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REVIEW

Narrative Review: Fibrotic Diseases: Cellular and Molecular Mechanisms and Novel Therapies

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Abnormal and exaggerated deposition of extracellular matrix is the hallmark of many fibrotic diseases, including systemic sclerosis and pulmonary, liver, and kidney fibrosis. The spectrum of affected organs, the usually progressive nature of the fibrotic process, the large number of affected persons, and the absence of effective treatment pose an enormous challenge when treating fibrotic diseases. Delineation of the central role of transforming growth factor-β (TGF-β) and identification of the specific cellular receptors, kinases, and other mediators involved in the fibrotic process have provided a sound basis for development of effective therapies. The inhibition of signaling pathways activated by TGF-β represents a novel therapeutic approach for the fibrotic disorders. One of these TGF-β pathways results in the activation of the nonreceptor tyrosine kinase cellular Abelson (c-Abl), and c-Abl inhibitors, including imatinib mesylate, diminishing the fibrogenic effects of TGF-β. Thus, recently acquired basic knowledge about the pathogenesis of the fibrotic process has enabled the development of novel therapeutic agents capable of modifying the deleterious effects of the fibrotic diseases.

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The fibrotic diseases encompass a wide spectrum of clinical entities, including multisystemic diseases, such as systemic sclerosis (1–4), multifocal fibrosclerosis (5–7), sclerodermatous graft-versus-host disease in bone marrow transplant recipients (8), and the recently recognized nephrogenic systemic fibrosis (see Glossary) (9–15), as well as organ-specific disorders, such as pulmonary, liver, and kidney fibrosis (16–23). Although their etiology and causative mechanisms differ, the fibrotic diseases share the common feature of disordered and exaggerated deposition of extracellular matrix (see Glossary) in affected tissues (24–31). Elevated expression of genes encoding extracellular matrix proteins is a common and characteristic feature of these conditions, and the resulting fibrosis disrupts the normal architecture of the affected organs, which ultimately leads to their dysfunction and failure. The persistent activation of fibroblastic cells distinguishes controlled repair, such as that occurring during normal wound healing, from the uncontrolled fibrosis that is the hallmark of this group of diseases. Fibrotic diseases affect a wide spectrum of organs and a large number of persons, and their devastating effects cause an enormous burden on health resources with severe economic consequences. The usually progressive nature of these diseases and the absence of effective treatment compound the seriousness of the problem.

The recent recognition of novel mechanisms and the elucidation of crucial regulatory pathways in the development of the fibrotic response have provided a rational basis for the development of novel and effective therapies. The delineation of the crucial function of transforming growth factor-β (TGF-β) (see Glossary) in the pathogenesis of tissue fibrosis and the identification of specific cellular receptors, kinases, and intracellular transduction pathways that participate in the earliest stages of pathologic fibrosis have been most significant.

Molecular Mechanisms of TGF-β Induction of Tissue Fibrosis

Transforming growth factor-β exerts several cellular effects and participates in the pathogenesis of many human diseases (32–34). It is now considered a key molecule in the pathogenesis of tissue fibrosis in the fibrotic diseases (35–41). One important effect of TGF-β is the remarkable stimulation of the production of various col-

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Key Summary Points

Abnormal and exaggerated deposition of extracellular matrix is the most typical feature of fibrotic diseases.

Fibrotic disorders affect several organs, are usually progressive, and cause serious functional alterations.

Currently, no effective treatment exists, although recent elucidation of crucial regulatory pathways involved in the fibrotic response has provided a sound basis for the development of novel therapies.

Transforming growth factor-β (TGF-β) plays a crucial role in the pathogenesis of tissue fibrosis; thus, components of its complex signaling pathway represent potentially important therapeutic targets.

Activation of the nonreceptor tyrosine kinase c-Abl is an important TGF-β–dependent profibrotic pathway, and c-Abl inhibitors markedly diminish the fibrogenic response to TGF-β.

Inhibition of other tyrosine kinases and other molecular pathways involved in tissue fibrosis also holds promise for treatment of the fibrotic diseases.

lagens and other extracellular matrix proteins by mesenchymal cells. Transforming growth factor-β_1 also decreases the synthesis of collagen-degrading metalloproteinases and stimulates production of tissue inhibitors of metalloproteinases (42). It sensitizes fibroblasts to their own effects and maintains them in a persistently activated state by an autocrine mechanism that causes further production of TGF-β (43, 44).

Various cells, including fibroblasts and macrophages, produce TGF-β, and most cells in the body have receptors for TGF-β. Transforming growth factor-β is initially produced in an inactive form (pro–TGF-β) that is composed of 2 identical peptides. Pro–TGF-β undergoes complex proteolytic and conformational events leading to activation (45–48). Once activated, TGF-β binds to a serine threonine transmembrane kinase known as the *TGF-β type II receptor* (TGF-βRII), which is intrinsically active. The signaling events that follow are complex and involve several intracellular molecules and pathways (49–53). **Figure 1** shows the pathways that are most relevant to this discussion.

Classic TGF-β Signaling Pathways

In these pathways, the complex of TGF-β and TGF-βRII recruits another receptor, TGF-β type I receptor (TGF-βRI), to form a larger complex. The other receptor is a member of a family of at least 7 proteins called *activin-like kinase* (ALK) *proteins* (49–51) (see Glossary). The most important and common protein is ALK-5. It is phosphorylated on 3 to 5 serine and threonine residues in a 30–amino acid regulatory sequence. Signaling from the TGF-β receptor complex to the nucleus then occurs through proteins called *Smads* (see Glossary), which mediate the activity of TGF-β (52–57). Smad2 or Smad3 binds to the activated TGF-β receptor complex, becomes phosphorylated, and then forms a complex with Smad4 that allows the Smad complex to translocate across the nuclear membrane. In the nucleus, the Smad complex binds to specific DNA-binding sites in the promoter regions of target genes with the help of intranuclear transcriptional partners. This binding modulates the transcriptional activity of genes that encode extracellular matrix proteins, such as the α_1 and α_2 chains of type I collagen, or of extracellular matrix regulatory proteins, such as connective tissue growth factor.

Fine-tuning of TGF-β activity is achieved through a balance of positive- and negative-effector molecules. Smad7 is important because it inhibits TGF-β signaling by binding to ALK-5. This binding prevents the recruitment and phosphorylation of other Smads, and it facilitates the degradation of ALK-5, thus inhibiting Smad signaling (57–60). Another recently identified mechanism of regulation and fine-tuning of TGF-β activity involves caveolin-1 (see Glossary). Caveolin-1 is a member of the family of proteins involved in the formation of caveolae or lipid rafts, which are cholesterol- and sphingolipid-

Glossary

ALK (activin-like kinase): Serine or threonine kinase receptor for members of the TGF-β family. ALK-1 and ALK-5 are the cellular receptors for TGF-β.

c-Abl (cellular Abelson nonreceptor kinase): Nonreceptor tyrosine kinase implicated in cell differentiation, cell division, cell adhesion, and stress response. Translocation of the genomic region containing the gene encoding c-Abl with chromosome 22 causes chronic myelogenous leukemia and creates the Philadelphia chromosome.

Caveolin-1: Member of the caveolin family of proteins involved in receptor-independent endocytosis. Caveolin-1 associates with lipid rafts, leading to the formation of caveolae, 50-nm hairpin loop invaginations of the plasma membrane that regulate signal transduction.

Extracellular matrix: Interstitial matrix present between cells, composed of collagens, polysaccharides, and other fibrous proteins.

Imatinib mesylate: Mesylate salt used to treat chronic myelogenous leukemia, gastrointestinal stromal tumors, and other types of cancer, which acts by inhibiting c-Abl and specific tyrosine kinase enzymes.

Integrins: Receptors that mediate cell attachment and play a role in cell signaling, thereby defining cellular shape and mobility and regulating the cell cycle.

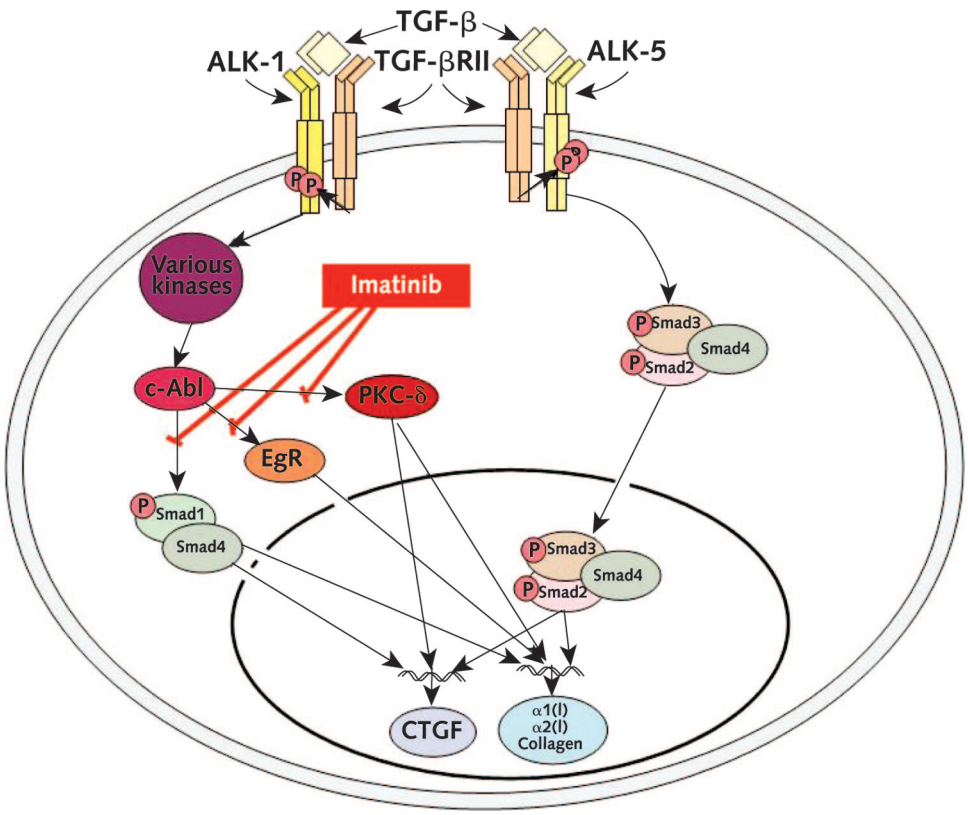
Nephrogenic systemic fibrosis: Recently described disorder characterized by pathologic fibrosis of skin, joints, tendons, muscles, and numerous internal organs in patients with renal insufficiency after exposure to gadolinium-based contrast agents used for magnetic resonance imaging.

PKC-δ (protein kinase C-δ): Serine- and threonine-specific protein kinase involved in cell signaling and in the regulation of growth, apoptosis, and differentiation of various cell types.

Smads: Class of intracellular proteins that mediate the activity of TGF-β ligands by forming complexes, often with other Smads, and entering the nucleus to serve as transcription factors.

TGF-β (transforming growth factor-β): Autocrine signaling protein that controls cell proliferation, differentiation, and several other functions in most cells. It also plays a role in immunity, cancer, heart disease, diabetes, the Marfan syndrome, wound healing, and pathologic fibrosis.

Figure 1. **TGF-β signaling pathways critical for the fibrotic response.**



This figure illustrates classic and nonclassic pathways originating from 2 representative tetrameric TGF-β receptors. After TGF-β binding, TGF-βRII recruits a TGF-βRI (either ALK-1 or ALK-5) and activates it by phosphorylation. ALK-5 then specifically phosphorylates receptor-regulated Smad2 and Smad3, which then complex with co-Smad4, resulting in their transport to the nucleus, where they cooperate with other factors to regulate transcription of critical genes, here represented by genes encoding CTGF and α1 and α2 type I collagens. Also illustrated are several nonclassic pathways. An important one involves the phosphorylation and activation of c-Abl by ALK-1, causing activation of several downstream critical factors, including Smad1; the transcription factor, EgR, and PKC-δ, all of which contribute to the fibrotic response. As pictured, imatinib blocks the activity of c-Abl, effectively inhibiting the fibrotic response by preventing the activation of downstream effectors. ALK = activin-like kinase; c-Abl = cellular Abelson nonreceptor kinase; CTGF = connective tissue growth factor; EgR = early growth response protein; P = phosphorylation; PKC-δ = protein kinase C-δ; TGF-β = transforming growth factor-β; TGF-βRII = TGF-β type II receptor.

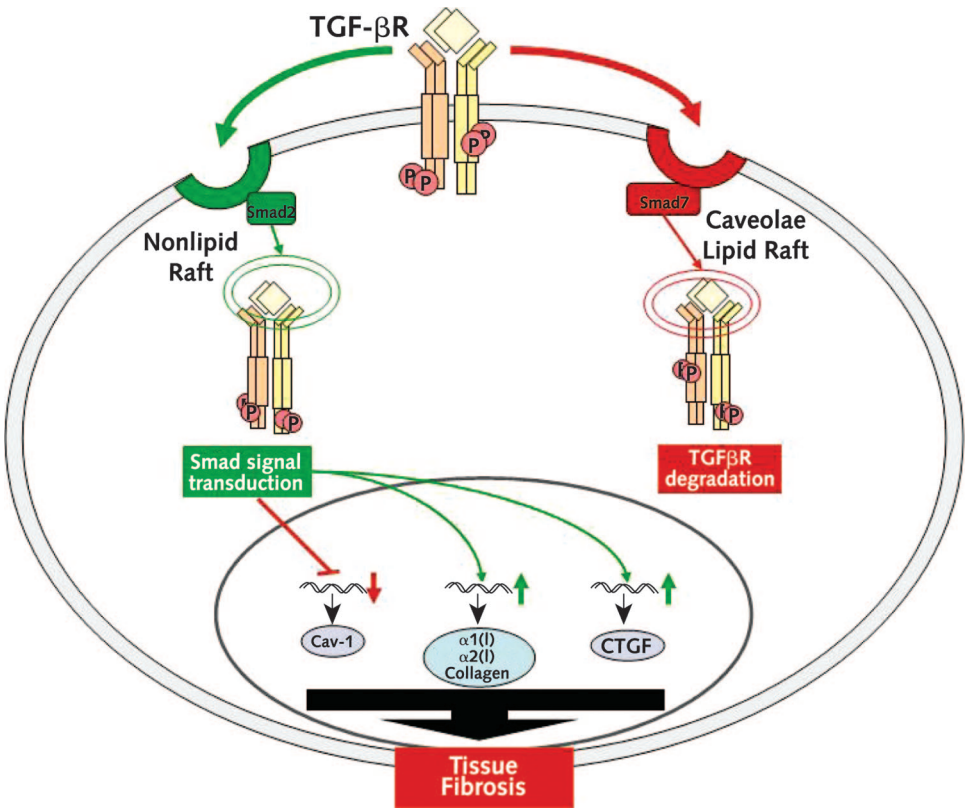
enriched microdomains in cell membranes that serve as organizing centers for the assembly of signaling molecules. Caveolin-1 plays an important role in TGF-β–signaling regulation because it is involved in the internalization of TGF-β receptors (**Figure 2**). Non–caveolin-associated internalization increases TGF-β signaling, but caveolin-associated internalization increases the degradation of TGF-β receptors, thereby decreasing or abolishing TGF-β signaling (61, 62). This novel mechanism of regulation of the activity of TGF-β receptors occurs after TGF-β binds to its receptors. Thus, caveolin-1 has emerged as a crucial regulator of TGF-β intracellular signaling and degradation of TGF-β receptors, and alterations in these mechanisms play a role in the pathogenesis of idiopathic pulmonary fibrosis and systemic sclerosis (63–65).

Other Pathways Activated by TGF-β

Signaling cascades independent of Smad2 and Smad3 mediate important TGF-β effects (**Figure 1**). These pathways may become activated in a cell-specific and stimulus-dependent manner (66–70). The nonclassic pathways activated by TGF-β involve various important kinases. Among these pathways is the one involving the nonreceptor tyrosine kinase cellular Abelson (c-Abl) (see Glossary) (69, 70). Although the signaling events downstream of c-Abl need further clarification, c-Abl is required for activation of Smad1 and for stimulation of collagen production through a signaling mechanism independent of Smad2 and Smad3 (71).

Another TGF-β pathway involves protein kinase C-δ (PKC-δ) (see Glossary). In response to TGF-β, PKC-δ is phosphorylated; phosphorylated PKC-δ then removes inhibitory factors from the collagen gene promoter in the nucleus, which increases the transcriptional activity of the collagen gene. These observations are consistent with earlier studies demonstrating that inhibition of PKC-δ by pharmacologic or molecular biological techniques diminished the in-

Figure 2. **Model for involvement of caveolae in TGF-β signal transduction and downregulation resulting in fibrosis and caveolin-1 downregulation.**



Receptors activated by TGF-β can be internalized through 2 distinct endocytic pathways. The nonlipid raft pathway (*green*) increases TGF-β–related signal transduction, leading to tissue fibrosis and simultaneous transcriptional downregulation of Cav-1 gene expression, thus generating a vicious cycle that increases and perpetuates tissue fibrosis. In contrast, the Cav-1–positive lipid raft compartment (*red*) drives TGF-β receptor degradation, which prevents tissue fibrosis. Cav-1 = caveolin-1; CTGF = connective tissue growth factor; P = phosphorylation; TGF-β = transforming growth factor-β; TGF-βR = TGF-β receptor.

creased collagen gene expression induced by TGF-β and that of cultured systemic sclerosis fibroblasts (72).

Novel Antifibrotic Approaches

Transforming growth factor-β signaling is an attractive therapeutic target for controlling fibrosis because of its prominent role in extracellular matrix regulation. However, the number and complexity of the TGF-β pathways make selection of appropriate therapeutic targets difficult; furthermore, targeting a single component in any one pathway may not be effective. Various strategies have been developed to block TGF-β effects, including the use of soluble TGF-βRII fragments; TGF-β–neutralizing antibodies; TGF-βRII kinase inhibitors; RNA expression inhibitors, such as antisense and small inhibitory RNA; and blocking oligonucleotides. However, intensive investigations applying these strategies in vitro as well as in vivo, including some human studies, have not been effective or are still in development, which makes them unavailable for clinical use.

Inhibition of TGF-β Signaling With Tyrosine Kinase Inhibitors

Protein kinase inhibitors are a relatively new class of therapeutic agents shown to be effective in several oncologic and chronic inflammatory diseases. Of particular interest is the recent demonstration that c-Abl is a critical participant in TGF-β signaling. The initial observations leading to this important discovery described a remarkable reduction of bone marrow fibrosis in patients who received treatment for chronic myelogenous leukemia with imatinib mesylate (see Glossary) (73–75). Imatinib mesylate is a small molecule that specifically inhibits several tyrosine kinases, including c-Abl, by blocking the binding of adenosine triphosphate to the active kinase site (**Figure 3**). As seen in **Figure 1**, inactivating c-Abl blocks several downstream effector molecules required for the full TGF-β fibrotic response, an effect documented in vitro in systemic sclerosis fibroblasts (76). In addition, imatinib mesylate has been shown to effectively prevent the development of organ fibrosis in the kidney, lung, liver, and skin in several

animal models (77–84). More recently, it has been used for treating fibrotic diseases, including systemic sclerosis, nephrogenic systemic fibrosis, and sclerodermatous graft-versus-host disease (85–91) and may represent a truly novel therapeutic approach for these diseases (92). However, this promising outlook is tempered by a wide spectrum of side effects, including congestive heart failure, edema, muscle cramps, diarrhea, anemia, neutropenia, and thrombocytopenia. Furthermore, a recent clinical trial of imatinib in patients with idiopathic pulmonary fibrosis (ClinicalTrials.gov registration number: NCT00131274) did not accomplish the primary outcome goals. Because a substantial number of patients with fibrotic diseases might not tolerate imatinib treatment, second-generation inhibitors with similar mechanisms of action but improved adverse effect profiles, such as dasatinib and nilotinib, are currently under investigation.

Blocking TGF-β Production and Activation

Pirfenidone has been studied in both experimental models of pulmonary fibrosis and clinical therapeutic trials of idiopathic pulmonary fibrosis (93–99). In experimental models of lung fibrosis, pirfenidone significantly reduced the influx of inflammatory cells, levels of TGF-β in lavage fluid, and tissue levels of TGF-β mRNA by suppressing TGF-β gene expression at the transcriptional level. In clinical trials of idiopathic pulmonary fibrosis, pirfenidone treatment produced a statistically significant improvement in the decline of vital capacity, although no difference was found between the pirfenidone and placebo groups in total lung capacity, diffusing capacity, or resting arterial oxygen levels.

The integrin (see Glossary) $\alpha v\beta 6$ expressed on epithelial cells is capable of activating matrix-bound latent TGF-β in the local environment (48, 100). Antibodies to $\alpha v\beta 6$ blocked the activation of TGF-β and prevented the development of experimental lung fibrosis (101–103). If this approach proved to be effective in humans, it would have the advantage of confining the blocking activity to sites of injury where $\alpha v\beta 6$ integrin is expressed without compromising the more general homeostatic effects of TGF-β.

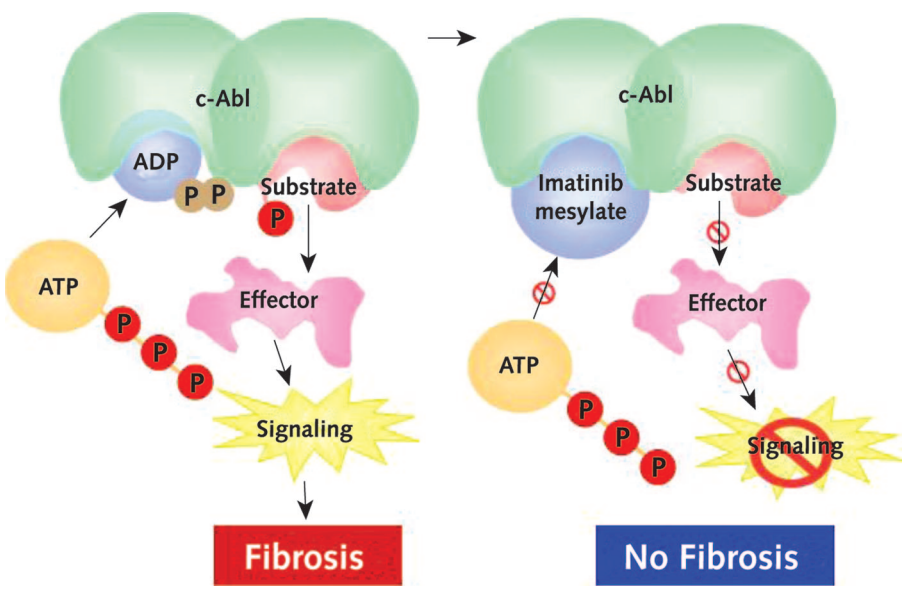
Inhibition of PKC-δ and Other Kinases

The demonstration of an important role of PKC-δ and other kinases in the exaggerated production of collagen and other extracellular matrix proteins in tissue fibrosis indicates that specific inhibitors of their functional activities may be effective therapeutic agents for the fibrotic diseases. Although still in the experimental stage, specific inhibitors of PKC-δ, such as cell-permeable peptides (104–107), or small-molecule inhibitors of other kinases that participate in tissue fibrosis (108–111) may prove to be highly selective and effective antifibrotic drugs in the future. Furthermore, these inhibitors may synergistically potentiate the antifibrotic effects of imatinib mesylate, as already shown in malignant cells (112).

Angiotensin Inhibitors

In certain circumstances, angiotensin II has remarkable profibrotic properties, acting at least in part through stimulation of TGF-β production (113–115). Although angiotensin-converting enzyme and inhibitors of the angiotensin-I receptor can block experimental models of

Figure 3. **Model illustrating the inhibition of c-Abl by imatinib.**



In the absence of imatinib, c-Abl binds ATP and activates substrate effector molecules by phosphorylation. Imatinib blocks the ATP binding site of c-Abl, effectively inhibiting its kinase activity. ADP = adenosine diphosphate; ATP = adenosine triphosphate; P = phosphorylation.

lung fibrosis, to our knowledge no published studies on the use of these drugs in humans with pulmonary fibrosis exist. Therefore, it is still unclear whether angiotensin-II inhibition would have beneficial effects on lung fibrosis or other fibrotic conditions, although the complex interactions between TGF-β and the angiotensin system (116, 117) suggest that pharmacologic inhibition of both pathways may yield synergistic effects in the treatment of fibrotic diseases.

Summary and Future Directions

Abnormal and exaggerated deposition of extracellular matrix is the hallmark of many fibrotic diseases, including systemic sclerosis and pulmonary, liver, and kidney fibrosis. The spectrum of affected organs, the usually progressive nature of the fibrotic process, and the large number of affected persons pose an enormous challenge and a serious economic burden to health care systems worldwide. Although no effective treatment exists at this time, recent research has uncovered an important and promising approach dependent on the role that TGF-β plays in pathways involving specific receptors and signaling molecules to stimulate extracellular matrix production. Studies have identified several potential targets, especially in nonclassic TGF-β signaling pathways, which are particularly attractive because their inhibition may downregulate the fibrotic response without impairing other important TGF-β functions. The best example of this approach is the use of imatinib mesylate to inhibit c-Abl. Recently acquired basic knowledge about the pathogenesis of the fibrotic process may lead to identification of novel drugs capable of affecting other elements of the complex pathways that lead to tissue fibrosis, such as inhibitors of PKC-δ or activators of caveolin-1 function, which could promptly translate into effective therapies for fibrotic diseases.

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