleomycin-induced pulmonary fibrosis in mice. PDGFs induce fibroblast proliferation and chemotaxis through cell surface receptor signaling. The authors reported that during bleomycin-induced lung fibrosis in mice, the levels of PDGF-C mRNA increased significantly, whereas the level of PDGF-D mRNA decreased. The increased levels of PDGF-C mRNA were localized by *in situ* hybridization to areas of lung fibrosis and were not observed in the lungs of bleomycin-resistant BALB/c mice. The authors suggested that PDGF-C is involved in the development of bleomycin-induced pulmonary fibrosis through binding to its receptor.

Systemic sclerosis model induced by spleen cell injections in immunodeficient mice

Ruzek *et al.* [13] described a modified model of induced SSc that demonstrates all major aspects of the human disease. The authors transferred donor B10.D2 spleen cells into RAG-2 knockout mice to induce a graft-versus-host response. RAG-2 knockout mice are genetically deficient in mature T and B cells and therefore cannot generate an antigen-specific immune response. The results were similar to a graft-versus-host model using irradiated mice. In the treated mice, dermal thickening developed, primarily of the extremities, with less pronounced dermal thickening in dorsal or abdominal skin. The dermal thickening peaked at 3 to 5 weeks and began to decline by 6 weeks but did not completely disappear even by 22 weeks. Fibrosis of internal organs including the kidneys, the intestinal tract, and the liver was also observed, and, in contrast to the dermis, the visceral fibrosis continued to increase over time. Overexpression of both type VII and type III collagens in the skin and type III collagen in the kidneys was also observed. The authors also reported a significant decrease in the luminal ratio of blood vessels in both skin and kidney, indicating vasoconstriction and occlusion, which was progressive over the course of the disease. In addition, the expression of the potent vasoconstrictor ET-1 was increased. The smooth muscle marker αSMA showed that the cells surrounding vessels bearing this marker had morphologic changes that likely contributed to occlusion of the vessel lumen. The mice also showed a prominent early immune response up to 3 weeks, which resolved by 6 weeks. CD4+, CD8+ T cells and macrophages were shown to participate: they increased tenfold, sixfold, and eightfold, respectively, in the ear dermis. CD4+ and CD8+ cells also increased in the kidney. Of remarkable importance was the observation that ANAs with a speckled pattern developed in the mice and that Scl-70 antibodies were present in more than 90% of these mice. The authors concluded that this modified model of graft-versus-host—induced SSc shows all the major components of the human disease and can be used to test effective therapeutic agents.

Models of pulmonary fibrosis

Kuroki *et al.* [14] investigated the role of TNF-α in pulmonary inflammation and fibrosis induced by intratracheal injection of bleomycin. They used TNF-α knockout (TNF-α–/–) mice in this study. They demonstrated that the number of inflammatory cells in the bronchoalveolar lavage fluid (BALF) peaked at 7 days in TNF-α+/+ mice and then decreased. By contrast, in TNF-α–/– mice the number of inflammatory cells in BALF was persistently increased to 35 days after the instillation of bleomycin. The predominant cells present in the BALF of TNF-α+/+ mice were macrophages, whereas in TNF-α–/– mice they were lymphocytes. When cells from lung tissue were taken 21 days after bleomycin instillation, the total cell and lymphocyte numbers were higher in the TNF-α–/– mice than in the TNF-α+/+ mice. Histologic examination of TNF-α+/+ and TNF-α–/– mouse lungs revealed lymphocytic and neutrophilic infiltration, thickening of the alveolar septa, and proliferation of fibroblasts in both mouse strains 14 days after bleomycin instillation. At day 21 no difference in hydroxyproline content of the lungs between either strain was observed. However, in the TNF-α+/+ mice the inflammatory response gradually subsided, whereas in the TNF-α–/– mice massive infiltration of lymphocytes persisted, and a fibrotic and honeycomb tissue morphology was observed 75 days after bleomycin instillation. TNF-α production was measured in the TNF-α+/+ mice in response to bleomycin instillation and was shown to be biphasic reaching an initial peak after 12 hours, declining until 7 days and then rising again and remaining persistently elevated up to 50 days after instillation. Flow cytometry revealed that markers for the TNFα receptor were upregulated on inflammatory cells in BALF from TNF-α+/+ and TNF-α–/– mice 14 days after bleomycin instillation. Further flow cytometric analysis revealed that significant numbers of the inflammatory cells in BALF from the TNF-α+/+ mice were apoptotic, whereas fewer apoptotic cells were observed in the BALF from TNF-α–/– mice. When TNF-α–/– mice were treated with murine rTNF-α, the levels of apoptotic inflammatory cells increased, and two weekly treatments with the protein effectively diminished the pulmonary inflammatory response. The authors concluded that TNF-α is essential

for repressing pulmonary inflammation and fibrosis in bleomycin-induced pneumopathy and that this effect is mediated through induction of apoptosis of inflammatory cells.

A very interesting study examined the role of viruses as a cofactor in the development of pulmonary fibrosis in bleomycin-resistant mice. Lok *et al.* [15] studied the development of fibrosis in BALB/c mice injected intraperitoneally with bleomycin. This mouse strain is normally resistant to bleomycin-induced fibrosis. The mice received, in addition to the intraperitoneal injections of bleomycin, transnasal dosing with murine gammaherpes virus 68. Control mice received no virus and only phosphate-buffered saline injections. In mice that received both the virus and bleomycin, significantly more severe lung inflammation developed than in mice that received virus alone, bleomycin alone, or saline. In addition, the collagen content of the lung followed a similar pattern. The authors concluded that the virus does not cause pulmonary fibrosis by itself but can act as a cofactor in the development of lung fibrosis or in its progression.

Studies in transgenic mice

In a very interesting study, Denton *et al.* [16] examined TGF-β signaling pathways involved in tissue fibrosis, using transgenic mice. The authors reported the development of transgenic mice that express a kinase-deficient human type II TGF-β receptor (TβRIIΔk) in fibroblasts. In previous work, TβRIIΔk was shown to act as a dominant negative inhibitor of TGF-β signaling. However, in the present study, the authors demonstrated that in adult mice that expressed this receptor, pulmonary and dermal fibrosis developed. Neonatal dermal fibroblasts cultured from the transgenic mice proliferated more rapidly than control cells and produced more ECM. Several markers of TGF-β signaling were upregulated, including plasmogen activator inhibitor-1, CTGF, and Smads 3, 4, and 7. Microarray experiments showed that the transgenic fibroblasts had a similar gene expression profile to that of littermate control fibroblasts that had been treated with TGF-β1. TGF-β was not able to further stimulate the transgenic fibroblasts. However, overexpression of type II TGF-β receptors partially restored the responsiveness of these transgenic fibroblasts to TGF-β stimulation. Additional studies of the mitogen-activated protein kinase pathways (another TGF-β signaling pathway separate from the Smad signaling pathway) showed that they were less perturbed by recombinant TGF-β, and the authors concluded that this pathway is less affected in the transgenic mice than the Smad pathways. Therefore, this mouse appears to be a model for the study of TGF-β overexpression and signaling and the molecular pathways leading to tissue fibrosis.

Potential therapeutic approaches

Animal models have also been used in numerous studies to examine putative therapeutic interventions that may be effective to modulate or improve the pathologic alterations typically present in patients with SSc.

McGaha *et al.* [17] examined the effects of halofuginone, a drug with antifibrotic properties, in preventing the appearance of dermal fibrosis in the Tsk1/+ mouse. They reported that 1 μg of halofuginone injected intraperitoneally every other day for 60 days prevented the development of dermal fibrosis in both neonates and adult Tsk1/+ mice, as demonstrated by histologic analysis. The thickness of the skin of Tsk1/+ mice that had been treated with halofuginone was less than 70% of the untreated animal skin thickness. Dermal fibroblasts derived from these mice were also sensitive to the drug, which caused reduced type I collagen synthesis. The drug appeared to cause this effect by affecting the promoter activity of type I collagen genes. The site of action of halofuginone on the Col1a2 promoter was localized to a region between –3200 and +54 bp. The mechanism by which halofuginone exerted this inhibitory effect on collagen synthesis appeared to be through the TGF-β signaling pathways by blocking the phosphorylation of Smad3.

In a parallel paper, McGaha *et al.* [18] examined the effect of halofuginone on the development of the Tsk1/+ phenotype. The authors administered halofuginone intraperitoneally to newborn (1-day-old) and 1-month-old Tsk1/+ mice every other day for 60 days. They confirmed their earlier work, which showed that this drug significantly reduces collagenous material in Sirus-red stained skin sections. The collagen content of skin was decreased by 20 to 25% when drug treatment was begun in 1-month-old mice and by almost 50% when neonates were treated. *In situ* hybridization studies indicated that halofuginone-treated neonate mice had a significantly lower number of cells expressing type I collagen mRNA and that this number was indistinguishable from the number detected in C57BL/6 pa/pa normal control mice. The authors concluded that the decreased collagen content observed in halofuginone-treated Tsk1/+ mice is due to decreased numbers of cells expressing collagen in the dermis. The authors also presented data showing that halofuginone had no effect on the emphysema that develops in Tsk1/+ mice. An interesting observation was that when adult Tsk1/+ mice were treated with the drug, the level of anti–topoisomerase-1 and anti–fibrillin-1 antibodies typically present in these animals decreased to control levels in adult Tsk1/+ mice but not in neonates.

A third study by McGaha *et al.* [19] examined the mechanisms of the inhibitory effects of halofuginone on fibrosis. They showed that the inhibition of collagen gene promoter activity caused by the drug is mediated

through transcription factors that regulate type I collagen gene expression. Using mouse-cultured fibroblasts, the authors found that TGF-β1, PDGF, and PMA induced rapid phosphorylation of c-Jun, a component of a dimeric transcription factor that binds with highest affinity to the AP-1 regulatory element. In the presence of halofuginone, the induced phosphorylation of c-Jun was higher than in its absence, indicating that the mechanism that suppresses collagen synthesis may involve c-Jun. Further experiments showed that TGF-β1–induced AP-1 binding activity was greatly increased in intensity, and the time over which the increase occurred was lengthened in the presence of halofuginone. Transfection assays also showed that there was a synergistic effect of TGF-β1 and halofuginone on the activation of AP-1 activity and that this AP-1 complex induced by TGF-β1 in the presence of halofuginone is a potential antagonist of type I collagen gene activity. Halofuginone abrogated the TGF-β1–induced upregulation of the Col1a2 promoter and the reduction of Col1a2 mRNA levels through a c-Jun dependent mechanism. In additional tests on Tsk1/+ mice, the authors showed that the topical application of halofuginone led to increased amounts of phospho-c-Jun in the stratum and granular basal layer of the epidermis.

Another potential therapeutic agent was studied by Gong *et al.* [20]. The authors showed that the recently described hepatocyte growth factor (HGF) modulates matrix metalloproteinases and plasminogen activator/plasmin proteolytic pathways in progressive renal interstitial fibrosis. HGF increased collagen catabolism in human proximal tubular epithelial cells (HKC) treated with TGF-β1 associated with increased MMP activity and plasminogen activator/plasmin proteolytic pathway enhancement. HGF also induced the production of MMP-9 and prevented TGF-β1–induced production of tissue inhibitor of metalloproteinase (TIMP)-2 and plasminogen activator inhibitor (PAI)-1. Continuous infusion of HGF in the rat remnant kidney model ameliorated renal fibrosis and tubulo-interstitial collagen deposition. In this model the authors also reported increased tubular expression of MMP-9, enhanced *in situ* gelatinolytic activity, restoration of plasmin activity, and decreased expression of TIMP-2 and PAI-1. There was an overall increase in TIMP-3 expression. Anti-HGF antibodies caused increased renal fibrosis and exaggerated accumulation of interstitial collagen deposition. Accompanying these changes were decreased tubular expression of MMP-9 and elevated TIMP-2 and PAI-1 expression. The authors concluded that HGF ameliorates renal fibrosis by enhancing ECM catabolism through the MMP and PA/plasmin proteolytic pathways.

An additional study examined the possibility that HGF may have antifibrotic effects. Wu *et al.* [21] showed that HGF both prevents and ameliorates bleomycin-induced dermal sclerosis. These authors transfected a construct encoding human HGF into mouse skeletal muscle and showed that expression of HGF had a marked effect on preventing dermal sclerosis in mice injected with bleomycin. The HGF was effective even when injected 4 weeks after bleomycin treatment. Levels of HGF mRNA and protein were higher in skin, lung, muscle, and serum after two transfections. It appears that the effect of HGF in this model involves TGF-β, because levels of TGF-β were reduced. Transfections of HGF also ameliorated lung fibrosis. The authors suggest that gene therapy with HGF may be useful to prevent even established tissue fibrosis.

Conclusion

Most of the work published over the past 2 years indicates that the TGF-β signaling pathway is a common mechanism by which fibrosis occurs. However, there are numerous other molecules that can either modulate this pathway or have a direct effect on fibrosis. Two compounds, HGF and halofuginone, have been shown to be effective in preventing or ameliorating fibrosis, and it appears that perturbation of TGF-β pathways was involved in the mechanism of action of these drugs.

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