Published in final edited form as:
Curr Opin Rheumatol. 2009 November ; 21(6): 649–655. doi:10.1097/BOR.0b013e328330da9b.

Innovative Approaches to the Therapy of Fibrosis

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Abstract

Purpose of review—The lung in systemic sclerosis (scleroderma) is susceptible to fibrosis and the ensuing respiratory insufficiency contributes to significant morbidity and mortality in this disease. The lack of effective therapies for pulmonary fibrosis has spurred a re-evaluation of pathobiological paradigms and therapeutic strategies in scleroderma-associated interstitial lung disease and in idiopathic pulmonary fibrosis. The purpose of this review is to examine emerging new therapeutic targets that modulate pro-fibrotic phenotypes of tissue-resident cells and the associated aberrant tissue remodeling responses in fibrotic disorders.

Recent findings—Progressive forms of tissue fibrosis, including scleroderma, are characterized by an accumulation of activated mesenchymal cells and their secreted extracellular matrix proteins in association with dysrepair of epithelial and endothelial cells. Recent studies suggest that emergence of cellular phenotypes that perpetuate loss of cellular homeostasis is characteristic of many fibrosis-related clinical syndromes.

Summary—Therapeutic strategies that modulate the fate/phenotype of reparative structural cells, including epithelial, endothelial, and mesenchymal cells, offer new opportunities for the development of more effective drugs for the treatment of fibrosis.

Keywords

Epithelial Cells; Mesenchymal Stem Cells; Fibroblasts; Apoptosis; Protein Kinase Inhibitors; PPAR gamma; Losartan; Bosentan; Hydroxymethylglutaryl CoA Reductase Inhibitors

Introduction

The focus on developing new treatments for fibrosis has shifted from anti-inflammatory and immune-modulating agents to therapeutic strategies that more directly target the fibrogenic process. The paradigm that inflammation leads to fibrosis (in a “serial” pathway) has been supplanted by the concept that inflammation and fibrosis may be independent of each other (by “parallel”, albeit interacting, pathways) [1,2]. This paradigm shift has been spurred, in large part, by clinical trials that have demonstrated minimal clinical benefit with agents that target immune/inflammatory pathways, both in scleroderma [3] and idiopathic pulmonary fibrosis (IPF) [4]. For example, a large multicenter randomized placebo-controlled study of cyclophosphamide versus placebo in patients with relatively early interstitial lung disease secondary to scleroderma yielded modest beneficial effects on lung function over a 12 month follow-up period [3].

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In this review, we will focus our discussion on pathophysiologic mechanisms underlying fibrogenesis, and novel anti-fibrotic therapies that are currently in various stages of preclinical/clinical development. We will also focus this discussion on pulmonary fibrosis, in particular scleroderma and IPF, which share several key pathophysiological mechanisms.

Fibrosis: Aberrant Cellular Phenotypes and Loss of Homeostasis

The pathogenesis of tissue fibrosis is complex. Deciphering mechanisms underlying this chronic disorder requires integration of information from molecular/cellular biology, animal models of injury-provoked fibrosis, studies with disease-specific fibrotic cells/tissues, and clinical behavior/responses of the disease in human subjects. A logical starting point is to carefully examine the histopathology of tissue fibrosis in humans. In electron microscopy ultrastructural studies, there is evidence for an apparent defect in epithelial regeneration in association with expanded populations of activated mesenchymal cells [5]. Typically, there is increased rate of apoptosis in alveolar epithelial cells, while mesenchymal cells themselves, in particular myofibroblasts, acquire an apoptosis-resistant phenotype [6].

A number of different mechanisms for alveolar epithelial cell apoptosis have been proposed [6]. Recent reports suggest an important role for endoplasmic reticulum (ER) stress in apoptosis of type 2 alveolar epithelial cells (AT2 cells) [7,8]. ER stress and activation of the unfolded protein response in AT2 cells lining areas of fibrotic remodeling in IPF lungs are reported, both in patents with mutations in surfactant protein-C (SP-C) and in sporadic cases in which such mutations were absent [7]. ER stress pathway proteins and markers of apoptosis are expressed in AT2 cells near dense zones of fibrosis in IPF, but not in the apoptosis of alveolar epithelium associated with chronic obstructive pulmonary disease (COPD) [8]. Another mechanism relevant to apoptosis-susceptibility, which may also contribute to impaired epithelial regeneration, is the role of telomere maintenance and senescence of adult stem cells [9-11].

In contrast to epithelial/endothelial cells which appear to be injure-prone and apoptosis-susceptible, (myo)fibroblasts become resistant to apoptosis, both in IPF [6,12] and in scleroderma [13-15]. We believe studies aimed at understanding mechanisms for the apparent differential susceptibilities to apoptosis of epithelial/endothelial cells versus mesenchymal cells will provide important insights into the progressive nature of this fatal lung disease. Importantly, such studies have the potential to uncover novel therapeutic targets that regulate the pro-fibrotic phenotype/fates of tissue-resident cells.

Therapeutic Strategies to Promote Epithelial/Endothelial Regeneration and Repair

Epithelial lining cells and vascular endothelial cells (to varying degrees) of organ systems that develop fibrosis are typically in a state of dysrepair. In classic studies with explanted murine lungs, it has been demonstrated that injury to the alveolar epithelium, when associated with ineffective repair, is sufficient to disturb normal epithelial-mesenchymal interactions and promote fibrogenesis [16]; that this occurs in a blood-free environment supports a direct role for tissue-resident cells in the fibrogenic process. A diverse number of phenotypes and fates of alveolar epithelial cells (AECs) have been described in fibrotic tissues; this includes hyperplastic epithelium [17], apoptosis [18], and epithelial-mesenchymal transition (EMT) [19,20]. Together, this suggests that the process of alveolar regeneration that involves differentiation of tissue-resident facultative stem cells of the alveolar epithelium, the AT2 cells, to type 1 alveolar epithelial (AT1) cells fails to proceed normally in the fibrotic lung. Thus, therapeutic strategies which promote normal alveolar regeneration may be expected to be of benefit in pulmonary fibrosis. A few such strategies are discussed here.

Hepatocyte growth factor (HGF)

HGF signaling via its receptor, c-met, modulates pro-fibrotic phenotypes of potentially several cell types, including epithelial cells. In addition to functioning as a mitogen, motogen, and survival factor for epithelial cells, a number of recent studies indicate that HGF prevents EMT by interfering with transforming growth factor-β (TGF-β) signaling [21]. The ability of HGF to alter the fate of other cell types such as to promote myofibroblast apoptosis may also contribute to its *in vivo* anti-fibrotic effects [22]. In animal models of bleomycin injury-induced lung fibrosis, administration of HGF even when administered in a delayed manner has been shown to protect against fibrosis [23-26]. The optimal strategies for delivery of HGF to target tissues need to be determined, although recombinant forms of HGF are currently undergoing Phase I/II trials for acute liver failure (www.clinicaltrials.gov; NCT00225901) and chronic venous leg ulcers (www.clinicaltrials.gov; NCT00797706).

Keratinocyte growth factor (KGF)

KGF is produced primarily by mesenchymal cells and is known to be a potent paracrine mediator of proliferation, migration and differentiation of AT2 epithelial cells. Interestingly fibroblasts isolated from lungs of IPF patients have diminished capacity to induce KGF secretion [27]. Similar to studies with HGF, administration of KGF protects against fibrosis in animal models of bleomycin lung injury [28-30]. Human recombinant KGF (palifermin) has been approved for the treatment of severe oral mucositis complicating myelotoxic therapy and hematopoietic stem cell support in patients with hematological malignancies. To our knowledge, no clinical trials of KGF for fibrotic disorders have been undertaken.

Nitric Oxide (NO)

In addition to its well recognized vasodilatory and vasculoprotective actions, NO may mediate anti-fibrotic effects by actions on epithelial cells and/or mesenchymal cells. Studies in rat AECs suggest that endogenous generation of NO may prevent AECs from undergoing an EMT-like phenotype and exogenous NO suppresses TGF-β1-induced EMT [31]. NO has been shown to mediate anti-fibrotic effects *in vivo*, as demonstrated in a baboon model of chronic lung disease associated with premature birth [32]. We are unaware of any clinical trials of NO for fibrotic disorders (excluding cystic fibrosis); however, the NIH-sponsored IPF Clinical Research Network (IPFnet) is conducting a Phase III trial of sildenafil, a phosphodiesterase type 5 (PDE5) inhibitor that augments NO-cGMP signaling, to test exercise performance in patients with advanced IPF (www.clinicaltrials.gov; NCT00517933).

eIF4E inhibitor-1 (4Ei-1)

eIF4E is the rate-controlling component of the translation initiation complex e1F4F that associates with the cap structure of mRNA to initiate protein synthesis in eukaryotic cells, essential for growth and survival of neoplastic and fibrogenic cells. Ghosh and colleagues [33] sought to explore the effect of a recently synthesized inihibitor of the association of elF4E with the mRNA cap (4Ei-1) in a zebrafish EMT model. 4Ei-1 inhibited cap-dependent translation in a dose-dependent manner in zebrafish embryos without evidence of developmental abnormalities. Furthermore, embryo explants injected with elF4E underwent EMT whereas embryos co-injected with 4Ei-1 had complete abrogation of EMT without evidence of toxicity defined by morphological/pathological parameters [33]; compounds designed to inhibit cap-dependent translation deserve further exploration in pre-clinical disease models of cancer and fibrosis.

Cell-based therapies

There has been recent excitement over the prospects of cell-based therapies for the treatment of chronic degenerative diseases. If such therapies can be designed to enhance normal epithelial

regeneration in fibrotic organs, this enthusiasm may be justifiable. Two cell-based therapeutic approaches that have garnered significant interest in this regard include mesenchymal stem cells (MSCs) and induced pluripotent stem cells (iPSCs). Murine bone marrow-derived MSCs have been shown to home to the lung, adopt an epithelium-like phenotype, and reduce inflammation and collagen deposition in lungs of bleomycin-injured mice [34]. Subsequently, the protective effect of these exogenously administered MSCs appears to be mediated by the release of ant-inflammatory paracrine mediators [35]. It appears that the ability of bone marrow cells to reconstitute and engraft lung epithelium is more limited than initially estimated [36]. There is evidence for tissue-resident MSCs in adult organ systems, including the lung [37-39]. The behavior and phenotype/fate of tissue-resident MSCs deserve further study, perhaps even before embarking on clinical trials of exogenous MSCs for fibrotic diseases.

The potential for generating organ-specific epithelial cells from embryonic stem cells (ESCs) or iPSCs affords another opportunity to regenerate epithelium which is invariably anarchic in fibrotic disorders. Strategies for generating pure populations of AT2 cells from human ESCs have been described [40]. Here again, one would like to know why the endogenous hyperplastic AT2 cells of the fibrotic IPF lung fail to differentiate normally into AT1 cells; if it were related to an altered (differentiation-prohibitive) cellular microenvironment, such cell-based therapies would be predicted to fail. On the other hand, if relative insufficiency or inherent defect of endogenous AT2 cells (e.g. a genetic defect that results in a protein trafficking abnormality and ER stress) prevent their ability to differentiate into AT1 cells, exogenous delivery of “normal” AT2 cells, when successfully engrafted, may prove successful.

Therapeutic Strategies that Target Mesenchymal Cell Activation and Survival

Activated mesenchymal cells are effectors of the exuberant extracellular matrix (ECM) production and architectural tissue remodeling, characteristic features of progressive fibrotic disorders. Myofibroblasts represent a differentiated/specialized cell type capable of mediating enhanced ECM synthesis and tissue contracture [41]. Myofibroblast differentiation is critically dependent on TGF-β1 [42,43], in addition to biomechanical tension signaling [44]. While a number of pharmaceutical companies are developing strategies to interfere with TGF-β1 signaling in cancer and fibrosis, more specific strategies downstream of TGF-β ligand(s)/receptor(s) to inhibit myofibroblast differentiation are also being developed; we will discuss a few of these here along with selected agents designed to inhibit myofibroblast contractility and/or survival.

Peroxisome proliferator-activated receptor-γ (PPAR-γ) agonists

PPAR-γ is one of the three subtypes of PPARs and is a member of the nuclear hormone receptor superfamily. PPARs were originally found to be important regulators of lipid and glucose homeostasis; however, more recently, PPAR-γ has been implicated in the regulation of inflammation, fibrosis, and cancer. The thiazolidinediones (TZDs) such as rosiglitazone, pioglitazone and troglitazone are synthetic PPAR-γ agonists used successfully for the treatment of diabetes type II since the 1990s [45]. Both natural and synthetic PPAR-γ agonists have been shown to decrease lung injury/fibrosis induced by intratracheal administration of bleomycin in mice [46]. More recently, fibroblasts isolated from patients with different types of fibrotic lung disease when stimulated with PPAR-γ agonists demonstrate reduced proliferative responses to mitogens and attenuated TGF-β1-induced myofibroblast differentiation [47]; additionally, troglitazone administered 10 days after the intratracheal instillation of bleomycin in mice was effective in inhibiting lung fibrosis. In a murine model of subcutaneous bleomycin-induced scleroderma, the concomitant intraperitoneal injection of rosiglitazone inhibited early inflammation responses and abrogated skin fibrosis, local collagen accumulation, lipoatrophy and reduced tissue accumulation of myofibroblasts [48]. A subset of mice in this study did not receive rosiglitazone until 7 days after bleomycin injury and, despite this, there was evidence

of reduced skin fibrosis [48], supporting the notion that PPAR-γ agonists may abrogate fibrosis through more direct "anti-fibrotic" mechanisms rather than through its recognized anti-inflammatory effects.

Protein kinase inhibitors

Protein kinases have become an important target for drug development [49]. This enthusiasm has been largely driven by the success of imatinib mesylate (Gleevec™), the first orally administered protein kinase inhibitor (PKI) approved for the treatment of chronic myelogenous leukemia (CML) [50]. Imatinib inhibits the Bcr-Abl tyrosine kinase which is constitutively activated in CML [51]. However, although imatinib is a relatively specific inhibitor of Bcr-Abl kinase, it also inhibits the c-Kit and PDGF receptor tyrosine kinases [52]. Based on its ability to inhibit non-SMAD TGF-β signaling via c-Abl tyrosine kinase [53] and PDGF receptor signaling [52], imatinib appears to mediate *in vivo* anti-fibrotic effects in animal models of pulmonary fibrosis [53] and dermal fibrosis [54,55]. Some studies indicate that imatinib may arrest and even reverse established fibrosis [55,56], while others indicate that delayed administration of imatinib during the post-inflammatory phase may not be as effective [57,58]. A potential concern with imatinib is its detrimental effects on epithelial cells, which may interfere with regenerative capacity of epithelium, and counterbalance putative beneficial effects on activated mesenchymal cells [58]. A Phase II multicenter double-blind, randomized and placebo controlled trial of imatinib in IPF was completed in 2007; however, results have not been published (www.clinicaltrials.gov; NCT00131274). Another Phase II study of the efficacy and tolerability of imatinib in dermal fibrosis associated with scleroderma has completed enrollment and results will be available in early 2010 (www.clinicaltrials.gov; NCT00613171). PKIs that target pro-survival signaling pathways in mesenchymal cells may also have utility in the treatment of fibrotic disorders [59].

Modulation of the contractile myofibroblast phenotype

Pharmacologic agents that modulate the contractile phenotype of myofibroblasts may be particularly effective due to a central role of this cellular phenotype in chronic fibro-contractive disorders. Such agents might include inhibitors of endothelin-1 (ET-1) and Rho kinase (ROCK), a more downstream mediator of ligand-receptor binding/activation. ET-1 is a potent endogenous vasoconstrictor that is implicated in the pathogenesis of pulmonary arterial hypertension (PAH), and the dual specificity ET receptor antagonist, bosentan, is FDA-approved for treatment of PAH [60]. Pre-clinical studies support a potential role for bosentan as an anti-fibrotic agent [61,62]. A randomized placebo-controlled clinical trial enrolled 158 patients with relatively early IPF to receive either placebo or bosentan for 12 months [63]. This study failed to demonstrate efficacy based on the primary endpoint of six-minute walk distance at 12 months; however, a *post hoc* analysis of patients that were diagnosed via a surgical lung biopsy demonstrated both a survival and a quality of life advantage in favor of bosentan. A larger multicenter, placebo-controlled, randomized study with bosentan in IPF patients diagnosed via a surgical lung biopsy and with minimal fibrotic changes on HRCT of the chest is underway [64].

The RhoA/ROCK pathway is a critical regulator of cellular contractility [65]. A small molecule inhibitor of ROCK, fasudil, is approved for the treatment of cerebral vasospasm in Japan. Fasudil has also been also recently been studied in U.S. populations for other disease indications (www.clinicaltrials.gov). One of these, a Phase III study of fasudil in the treatment of Raynaud's phenomenon is currently ongoing enrolling (www.clinicaltrials.gov; NCT00498615). To our knowledge, fasudil has not been studied for the treatment of fibrotic diseases. The 3-hydroxy-3-methylglutaryl CoenzymeA (HMG-CoA) reductase inhibitors (statins), in addition to their cholesterol-lowering properties, inhibit RhoA/ROCK activation [66]. Simvastatin inhibits myofibroblast differentiation and contractility in human lung

fibroblasts derived from IPF patients [67]. Mice treated with simvastatin, *prior* to bleomycin-induced lung fibrosis and for the duration of the study, demonstrate a reduction in inflammatory cell influx and lung collagen accumulation in association with downregulation of CTGF and TGF-β1 expression [68]. Testing a potential role of statins in subgroups of patients with fibrosis may be warranted.

Modulators of oxidative stress and redox signaling

Oxidative stress has been implicated in the pathogenesis of tissue fibrosis [69,70]. Additionally, there is now ample evidence that reactive oxygen species (ROS) function as signaling molecules when they are generated in a compartmentalized and regulated manner [71,72]. The NADPH oxidase (NOX) family of enzymes, which catalyze the reduction of O_2 to form ROS, are likely to have roles in normal cellular physiology; indeed, the number of NOX enzymes have increased during eukaryotic evolution with seven NOX isoforms identified in mammals [73-75]. However, the roles of individual NOX family members in normal physiology and mechanisms by which they contribute to disease pathogenesis are only beginning to be elucidated.

Myofibroblasts generate ROS in response to TGF-β1 and the NOX4 isoform has been identified as a source of TGF-β1-induced ROS generation in cardiac and lung myofibroblasts; NOX4 is implicated in the induction of myofibroblast differentiation [76,77]. A recent study reported that NOX4 is upregulated in lungs of patients with IPF and in mice subjected to non-infectious lung injury. In lung mesenchymal cells, NOX4-dependent generation of H_2O_2 is required for TGF-β1-induced myofibroblast differentiation, extracellular matrix (ECM) production, and contractility. Furthermore, genetic or pharmacologic targeting of NOX4 inhibited fibrogenesis in two different murine models of lung injury [77]. NOX4-dependent extracellular generation of H_2O_2 by lung myofibroblasts may mediate additional fibrogenic effects in tissues by inducing epithelial cell apoptosis [78] and/or epithelial-mesenchymal transition [79], or by inducing ECM crosslinking reactions in the presence of extracellular heme peroxidases [80]. These studies support a role for NOX4 in tissue repair functions of myofibroblasts and fibrogenesis as well as provide proof-of-concept for therapeutic targeting of NOX4 in abrogating lung fibrosis. The development of small molecule inhibitors and/or other strategies targeting NOX4 offers substantial promise for the treatment of recalcitrant fibrotic disorders, such as IPF and scleroderma.

Conclusion

A number of different mechanisms have been implicated in the pathogenesis of progressive fibrosing disorders. Contemporary concepts regarding fibrogenic mechanisms have converged on a paradigm of epithelial-mesenchymal disrepair and failure of tissue regeneration. Novel therapeutic agents based on this paradigm are beginning to produce promising results in pre-clinical disease-specific cell culture models and animal models of fibrosis. However, promising pre-clinical results, and even preliminary (Phase I/II) clinical studies, do not always translate into clear benefit for patients with the disease. Future well-conducted and controlled clinical trials are necessary before these novel agents can be recommended for the treatment of progressive fibrosing disorders.

Acknowledgments

This work is supported in part by National Institutes of Health grants, U10 HL080510 (Gulf South IPF Clinical Research Network), and R01 HL067967.

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