

NANOTECHNOLOGY IN MEDICINE

Microvascular Targets for Anti-Fibrotic Therapeutics

Kai-Ming T. Pu^a, Parid Sava^b, and Anjelica L. Gonzalez^{b*}

^a*Department of Molecular, Cellular, and Developmental Biology and* ^b*Department of Biomedical Engineering, Yale University, New Haven, Connecticut*

Fibrosis is characterized by excessive extracellular matrix deposition and is the pathological outcome of repetitive tissue injury in many disorders. The accumulation of matrix disrupts the structure and function of the native tissue and can affect multiple organs including the lungs, heart, liver, and skin. Unfortunately, current therapies against the deadliest and most common fibrosis are ineffective. The pathogenesis of fibrosis is the result of aberrant wound healing, therefore, the microvasculature plays an important role, contributing through regulation of leukocyte recruitment, inflammation, and angiogenesis. Further exacerbating the condition, microvascular endothelial cells and pericytes can transdifferentiate into matrix depositing myofibroblasts. The contribution of the microvasculature to fibrotic progression makes its cellular components and acellular products attractive therapeutic targets. In this review, we examine many of the cytokine, matrix, and cellular microvascular components involved in fibrosis and discuss their potential as targets for fibrotic therapies with a particular focus on developing nanotechnologies.

*To whom all correspondence should be addressed: Anjelica L. Gonzalez, Yale University, PO Box 208103, New Haven, CT 06520-8103; Tele: 203-436-2971; Fax: 203-432-0300; Email: anjelica.gonzalez@yale.edu.

†Abbreviations: AECA, anti-endothelial cell antibody; BALF, bronchoalveolar lavage fluid; bFGF, basic fibroblast growth factor; BM, basement membrane; CCL, CC ligand; CCN, Cyr61 (cysteine-rich protein 61), CTGF, and NOV (nephroblastoma overexpressed gene); CCR, CC receptor; CTGF, connective tissue growth factor; CXCL, CXC ligand; EC, endothelial cell; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; endo-MT, endothelial-mesenchymal transition; FAK, focal adhesion kinase; FN, fibronectin; HA, hyaluronan; HSC, hepatic stellate cell; ICAM, intercellular adhesion molecule; IL, interleukin; IPF, idiopathic pulmonary fibrosis; LAP, latency-associated protein; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; PC, pericyte; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PECAM, platelet-endothelial cell adhesion molecule; PGE₂, prostaglandin E₂; shRNA, short hairpin RNA; SMA, smooth muscle actin; TGF, transforming growth factor, TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; VE, vascular endothelial; VEGF, vascular endothelial growth factor.

Keywords: fibrosis, microvasculature, nanomedicine, nanoparticle, TGFβ, fibrocyte

INTRODUCTION

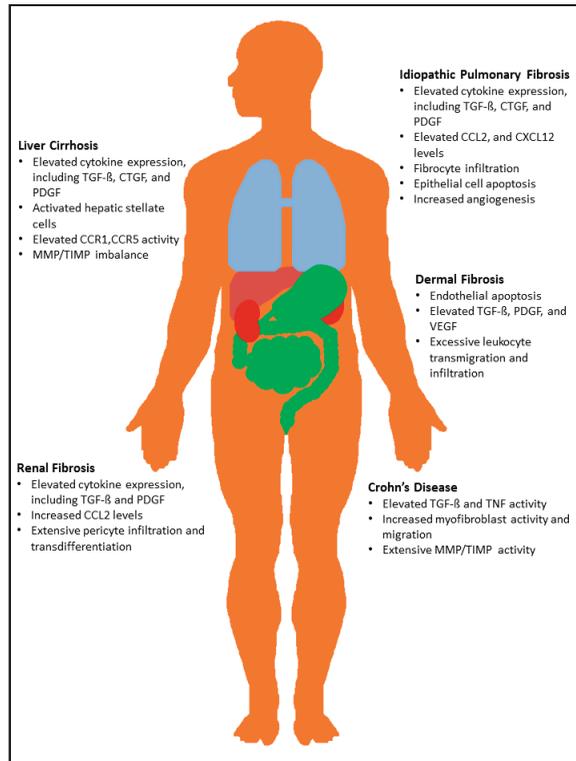
Following insult through injury or pathogenesis, the synthesis and remodeling of the extracellular matrix (ECM†) is a critical step in the wound healing process. If this pathway is continuously activated through chronic inflammation, repetitive injury, or dysregulation, excessive ECM components, including collagen I, fibronectin, and hyaluronan, accumulate in the surrounding tissue. This accumulation is defined as fibrosis and is damaging to the tissues surrounding the site of injury. Unchecked, fibrosis can cause permanent organ damage and can be potentially fatal. Fibrosis can affect many different organ systems and is a hallmark of diseases such as liver cirrhosis, rheumatoid arthritis, Crohn's disease, interstitial lung disorders, scleroderma, and ulcerative colitis (Figure 1) [1]. As many as 45 percent of all natural deaths in the United States can be attributed to fibrotic disorders [2]. Recent advances have furthered our understanding of the mechanisms contributing to fibrosis; however, few, if any, effective treatments exist for some of the most common and lethal forms of fibrosis such as idiopathic pulmonary fibrosis (IPF). In this review, we highlight a number of potential microvascular targets for anti-fibrotic therapeutics (Table 1). In particular, we focus on the emergence of nanomedicine as an avenue for the improvement and development of existing and novel therapies.

The use of nanoscale (1-100 nm) particles to deliver therapies or as diagnostic tools has numerous advantages over traditional methods. In particular, nanoparticles such as liposomes, polymers, and dendrimers can significantly improve drug delivery via high specificity targeting, controlled release and activation, increased drug stability, and by passing through physiological barriers [3,4]. For example, coating nanoparticles in ligands of specialized cell receptors or monoclonal antibodies can target them for drug delivery to a specific cell type, e.g., cancer cells [5]. Such targeting can be especially advantageous in fibrotic disorders that are localized to specific organ systems or tissues.

Despite their heterogeneous origins, different fibrotic disorders, including those of the liver, heart, lungs, and kidney, all involve the activation of myofibroblasts [6-10]. Myofibroblasts are specialized fibroblasts that are responsible for the majority of the ECM remodeling and synthesis that accompanies wound healing and fibrosis. Importantly, myofibroblasts may originate from several different cellular sources. Endothelial cells and pericytes from the microvasculature and epithelium in organs like the lung can lose their tissue specific markers, transdifferentiating into myofibroblasts [11,12]. Transdifferentiation can be initiated by transforming growth factor- β (TGF- β), while specific ECM proteins can recruit myofibroblasts into injured tissue [13]. Notably, circulating fibrocytes can be recruited to the site of injury and will subsequently transdifferentiate into myofibroblasts [14]. Fibrocyte recruitment into the extravascular space requires transmigration through the microvascular post-capillary structure. Differing from the large vessel, the post-capillary venule is composed of luminal endothelial cells and perivascular pericytes, both serving as barriers to cellular diapedesis until cytokine-activation occurs. During fibrosis, the abnormal structure of the microvasculature is marked by endothelial swelling, necrosis, and detachment and by a thickening of the vascular basement membrane [15,16]. These changes suggest that clear correlations between microvascular dysregulation, leukocyte recruitment, and fibrosis could provide mechanisms for anti-fibrotic therapeutics.

The recruitment, subsequent transdifferentiation, and activation of myofibroblast precursors are acutely tied to immune and wound healing responses. As such, the cells, matrix, and signaling molecules of the microvasculature play an important role in the pathogenesis of fibrotic disorders (Figure 2). However, the microvasculature does not act in isolation. Fibrosis is a complex disease, and fibrotic pathogenesis involves many systems and pathways acting in concert. While an abundance of research has been done to identify the involvement of each system in fibrosis, we have dedicated the scope of this review to the important mi-

Figure 1. Pathological and molecular markers of fibrosis. Although the pathogenesis of many fibrotic disorders is poorly understood, each is characterized by specific pathological or molecular changes. While many of these changes – such as extensive pericyte-to-myofibroblast transdifferentiation in renal fibrosis – are unique to a subset of fibrosis, there are common features as well. Notably, elevated TGF- β is characteristic of all fibrosis, underscoring its role in fibrotic pathogenesis. There are few, if any, effective anti-fibrotic therapies (Table 1), however many of the current and developing therapies target common fibrotic pathways such as TGF- β . Thus, although developed with a specific fibrosis in mind, some drugs, like pirfenidone, might effectively treat multiple fibrosis.



crovascular components involved in fibrosis, and we highlight their utility as targets for anti-fibrotic nano-based therapies.

CYTOKINES

TGF- β 1

Transforming growth factor- β has been implicated in most fibrotic disorders in human disease and confirmed in animal models [17]. The three isoforms of TGF- β , TGF- β 1, - β 2, and - β 3, have biologically similar functions, though TGF- β 1 plays a larger role in fibrotic disorders [18]. TGF- β 1 is a prolific cytokine, and depending on the context, it can inhibit or stimulate cell proliferation, act as an immunosuppressant, and induce ECM production [17]. During wound healing and in the pathogenesis of fibrotic disorders, TGF- β 1 induces fibroblast to myofibroblasts transdifferentiation and induces the production of ECM components (Figure 3) [19,20]. Additionally, it has been demonstrated to be a primary mediator of epithelial cell, endothelial cell, and pericyte transdifferentiation into myofibroblasts [21-

23]. During fibrotic progression, TGF- β 1 binds a heterodimeric receptor consisting of a TGF- β 1 type I and a TGF- β 1 type II receptor, subsequently activating Smad transcription factors. Smad2 and, to a lesser degree, Smad4 upregulate α -smooth muscle actin (α -SMA), and Smad3 upregulates pro-fibrotic genes including procollagen, fibronectin, and connective tissue growth factor (CTGF) [24-26]. TGF- β 1 alone is insufficient to induce fibroblast transdifferentiation. *In vitro* and *in vivo* studies have demonstrated that ED-A fibronectin (FN), an alternatively spliced FN, and integrin signaling at focal adhesions (via focal adhesion kinase [FAK]) are necessary for TGF- β 1-induced transdifferentiation [27,28].

During wound healing, platelets initially release TGF- β 1 and other factors like platelet derived growth factor (PDGF) into the site of injury. This both recruits necessary cells and induces additional TGF- β 1 synthesis [17]. The autoinduction of TGF- β 1 appears to be controlled by Smad3, with input from the MKK4/Sapk and MEK/Erk pathways [29]. TGF- β 1 is secreted in the latent (inactive) form, non-covalently bound

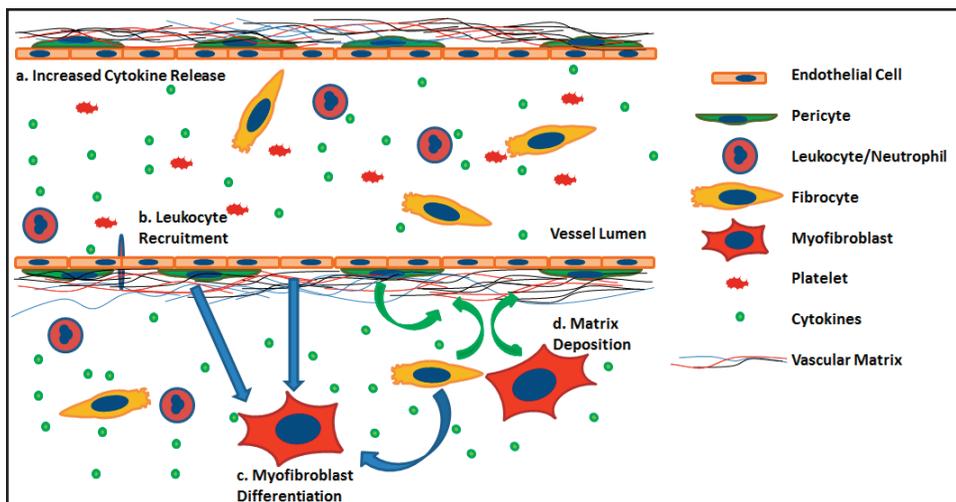


Figure 2. The microvasculature in fibrosis. The microvasculature plays multiple roles in the pathogenesis of fibrosis. a) Cytokines such as TGF- β , PDGF, CTGF, CCL2, IL-8, and IL-1 β activate endothelial cells and pericytes – inducing pro-fibrotic activities including cytokine production, angiogenesis, transdifferentiation, and leukocyte recruitment. b) During leukocyte recruitment, IL-1 β and TNF- α activate endothelial cells, enabling capture of circulating leukocytes, subsequently initiating leukocyte transmigration through the vessel wall and into the surrounding tissue. This process regulates the recruitment of fibrocytes and leukocytes and is crucial for the pathogenesis of some fibrosis. c) Endothelial cells, pericytes, and fibrocytes can transdifferentiate into myofibroblasts under the influence of TGF- β . Notably, pericytes and fibrocytes appear to be major sources of myofibroblasts in renal and idiopathic pulmonary fibrosis respectively. d) During fibrogenesis activated myofibroblasts deposit excessive collagen into the surrounding tissue. Pericytes also produce matrix, depositing it into the basement membrane and contributing to the thickening of the basement membrane and deformation of microvasculature during fibrosis.

by latency-associated protein (LAP). At the site of injury, dissociation of LAP is catalyzed by cellular, vascular, and ECM proteins, including plasmin, integrin $\alpha_v\beta_6$, matrix metalloproteinase-9 (MMP-9), MMP-2, and thrombospondin [30-32]. Because TGF- β 1 is prominently featured in the pathogenesis of fibrotic disorders, it is considered a promising target for anti-fibrotic therapies. However, because it is so prolific, targeting TGF- β 1 during fibrosis without disrupting its other physiological functions — including its tumor suppressor activity and its role as a leukocyte chemokine — has proven to be a challenge [33,34].

There are several drugs in various phases of development or approval that are designed to target multiple parts of the TGF- β 1 pathway. For example, pirfenidone (InterMune), a small molecule drug, suppresses TGF- β 1 transcription and subsequent collagen accumulation and was recently approved

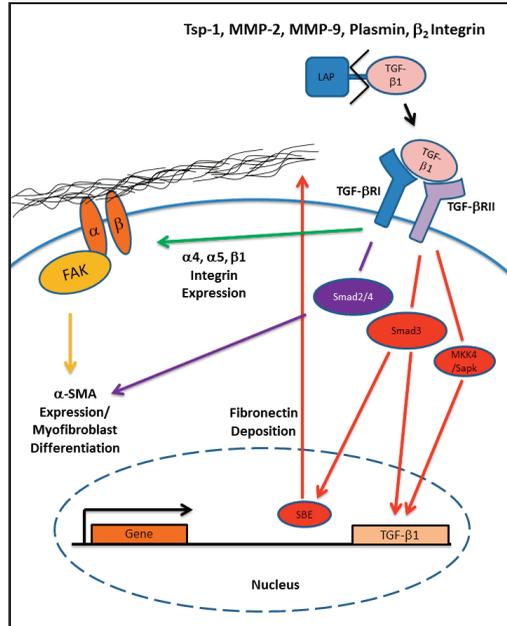
to treat IPF in the European Union and Japan (as well as several other countries) [35]. In the United States, pirfenidone is currently being evaluated in a phase III clinical trial. STX-100 (Stromedix) is a monoclonal antibody that targets integrin $\alpha_v\beta_6$ and neutralizes its TGF- β 1 activating activity. STX-100 is also designed to treat IPF and is currently entering phase II clinical trials [36].

Increasingly novel methods of targeting TGF- β 1 occur through nanoparticle delivery of inhibiting and neutralizing reagents. Using pirfenidone-loaded poly(lactide-co-glycolide) nanoparticles significantly increased drug retention in the lungs (versus a pirfenidone solution) and increased the overall anti-fibrotic efficacy of the drug [37]. Prostaglandin E2 (PGE2) has also been shown to attenuate bleomycin-induced fibrosis. Its exact mechanism of action is unknown, but it inhibits lung fibroblast transdifferentiation to myofibroblasts, hint-

Figure 3. The TGF- β fibrotic pathway.

TGF- β 1 is the most ubiquitous fibrotic cytokine, and it can act in several ways to induce ECM production. TGF- β 1 is activated when extracellular or membrane bound proteins including MMPs, plasmin, and integrins cleave the bound latency-associated protein. Activated-TGF- β 1 binds its heterodimeric receptor, initiating two Smad signaling cascades.

In myofibroblasts, Smad3 along with input from the MKK4/Sapk pathway activates production of additional TGF- β 1 and extracellular matrix components including collagen and fibronectin. In non-myofibroblasts, Smad2 and Smad4 ultimately control transdifferentiation into myofibroblasts, upregulating α -smooth muscle actin. Transdifferentiation also requires integrin signaling via focal adhesion kinase and the alternatively spliced ED-A fibronectin.



ing that it might act on parts of the TGF- β pathway. It was recently shown that using nanoscale liposomes to deliver PGE2 to the lungs via inhalation effectively diminished bleomycin-induced fibrosis, overcoming previous difficulties of specifically delivering PGE2 to the lungs [38]. Wang et al. (2009) used chitosan nanoparticles to deliver anti-TGF- β 1 short hairpin RNA (shRNA), successfully knocking down TGF- β 1 expression in rhabdomyosarcoma cells [39]. Using a similar strategy, Liu et al. (2010) demonstrated that specifically blocking miR-21, a miRNA regulator of the Smad and thus TGF- β , with small antisense probes successfully attenuated TGF- β 1 activity in bleomycin-induced fibrosis in mice [40]. If combined with recent developments in RNA delivery to specific lung cells, this method could prove an effective therapy for targeted inhibition of TGF- β 1 signaling in myofibroblasts [41].

Connective Tissue Growth Factor

Connective tissue growth factor (CTGF) is a cytokine that is associated with most types of fibrosis. A member of the CCN protein family (CCN is an acronym derived from the names of the first three members of the family: Cyr61 [cysteine-rich protein 61], CTGF, and NOV [nephroblastoma overex-

pressed gene]), CTGF is produced by fibroblasts and endothelial cells following TGF- β stimulation [42,43]. Like TGF- β , CTGF stimulates cell proliferation, transdifferentiation, apoptosis, ECM production, and potentially angiogenesis [44,45]. CTGF is generally considered a downstream mediator of TGF- β activity. It enhances TGF- β signaling by directly binding TGF- β and increasing its affinity for its numerous receptors. Blocking CTGF production decreases TGF- β -induced ECM production [18,46]. Additionally, it has been reported that CTGF is important for epithelial and endothelial cell transdifferentiation into myofibroblasts, also known as the epithelial-mesenchymal (EMT) and endothelial-mesenchymal (endo-MT) transition [47]. Alone, CTGF signaling is not sufficient to cause fibrosis; however, overexpression does increase fibrotic susceptibility in bleomycin mouse models [48]. Because the scope of CTGF's activity is more limited than TGF- β , it has good potential as a therapeutic target, though its pleiotropic effects should be fully considered. The CTGF targeting antibody FG-3019 recently completed phase I trials and is currently entering phase II trials as a treatment for advanced kidney disease and other fibrotic disorders [49]. Furthermore, the use of cationic solid lipid nanoparticles to deliver

Table 1. Summary of anti-fibrotic therapies.

Name (Manufacturer)*	Target	Class	Target Disease	Phase
Pirfenidone (Intermune)	TGF- β 1 synthesis	Small molecule	IPF, renal fibrosis, and hepatic fibrosis	III/In use
Maraviroc (Pfizer)	CCR5	Small molecule	Hepatic fibrosis	IV
Imatinib (Novartis)	PDGF	Small molecule	Nephrogenic systematic fibrosis, Crohn's Disease	III
BIBF 1120 (Boehringer Ingelheim)	VEGFR, PDGFR, and FGFR inhibitor	Small molecule	IPF, Crohn's Disease	III
STX-100 (Stromedix)	Integrin α V β 6 /TGF- β 1 activity	Monoclonal antibody	IPF	II
Carlumab (CNTO-888) (Centocor/Janssen)	CCL2	Monoclonal antibody	IPF	II
FG-3019 (Fibrogen)	CTGF	Monoclonal antibody	Kidney disease, IPF	I/II
Tetrathiomolybdate (Pipex)	Angiogenic pathways	Small molecule	IPF	I/II
Pirfenidone nanoparticles	TGF- β 1 synthesis	Poly(lactide-co-glycolide) nanoparticle	IPF	Research
CTGF siRNA	CTGF	Cationic solid lipid nanoparticles	Hepatic Fibrosis	Research
Recombinant Flt23k intraceptor plasmid	Angiogenesis/ VEGF	Targeted nanoparticle	Macular degeneration related fibrosis	Research
Prostaglandin E2	Fibroblast/myofibroblasts activity	Liposome nanoparticle	IPF	Research
TGF- β 1 shRNA	TGF- β 1 synthesis	Chitosan nanoparticle	Cancer/IPF	Research
Anti-miR-21 antisense probes	TGF- β 1 activity via Smad	Targeted nanoparticle	IPF	Research
CCR5 RNAi	CCR5 synthesis	Targeted nanoparticle	HIV/Hepatic Fibrosis	Research
P-selectin antagonist	Leukocyte recruitment via P-selectins	Polymerized liposomes	Pulmonary fibrosis/IPF, and Dermal fibrosis	Research

*Manufacturer listed for drugs in clinical development

CTGF siRNA was shown effective in treatment of rat hepatic fibrosis, highlighting the utility of emerging nanotechnologies for targeting cytokine signaling at the translational level [50].

Platelet-Derived Growth Factor

Platelet-derived growth factor (PDGF) acts as a powerful mitogen and chemoattractant for pro-fibrotic cells including myofibroblasts. It plays an important role in the

pathogenesis of pulmonary, hepatic, renal, cardiac, dermal, and intestinal fibrosis [51]. The PDGF isoforms, PDGF-A and PDGF-B, bind and dimerize either the PDGF α or PDGF β tyrosine-kinase receptors (PDGFRs). PDGF-C and PDGF-D also act through the PDGFRs. Their part in fibrosis is less clear, but potential roles being considered include the regulation of ECM degradation [52,53]. PDGF signal transduction follows the Ras and ERK/MAP kinase pathways. In addition to its mitogenic activity, PDGF stimulates the production of collagen and other ECM components and promotes cell adhesion [51]. In IPF and other pulmonary fibrosis, alveolar macrophages are a major source of excess PDGF. They primarily produce PDGF-B and lung myofibroblasts produce PDGF-A in an autocrine feedback loop [54]. *In vitro* and *in vivo* studies have shown that multiple isoform PDGF overexpression can lead to accumulation of ECM components; however, additional factors such as TGF- β are necessary to sustain the fibrotic state [55].

PDGF activity is regulated through interactions with its receptors and through synergism with other cytokines, and extracellular proteins including ECM components contribute significantly to its functional effects [56]. For example, interleukin 1 β (IL-1 β) is produced by macrophages and upregulates PDGFR α , enhancing the mitogenic and chemotactic effect of PDGF on myofibroblasts [57]. Additionally, TGF- β activity down-regulates PDGFR α expression, suppressing cell growth in favor of collagen/ECM production [58].

Currently, there are several fibrosis therapies in development that target PDGF activity. Perhaps the most promising is imatinib (also known as Gleevec), a small molecule tyrosine-kinase inhibitor. Imatinib is thought to block PDGF activity and has shown promise as an anti-fibrotic drug and recently completed a phase III trial to treat nephrogenic systemic fibrosis, results pending [59,60].

CC and CXC Chemokines

Historically, the role of chemokines in fibrosis was limited to the initial recruitment of

immune cells to the site of injury. However, recent studies have demonstrated that they play a far larger role in fibrosis, initiating angiogenesis and acting as mediators of the fibrotic response. For example, the pro-fibrogenic CC chemokine CCL2 (CC Ligand 2, or MCP-1) has been implicated in hepatic, renal, dermal, and pulmonary fibrosis. Along with CCL7, CCL8, CCL13, and CCL16, CCL2 binds the CCR2 receptor (CC Receptor 2) [61]. In scleroderma, fibroblasts spontaneously express CCL2, which engages an autocrine feedback loop, stimulating further CCL2 production and attracting monocytes [62]. In addition to recruiting immune cells, CCL2 stimulates TGF- β production in hepatic and pulmonary fibroblasts, contributing to the accumulation of collagen [63,64]. CCL2 levels are elevated in the serum and bronchoalveolar lavage fluid (BALF) of patients with IPF. Murine models deficient in the CCL2 receptor CCR2 were partially protected against renal, pulmonary, and hepatic fibrosis in various fibrotic disorder models [61,65-67].

CCR1 and CCR5, and their shared ligands CCL3 and CCL5, have also been shown to be important pro-fibrotic mediators. In hepatic fibrosis, CCR1 and CCR5 levels are highly elevated and blocking the receptors reduces the fibrotic response. CCR1 and CCR5 are co-expressed on many different cell types, but they differentially activate cell populations. CCR1 is a pro-fibrotic mediator of bone marrow-derived cells and CCR5 acts on resident liver cells. The ultimate effect of their action is the recruitment and activation of hepatic stellate cells, the primary fibrogenic cell in the liver [68,69]. Eliminating CCR1 and CCR5 signaling in mice via receptor knockout lessens the impact of induced pulmonary fibrosis, suggesting an additional pro-fibrotic role of these receptors in the lung [70,71]. However, it does not appear that CCR1 and CCR5, or their ligands, directly activate lung mesenchymal cells as in hepatic fibrosis, but instead play an important role in regulating the balance of pro/anti-fibrotic cytokines and immune cell infiltration [61,72].

In addition to their role in immune activation, CXC chemokines are important

angiogenic/static regulators. For example, endothelial-derived CXCL8 (IL-8) is a potent neutrophil chemoattractant and important to the transendothelial and transpericyte migration of leukocytes. It has also been shown to be a powerful angiogenic factor [73-75]. The recruitment of neutrophils to the early fibrotic lung results in the release of cytokines, including TNF- α and IL-1 β , elastases, and reactive oxygen species that cause further damage to the tissue [76,77]. During wound healing and the pathogenesis of fibrosis, metabolic demand increases and angiogenesis is required to supply the tissue with requisite nutrients and for tissue remodeling [78]. CXC chemokines with the ELR motif (ELR+) are angiogenic and primarily bind endothelial CXCR2. ELR-CXC chemokines are angiostatic and they bind CXCR3 [78,79]. Examining BALF, serum, and tissue samples from patients with IPF and hepatic fibrosis has shown that fibrosis is correlated with an imbalance of ELR+ and ELR- chemokines [78,80]. In addition to regulating angiogenesis, CXC chemokines contribute to fibrotic pathogenesis by mediating fibrocyte extravasation and tissue infiltration, increasing the myofibroblasts population and matrix deposition [81].

Although targeting chemokines has shown potential *in vitro* and animal models, successful chemokine directed human anti-fibrotic therapies have yet to be developed. However, several drugs developed to treat other diseases are currently being investigated for their pleotropic anti-fibrotic properties. The CCL2 targeting monoclonal antibody carlumab (CNTO-888) (Centocor/Janssen) is in phase 2 trials to assess its safety and effects in individuals with IPF. Maraviroc is a CCR5 antagonist developed as an antiretroviral HIV treatment and is currently entering a phase 4 clinical trial (NCT01327547) to investigate its effect on liver fibrosis in HIV/HCV co-infected individuals. A recent study also targeted HIV with CCR5 RNAi delivered via nanoparticles; however, this method's utility in treating fibrosis has not been evaluated [82].

EXTRACELLULAR MATRIX COMPONENTS

General ECM Changes

The pathological outcome of fibrosis is an excessive accumulation of ECM components. Depending on the tissue, the amount and composition of ECM accumulation differs but can be generalized as an increase in fibrillar and non-fibrillar collagens, fibronectin, and proteoglycans [83-85]. Notably, in decellularized human IPF lungs versus healthy lungs, there is a 21-fold increase in hyaluronan, a 20-fold increase of matrix gla protein, a 16-fold increase in latent-TGF- β -binding protein 1, and a 3-fold increase in collagen III chains [85]. This accumulation of large and small matrix protein increases tissue stiffness and inhibits normal tissue function.

Hyaluronan

Hyaluronan (HA), a glycosaminoglycan, is one of the chief components of ECM, providing structural support by binding and aggregating proteoglycan chains in connective tissue [86]. HA is produced by a wide range of cells, but fibroblasts are the most productive source. HA is an important regulator of the immune response and myofibroblasts activity, including cell adhesion, chemoattraction, and signaling for transdifferentiation. [87]. While HA and HA fragments interact with numerous receptors, CD44 is the principle HA receptor and can be found on most cell types, including fibroblasts, fibrocytes, endothelial cells, epithelial cells, lymphocytes, and leukocytes [88-91]. CD44-HA interactions can induce a broad range of activity including cell adhesion, migration, transdifferentiation, and protein expression [92]. In response to injury and factors like IL-1 β and TNF- α , fibroblasts secrete HA, which associates with CD44 to form a pericellular coat. This pericellular coat facilitates cell adhesion/de-adhesion and shape changes required for cell motility and proliferation [93]. Furthermore, continual HA secretion appears to be required for transdifferentiation to and maintenance of the myofibroblasts phenotype [94,95]. Inhibiting HA synthesis prevents

TGF- β mediated myofibroblasts transdifferentiation; however, adding HA alone to fibroblasts fails to induce transdifferentiation, indicating that HA modulates TGF- β induced transdifferentiation [95,96].

Through the research cited above, it has become increasingly clear that HA is an important player in the pathogenesis of fibrosis and is a potential therapeutic target. However, few, if any, therapies targeting HA are being developed or proposed. The HA receptor CD44 is also being studied as a target for metastatic tumor therapies, though a safe therapy has yet to be developed.

MMPs and TIMPs

Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are enzymes responsible for the turnover and regulation of ECM components. The 22 different MMPs are zinc-dependent proteolytic enzymes that degrade specific ECM components, and the four identified TIMPs can inhibit the activity or activation of multiple MMPs [97,98]. MMPs are produced as pro-MMPs — latent forms of the enzymes that require the cleavage of a propeptide for activation [99]. The balance of MMPs and TIMPs is critical for the maintenance of homeostasis and during fibrotic pathogenesis. It is generally accepted that early MMP activity is needed for fibrogenesis, while persistence of some MMPs can contribute to prolonged fibrosis [99]. There are various levels of imbalance that can lead to either prolonged or self-resolving fibrosis. For example, the progression of hepatic fibrosis is initially marked by elevated MMP-1, MMP-3, and MMP-13 activity. This is thought to degrade existing ECM in preparation for de-novo ECM synthesis and help to activate ECM producing hepatic stellate cells (HSCs) (liver pericytes) [99-101]. This brief period of ECM degradation is followed by an upregulation of TIMP-1 and TIMP-2, which inhibits further matrix degradation and tips the balance in favor of fibrosis [102]. Interestingly, expression of the collagenase (type IV) MMP-2 and its membrane-bound activator MMP-14 also increases. Pro-MMP-2 is activated at the cell membrane through a

complex with MMP-14 and TIMP-2, and its local activity, degrading periceullar matrix, likely facilitates the proliferative, pro-fibrotic HSC phenotype [99,100,103]. Similar matrix degrading yet pro-fibrotic activity has been observed in kidney and pulmonary fibrosis and is important for EMT and sustained fibrosis [104,105]. Additionally, infiltrating neutrophils also release MMPs, including MMP-2, into the extravascular tissue during the wound healing response, but the extent to which MMPs contribute to fibrotic pathogenesis is still being defined [106].

Because MMPs and TIMPs play a role in both fibrogenesis and fibrolysis, they are attractive anti-fibrotic targets. Upregulating MMPs, downregulating TIMPs, or some combination of the two should help control and reverse fibrotic pathogenesis. To this effect, there have been many studies examining possible MMP/TIMP based therapies (See [99] for review in Hepatic Fibrosis) in animal and human models. Blocking TIMP-1 via adenovirus-delivered mutant MMP-9 suppressed HSC activation and inhibited fibrosis in mice [107]. In rats, the plant alkaloid halofuginone has been shown to upregulate anti-fibrotic MMP-3 and MMP-13 while downregulating MMP-2 and TIMP-1 [108]. Furthermore, many MMPs and TIMPs are in the TGF- β pathway, so therapies targeting TGF- β are likely to affect MMPs and TIMPs in an anti-fibrotic manner [98].

MICROVASCULAR CELLS

Endothelial Cells

Endothelial cells (EC) mediate or participate in many biological processes important to fibrotic pathogenesis, including the inflammatory/immune response, angiogenesis, and myofibroblasts transdifferentiation. They respond to a broad range of stimuli and produce proteins and factors including ECM, TGF- β , PDGF, CTGF, CCL2, IL-8 (CXCL8), and MMPs [109]. As the physical barrier separating the blood stream from the extravascular space, ECs regulate cellular and molecular access to the underlying tissue.

Circulating leukocytes and lymphocytes must pass through the EC monolayer during the inflammatory response that occurs early in the pathogenesis of many fibrotic diseases [110]. Furthermore, leukocyte accumulation is a hallmark of scleroderma and other dermal fibrosis like psoriasis, contributing to tissue remodeling and skin damage [111,112]. Transendothelial migration occurs when ECs are activated by cytokines like IL-1 β or TNF- α and capture circulating leukocytes via selectins. Subsequent migration through the EC monolayer, subendothelial basement membrane, and vascular pericytes (PC) is facilitated by adhesion molecules such as intercellular adhesion molecule-1 and -2 (ICAM-1, -2), platelet-endothelial cell adhesion molecule (PECAM-1), and neutrophil Mac-1 (CD11b/CD18) [75,113].

As previously mentioned, angiogenesis is required to sustain the elevated metabolic activity associated with fibrotic pathogenesis [78]. EC are activated from their quiescent state by a number of different angiogenic factors, including tumor necrosis factor- α (TNF- α), IL-8, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) [114]. In their activated state, EC proliferate and produce angiogenesis-promoting proteins such as ECM, MMPs, and integrins. Integrin signaling, in particular, is an important mediator of angiogenesis because it regulates migration, adhesion, and cell survival [115]. If the proper balance of integrin ligands (predominantly ECM molecules including collagens, laminin, and fibronectin) is not present in the provisional matrix, integrin signaling prevents EC proliferation and may induce apoptosis [114,115].

In addition to their proliferation during angiogenesis, EC proliferation and subsequent transdifferentiation represents another source of myofibroblasts. During the endothelial-to-mesenchymal transition (endo-MT), ECs lose the cell markers CD31 and vascular endothelial cadherin (VE-cadherin) and expresses the mesenchymal markers including procollagen 1, fibroblast-specific protein-1, and α -SMA [116]. Like fibroblast transdifferentiation, endo-MT is regulated by TGF- β via the SMAD pathway with some ev-

idence suggesting that Ras plays a role in maintenance of the myofibroblasts phenotype [117,118]. The extent to which endo-MT contributes to fibrosis is still unclear, though the process has been identified as a myofibroblast source in mouse models of cardiac, kidney, and pulmonary fibrosis [117-119].

Apoptosis of vascular EC also plays an important role in the pathogenesis of dermal fibrosis, including scleroderma. Although the mechanism regulating pathogenesis of scleroderma remains unknown, it is thought that anti-endothelial cell antibody (AECA)-induced apoptosis of EC early in the progression of the disease is a key event [120,121]. Treatment with an anti-Fas ligand antibody can block AECA induced apoptosis *in vitro*, demonstrating that AECA acts with the Fas pathway, thereby suggesting a potential therapeutic target for treatment of scleroderma [122].

Perhaps the most promising anti-fibrotic strategy targeting endothelial cells is anti-angiogenic drugs. Drugs like tetrathiomolybdate and BIBF 1120 (Boehringer Ingelheim Pharmaceuticals) target angiogenic pathways controlled by VEGF, PDGF, and FGF. Both have shown promise in treating bleomycin induced fibrosis in mice and are currently in early clinical trials [123-125]. Integrins have been the focus of much anti-angiogenic cancer research and several, including $\alpha_v\beta_3$ and $\alpha_v\beta_5$, have been identified as therapeutic targets. Recently, the small molecule $\alpha_v\beta_3$ and $\alpha_v\beta_5$ antagonist cilengitide (Merck) completed phase 2 trials and entered phase 3, showing positive results as a glioblastoma therapy [126]. However, when used to treat hepatic fibrosis in rats, cilengitide both suppressed angiogenesis and aggravated fibrosis [127]. Thus, further studies are needed to understand the full effects of integrin-targeted anti-angiogenic therapies in varying models of fibrosis. Integrins can also be used to target delivery systems in a non-invasive manner. Nanoparticles coated in integrin-binding RGD peptide successfully delivered an anti-angiogenic and anti-fibrotic gene-therapy plasmid to treat age-related macular degeneration in rats [128]. Nanoparticles have also been used to target activated endothelium and

inhibit leukocyte recruitment, attenuating inflammation in airway diseases. Coating polymerized liposome nanoparticles with a P-selectin antagonist bound activated endothelium and inhibited leukocyte recruitment to the extravascular space [129]. This strategy could be beneficial in treating pulmonary and dermal fibrosis, and warrants further examination.

Current animal models suggest that the endo-MT is a valuable target for anti-fibrotic therapies. As in other myofibroblasts transdifferentiation pathways, TGF- β is a key player, so many of the TGF- β targeting drugs and strategies previously discussed are candidates for inhibiting endo-MT. However, our understanding of the molecular basis of endo-MT and its role in human fibrosis is incomplete and must be expanded to aid in the development of novel therapies targeting the process of endo-MT.

Pericytes

PC are often thought of as the microvascular equivalent of smooth muscle cells; however, they have distinct localization, morphology, and marker expression. Embedded in the microvascular basement membrane (BM), PC are in intimate contact with the luminal EC via cell-cell contact, providing structural support and integrating EC signaling [130]. They are an integral part of the microvasculature and are involved in many of the same processes as EC, including leukocyte transmigration, angiogenesis, and myofibroblasts proliferation [75,131,132]. In fact, PC regulate many of those processes through direct signaling (cell-to-cell), as is the case in leukocyte transmigration and angiogenesis or through production cytokines and basement membrane proteins. Thus, PC are a possible therapeutic target, especially in fibrotic disorders that present with morphologic changes to the microvasculature such as scleroderma or psoriasis [75,133,134].

In addition to the role of PC, or hepatic stellate cells, in liver fibrosis, PC were also recently identified as an additional source of pro-fibrotic myofibroblasts in renal fibrosis [135]. Using genetic fate tracing, Lin et al. (2008) observed that in response to kidney in-

jury, PC populations expanded 15-fold and possessed a myofibroblasts like phenotype. Additionally, nearly all myofibroblasts in the mouse model of renal fibrosis were pericyte-derived. This finding is supported by additional genetic fate tracing studies that found no evidence for epithelial-derived myofibroblasts in renal fibrosis [136,137]. The exact mechanism for PC transdifferentiation is unclear. *In vivo* experiments showed that inhibiting PDGF signaling decreased pericyte transdifferentiation, but PDGF failed to stimulate transdifferentiation in *in vitro* experiments, though TGF- β did [12]. It is likely that pericyte transdifferentiation involves TGF- β signaling through both the normal TGF- β pathways and as a mediator for PDGF signaling.

Along with VEGF, PDGF is also an important mediator of EC/PC crosstalk that helps regulate angiogenesis. Ablation of endothelial PDGF inhibits pericytes recruitment to microvasculature vessels, ultimately leading to defective tissues [138]. Additionally, inhibiting pericyte-associated VEGF induced EC apoptosis [139]. Thus, it is believed that EC/PC crosstalk via VEGF and PDGF is necessary for vascular integrity and angiogenesis. Interestingly, blocking the receptors VEGFR2 and PDGFR β , in turn blocking pericyte migration, reduces both intestinal fibrosis and capillary rarefaction in models of mouse renal fibrosis [140].

We have only recently begun to understand the role of PC in fibrotic pathogenesis; however, it is clear that they are a significant player. The transdifferentiation of PC to myofibroblasts and EC/PC crosstalk via VEGFR and PDGFR are promising novel targets with especially significant implications for renal fibrosis therapies.

Fibrocytes

First described in 1994, fibrocytes are circulating mesenchymal cells that share both macrophage and fibroblast characteristics. Arising from monocyte populations, fibrocytes express CD45 and CD34, produce collagen, and can transdifferentiate into myofibroblasts [14,141]. During wound healing, fibrocytes transmigrate through the microvasculature,

aiding wound repair by differentiating and secreting a number of cytokines, growth factors, and ECM proteins [142]. Fibrocytes are now thought to contribute to fibrotic pathogenesis. In particular, they appear to play an important role in pulmonary fibrosis. Fibrocytes migrate to injured lung via the CXCR4/CXCL12 receptor-ligand pair, and CXCL12 can be detected in the BALF of many IPF patients (40 percent), but it is not present in normal patients [81,143,144]. Furthermore, while fibrocytes normally comprise <1 percent of circulating leukocytes, that number increases by an order of magnitude in patients with IPF [145]. Like the other myofibroblasts transdifferentiation pathways previously discussed, the process of fibrocyte-to-myofibroblast transdifferentiation is controlled by TGF- β 1 activity [14].

The CXCR4/CXCL12 axis is the most likely candidate for a fibrocyte targeting fibrosis therapy. Anti-CXCL12 antibodies successfully inhibited fibrocyte infiltration and attenuated bleomycin-induced pulmonary fibrosis in mice [81]. Furthermore, targeting the CXC pathways may have the additional benefit of inhibiting pro-fibrotic angiogenesis.

CONCLUSION AND OUTLOOK

Fibrosis represents a diverse range of diseases with distinct molecular mechanisms and pathogenic routes. The cells, signaling molecules, and proteins that comprise the microvasculature are major regulators of many fibrotic diseases. This review examines some of the developing anti-fibrotic therapies that target the microvasculature and suggests additional avenues to further identify novel targets and strategies. While current clinical trials targeting fibrosis utilize small molecule drugs and monoclonal antibodies, there are several nanoparticle-based technologies that are currently in the research phase.

Well-designed nanoparticle-based therapeutics offer a safe method to improve delivery, specificity, uptake, stability, and release of traditional and novel reagents [146-148]. Nanoparticles have proven optimal for delivery of emerging nucleotide-based therapies, including RNAi/siRNA/shRNA that specifi-

cally target pro-fibrotic pathways, e.g., TGF- β 1 pathway. Current therapeutic strategies utilize nanoparticles to target inflammatory cytokines and growth factors, such as VEGF, CTGF, TNF- α and TGF- β 1 signaling, through the delivery of pirfenidone, PGE2, and IFN- γ , among others outlined in Table 1 [37,149]. Other examples include nanoparticle encapsulation of anti-sense oligonucleotides of Smad regulating miRNAs and TGF-1 shRNAs. Nanoparticles are especially suited for targeting two of the most common types of fibrosis — pulmonary and hepatic — due to natural trafficking of nanoparticles to the liver and lungs [150,151]. Therefore, nanoparticle delivery of siRNAs against CCR5, miR-21, or TGF- β 1 are logical candidates for nano-therapeutic development.

There are still large gaps in our understanding and ability to treat fibrosis. Increased research into the basic mechanisms of fibrogenesis and how it relates to microvascular remodeling and inflammation will help elucidate the most effective anti-fibrotic targets. An improved understanding of pathogenesis and pathological progression in conjunction with developing therapeutic strategies will help us to halt the progression of fibrosis and also restore normal tissue function, improving the outlook of all fibrotic disorders.

REFERENCES

1. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med.* 2012;18(7):1028-40.
2. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol.* 2004;4(8):583-94.
3. Sandhiya S, Dkhar SA, Surendiran A. Emerging trends of nanomedicine--an overview. *Fundam Clin Pharmacol.* 2009;23(3):263-9.
4. Kim BY, Rutka JT, Chan WC. Nanomedicine. *N Engl J Med.* 2010;363(25):2434-43.
5. Mitra A, Nan A, Line BR, Ghandehari H. Nanocarriers for nuclear imaging and radiotherapy of cancer. *Curr Pharm Des.* 2006;12(36):4729-49.
6. Gabbiani G. The myofibroblast in wound healing and fibrocontractive diseases. *J Pathol.* 2003;200(4):500-3.
7. Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: one function, multiple origins. *Am J Pathol.* 2007;170(6):1807-16.

8. Desmouliere A, Darby IA, Gabbiani G. Normal and pathologic soft tissue remodeling: role of the myofibroblast, with special emphasis on liver and kidney fibrosis. *Lab Invest.* 2003;83(12):1689-707.
9. Virag JL, Murry CE. Myofibroblast and endothelial cell proliferation during murine myocardial infarct repair. *Am J Pathol.* 2003;163(6):2433-40.
10. Phan SH. The myofibroblast in pulmonary fibrosis. *Chest.* 2002;122(6 Suppl):286S-9S.
11. Li J, Bertram JF. Review: Endothelial-myofibroblast transition, a new player in diabetic renal fibrosis. *Nephrology (Carlton).* 2010;15(5):507-12.
12. Chen YT, Chang FC, Wu CF, Chou YH, Hsu HL, Chiang WC, et al. Platelet-derived growth factor receptor signaling activates pericyte-myofibroblast transition in obstructive and post-ischemic kidney fibrosis. *Kidney Int.* 2011;80(11):1170-81.
13. Hinz B. Formation and function of the myofibroblast during tissue repair. *J Invest Dermatol.* 2007;127(3):526-37.
14. Abe R, Donnelly SC, Peng T, Bucala R, Metz CN. Peripheral blood fibrocytes: differentiation pathway and migration to wound sites. *J Immunol.* 2001;166(12):7556-62.
15. Magro CM, Ross P, Marsh CB, Allen JN, Liff D, Knight DA, et al. The role of anti-endothelial cell antibody-mediated microvascular injury in the evolution of pulmonary fibrosis in the setting of collagen vascular disease. *Am J Clin Pathol.* 2007;127(2):237-47.
16. Magro CM, Waldman WJ, Knight DA, Allen JN, Nadasdy T, Frambach GE, et al. Idiopathic pulmonary fibrosis related to endothelial injury and antiendothelial cell antibodies. *Hum Immunol.* 2006;67(4-5):284-97.
17. Border WA, Noble NA. Transforming growth factor beta in tissue fibrosis. *N Engl J Med.* 1994;331(19):1286-92.
18. Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. *Cardiovasc Res.* 2007;74(2):196-206.
19. Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. *J Clin Invest.* 1997;100(4):768-76.
20. Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G. Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol.* 1993;122(1):103-11.
21. Arciniegas E, Sutton AB, Allen TD, Schor AM. Transforming growth factor beta 1 promotes the differentiation of endothelial cells into smooth muscle-like cells in vitro. *J Cell Sci.* 1992;103(Pt 2):521-9.
22. Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, et al. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: potential role in idiopathic pulmonary fibrosis. *Am J Pathol.* 2005;166(5):1321-32.
23. Rajkumar VS, Howell K, Csiszar K, Denton CP, Black CM, Abraham DJ. Shared expression of phenotypic markers in systemic sclerosis indicates a convergence of pericytes and fibroblasts to a myofibroblast lineage in fibrosis. *Arthritis Res Ther.* 2005;7(5):R1113-23.
24. Evans RA, Tian YC, Steadman R, Phillips AO. TGF-beta1-mediated fibroblast-myofibroblast terminal differentiation-the role of Smad proteins. *Exp Cell Res.* 2003;282(2):90-100.
25. Verrecchia F, Chu ML, Mauviel A. Identification of novel TGF-beta/Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. *J Biol Chem.* 2001;276(20):17058-62.
26. Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A. CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. *J Biol Chem.* 2001;276(14):10594-601.
27. Serini G, Bochaton-Piallat ML, Ropraz P, Geinoz A, Borsi L, Zardi L, et al. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. *J Cell Biol.* 1998;142(3):873-81.
28. Thannickal VJ, Lee DY, White ES, Cui Z, Larios JM, Chacon R, et al. Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase. *J Biol Chem.* 2003;278(14):12384-9.
29. Mulder KM. Role of Ras and Mapks in TGF-beta signaling. *Cytokine Growth Factor Rev.* 2000;11(1-2):23-35.
30. Schultz-Cherry S, Murphy-Ullrich JE. Thrombospondin causes activation of latent transforming growth factor-beta secreted by endothelial cells by a novel mechanism. *J Cell Biol.* 1993;122(4):923-32.
31. Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev.* 2000;14(2):163-76.
32. Khalil N, Corne S, Whitman C, Yacyshyn H. Plasmin regulates the activation of cell-associated latent TGF-beta 1 secreted by rat alveolar macrophages after in vivo bleomycin injury. *Am J Respir Cell Mol Biol.* 1996;15(2):252-9.
33. Scotton CJ, Chambers RC. Molecular targets in pulmonary fibrosis: the myofibroblast in focus. *Chest.* 2007;132(4):1311-21.
34. Fava RA, Olsen NJ, Postlethwaite AE, Broadley KN, Davidson JM, Nanney LB, et al. Transforming growth factor beta 1 (TGF-beta 1) induced neutrophil recruitment to syn-

- ovial tissues: implications for TGF-beta-driven synovial inflammation and hyperplasia. *J Exp Med.* 1991;173(5):1121-32.
35. Di Sario A, Bendia E, Svegliati Baroni G, Ridolfi F, Casini A, Ceni E, et al. Effect of pirfenidone on rat hepatic stellate cell proliferation and collagen production. *J Hepatol.* 2002;37(5):584-91.
 36. Horan GS, Wood S, Ona V, Li DJ, Lukashev ME, Weinreb PH, et al. Partial inhibition of integrin alpha(v)beta6 prevents pulmonary fibrosis without exacerbating inflammation. *Am J Respir Crit Care Med.* 2008;177(1):56-65.
 37. Trivedi R, Redente EF, Thakur A, Riches DW, Kompella UB. Local delivery of biodegradable pirfenidone nanoparticles ameliorates bleomycin-induced pulmonary fibrosis in mice. *Nanotechnology.* 2012;23(50):505101.
 38. Ivanova V, Garbuzenko OB, Reuhl KR, Reimer DC, Pozharov VP, Minko T. Inhalation treatment of pulmonary fibrosis by liposomal prostaglandin E2. *Eur J Pharm Biopharm.* 2013;84(2):335-44.
 39. Wang SL, Yao HH, Guo LL, Dong L, Li SG, Gu YP, et al. Selection of optimal sites for TGFβ1 gene silencing by chitosan-TPP nanoparticle-mediated delivery of shRNA. *Cancer Genet Cytogenet.* 2009;190(1):8-14.
 40. Liu G, Friggeri A, Yang Y, Milosevic J, Ding Q, Thannickal VJ, et al. miR-21 mediates fibrogenic activation of pulmonary fibroblasts and lung fibrosis. *J Exp Med.* 2010;207(8):1589-97.
 41. Li S-D, Huang L. Targeted Delivery of Antisense Oligodeoxynucleotide and Small Interference RNA into Lung Cancer Cells. *Mol Pharm.* 2006;3(5):579-88.
 42. Riser BL, Denichilo M, Cortes P, Baker C, Grondin JM, Yee J, et al. Regulation of Connective Tissue Growth Factor Activity in Cultured Rat Mesangial Cells and Its Expression in Experimental Diabetic Glomerulosclerosis. *J Am Soc Nephrol.* 2000;11(1):25-38.
 43. Bork P. The modular architecture of a new family of growth regulators related to connective tissue growth factor. *FEBS Lett.* 1993;327(2):125-30.
 44. Hall-Glenn F, De Young RA, Huang BL, van Handel B, Hofmann JJ, Chen TT, et al. CCN2/connective tissue growth factor is essential for pericyte adhesion and endothelial basement membrane formation during angiogenesis. *PLoS One.* 2012;7(2):e30562.
 45. Grotendorst GR. Connective tissue growth factor: a mediator of TGF-beta action on fibroblasts. *Cytokine Growth Factor Rev.* 1997;8(3):171-9.
 46. Ruperez M, Lorenzo O, Blanco-Colio LM, Esteban V, Egido J, Ruiz-Ortega M. Connective tissue growth factor is a mediator of angiotensin II-induced fibrosis. *Circulation.* 2003;108(12):1499-505.
 47. Burns WC, Twigg SM, Forbes JM, Pete J, Tikellis C, Thallas-Bonke V, et al. Connective tissue growth factor plays an important role in advanced glycation end product-induced tubular epithelial-to-mesenchymal transition: implications for diabetic renal disease. *J Am Soc Nephrol.* 2006;17(9):2484-94.
 48. Bonniaud P, Margetts PJ, Kolb M, Haberberger T, Kelly M, Robertson J, et al. Adenoviral gene transfer of connective tissue growth factor in the lung induces transient fibrosis. *Am J Respir Crit Care Med.* 2003;168(7):770-8.
 49. Mageto Y, Flaherty K, Brown K, Fong A, Raghu G. Safety and tolerability of human monoclonal antibody fg-3019, anti-connective tissue growth factor, in patients with idiopathic pulmonary fibrosis. *CHEST Journal.* 2004;126(4_MeetingAbstracts):773S-a.
 50. Kong WH, Park K, Lee MY, Lee H, Sung DK, Hahn SK. Cationic solid lipid nanoparticles derived from apolipoprotein-free LDLs for target specific systemic treatment of liver fibrosis. *Biomaterials.* 2013;34(2):542-51.
 51. Bonner JC. Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev.* 2004;15(4):255-73.
 52. Heldin CH, Eriksson U, Ostman A. New members of the platelet-derived growth factor family of mitogens. *Arch Biochem Biophys.* 2002;398(2):284-90.
 53. Jinnin M, Ihn H, Mimura Y, Asano Y, Yamane K, Tamaki K. Regulation of fibrogenic/fibrolytic genes by platelet-derived growth factor C, a novel growth factor, in human dermal fibroblasts. *J Cell Physiol.* 2005;202(2):510-7.
 54. Lasky JA, Coin PG, Lindroos PM, Ostrowski LE, Brody AR, Bonner JC. Chrysotile asbestos stimulates platelet-derived growth factor-AA production by rat lung fibroblasts in vitro: evidence for an autocrine loop. *Am J Respir Cell Mol Biol.* 1995;12(2):162-70.
 55. Yi ES, Lee H, Yin S, Piguet P, Sarosi I, Kaufmann S, et al. Platelet-derived growth factor causes pulmonary cell proliferation and collagen deposition in vivo. *Am J Pathol.* 1996;149(2):539-48.
 56. Bonner JC, Brody AR. Cytokine-binding proteins in the lung. *Am J Physiol.* 1995;268(6 Pt 1):L869-78.
 57. Lindroos PM, Coin PG, Osornio-Vargas AR, Bonner JC. Interleukin 1 beta (IL-1 beta) and the IL-1 beta-alpha 2-macroglobulin complex upregulate the platelet-derived growth factor alpha-receptor on rat pulmonary fibroblasts. *Am J Respir Cell Mol Biol.* 1995;13(4):455-65.
 58. Bonner JC, Badgett A, Lindroos PM, Osornio-Vargas AR. Transforming growth factor beta 1 downregulates the platelet-derived growth factor alpha-receptor subtype on human lung fibroblasts in vitro. *Am J Respir Cell Mol Biol.* 1995;13(4):496-505.
 59. Chung L, Fiorentino DF, Benbarak MJ, Adler AS, Mariano MM, Paniagua RT, et al. Molecular framework for response to imatinib

- mesylate in systemic sclerosis. *Arthritis Rheum.* 2009;60(2):584-91.
60. Rosenbloom J, Jimenez SA. Molecular ablation of transforming growth factor beta signaling pathways by tyrosine kinase inhibition: the coming of a promising new era in the treatment of tissue fibrosis. *Arthritis Rheum.* 2008;58(8):2219-24.
 61. Sahin H, Wasmuth HE. Chemokines in tissue fibrosis. *Biochim Biophys Acta.* 2013;1832(7):1041-8.
 62. Yamamoto T, Eckes B, Krieg T. High expression and autoinduction of monocyte chemoattractant protein-1 in scleroderma fibroblasts. *Eur J Immunol.* 2001;31(10):2936-41.
 63. Gharaee-Kermani M, Denholm EM, Phan SH. Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. *J Biol Chem.* 1996;271(30):17779-84.
 64. Gharaee-Kermani M, Phan SH. Molecular mechanisms of and possible treatment strategies for idiopathic pulmonary fibrosis. *Curr Pharm Des.* 2005;11(30):3943-71.
 65. Moore BB, Paine R 3rd, Christensen PJ, Moore TA, Sitterding S, Ngan R, et al. Protection from pulmonary fibrosis in the absence of CCR2 signaling. *J Immunol.* 2001;167(8):4368-77.
 66. Seki E, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, et al. CCR2 promotes hepatic fibrosis in mice. *Hepatology.* 2009;50(1):185-97.
 67. Suga M, Iyonaga K, Ichiyasu H, Saita N, Yamasaki H, Ando M. Clinical significance of MCP-1 levels in BALF and serum in patients with interstitial lung diseases. *Eur Respir J.* 1999;14(2):376-82.
 68. Seki E, De Minicis S, Gwak GY, Kluwe J, Inokuchi S, Bursill CA, et al. CCR1 and CCR5 promote hepatic fibrosis in mice. *J Clin Invest.* 2009;119(7):1858-70.
 69. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev.* 2008;88(1):125-72.
 70. Ishida Y, Kimura A, Kondo T, Hayashi T, Ueno M, Takakura N, et al. Essential roles of the CC chemokine ligand 3-CC chemokine receptor 5 axis in bleomycin-induced pulmonary fibrosis through regulation of macrophage and fibrocyte infiltration. *Am J Pathol.* 2007;170(3):843-54.
 71. Tokuda A, Itakura M, Onai N, Kimura H, Kuriyama T, Matsushima K. Pivotal role of CCR1-positive leukocytes in bleomycin-induced lung fibrosis in mice. *J Immunol.* 2000;164(5):2745-51.
 72. Capelli A, Di Stefano A, Gnemmi I, Donner CF. CCR5 expression and CC chemokine levels in idiopathic pulmonary fibrosis. *Eur Respir J.* 2005;25(4):701-7.
 73. Gonzalez AL, El-Bjeirami W, West JL, McIntire LV, Smith CW. Transendothelial migration enhances integrin-dependent human neutrophil chemokinesis. *J Leukoc Biol.* 2007;81(3):686-95.
 74. Keane MP, Arenberg DA, Lynch JP 3rd, Whyte RI, Iannettoni MD, Burdick MD, et al. The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. *J Immunol.* 1997;159(3):1437-43.
 75. Ayres-Sander CE, Lauridsen H, Maier CL, Sava P, Pober JS, Gonzalez AL. Transendothelial migration enables subsequent transmigration of neutrophils through underlying pericytes. *PLoS One.* 2013;8(3):e60025.
 76. Takahashi T, Hato F, Yamane T, Fukumasu H, Suzuki K, Ogita S, et al. Activation of human neutrophil by cytokine-activated endothelial cells. *Circ Res.* 2001;88(4):422-9.
 77. Jones HA, Schofield JB, Krausz T, Boobis AR, Haslett C. Pulmonary fibrosis correlates with duration of tissue neutrophil activation. *Am J Respir Crit Care Med.* 1998;158(2):620-8.
 78. Strieter RM, Gomperts BN, Keane MP. The role of CXC chemokines in pulmonary fibrosis. *J Clin Invest.* 2007;117(3):549-56.
 79. Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, et al. CXC chemokines in angiogenesis. *J Leukoc Biol.* 2000;68(1):1-8.
 80. Wasmuth HE, Lammert F, Zaldivar MM, Weiskirchen R, Hellerbrand C, Scholten D, et al. Antifibrotic effects of CXCL9 and its receptor CXCR3 in livers of mice and humans. *Gastroenterology.* 2009;137(1):309-19, 19 e1-3.
 81. Phillips RJ, Burdick MD, Hong K, Lutz MA, Murray LA, Xue YY, et al. Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest.* 2004;114(3):438-46.
 82. Kim SS, Peer D, Kumar P, Subramanya S, Wu H, Asthana D, et al. RNAi-mediated CCR5 silencing by LFA-1-targeted nanoparticles prevents HIV infection in BLT mice. *Mol Ther.* 2010;18(2):370-6.
 83. Grigorescu M. Noninvasive biochemical markers of liver fibrosis. *J Gastrointest Liver Dis.* 2006;15(2):149-59.
 84. Hernandez-Gea V, Friedman SL. Pathogenesis of liver fibrosis. *Annu Rev Pathol.* 2011;6:425-56.
 85. Booth AJ, Hadley R, Cornett AM, Dreffs AA, Matthes SA, Tsui JL, et al. Acellular normal and fibrotic human lung matrices as a culture system for in vitro investigation. *Am J Respir Crit Care Med.* 2012;186(9):866-76.
 86. Laurent TC, Fraser JR. Hyaluronan. *FASEB J.* 1992;6(7):2397-404.
 87. Jiang D, Liang J, Noble PW. Hyaluronan as an immune regulator in human diseases. *Physiol Rev.* 2011;91(1):221-64.
 88. Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell

- surface receptor for hyaluronate. *Cell*. 1990;61(7):1303-13.
89. Maharjan AS, Pilling D, Gomer RH. High and low molecular weight hyaluronic acid differentially regulate human fibrocyte differentiation. *PLoS One*. 2011;6(10):e26078.
 90. Sherman L, Sleeman J, Herrlich P, Ponta H. Hyaluronate receptors: key players in growth, differentiation, migration and tumor progression. *Curr Opin Cell Biol*. 1994;6(5):726-33.
 91. Xu H, Manivannan A, Liversidge J, Sharp PF, Forrester JV, Crane IJ. Involvement of CD44 in leukocyte trafficking at the blood-retinal barrier. *J Leukoc Biol*. 2002;72(6):1133-41.
 92. Lesley J, Hyman R, Kincade PW. CD44 and its interaction with extracellular matrix. *Adv Immunol*. 1993;54:271-335.
 93. Evanko SP, Tammi MI, Tammi RH, Wight TN. Hyaluronan-dependent pericellular matrix. *Adv Drug Deliv Rev*. 2007;59(13):1351-65.
 94. Webber J, Jenkins RH, Meran S, Phillips A, Steadman R. Modulation of TGFbeta1-dependent myofibroblast differentiation by hyaluronan. *Am J Pathol*. 2009;175(1):148-60.
 95. Webber J, Meran S, Steadman R, Phillips A. Hyaluronan orchestrates transforming growth factor-beta1-dependent maintenance of myofibroblast phenotype. *J Biol Chem*. 2009;284(14):9083-92.
 96. Meran S, Thomas D, Stephens P, Martin J, Bowen T, Phillips A, et al. Involvement of hyaluronan in regulation of fibroblast phenotype. *J Biol Chem*. 2007;282(35):25687-97.
 97. Somerville RP, Oblander SA, Apte SS. Matrix metalloproteinases: old dogs with new tricks. *Genome Biol*. 2003;4(6):216.
 98. Iredale JP. Tissue inhibitors of metalloproteinases in liver fibrosis. *Int J Biochem Cell Biol*. 1997;29(1):43-54.
 99. Hemmann S, Graf J, Roderfeld M, Roeb E. Expression of MMPs and TIMPs in liver fibrosis — a systematic review with special emphasis on anti-fibrotic strategies. *J Hepatol*. 2007;46(5):955-75.
 100. Benyon RC, Arthur MJ. Extracellular matrix degradation and the role of hepatic stellate cells. *Semin Liver Dis*. 2001;21(3):373-84.
 101. Mormone E, George J, Nieto N. Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches. *Chem Biol Interact*. 2011;193(3):225-31.
 102. Benyon RC, Iredale JP, Goddard S, Winwood PJ, Arthur MJ. Expression of tissue inhibitor of metalloproteinases 1 and 2 is increased in fibrotic human liver. *Gastroenterology*. 1996;110(3):821-31.
 103. Strongin AY, Collier I, Bannikov G, Marmer BL, Grant GA, Goldberg GI. Mechanism of cell surface activation of 72-kDa type IV collagenase. Isolation of the activated form of the membrane metalloprotease. *J Biol Chem*. 1995;270(10):5331-8.
 104. Zeisberg M, Bonner G, Maeshima Y, Colorado P, Muller GA, Strutz F, et al. Renal fibrosis: collagen composition and assembly regulates epithelial-mesenchymal transdifferentiation. *Am J Pathol*. 2001;159(4):1313-21.
 105. Selman M, Ruiz V, Cabrera S, Segura L, Ramirez R, Barrios R, et al. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? *Am J Physiol Lung Cell Mol Physiol*. 2000;279(3):L562-74.
 106. Padmanabhan J, Gonzalez AL. The effects of extracellular matrix proteins on neutrophil-endothelial interaction—a roadway to multiple therapeutic opportunities. *Yale J Biol Med*. 2012;85(2):167-85.
 107. Roderfeld M, Weiskirchen R, Wagner S, Berres ML, Henkel C, Grotzinger J, et al. Inhibition of hepatic fibrogenesis by matrix metalloproteinase-9 mutants in mice. *FASEB J*. 2006;20(3):444-54.
 108. Popov Y, Patsenker E, Bauer M, Niedobitek E, Schulze-Krebs A, Schuppan D. Halofuginone induces matrix metalloproteinases in rat hepatic stellate cells via activation of p38 and NFkappaB. *J Biol Chem*. 2006;281(22):15090-8.
 109. Sumpio BE, Riley JT, Dardik A. Cells in focus: endothelial cell. *Int J Biochem Cell Biol*. 2002;34(12):1508-12.
 110. Wick G, Grundtman C, Mayerl C, Wimpfissinger TF, Feichtinger J, Zelger B, et al. The immunology of fibrosis. *Annu Rev Immunol*. 2013;31:107-35.
 111. Zhang C, Hekmatfar S, Ramanathan A, Karuri NW. PEGylated human plasma fibronectin is proteolytically stable, supports cell adhesion, cell migration, focal adhesion assembly, and fibronectin fibrillogenesis. *Biotechnol Prog*. 2013;29(2):493-504.
 112. Petzelbauer P, Bender JR, Wilson J, Pober JS. Heterogeneity of dermal microvascular endothelial cell antigen expression and cytokine responsiveness in situ and in cell culture. *J Immunol*. 1993;151(9):5062-72.
 113. Woodfin A, Voisin MB, Imhof BA, Dejana E, Engelhardt B, Nourshargh S. Endothelial cell activation leads to neutrophil transmigration as supported by the sequential roles of ICAM-2, JAM-A, and PECAM-1. *Blood*. 2009;113(24):6246-57.
 114. Avraamides CJ, Garmy-Susini B, Varner JA. Integrins in angiogenesis and lymphangiogenesis. *Nat Rev Cancer*. 2008;8(8):604-17.
 115. Stupack DG, Cheresh DA. ECM remodeling regulates angiogenesis: endothelial integrins look for new ligands. *Sci STKE*. 2002;2002(119):pe7.
 116. Goumans MJ, van Zonneveld AJ, ten Dijke P. Transforming growth factor beta-induced endothelial-to-mesenchymal transition: a switch to cardiac fibrosis? *Trends Cardiovasc Med*. 2008;18(8):293-8.

117. Hashimoto N, Phan SH, Imaizumi K, Matsuo M, Nakashima H, Kawabe T, et al. Endothelial-mesenchymal transition in bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol.* 2010;43(2):161-72.
118. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med.* 2007;13(8):952-61.
119. Zeisberg EM, Potenta SE, Sugimoto H, Zeisberg M, Kalluri R. Fibroblasts in kidney fibrosis emerge via endothelial-to-mesenchymal transition. *J Am Soc Nephrol.* 2008;19(12):2282-7.
120. Sgonc R, Gruschwitz MS, Dietrich H, Recheis H, Gershwin ME, Wick G. Endothelial cell apoptosis is a primary pathogenetic event underlying skin lesions in avian and human scleroderma. *J Clin Invest.* 1996;98(3):785-92.
121. Winn RK, Harlan JM. The role of endothelial cell apoptosis in inflammatory and immune diseases. *J Thromb Haemost.* 2005;3(8):1815-24.
122. Sgonc R, Gruschwitz MS, Boeck G, Sepp N, Gruber J, Wick G. Endothelial cell apoptosis in systemic sclerosis is induced by antibody-dependent cell-mediated cytotoxicity via CD95. *Arthritis Rheum.* 2000;43(11):2550-62.
123. Hamada N, Kuwano K, Yamada M, Hagimoto N, Hiasa K, Egashira K, et al. Anti-vascular endothelial growth factor gene therapy attenuates lung injury and fibrosis in mice. *J Immunol.* 2005;175(2):1224-31.
124. Pan Q, Kleer CG, van Golen KL, Irani J, Bottema KM, Bias C, et al. Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. *Cancer Res.* 2002;62(17):4854-9.
125. Datta A, Scotton CJ, Chambers RC. Novel therapeutic approaches for pulmonary fibrosis. *Br J Pharmacol.* 2011;163(1):141-72.
126. Mas-Moruno C, Rechenmacher F, Kessler H. Cilengitide: the first anti-angiogenic small molecule drug candidate design, synthesis and clinical evaluation. *Anticancer Agents Med Chem.* 2010;10(10):753-68.
127. Patsenker E, Popov Y, Sticker F, Schneider V, Ledermann M, Sagesser H, et al. Pharmacological inhibition of integrin α v β 3 aggravates experimental liver fibrosis and suppresses hepatic angiogenesis. *Hepatology.* 2009;50(5):1501-11.
128. Luo L, Zhang X, Hirano Y, Tyagi P, Barabas P, Uehara H, et al. Targeted intraceptor nanoparticle therapy reduces angiogenesis and fibrosis in primate and murine macular degeneration. *ACS Nano.* 2013;7(4):3264-75.
129. John AE, Lukacs NW, Berlin AA, Palecanda A, Bargatze RF, Stoolman LM, et al. Discovery of a potent nanoparticle P-selectin antagonist with anti-inflammatory effects in allergic airway disease. *FASEB J.* 2003;17(15):2296-8.
130. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res.* 2005;97(6):512-23.
131. Raza A, Franklin MJ, Dudek AZ. Pericytes and vessel maturation during tumor angiogenesis and metastasis. *Am J Hematol.* 2010;85(8):593-8.
132. Schrimpf C, Duffield JS. Mechanisms of fibrosis: the role of the pericyte. *Curr Opin Nephrol Hypertens.* 2011;20(3):297-305.
133. Mostafa M, Tairag E, Chollet-Martin S, Oudghiri M, Laquay N, Jacob MP, Michel JB, et al. Effects of interleukin-10 on monocyte/endothelial cell adhesion and MMP-9/TIMP-1 secretion. *Cardiovasc Res.* 2001;49(4):882-90.
134. Zhang C, Hekmatfar S, Karuri NW. A comparative study of polyethylene glycol hydrogels derivatized with the RGD peptide and the cell-binding domain of fibronectin. *J Biomed Mater Res A.* 2013 Apr 24. Epub ahead of print.
135. Lin SL, Kisseleva T, Brenner DA, Duffield JS. Pericytes and perivascular fibroblasts are the primary source of collagen-producing cells in obstructive fibrosis of the kidney. *Am J Pathol.* 2008;173(6):1617-27.
136. Li L, Zepeda-Orozco D, Black R, Lin F. Autophagy is a component of epithelial cell fate in obstructive uropathy. *Am J Pathol.* 2010;176(4):1767-78.
137. Humphreys BD, Lin SL, Kobayashi A, Hudson TE, Nowlin BT, Bonventre JV, et al. Fate tracing reveals the pericyte and not epithelial origin of myofibroblasts in kidney fibrosis. *Am J Pathol.* 2010;176(1):85-97.
138. Bjarnegard M, Enge M, Norlin J, Gustafsdottir S, Fredriksson S, Abramsson A, et al. Endothelium-specific ablation of PDGFB leads to pericyte loss and glomerular, cardiac and placental abnormalities. *Development.* 2004;131(8):1847-57.
139. Darland DC, Massingham LJ, Smith SR, Piek E, Saint-Geniez M, D'Amore PA. Pericyte production of cell-associated VEGF is differentiation-dependent and is associated with endothelial survival. *Dev Biol.* 2003;264(1):275-88.
140. Lin SL, Chang FC, Schrimpf C, Chen YT, Wu CF, Wu VC, et al. Targeting endothelium-pericyte cross talk by inhibiting VEGF receptor signaling attenuates kidney microvascular rarefaction and fibrosis. *Am J Pathol.* 2011;178(2):911-23.
141. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med.* 1994;1(1):71-81.
142. Herzog EL, Bucala R. Fibrocytes in health and disease. *Exp Hematol.* 2010;38(7):548-56.
143. Strieter RM, Keeley EC, Burdick MD, Mehrad B. The role of circulating mesenchymal progenitor cells, fibrocytes, in promoting pulmonary fibrosis. *Trans Am Clin Climatol Assoc.* 2009;120:49-59.

144. Andersson-Sjoland A, de Alba CG, Nihlberg K, Becerril C, Ramirez R, Pardo A, et al. Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol.* 2008;40(10):2129-40.
145. Mehrad B, Burdick MD, Zisman DA, Keane MP, Belperio JA, Strieter RM. Circulating peripheral blood fibrocytes in human fibrotic interstitial lung disease. *Biochem Biophys Res Commun.* 2007;353(1):104-8.
146. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv Drug Deliv Rev.* 2003;55(3):329-47.
147. Song Y, Li X, Du X. Exposure to nanoparticles is related to pleural effusion, pulmonary fibrosis and granuloma. *Eur Respir J.* 2009;34(3):559-67.
148. Ren H, Huang X. Polyacrylate nanoparticles: toxicity or new nanomedicine? *Eur Respir J.* 2010;36(1):218-21.
149. Badea I, Virtanen C, Verrall RE, Rosenberg A, Foldvari M. Effect of topical interferon-gamma gene therapy using gemini nanoparticles on pathophysiological markers of cutaneous scleroderma in Tsk/+ mice. *Gene Ther.* 2012;19(10):978-87.
150. Card JW, Zeldin DC, Bonner JC, Nestmann ER. Pulmonary applications and toxicity of engineered nanoparticles. *Am J Physiol Lung Cell Mol Physiol.* 2008;295(3):L400-11.
151. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine.* 2008;3(2):133-49.