

Frontiers of antifibrotic therapy in systemic sclerosisJörg H.W. Distler¹, Carol Feghali-Bostwick², Alina Soare¹, Yoshihide Asano³, Oliver Distler⁴,David J. Abraham⁵

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Abstract

Although fibrosis is becoming increasingly recognized as a major cause of morbidity and mortality in modern societies, targeted anti-fibrotic therapies are still not approved for most fibrotic disorders. However, intense research over the last decade has improved our understanding of the underlying pathogenesis of fibrotic diseases. We now appreciate fibrosis as the consequence of a persistent tissue repair responses, which, in contrast to normal wound healing, fails to be effectively terminated. Profibrotic mediators released from infiltrating leukocytes, activated endothelial cells and degranulated platelets may predominantly drive fibroblast activation and collagen release in early stages, whereas endogenous activation of fibroblasts due epigenetic modifications and biomechanical or physical factors such as stiffening of the extracellular matrix and hypoxia may play pivotal role for disease progression in later stages. In the present review, we discuss novel insights into the pathogenesis of fibrotic diseases using systemic sclerosis (SSc) as example for an idiopathic, multisystem disorder. We set a strong translational focus and predominantly discuss approaches with very high potential for rapid transfer from bench-to-bedside. We highlight the molecular basis for ongoing clinical trials in SSc and also provide an outlook on upcoming trials.

Introduction

Fibrosis is defined as excessive deposition of fibrous connective tissue in an organ or tissue initiated by tissue repair programs in response to injury. The accumulation of extracellular matrix (ECM) often disrupts the physiological architecture, and can lead to organ malfunction. Normal wound healing and fibrotic diseases share many similarities. In both cases, an initial injury initiates a series of reparative processes in damaged tissues to restore organ integrity. These processes involve leukocyte activation and infiltration and culminate in the accumulation of myofibroblasts, which release ECM and contract the damaged tissue (Figure 1). Myofibroblasts are a heterogeneous cell population, characterized by the expression of contractile proteins and high capacity for ECM synthesis. Myofibroblasts may be primarily derived from transdifferentiation of resident fibroblasts and pericytes, but other tissue resident cell types such as epithelial cells, endothelial cells, smooth muscle cells, adipocytes as well as bone-marrow-derived fibrocytes and other precursor and progenitor cell populations may also contribute to the pool of myofibroblasts (Figure 1). However, while normal reparative responses are terminated after sufficient repair of damage, tissue remodeling and fibroblast activation persist in fibrotic diseases. Fibrotic diseases may thus be considered as exaggerated and prolonged wound healing responses (1). In consequence, impaired termination may also drive the progression of fibrotic diseases in addition to pathological activation of tissue repair responses (Figure 1).

Fibrotic diseases can affect virtually every organ system. In addition to classic fibrotic diseases, tissue fibrosis is a common outcome of almost all chronic diseases. Fibrotic tissue responses can make an important contribution to morbidity or disease progression, even in diseases not commonly associated with fibrosis. For example, fibrotic tissue responses mediate vascular remodeling in atherosclerosis, myocardial remodeling in chronic heart failure, and small-airway remodeling in chronic obstructive pulmonary disease or in asthma.

Fibrotic tissue remodeling also influences tumor invasion and metastasis. Indeed, up to 45% of all deaths in the developed world have been estimated to be attributed to some type of chronic fibroproliferative disease (2). In general, the global incidence of fibrosis as well as the associated health-care burden are further increasing and therefore, fibrosis is increasingly recognized as one of today's major healthcare challenges (2, 3). As there are only few antifibrotic therapies and even fewer targeted approaches, there remains a major need for continued intensive research and active drug discovery.

Contrary to the held perception that fibrosis is permanent, multiple lines of evidence point to considerable plasticity of fibrotic tissues. Although this plasticity varies between different organs and individual diseases, the accumulation of extracellular matrix is not irreversible, but can regress, when major effector cells such as myofibroblasts are eliminated and the balance of matrix synthesis and degradation is shifted towards degradation (3).

While originally pursued as different diseases, it is now clear that several common pathways orchestrate fibrotic tissue responses of all organs (4). This perception together with the identification and characterization of several key regulators of fibrosis stimulated increasing interest of pharmaceutical companies in the development of targeted antifibrotic therapies.

Those efforts lead to the approval of the first two antifibrotic drugs (nintedanib and pirfenidone, both for idiopathic pulmonary fibrosis), a rapidly growing number of clinical trials with antifibrotic drugs and numerous promising small molecules in the earlier stages of clinical development. The most advanced approaches in the context of systemic sclerosis (SSc) are reviewed here.

Vascular damage and platelet degranulation

Vascular alterations in fibrotic diseases are particularly prominent in SSc and are associated with profound imbalances between pro- and anti-angiogenic mediators as well

vasoconstrictive and vasodilatory factors. First vascular changes occur early in the pathogenesis of SSc and precede fibrotic manifestations in humans and in murine models (5).

The exposure of sub-endothelial ECM and the reduced blood flow rates in damaged vessels lead to activation and degranulation of platelets in SSc (6). Platelet granules contain large amounts of serotonin (5-hydroxytryptophan, 5-HT). More than 90% of the serotonin in the human body is stored in platelets. Consistent with the increased activation of platelets in SSc, the levels of 5-HT are elevated in the blood of SSc patients. Inhibition of platelet activation by high doses of clopidogrel ameliorates fibrosis induced by bleomycin and in tight-skin-1 (Tsk-1) mice (7). Anti-platelet therapy has also been shown to ameliorate fibrosis of the heart, the aortic valve, kidneys and liver (8, 9). Further studies demonstrated that mice deficient in tryptophan hydroxylase 1 (Tph-1), which are characterized by very low levels of 5-HT in platelets, are also protected from experimental fibrosis (7). 5-HT directly stimulates the release of collagen in cultured human fibroblasts, human mesangial cells or murine cardiac fibroblast. Pharmacologic and genetic approaches demonstrate that these stimulatory effects on fibroblasts are mediated by the 5-HTR2B. 5-HTR2B deficient mice are protected from experimental skin fibrosis (7). Treatment with the non-selective 5-HTR2 inhibitors terguride and cyproheptadine, both of which are in clinical use, as well as the selective 5-HTR2B inhibitor SB204741 also exerted potent anti-fibrotic effects. Selective inhibition of 5-HTR2B also showed therapeutic potential in mouse models of liver fibrosis (10).

The effects of terguride in SSc patients were evaluated in a small, unblinded proof-of-concept trial. SSc patients treated with terguride were found to have lower modified Rodnan Skin Scores (mRSS) as compared to untreated controls. Moreover, the dermal thickness, myofibroblasts counts, the mRNA levels of type I collagen, of TGF- β 1 and of the recently proposed four gene biomarker set (11) were reduced by treatment with terguride (12). These results prompted the initiation of randomized, placebo-controlled confirmatory phase III

clinical trial with terguride in patients with early, diffuse cutaneous SSc, which will start recruitment in autumn 2016.

5-HT is not the only pro-fibrotic mediator stored in platelets. Platelet granules contain an array of other pro-fibrotic mediators including growth factors such as transforming growth factor-beta (TGF- β), platelet-derived growth factor (PDGF), fibroblast growth factors (FGF) and vascular endothelial growth factor (VEGF) and bioactive lipids such as lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) (13). Several of those mediators such and ongoing clinical programs to target those mediators are discussed in more detail below. The release of a plethora of profibrotic mediators from activated platelets at sites of vascular damage thus provides a direct pathophysiologic link between vascular injury and tissue fibrosis.

Activation of the coagulation cascade upon tissue injury may also promote fibrosis in SSc as well as in other fibrotic diseases such as IPF (14). Thrombin enhances the proliferative effects of fibrinogen on fibroblasts, induces the expression of profibrotic growth factors, stimulates the release of extracellular matrix proteins and promotes transdifferentiation of resting fibroblasts into myofibroblasts. Thrombin levels are elevated in patients with SSc, and findings suggest that patients are in a hypercoagulable state (15). Direct thrombin inhibitors such as dabigatran reduce the activated phenotype of SSc fibroblasts and demonstrated antifibrotic effects model of bleomycin-induced lung fibrosis in the both preventive and therapeutic settings (16). Further, thromboxane A₂ and its receptor (TXAR) have also been implicated in impaired angiogenesis in SSc. Patients have elevated circulating levels of 8-isoprostane which activates TXAR (17) and increased expression of thromboxane synthase in leukocytes that results in increased serum levels of thromboxane A₂ (18). Anticoagulation has been controversial in SSc-associated PAH and does not seem to offer a benefit advantage over other available PAH therapies (19).

Leukocyte activation and cytokine release

A characteristic feature of SSc is the presence of perivascular inflammatory infiltrates associated with regions of activated fibroblasts and matrix remodeling. Inflammatory infiltrates are seen early in the course of SSc (5). These inflammatory infiltrates are thought to induce fibroblast activation and to initiate tissue repair responses in SSc. While research initially focused mainly on adaptive immune responses, accumulating evidence points to a key-role of innate immune responses in fibrotic diseases such as SSc and toll-like receptors as well as innate lymphocyte cells have gained increasing interest (20, 21). The central role of aberrant immune activation for fibrosis in SSc is particularly highlighted by the success of high dose immunosuppression with subsequent autologous stem cell transplantation (22). Although a plethora of inflammatory mediators might have the potential for targeted intervention, we focus here on candidates with ongoing clinical trial programs. Further potential targets are briefly highlighted in the Supplementary Table 1.

Interleukin-6 (IL-6) is a central regulator of acute phase responses and also plays a key role for the transition from innate to acquired immunity. IL-6 signals are transduced into the nucleus by IL-6 receptor alpha (IL-6R α) and gp130 via JAK/STAT (23). Among its pleiotropic effects, IL-6 has been implicated in fibroblast activation and collagen release. Serum levels of IL-6 are elevated in SSc patients and positively correlate with skin thickness (24). Increased amounts of IL-6 are also detected in lesional skin of SSc patients (25). Although IL-6R α is not expressed on the surface of dermal fibroblasts, gp130 is expressed and IL-6 activates fibroblasts via trans-signaling through an association between soluble IL-6 receptor, IL-6 and gp130 (26). Genetic deletion of the IL-6 gene in mice or treatment with anti-mouse IL-6 receptor antibodies attenuates bleomycin-induced fibrosis (27). A phase 2, randomized, placebo-controlled, double-blind trial of subcutaneous tocilizumab in patients

with early diffuse SSc and enriched for patients with inflammatory features, failed to meet its primary endpoint, but showed a clear trend towards improvement in the mRSS score. Moreover, several secondary and exploratory endpoints improved in a statistically significant manner (28, 29). Those results promoted a phase III, follow-up trial of tocilizumab in SSc, which is currently recruiting participants (NCT02453256).

A central paradigm of fibrotic diseases is a Th2 bias in T cell differentiation. High levels of T helper (Th)2 cytokines such as IL-4 and IL-13 are reported in serum of SSc patients (30). IL-13 targeting therapies attenuates experimental pulmonary and hepatic fibrosis (31, 32). However, a phase II double-blind, placebo-controlled trial of an anti-IL-13 antibody (QAX576, Novartis, Switzerland) in patients with IPF was closed preterm in 2013. Other approaches to target Th2 differentiation or cytokines are still at the level of preclinical or earlier clinical development (see supplementary table 1).

B cells are part of the inflammatory infiltrates of fibrotic skin and the presence of autoantibodies in sera of patients with fibrotic diseases suggest that B cells are involved in the pathogenesis of fibrosis (33, 34). Chronically abnormal B cell activity might contribute to an imbalance of cytokines, which promotes Th2-predominance and fibrosis. In addition, certain autoantibodies e.g. against PDGF- (35), endothelin- and angiotensin-receptors (36) might directly stimulate the release of collagen from fibroblasts. The role of B cells in the pathogenesis of SSc is further supported by studies demonstrating the mice deficient in CD19 are protected from fibrosis in the Tsk-1 mice as well as in bleomycin-induced fibrosis (37). A retrospective analyses within the EUSTAR cohort reported greater improvement of the mRSS and better forced vital capacity (FVC) in SSc patients treated with the B cell depleting CD20 antibody rituximab as compared to controls (38). Prospective randomized-controlled trials are needed to confirm these encouraging results. Two trials with rituximab in SSc are currently ongoing: A phase II randomized, double blind, placebo controlled trial recruiting patients with SSc-PAH (NCT01086540) and a trial with SSc-associated arthritis (NCT01748084).

Another approach to target inflammation in SSc that is currently evaluated in a clinical trial is inhibition of the cannabinoid receptor CB2. Exogenous as well as endogenous cannabinoids transmit their signals by two different cell surface receptors, CB1 and CB2, both of which are heterotrimeric GTP binding protein coupled receptors. Cannabinoids can modulate fibrotic tissue responses via CB1 and CB2: While CB2 mainly regulates leukocyte infiltration, CB1 may also affect resident cells such as fibroblasts (39, 40). Besides skin fibrosis, cannabinoids also contribute to the pathogenesis of liver fibrosis (41). Potential therapeutic effects of targeting CB2 with the synthetic cannabinoid-mimetic resunab in SSc are currently investigated in a phase II clinical trial in patients with early, diffuse-cutaneous SSc NCT02465437.

Transforming growth factor- β signaling

TGF- β is a master-regulator of mesenchymal tissue responses. Although TGF- β 1 is the most abundantly expressed isoform, the other isoforms of TGF- β , TGF- β 2 and TGF- β 3, are also upregulated in SSc. In addition to its regulation on the transcriptional level, the availability of bioactive TGF- β is also modulated by cleavage of latent TGF- β and by sequestration in the extracellular matrix (for more details on TGF- β signaling in SSc, we refer to recent reviews such as (42)). Activation of TGF- β signaling, e.g. by fibroblast-specific overexpression of a constitutively active TGF- β receptor type 1 (TBR1^{CA}), is sufficient to induce fibrosis (43). The expression of a subset of TGF- β regulated genes correlates with the mRSS and with myofibroblast counts (11). Numerous preclinical studies further demonstrated that inhibition of TGF- β signaling exerts potent anti-fibrotic effects in various animal models across different organs (42). Nevertheless, the first attempt to inhibit TGF- β signaling in SSc using recombinant TGF- β 1-neutralizing antibodies (CAT-192) failed to show efficacy in a placebo-

controlled trial (44). The results of this trial, however, do not provide evidence against inhibition of TGF- β signaling in SSc, because CAT-192 had only insufficient affinity to TGF- β 1 *in vivo*. A more recent uncontrolled study evaluated the effect of a high-affinity neutralizing antibody that targets all three TGF- β isoforms (fresolimumab) in SSc. The authors reported a decrease in the mRSS early after the first infusion of fresolimumab. This decrease was accompanied by reduced mRNA levels of several TGF- β -regulated genes and decreased myofibroblast counts in fibrotic skin (45). However, some studies noted a high number of keratoacanthomas at multiple sites in patients treated with fresolimumab (46). Mechanistically, the appearance of keratoacanthomas can be explained by the fact that TGF- β inhibits the proliferation of keratinocytes. While potent, systemic long-term inhibition of all TGF- β isoforms or direct targeting of the TGF- β receptors may thus be prohibited by safety concerns, several strategies have been developed to target downstream mediators of TGF- β signaling.

A recently identified intracellular downstream mediator of TGF- β signaling with translational potential is the soluble guanylate cyclase (sGC). sGC catalyzes the production of cyclic guanosine monophosphate (cGMP) upon binding of nitric oxide (NO). Increasing levels of cGMP inhibit TGF- β dependent fibroblast activation (47). sGC stimulators do not interfere with canonical TGF- β signaling, but may exert their anti-fibrotic effects by inhibiting TGF- β dependent activation of Erk (48). sGC stimulators demonstrated antifibrotic effects in various experimental mouse models of SSc (47, 49). The sGC stimulator riociguat has been approved in 2015 for the treatment of pulmonary arterial hypertension. Riociguat is currently evaluated in a large randomized, placebo-controlled study in patients with early, diffuse cutaneous SSc.

Nuclear receptors compose a superfamily of transcriptional regulators that are increasingly recognized as regulators of mesenchymal tissue responses. Several nuclear receptors have recently been suggested to be potential targets for anti-fibrotic therapies (50-54). Most advanced are clinical programs with agonists of peroxisome proliferator-activated receptors

(PPARs). In particular PPAR- γ has been linked to fibroblast activation in SSc. The expression of PPAR- γ is decreased in fibrotic tissues of SSc (55). PPAR- γ agonists such as thiazolidinediones inhibit TGF- β -induced myofibroblast differentiation and collagen release *in vitro* by interfering with the recruitment of the coactivator p300 to Smad binding sites (56).

Thiazolidinediones also ameliorate bleomycin-induced skin fibrosis. However, selective PPAR- γ agonists have been associated with elevated LDL levels and rosiglitazone was suspended from allowed use in Europe due to an increased risk of heart attack and stroke. Combined activation of all members of the PPAR family may overcome the safety limitations of thiazolidinediones. The pan-PPAR agonist IVA337 did not increase the levels of LDL and also demonstrated potent anti-fibrotic effects in several preclinical models of SSc (57). A randomized, controlled phase II trial with IVA337 in diffuse-cutaneous SSc has been started in October 2015.

Vitamin D receptor (VDR) also regulates fibrotic tissue responses and has potential for rapid transfer from bench-to-bedside. Decreased levels of vitamin D have been observed in various autoimmune diseases including SSc (58). In addition to a lack of vitamin D, the expression of VDR is downregulated in SSc skin (51). VDR directly regulates the sensitivity of fibroblasts to the profibrotic effects of TGF- β : While knockdown of VDR enhanced TGF- β -induced myofibroblast differentiation and collagen release, synthetic VDR agonists reduced the stimulatory effects of TGF- β . Mechanistically, VDR binds to phosphorylated Smad3 to inhibit its transcriptional activation. Preventive and therapeutic treatment with VDR agonists ameliorated bleomycin- as well as TBRI^{CA}-induced fibrosis. Moreover, VDR null mice spontaneously develop liver and vitamin D analogs prevent carbon tetrachloride (CCl₄)-induced liver fibrosis and renal fibrosis induced by unilateral ureteral obstruction (UUO) (59, 60). Numerous compounds that modulate VDR signaling are currently in clinical use and would be available for clinical trials.

Bioactive lipid mediators such as lysophosphatidic acid (LPA) and shingosine-1-phosphate have also been identified as important regulators of TGF- β signaling in fibrotic diseases (61, 62). The levels of LPA are elevated in the serum of SSc patients (63). LPA is chemotactic for fibroblasts, induces the expression of TGF- β and CTGF and promotes myofibroblast differentiation. Knockout of the lysophosphatidic acid-1 receptor (LPA-1) or treatment with antagonist of LPA1 protected from bleomycin-induced pulmonary and dermal fibrosis and ameliorated fibrosis in Tsk-1 mice (64). An orally available LPA1 receptor antagonist (SAR100842) was evaluated in a phase 2a clinical trial in patients with diffuse cutaneous SSc. Although no significant differences in the mRSS were observed between SAR100842 and placebo, effective target engagement was demonstrated and the levels of two potential biomarkers of skin fibrosis, COMP and TSP1, were reduced (65). Clinical trials with LPA antagonists (either LPA1 or LPA1-3) in SSc and in other fibrotic diseases are currently under consideration.

Pirfenidone is approved for IPF based on reduced loss of FVC in two out of three trials as compared to placebo-treated patients. Although the molecular mechanisms underlying the antifibrotic effects of pirfenidone are incompletely understood, it is thought to work predominantly by modulating TGF- β and TNF α signaling. An open label phase II trial with pirfenidone in SSc-ILD has recently been completed (66). Although exploratory disease outcomes remained largely unchanged, a follow-up trial in patients with SSc-ILD is planned [NCT01933334].

Reactive oxygen species (ROS) produced by the NADPH oxidase (Nox) system are believed to play important roles in tissue injury and fibrosis (67). Several Nox isoforms have been identified with Nox2 and Nox4 being elevated in SSc and the most relevant to fibrosis (68). Inhibiting NOX4 by targeting using a small molecule (GKT137831) or deficiency of Nox4 has been shown to reduce fibroblast activation in lung and liver (69, 70). The selective Nox

inhibitor, GKT137831 has completed safety trials [NCT02010242] and has recently been given orphan drug designation for development as a treatment in SSc.

Integrins, mechanical forces and extracellular matrix

The composition of the ECM, mechanical forces and ECM stiffness alter the activation and the bioavailability of several growth factors including TGF- β (71). Enhanced activation of latent TGF- β is thought to contribute to aberrant TGF- β signaling in fibrotic diseases. Several members of the integrin family of transmembrane receptors may contribute to enhanced activation of latent TGF- β in SSc. Alpha-V (α_v) integrins are recognized as potent activators of TGF- β 1 and TGF- β 3. Deletion of *Itgb6*, which encodes integrin $\alpha_v\beta_6$ and is upregulated in SSc-associated ILD, leads to resistance to bleomycin-induced pulmonary fibrosis (42, 72). Integrin $\alpha_v\beta_8$ may also play important roles in TGF- β activation and fibrotic tissue remodeling (73). Furthermore, ECM proteins such as fibronectin and thrombospondin-1 can activate latent TGF- β .

Fibroblasts do not only synthesize and remodel the ECM, but the composition and the mechanical properties of the ECM in turn influence fibroblast activation. Fibroblasts sense the stiffness of the ECM via integrins (74). β_1 integrin is overexpressed on SSc fibroblasts. Mice with fibroblast-specific loss of β_1 integrin are protected from bleomycin-induced fibrosis (75). Moreover, treatment with blocking antibodies against β_1 integrin ameliorated fibrosis in a transgenic mouse model harboring a mutation in the fibrillin gene associated with stiff skin syndrome (76). These findings stimulated the initiation of clinical trials with blocking β_1 integrin antibodies in IPF and other indications such as SSc are currently under consideration. Besides targeting individual integrin subunits, inhibitors targeting selective heterodimers might be a promising and more specific approach as recently shown with a small molecule $\alpha_v\beta_1$ inhibitor (77).

The fibrils of collagen are crosslinked during collagen maturation to enhance their stability. The crosslinking of type I collagens is mediated by lysyl oxidase and lysyl oxidase-like (LOXL) 1-4, which are overexpressed in SSc and in other fibrotic diseases (78). Targeting LOXL2 with inhibitory antibodies reduced TGF- β signaling, inhibited collagen release and ameliorated experimental liver and lung fibrosis models (79). Although we are not aware of clinical trials in SSc, a humanized monoclonal anti-LOXL-2 antibody (GS-6624) is currently in phase II clinical trials for IPF and hepatic fibrosis.

Several extracellular proteins are cleaved to release fragments, known as matrikines that exert biological activity. The non-collagenous carboxy terminal domain of collagen XVIII is cleaved by cathepsin L to release endostatin, a protein known for its anti-angiogenic activity (80). More recently, the anti-fibrotic activity of endostatin has emerged as one of its important functions. A peptide corresponding to the carboxy terminal region of endostatin was shown to exert anti-fibrotic activity in multiple pre-clinical models of skin and lung fibrosis (81). The mechanism of action of the peptide included reduction in the levels of the transcription factor, Egr-1, as well as reduction in extracellular matrix components and the cross-linking enzyme lysyl oxidase (82). Recombinant endostatin was shown to attenuate hepatic fibrosis induced by CCl₄, to improve interstitial fibrosis in a mouse model of renal injury, to reduce bleomycin-induced lung fibrosis and inhibited hypertrophic scarring (83-85). It is worth noting that conflicting data are reported for in a myocardial infarction model (86).

Stem cell pathways

Accumulating evidence demonstrates that morphogenic pathways including Wnt, Hedgehog, Notch and Hippo signaling also play important roles in fibrotic diseases.

The preclinical evidence for targeting Wnt, Hedgehog and Notch recently been reviewed in detail elsewhere (87-89). These studies may have direct translational implications as all pathways are drugable with several inhibitors either being either in advanced clinical

development or in already in clinical use (see supplementary table 1). While targeting of Notch signaling by inhibition of the γ -secretase complex maybe limited by gastrointestinal toxicity caused by a shift in differentiation from enterocytes in the intestinal crypts to mucosecreting goblet cells (90), first clinical trials suggest that targeting of canonical Wnt signaling with tankyrase- and porcupine inhibitors is better tolerated. Targeting of hedgehog signaling with inhibitors of smoothened also showed a favorable adverse event profile and vismodegib is already approved for the treatment of basal cell carcinoma. However, despite those encouraging results, potential toxicity to the stem cell compartment of inhibitors of hedgehog, Notch and i.p. Wnt signaling remains a major safety concern for long-term application. A potential approach to overcome safety concerns with systemic application is topical application. This approach is currently investigated for C-82, a small molecule that interferes with the interaction of β -catenin with the coactivator CBP (NCT02349009).

Epigenetics

Epigenetics refers to all heritable changes in phenotype or in gene expression states, which are not encoded by changes of the nucleotide sequence of the DNA. Epigenetic modifications are thought to contribute to the basic activation of SSc fibroblasts under cell culture conditions and may contribute to the persistent tissue repair responses in fibrotic diseases. Several aberrant epigenetic modifications have been implicated in the pathogenesis of SSc and other fibrotic diseases (91-93).

The most extensively studied target of aberrant DNA methylation in SSc is the Friend leukemia integration 1 (FLI1), a member of the ETS family of transcription factors. FLI1 is silenced due to increased promoter methylation in SSc fibroblasts (94). Knockdown of FLI1 in cultured fibroblasts stimulates the release of collagen. Moreover, targeted heterozygous

inactivation of Fli1 together with KLF5, another transcription factor epigenetically suppressed in SSc dermal fibroblasts, in mice (*Fli1^{+/-};Klf5^{+/-}* mice) reproduced the three cardinal pathological features of SSc, including immune abnormalities, vasculopathy, and fibrosis (95). Other genes downregulated by DNA methylation are suppressor of cytokine signaling 3 (SOCS3) and endogenous Wnt antagonists such as dickkopf-1 (DKK1) and secreted frizzled-related protein 1 (SFRP1) (96). Pharmacological inhibition of DNA methylation by 5-Aza-2'-deoxycytidine normalized the expression levels of those antifibrotic genes inhibited the collagen release by SSc fibroblasts and ameliorated fibrosis in several mouse models of SSc (96). Moreover, inhibitors of Dnmts also demonstrated antifibrotic effects in murine models of other fibrotic diseases (97).

Polypharmacologic approaches

Polypharmacology describes the simultaneous inhibition of several pathologically relevant targets by a single drug. Given the complex pathogenesis of fibrotic diseases, a single pathway intervention is unlikely to be curative and polypharmacological interventions are considered as promising antifibrotic approaches despite the theoretically increased risk of adverse effects. The most relevant example to date is the multi-tyrosine kinase inhibitor nintedanib. Nintedanib has been shown in two phase 3 replication trials (INPULSIS-1 and -2) to slow the loss of FVC in patients with IPF and was approved for the treatment of IPF in 2014/2015. Nintedanib blocks PDGFR α/β , fibroblast growth factor receptor (FGFR)-1, 2, 3, VEGFR-1, 2, 3 and the SRC-family kinases SRC, LYN and LCK by blocking the intracellular ATP-binding pocket (98). Several of the molecular targets of nintedanib such as PDGF-, VEGF- and SRC-signaling cascades have also been linked to the pathogenesis of fibrosis in SSc (99-103). Pathologic activation of PDGFRs and VEGFRs are both sufficient to induce fibrosis in mice (101, 102) and Src kinases serve as downstream mediators of several

profibrotic growth factors including TGF- β (103). Nintedanib thus offers an option for combined inhibition of several profibrotic pathways. Indeed, nintedanib inhibited proliferation, migration, myofibroblast differentiation and collagen release from cultured fibroblasts and showed anti-fibrotic effects in various *in vivo* models of SSc (104). The efficacy of nintedanib in SSc-ILD is currently evaluated in a phase III clinical trial (NCT02597933).

Summary and conclusion

Identification of potential targets is the initial step in the development of antifibrotic therapies. Increasing the framework for understanding the pathogenesis of fibrotic diseases will provide further insights and offer opportunities to develop effective antifibrotic treatment strategies. Indeed, our understanding of the molecular pathogenesis of fibrotic diseases is increasing and the list of potential molecular targets for the treatment of fibrosis is growing (supplementary table 1 and Figure 2). Several of those studies have direct translational implications as the identified mediators can be targeted pharmacologically with available drugs. Indeed, some of these studies have already been transferred from bench to bedside with clinical trials in preparation or ongoing (Table 1). The diversity of the available strategies raises hope to start into a new era of disease specific treatment strategies for fibrotic diseases.

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Drug	Target(s)	Target population	Phase	Clinical trial identifier	Status
Actemra	IL6R	Early inflammatory dcSSc	III	NCT02453 256	recruiting
Nintedanib	PDGFRs, FGFRs, VEGFRs, Src kinases	ILD-SSc	III	NCT02597 933	recruiting
IVA-337	PPARs	Early dcSSc	II	NCT02503 644	recruiting
Riociguat	sGC	Early dcSSc	II	NCT02283 762	recruiting
Terguride	5-HTR2	Early dcSSc	III	--	preparation
Rituximab	CD20 / B cells	SSc-PAH	II	NCT01086 540	recruiting
Rituximab	CD20 / B cells	SSc- arthritis	II	NCT01748 084	recruiting
IVIG	Fc receptors?	dcSSc	II	NCT01785 056	recruiting
Resunab	CB2	Early dcSSc	II	NCT02465 437	recruiting
Rilonacept	IL1	dcSSc	I/II	NCT01538	recruiting

C-82 (topical)	β - Catenin/CBP interaction	Early dcSSc	I/II	719 NCT02349 009	ongoing, not recruiting
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Table 1: Placebo-controlled, blinded clinical trials for the treatment of fibrosis in SSc that are currently ongoing or expected to start 2016.

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Figure 1: Physiologic and pathologic tissue responses.**Figure 2: Pathways and network targets for tissue fibrosis in SSc.**

The common events in tissue remodelling and excessive scarring leading to persistent fibrosis can be targeted at selective points in the fibrogenic process. Damage and chronic inflammation encourage the recruitment of progenitor populations into lesional tissue and stimulate the differentiation of resident fibroblasts. Activated myofibroblast contribute to the production, deposition and modification of the extracellular matrix (ECM). Abnormal ECM composition and structure enhance cell-matrix interactions which in the context of inflammation and a pro-fibrotic environment impede scar resolution promoting tissue fibrosis and persistence culminating in compromised organ function.

ALK5 - Activin receptor-like kinase 5, **ATX** – Autotaxin, **β-catenin** - Catenin beta -1, **C proteinase**- procollagen C-proteinase (aka bone morphogenetic protein 1), **CCR2** - C-C chemokine receptor type 2, **CCR5** - C-C chemokine receptor type 5, **CXCR4** - C-X-C chemokine receptor type 4, **Integrin (αβx)** – RGD receptors (e.g. Vitronectin, Fibronectin receptor), **Elastase** - A class of serine protease, **FAK**- Focal adhesion kinase
Lox - Lysyl oxidase, **LoxL2**- Lysyl oxidase like 2, **P4H**- Prolyl 4-hydroxylase, **PAI** - Plasminogen activator inhibitor-1, **PAR**- Protease-activated receptors, **ROCK1/2** - Rho-associated protein kinase, **RXFP1** - Relaxin/Insulin-Like Family Peptide Receptor 1, **S1PR** - Sphingosine-1-phosphate receptor, **TGM2** – Transglutaminase 2, **TNK1** - Tankyrase 1, **Wnt**- Evolutionary conserved signal pathway. Wnt being derived via a fusion of the name from the drosophila *wingless* gene and vertebrate homolog name, *integrated*

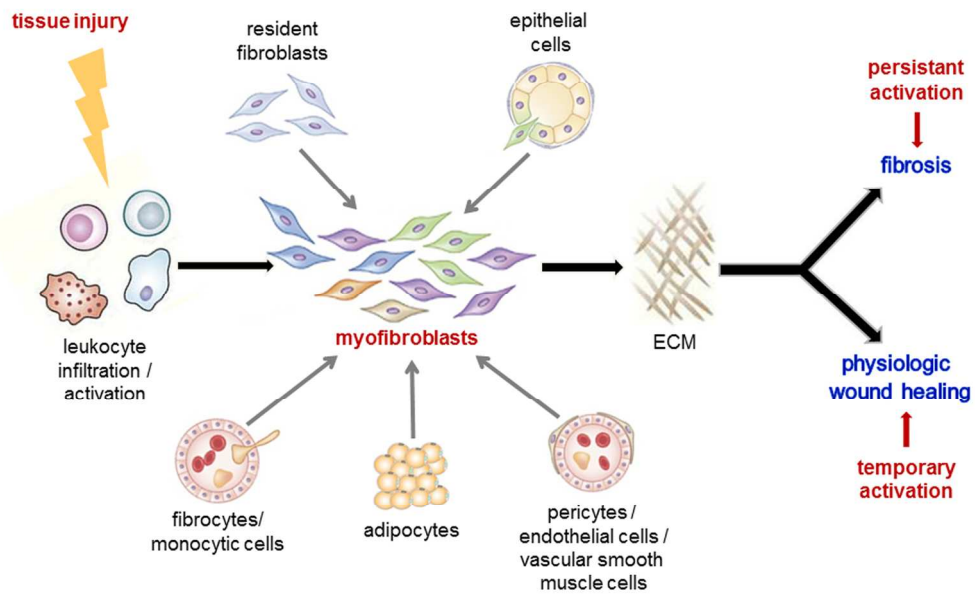


Figure 1
Figure 1
160x100mm (300 x 300 DPI)

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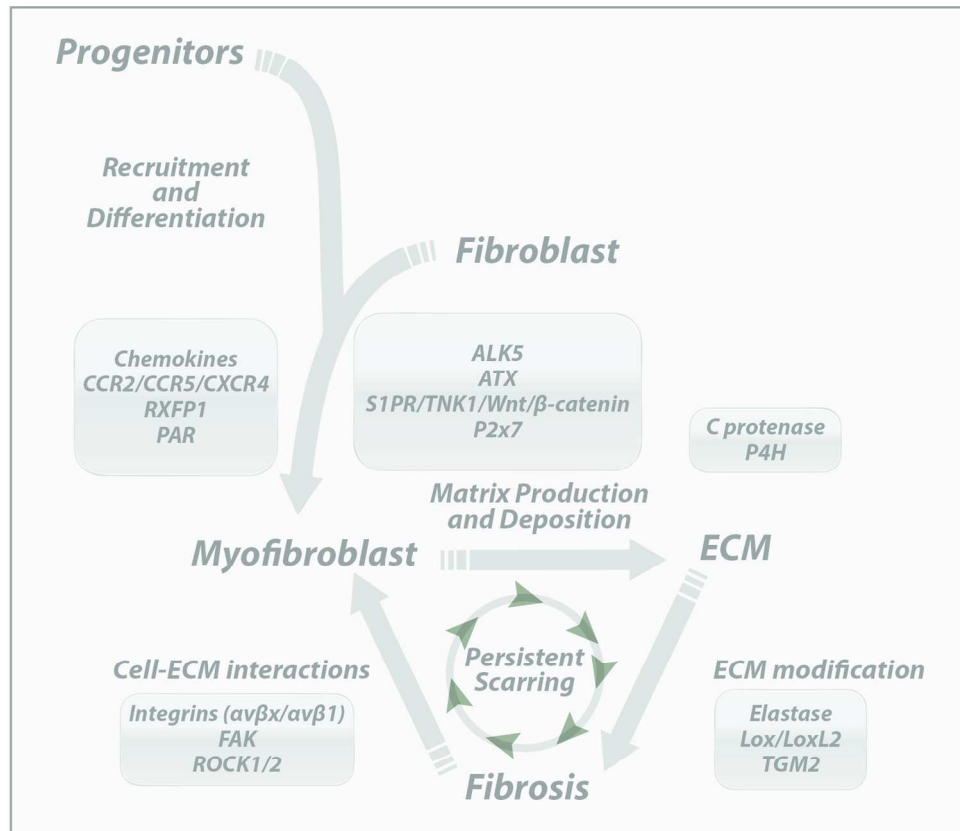


Figure 2
Figure 2
151x132mm (300 x 300 DPI)

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