This Review is part of a thematic series on Cardiac Fibroblasts, which includes the following articles:

- Intramyocardial Fibroblast–Myocyte Communication [Circ Res. 2010;106:47–57]

Potential Therapeutic Targets for Cardiac Fibrosis: TGFβ, Angiotensin, Endothelin, CCN2, and PDGF, Partners in Fibroblast Activation

Fate Mapping Cardiac Fibroblasts

Jeff Molkentin, Guest Editor

**Potential Therapeutic Targets for Cardiac Fibrosis**

**TGFβ, Angiotensin, Endothelin, CCN2, and PDGF, Partners in Fibroblast Activation**

Andrew Leask

Abstract: Fibrosis is one of the largest groups of diseases for which there is no therapy but is believed to occur because of a persistent tissue repair program. During connective tissue repair, “activated” fibroblasts migrate into the wound area, where they synthesize and remodel newly created extracellular matrix. The specialized type of fibroblast responsible for this action is the α-smooth muscle actin (α-SMA)–expressing myofibroblast. Abnormal persistence of the myofibroblast is a hallmark of fibrotic diseases. Proteins such as transforming growth factor (TGF)β, endothelin-1, angiotensin II (Ang II), connective tissue growth factor (CCN2/CTGF), and platelet-derived growth factor (PDGF) appear to act in a network that contributes to myofibroblast differentiation and persistence. Drugs targeting these proteins are currently under consideration as antifibrotic treatments. This review summarizes recent observations concerning the contribution of TGFβ, endothelin-1, Ang II, CCN2, and PDGF to fibroblast activation in tissue repair and fibrosis and the potential utility of agents blocking these proteins in affecting the outcome of cardiac fibrosis. (Circ Res. 2010;106:1675-1680.)

Key Words: PDGF ■ TGFβ endothelin ■ fibrosis ■ scarring

Hypertension and heart failure are major health problems worldwide. Patients with hypertensive heart disease possess cardiac fibrosis (that is, excessive deposition of scar tissue) that significantly reduces organ function. Thus, rationally designed antifibrotic therapies are likely to be invaluable in curbing this health problem. However, there is no therapy for fibrotic diseases in general, largely because the underlying basis of fibrosis is unclear.

However, numerous studies conducted over the past 30 years have suggested that chronic inappropriate increases in levels of circulating hormones such as angiotensin II and endothelin (ET)-1 and fibrogenic cytokines/proteins such as transforming growth factor (TGF)β, connective tissue growth factor (CTGF/CCN2), and platelet-derived growth factor (PDGF) are likely to be key driving forces culminating in fibrosis. Collectively, these hormones and cytokines result in the activation of mesenchymal cells (fibroblasts) within connective tissue. These activated mesenchymal cells are termed myofibroblasts because they express the highly contractile protein α-smooth muscle actin (α-SMA), which is organized into contractile microfilaments.1 These cells are also responsible for producing the elevated amounts of extracellular matrix (ECM) that characterizes scar tissue.1 The origin of myofibroblasts is unclear but may result from growth factor–mediated differentiation of resident mesenchymal cells or recruitment of microvascular pericytes. Although...
myofibroblasts are induced in response to normal tissue injury, these cells disappear, thereafter, probably by apoptosis. In fibrotic disease, however, myofibroblasts persist, resulting in the excessive production and remodeling of ECM.

Recent evidence has suggested that differentiation of resident fibroblasts occurs in response to TGFβ, ET-1, and angiotensin, which are all likely to play key roles in this process. These proteins are likely to act in concert with the matricellular protein connective tissue growth factor (CTGF/CCN2), which appears to augment signaling responses from other proteins. However, the protein PDGF appears to affect pericyte differentiation and recruitment. This review summarizes these recent observations.

### Transforming Growth Factor-β

TGFβ expression is elevated in response to injury. There is an extensive literature that discusses the basics of TGFβ signaling and its relationship to tissue repair and fibrosis. Briefly, there are 3 TGFβ isoforms, namely TGFβ1, TGFβ2, and TGFβ3. These are initially present within a complex containing latent TGFβ-binding proteins that are proteolytically removed to release active TGFβ. The TGFβ receptor is a heteromer of TGFβ type I (termed activin linked kinase [ALK] in the case of fibroblasts) and TGFβ type II receptors. ALK5 phosphorylates Smad2 and -3, which bind to Smad4, translocate into the nucleus, and activate transcription of target genes. Conversely, TGFβ signaling is inhibited by Smad3. TGFβ activates a variety of noncanonical signaling pathways including: ras/MEK/ERK, which requires the heparan sulfate–containing proteoglycan (HSPG) syndecan 4; p38, which requires the HSPG β-glycan; and c-Jun N-terminal kinase (JNK), which requires focal adhesion kinase and TGFβ activated kinase (TAK1). Thus, cell adhesion via integrins and HSPGs is likely to play a significant role in modulating TGFβ signaling responses in fibroblasts.

Substantial evidence supports a central role for TGFβ in fibroblast activation. When applied to fibroblasts, TGFβ directly induces ECM gene expression and promotes ECM deposition by simultaneously suppressing matrix metalloproteinase expression and inducing tissue inhibitors of matrix metalloproteinase gene expression (Figure). In vivo, TGFβ enhances closure of fetal wounds and induces granulation tissue formation. Wounds treated with anti-TGFβ antibodies or antisense oligonucleotides show reduced ECM synthesis and scarring. Smad3-/- mice show accelerated wound healing, reduced granulation tissue formation, increased epithelialization, and reduced inflammation. Experiments using Smad3-/- and Smad3+/+ fibroblasts have revealed that Smad3 is required for TGFβ to induce collagen and other ECM genes. For example, in isolated cardiac fibroblasts TGFβ-mediated induction of procollagen type III and tenascin-C is dependent on Smad3; infarcted Smad3-/- hearts possess decreased fibrosis compared to their wild-type counterparts. TGFβ also induces fibroblasts to differentiate into α-SMA expressing myofibroblasts (Figure), in a fashion which involves FAK, JNK and TAK1. In addition to α-SMA, TGFβ induces key markers and effectors of myofibroblast differentiation including connective tissue growth factor (CTGF, CCN2). Syndecan 4/HSPGs and extracellular signal-regulated kinase (ERK) are required for CCN2 expression. ERK is also required for type I collagen expression. Intriguingly, TAK1 has been shown to be required for vascular development including the formation of smooth muscle.

ALK5 inhibitors have been considered as potential antifibrotic compounds, although these small molecules have not yet been thoroughly investigated in vascular tissue. However, in fibrotic fibroblasts isolated from patients with the disease scleroderma, ALK5 inhibition reduces some aspects of the activated fibroblast phenotype, such as an elevated collagen production but does not reduce expression of either α-SMA or CCN2 protein. In addition to ALK5 inhibitors, anti-TGFβ antibodies have also been under consideration as potential antifibrotic agents. A neutralizing anti-TGFβ antibody administered before induction of experimental myocardial fibrosis inhibited fibroblast activation and prevented collagen mRNA induction but not myocyte hypertrophy, blood pressure, and systolic function. However, in a mouse model of myocardial infarction, a neutralizing anti-TGFβ antibody administered before or after coronary artery ligation resulted in increased mortality and worsened ventricular remodeling, correlating with a decrease in collagen production and an increase in matrix-metalloproteinase expression. On the other hand, in an experimental rat model of myocardial infarction, treatment with an ALK5 inhibitor significantly reduced TGFβ activity leading to the attenuation of systolic dysfunction and left ventricular remodeling. In this latter study, no side effects were reported. These results suggest that broad targeting of TGFβ ligand might not be a viable antifibrotic strategy, but ALK5 inhibition may be a useful approach.

### Angiotensin II

Elevated intracardiac angiotensin II (Ang II) production is found in overloaded hearts with fibrosis. Angiotensin is an oligopeptide that causes vasoconstriction and increased blood pressure. Recent studies indicate that Ang II and TGFβ1 do not act independently from one another but act as part of an integrated signaling network that promotes cardiac remodeling and possibly fibrosis. Ang II upregulates TGFβ1 expres-
sion through the angiotensin type 1 (AT1) receptor in cardiac myocytes and fibroblasts. In an important study, it was shown that Ang II was not able to induce cardiac hypertrophy and fibrosis in vivo in the absence of TGFβ (Figure). Providing further support for this notion, Ang II induces collagen in cardiac fibroblasts through TGFβ and ERK (Figure). Ang II and TGFβ1 appear to act in an autocrine loop, as TGFβ1 can directly stimulate AT1 receptor expression through ALK5 and Smads 2/3/4, providing a further indication of crosstalk between the TGFβ and Angiotensin pathways. Furthermore, p38 mitogen-activated protein kinase, JNK, and phosphatidylinositol 3-kinase signaling pathways appear to be also involved in TGFβ1-stimulated AT1 receptor density. Moreover, vascular smooth muscle cells, Ang II causes Smad2 phosphorylation, nuclear translocation of phosphorylated Smad2 and Smad4, and increased Smad DNA-binding activity in a fashion that is blocked by the AT1 antagonist losartan but not by blockade of endogenous TGFβ. These results indicate that Ang II and TGFβ pathways are likely to cooperate to drive fibrogenic responses in vivo.

Drugs that inhibit the angiotensin pathway, namely angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists, are widely used to treat hypertension and a variety of cardiomyopathies. Angiotensin receptor inhibitors such as losartan appear to be effective in reducing cardiac fibrosis in a variety of models in animals and in humans. Thus, compared to generally antagonizing TGFβ signaling, angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists may be useful approaches to control fibrotic disease.

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**Figure. Schematic diagram of interplay among profibrotic cytokines.** Ang II induces TGFβ, CCN2/CTGF, and ET-1 directly; TGFβ induces ET-1 and CCN2/CTGF; ET-1 induces CCN2/CTGF. TGFβ also induces the angiotensin receptor, thus augmenting Ang II signaling (for details, see text). Collectively, these proteins promote fibroblast activation and myofibroblast formation. In normal tissue repair, myofibroblasts disappear and organ function is restored. Myofibroblast persistence leads to disease. FAK indicates focal adhesion kinase; Akt, V-akt murine thymoma viral oncogene homolog 1.
Endothelin-1

Endothelin is a protein secreted from endothelial cells and is recognized as playing a central role in the pathogenesis of chronic heart failure. Endothelin is a powerful vasoconstrictor with mitogenic or comitogenic properties, which acts through the stimulation of 2 subtypes of receptors (ET receptor subtype A [ETA] and ET receptor subtype B [ETB]). There are 3 isoforms of ET, namely ET-1, ET-2, and ET-3. Of these, ET-1, the significant isoform in humans, is normally produced by endothelial cells and also can be expressed by epithelial cells, bone marrow mast cells, macrophages, polymorphonuclear leukocytes, cardiomyocytes, and fibroblasts. ET-1 is first produced in the form of a 212-aa precursor (prepro-ET-1) and then cleaved twice to form a biologically active 21-aa peptide, the last cleavage mediated by ET-converting enzyme.

ET-1 induces ECM production and myofibroblast differentiation in fibroblasts. TGFβ induces ET-1 via JNK, and ET-1 is a downstream mediator of the fibrogenic responses of TGFβ in normal fibroblasts (Figure). Constitutive ET signaling, operating through an ALK5-independent mechanism, is responsible for the persistent myofibroblast phenotype of fibrotic lung fibroblasts. Some studies have suggested that TGFβ works together with ET-1 to promote myofibroblast differentiation. Intriguingly, Ang II also induces ET-1 via ERK and reactive oxygen species. These results suggest that ET operates downstream of the TGFβ/Ang II system to drive fibroblast activation and fibrosis (Figure).

ET receptor antagonism might be considered as an appropriate therapy for the fibrosis. Inhibitors that block individual or both ET receptors exist. The first dual ETA/ETB receptor blocker, bosentan, has already been approved by the Food and Drug Administration for the treatment of pulmonary arterial hypertension. Studies are ongoing on the effects of selective ETA antagonists or dual ETA/ETB antagonists in fibrosis. It is interesting to note that dual-acting angiotensin II and ET receptor blockers have been shown to reduce systemic blood pressure in animal models and in hypertensive patients. Preliminary data in smaller human studies have shown that these agents are safe and well tolerated. Thus combination ET/Ang II therapies promise in controlling cardiac disease.

Connective Tissue Growth Factor

CCN2 is a matricellular protein which promotes angiogenesis. CCN2 promotes cell adhesion and enhances adhesive signaling in response to extracellular ligands. CCN2 acts through a variety of integrins and HSPGs or the receptor trkA. CCN2 is an excellent surrogate marker for activated fibroblasts in wound healing and in fibrosis; for example, in the process of cardiac remodeling, connective tissue growth factor (CTGF/CCN2) is significantly induced in cardiac myocytes. CCN2 is induced by TGFβ, Ang II and ET-1 and is a downstream mediator of these proteins. CCN2 acts as a cofactor with TGFβ to induce fibrogenesis. However, on its own, CCN2 is considered to only weakly promote fibrosis; rather, what CCN2 appears to do is to create an environment favorable for fibrogenic stimuli to act. CCN2 is not required for all of TGFβ's actions, but in mouse embryonic fibroblasts, which constitutively express CCN2, it appears to be required for TGFβ to maximally induce type I collagen and α-SMA mRNA and protein. Although direct evidence functionally linking CCN2 to cardiac disease is scant, CCN2 has been shown both in vitro and in vivo to cause hypertrophy of rat cardiomyocytes.

Drugs targeting the action of CCN2, such as small interfering RNAs or neutralizing antibodies, are currently under development. A CCN2 response element exists in the COL1A2 promoter, and recently it has been shown that blocking CCN2 action using an anti-CCN2 antibody or small interfering RNA reduces aspects of bleomycin-induced lung fibrosis. Overall, however, strong in vivo evidence directly supporting the notion of anti-CCN2 therapies in pathologies, including that or the heart, is lacking. However, it is possible that CCN2 may be a key selective modulator of tissue remodeling in the heart, operating downstream and in concert with TGFβ, ET-1, and Ang II.

Platelet-Derived Growth Factor

PDGF comprises a family of homo- or hetero-dimeric growth factors including PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD. There are 2 different PDGF receptors, α and β. Elevated PDGF expression is observed postwounding. PDGF causes neutrophils, macrophages, fibroblasts, and smooth muscle cells to proliferate and migrate into the wound site. PDGF also stimulates granulation tissue formation. In addition, PDGF stimulates fibroblasts to contract collagen matrices and differentiate into myofibroblasts in vitro. PDGF receptors are expressed by activated microvascular pericytes in patients with early systemic sclerosis, but not in those with late-stage scleroderma. Pericytes fail to develop in PDGF receptor deficient embryos. The PDGFβ receptor inhibitor imatinib mesylate delays wound closure, accompanied by a reduction in myofibroblast numbers and expression of fibronectin ED-A and collagen type I. Imatinib mesylate did not prevent the differentiation of myofibroblasts in vitro but potently inhibited fibroblast proliferation and migration and appeared to principally operate by blocking pericyte recruitment. Because a subset (~30%) of myofibroblasts in cutaneous mouse wounds are NG2-positive pericytes, this phenomenon is likely to lead to the reduction myofibroblasts in the wound. In a mouse model of bleomycin-induced dermal fibrosis, dasatinib and nilotinib potently reduced the dermal thickness, the number of myofibroblasts, and the collagen content of the skin in a dose-dependent manner at well-tolerated doses.

Parenchymal fibrosis and cardiac allograft vasculopathy characterized by neointimal growth are the leading causes of graft loss for heart transplant recipients. PDGFα receptor mRNA is upregulated in acutely rejecting cardiac allografts, and PDGF-A and PDGF-C mRNAs are upregulated in chronically rejecting cardiac allografts. When introduced into the heart using adenovirus-mediated delivery, significantly upregulated pro-fibrotic TGFβ1 mRNA and accelerated cardiac fibrosis and arteriosclerosis, indicating that PDGF may also act to promote fibrosis by elevating TGFβ levels. Atrial fibrillation, characterized by atrial fibrosis, is a common arrhythmia that
increases the risk of stroke and heart failure. Injection of neutralizing PDGF receptor-specific antibody attenuated atrial fibrosis. These results strongly suggest that PDGF may be a good target for antifibrotic therapy in the heart.

**Future Prospects and Conclusions**

Currently, drugs affecting TGFβ, ET-1, CCN2, Ang II, and PDGF are being considered as antifibrotic therapies. Although these proteins are likely to cooperate in driving tissue repair and fibrogenic responses in fibroblasts, each protein also has certain unique features, providing a rationale that therapies targeting individual molecules might be useful. However, of these, the most promising clinical data thus far have been provided by anti-Ang II- and anti-ET-1-based approaches, either alone or in combination, and approaches targeting both these pathways may be appropriate therapies in controlling cardiac disease.

In the future, additional targets that enhance this fibrogenic network might be developed for antifibrotic therapies. For example, the protein osteopontin might be a useful, novel antifibrotic target because it is required for TGFβ-induced collagen production by cardiac fibroblasts. In addition, recent evidence has suggested that interleukin-13 might be protective against fibrosis. One study found that hearts of mice deficient in interleukin-13 knockout mice showed severe dilated cardiomyopathy and possessed increased levels of TGFβ. Interleukin-13 protected BALB/c mice from myocardial fibrosis. Collectively, there is a wide range of possible antifibrotic treatments, albeit at various stages of development, that target the TGFβ, ET-1, CCN2, Ang II, and PDGF network. However, more clinical data are needed to properly evaluate the efficacy of all of these.

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**Disclosures**

None.

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