PROFIBROTIC GENE TARGETS IN FIBROSIS: A NARRATIVE REVIEW

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Abstract

Fibrosis is characterized by excessive accumulation of extracellular matrix components within organs and tissues. Genes play a vital role in determining the course of fibrosis. Numerous genes are either up-regulated or down regulated during the fibrotic disease process. Pro-fibrotic genes propel the disease towards fibrosis while antifibrotic genes down regulate the process of fibrosis. Knowledge regarding these genes could help in development of targeted strategies to alleviate fibrosis in various organs. The current article is prepared mainly using data collected from PubMed, cochrane and MEDLINE databases.

Keywords: Fibrosis, profibrotic genes, my of ibroblasts, TGFB1, SMAD, Collagen

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1. Introduction

Fibrosis is a pathological phenomenon involving excess deposition of ECM matrix in response to any form of chronic stimuli. Following any form of injury, the body responds in two ways to heal the injured site: Regeneration or repair. During the process of regeneration the damaged cells are substituted by new cells of the same type and is followed by reconstitution of the original cellular architecture. However during repair the injured tissues are replaced by fibrous tissue. Initially the process of repair is beneficial, nevertheless, when this process is not adequately regulated it results in excess deposition of ECM matrix wherein normal tissue is replaced by fibrous tissue ultimately resulting in scar tissue formation. Thus Fibrosis can be considered as an over-healing wound or an exaggerated wound healing process(1).

In any form of chronic injury, there is overexpression of pro-inflammatory mediators, cytokines and growth factors. These chemical mediators inturn interact with numerous signaling pathways and nuclear receptors, which switches the normal wound healing response to a pro fibrotic response. This pro-fibrotic signaling produces a domino effect resulting in the recruitment of more inflammatory cells and induces transformation of fibroblasts to myofibroblasts. Induction and activation of myofibroblast inturn promote the production of ECM. Prolonged myofibroblast activation produce large amounts of ECM which shifts the well balanced repair response to produce fibrosis(2,3).

Though sustained myofibroblast activation can induce excess ECM production, fibrosis per se occurs when the synthesis of new collagen exceeds the rate at which it is degraded by various MMPs, such that there is a increase in net total collagen overtime(2,3). Numerous additional factors may also confer the susceptibility to fibrosis. Genetic, epigenetic and age related alteration have all been implicated in the development and progression of fibrosis(4,5).

Genes play a vital role in determining the course of fibrosis. Numerous genes are either up-regulated or down regulated during the fibrotic disease process. Pro-fibrotic genes propel the disease towards fibrosis and include growth factors like transforming growth factor- beta (TGFβ), and fibroblast growth factor (FGF2), collagen genes like collagen type I alpha 1 (COL1A1), collagen type I alpha 2 (COL1A2), tumor necrosis factor (TNF), Connective tissue growth factor(CTGF), lysyl oxidase(LOX), tissue inhibitors of matrix metalloproteinases(TIMP), and matrix metalloproteinases (MMP11, MMP12, MMP19 and MMP23) (6–8).

Thus, the aim of the current study is to review the literature to assess the roles of various genes involved in fibrosis. A computerized literature search was carried out using MEDLINE, Cochrane and embase databases. Mesh phrases used were "fibrosis AND genes", "profibrotic AND genes", fibrosis, TGF- beta. Full text of the articles were examined and relevant articles were selected for the review.

Profibrotic Genes

Profibrotic genes initiate the signaling cascade that up-regulates collagen production or down regulates collagen degradation, ultimately, driving a disease towards fibrosis. Numerous genes with profibrotic potential have been identified in the literature. The most commonly involved profibrotic genes involved in organ fibrosis is briefly discussed below.

Tgfb1 Gene

This gene is located on the long arm of chromosome 19 at the position 13.2(19q13.2). The TGFB1 genes encodes for the transforming growth factor beta-1 protein. The TGF-β protein has three isoforms TGF-β1, TGF-β2, TGF-β3, and is involved in numerous cellular activities including proliferation, differentiation, motility and apoptosis of cells. Signal transduction for TGB-β begins with the binding of the protein to TGFβ receptor. This receptor has two subunits. Binding of the TGFβ protein phosphorylates the type 2 receptor kinase which inturn activates the TGFβ receptor type1 (TβRI). The activation of TGFβ signalling can occur through the canonical or non-canonical pathways. The canonical pathways is a SMAD-reliant pathway. SMADs are intracellular proteins which mediate the signal transduction of TGF-β. Phosphorylation of the TβRI leads to

phosphorylation of receptor specific SMAD (R-SMAD) proteins, which includes SMAD2 and SMAD3. Upon phosphorylation R-SMAD along with the SMAD 4, which acts as a co-SMAD, translocate to the nucleus and regulate gene expression(9). In the non-canonical pathway signal transcription occurs through other factors such as tumor necrosis factor, MAP kinase pathways, phosphatidylinositol-3-kinase/AKT pathways, Rho-like GTPase signaling pathways,JUN N-terminal kinase (JNK) or nuclear factor-κB (NF-κB)(10). The TGF-β superfamily of genes plays a vital role in initiating the fibrotic cascade. TGB-β promotes production of type I collagen, by stimulating the gene transcription of COL1A1 and *COL1A2*. TGB-β also influences MMP and TIMP expression which regulate the degradation of collagen. By down regulating the expression of MMPs and up-regulating the expression of TIMPs, TGF-β further promotes the fibrotic response(11,12).

Smad

SMAD are intracellular protein involved in TGF-β signaling. Eight SMAD proteins have been identified and have been mapped to four different chromosomes. SMAD 2, 4, 7 are located on 18q, SMAD 5, 6 on 15q, and SMAD 1, 8 are located on chromosome 4 and 13 respectively. SMAD 2, 3, 4 are involved in signal mediation while Smad6 and Smad7 are inhibitory SMADs, and compete with R-SMADs for binding to activated TβRI and disrupt signal transduction. When TGF-β bind to its receptors, the resulting signal transduction pathway in the cytoplasm involves activation and translocation of SMAD to the nucleus. The SMAD complex then binds to a specific segment of the DNA which activates the collagen genes to bring about fibrosis. Studies done on liver fibrosis models showed that deletion of SMAD3 resulted in inhibition of collagen expression suggesting that SMAD3 is profibrotic in liver fibrosis. On the other hand SMAD 7 was anti-fibrotic and negatively mediated the expression of SMAD3(12). Similarly in renal fibrinogenesis, hyperactivation of Smad3 and reduction of Smad7 is said to play the key pathological mechanism leading to fibrosis(13). In a study by Luong VH et al, human skin fibroblasts were stimulated with TGF-β that caused phosphorylation of Smad3 which inturn lead to the expression of α-SMA, Col1a2, FN1, and CTGF(14)

Serpine1: This gene is located on at the 7q22.1 position. The protein encoded by this plasminogen activator inhibitor 1 (PAI-1). PAI-1, is a potent inhibitor of fibrinolytic activity and is implicated in a variety of fibrotic diseases including liver, renal, pulmonary and skin fibrosis. PAI-1 controls the proteolytic activity of MMPs aswell as the migration of inflammatory cells and fibroblasts. Elevated levels of PAI-1 has been implicated to induce fibrosis by suppressing proteolytic activity thereby stabilizing the deposited collagen matrix(15,16). However in a study by Monfort et al they found that hepatic fibrosis was enhanced in PAI-$1^{-/-}$ mice after chronic CCl_4 administration and concluded that PAI-1 has both protective and damaging roles and that protection is conferred by helping maintain hepatocyte division after an injury(17).

Tnf Gene:

The TNF gene is located on the short arm of chromosome 6 at the 21.33 position. This gene encodes the Cytokines belonging to Tumor necrosis factor (TNF) superfamily. TNFα is mainly secreted by macrophages. It enhances HSC survival, hepatocyte death, and immune cell activation, which has been associated with enhanced liver fibrosis(18). Increased production of TNF-α in chronic kidney disease may also be involved in the progression to renal failure(19). Production of TNF by pro-fibrotic M2-cells, have also been implicated in cardiac inflammation and fibrosis.(20) However in contrast, TNF-α has an antifibrotic effect on dermal fibroblast and acts by inhibiting the transcription of fibronectin, type I collagen, and type III collagen(21).

Timp 1:

This gene is located on Xp11.3. The protein determined by this gene are also inhibitors of MMPs. They form complexes with MMPs, inactivating them irreversibly, thus promoting fibrosis. This gene has been implicated in a number of fibrotic disorders. In idiopathic pulmonary fibrosis there is a high expression of TIMPs when compared to collagenases supporting the notion of a non-degradable collagen matrix environment(22). In cardiac fibrosis models increased expression of TIMPs

were associated with increased αSMA levels and contributed to elevated collagen matrix production by cardiac myofibroblasts(23).

Lox:
This gene located on 5 q23 is responsible for encoding the enzyme lysyl oxidase. Lysyl oxidase plays a vital role in collagen crosslinking and has been implicated in a wide variety of fibrotic lesions. Excess lysyl oxidase results in collagen crosslinking and stabilization of collagen fibers making them resistant to degradation. The upregulation of LOX activity has been implicated in the development and progression of both cardiac and liver fibrosis through (24,25). LOX was also significantly higher in patients with diffuse Systemic sclerosis and authors have suggested its use as a biomarker for fibrosis in SSc(26). However, certain studies in IPF and glomerulosclerosis have shown that lysyl oxidase like enzymes LOXL1 AND LOXL2 contribute to collagen stabilization but not LOX(27,28).

Ccn2/ Ctgf:
The gene located on 6q23.2 encodes a mitogenic factor that is secreted by vascular endothelial cells. The production of CTGF/CCN2 is regulated primarily by TGF-beta. This protein is involved in the regulation of various signaling pathways involved in cell adhesion, angiogenesis, myofibroblast activation and differentiation and ECM deposition which together contribute to remodeling of tissues and ultimately fibrosis. CTGF is also involved in the modulation of factors like VEGF and BMPs which play an important role in the development and repair process, deregulation of which, can promote fibroplasia. CTGF inhibits BMP-7, which is a antifibrotic molecule that counteracts the pro-fibrotic effects of TGF-beta signaling. CTGF also promotes inflammatory cell infiltration via the NF-κB pathway which may contributes to fibrosis(29,30).

FGF2:
This gene that encodes the FGF family of protein is located in loci 4q28.1 and possess broad mitogenic and angiogenic activities. The role of FGF in fibrosis is controversial. In liver fibrosis FGF2-dependent induction of *CYGB* gene expression resulted in the deactivation of human HSCs thus attenuating fibrosis(31). In vitro lung fibrosis models, FGF 2 promotes myofibroblast differentiation and proliferation in cooperation with transforming growth factor-β1. However FGF2 acts as a protective growth factor after lung epithelial injury in vivo(32). In cardiac fibrosis models, FGF-2 attenuates human cardiac myofibroblast-mediated ECM thus reducing fibrosis. However in renal fibrosis FGF-2 contributes to autocrine fibroblast proliferation in post-inflammatory matrix synthesis(33,34).

NOX4:
This gene is located on chromosome 11 and codes for the NOX family of enzymes. It functions as the catalytic subunit of the NADPH oxidase complex. TGFβ is also a powerful regulator of NOX4 mRNA. These enzymes play a role in myofibroblast activation and have an essential role in initiating fibrosis through the production of reactive oxygen species(35). ROS when present at minimal concentration act as secondary messengers to cellular stimuli, however at high concentration it is toxic and induces cell death and promotes collagen production through various pathways which ultimately result in increased procollagen production.

Hif-1a:
This gene is located on chromosome14 and codes for a transcription factor that controls cellular responses to reduced oxygen concentrations within tissues. HIF-1α regulates numerous pro-fibrotic mediators and contributes to fibrosis. TGF-β1 induces HIF1A stabilization in fibroblasts even without prominent hypoxic conditions. HIF1A is suggested to target pyruvate dehydrogenase kinase that promotes glycolysis which inturn induces myofibroblast differentiation. Studies in lung fibroblasts have shown that deletion of HIF1A attenuated bleomycin induced fibrosis. Besides fibroblasts, HIF1A also promotes profibrotic responses in macrophages through increased production of mediators like interlukins(36).

Acta2
The ACTA2 gene located on 10q23.31 encodes one of the six actin proteins present within the cell. It encodes for smooth muscle actin which is involved in cell contractility and motility. A key feature of all fibrotic disorders,

is the transformation of fibroblasts to myofibroblasts, In fibrotic diseases there is an increased expression of alpha smooth muscle actin within these transformed myofibroblasts and these myofibroblasts are the ones that are shown to be the predominant source of type 1 collagen within the lesion. The expression of alpha smooth muscle actin has also been used as a reliable marker for hepatic stellate cells which precedes the deposition of fibrous matrix(37). However in renal fibrinogenesis it is said to suppress the profibrotic potential of myofibroblasts(38).

CCL11(C-C Motif Chemokine Ligand 11)

This gene encodes foe eotaxin, a chemokine which is a member of the CC subfamily. It functions as chemotactic agent for eosinophils. CCL11 and CCR3 are involved in the recruitments of inflammatory cells and are thought to play a role in bleomycin induced fibrosis in lungs(39). Plasma levels of eotaxin were also found to correlate with the progression of liver fibrosis(40). Thus by recruiting granulocytes to the site of injury this chemokine is thought to play a role in the initiation and progression of fibrosis.

Mmp

Proteins in this family are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis. The encoded preproprotein is proteolytically processed to generate the mature protease. This secreted protease breaks down the interstitial collagens, including types I, II, and III.

2. Conclusion

Numerous genes are involved in the process of fibrosis. Some are involved in the initiation process while other contribute to its progression. Currently, most of the treatment modalities aimed at treating or reversing fibrosis is organ specific and limited to particular tissue or organ. However, evidence demonstrates that the core molecular mechanism involved in fibrosis, is similar among different fibro-proliferative diseases, owing to the fact that the same genes are involved in initiating the pro-fibrotic responses across the range of fibrotic diseases. Hence, strategies aimed at silencing profibrotic genes or stimulating antifibrotic genes may thus, help alleviate fibrosis and prevent the subsequent sequele of scar tissue formation and organ failure.

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