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Fibroblast Growth Factor 2 as an Antifibrotic: Antagonism of Myofibroblast Differentiation and Suppression of Pro-Fibrotic Gene Expression

David M. Dolivo^a, Sara A. Larson^a, and Tanja Dominko, DVM, Ph.D.^{a,*}

^aWorcester Polytechnic Institute, Department of Biology and Biotechnology, 100 Institute Road, Worcester, MA, 01609

Abstract

Fibrosis is a pathological condition that is characterized by the replacement of dead or damaged tissue with a nonfunctional, mechanically aberrant scar, and fibrotic pathologies account for nearly half of all deaths worldwide. The causes of fibrosis differ somewhat from tissue to tissue and pathology to pathology, but in general some of the cellular and molecular mechanisms remain constant regardless of the specific pathology in question. One of the common mechanisms underlying fibrosis is the paradigm of the activated fibroblast, termed the “myofibroblast,” a differentiated mesenchymal cell with demonstrated contractile activity and a high rate of collagen deposition. Fibroblast growth factor 2 (FGF2), one of the members of the mammalian fibroblast growth factor family, is a cytokine with demonstrated antifibrotic activity in non-human animal, human, and *in vitro* models. FGF2 is highly pleiotropic and its receptors are present on many different cell types throughout the body, lending a great deal of variety to the potential mechanisms of FGF2 effects on fibrosis. However, recent reports demonstrate that a substantial contribution to the antifibrotic effects of FGF2 comes from the inhibitory effects of FGF2 on connective tissue fibroblasts, activated myofibroblasts, and myofibroblast progenitors. FGF2 demonstrates effects antagonistic towards fibroblast activation and towards mesenchymal transition of potential myofibroblast-forming cells, as well as promotes a gene expression paradigm more reminiscent of regenerative healing, such as that which occurs in the fetal wound healing response, than fibrotic resolution. With a better understanding of the mechanisms by which FGF2 alters the wound healing cascade and results in a shift away from scar formation and towards functional tissue regeneration, we may be able to further address the critical need of therapy for varied fibrotic pathologies across myriad tissue types.

*Corresponding Author: Tanja Dominko, Biology and Biotechnology Department, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609, Telephone: 508-831-4108, tdominko@wpi.edu.

Competing Interests

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Conflict of Interest Form

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Keywords

FGF2; Fibrosis; Fibroblast; Myofibroblast; TGF- β

Introduction

Fibrotic pathologies account for nearly half of all deaths worldwide, emphasizing the need for anti-fibrotic agents and therapies. Fibroproliferative diseases have been described in organs as varied as the lungs, kidney, liver, eye, heart, and skin, among others. Though each fibrotic pathology has causal factors and pathological manifestations specific to that organ and that condition, it appears that there are common mechanisms underlying many fibrotic pathologies, lending potential translational value to research focused on specific fibroses [1].

One common mechanism underlying fibrotic pathology is contraction of the wound area and subsequent deposition of large quantities of collagen by a cell type known as the myofibroblast [2]. Myofibroblasts are characterized by their morphology, functional properties, and gene expression. Compared to non-activated fibroblasts, myofibroblasts demonstrate formation of supermature focal adhesions, which are larger in area and thus are able to exert more stress, contributing to the maintenance of myofibroblastic phenotypes [3]. Myofibroblasts contain contractile bundles, known as stress fibers, rich in smooth muscle α -actin (α -SMA), which augments their ability to generate contractile force in the wound site. Myofibroblasts are also characterized as producing high quantities of extracellular matrix, particularly type I collagen and the fibronectin isoform known as extra domain A (ED-A) fibronectin [4].

Fibroblast activation to myofibroblasts is a normal part of wound healing. During the proliferative phase of wound healing, myofibroblasts are derived from activation of local connective tissue fibroblasts, and they can also arise from other sources including mesenchymal progenitor cells from the injured tissue or bone marrow, circulating fibrocytes, or even from neighboring epithelial cells via the epithelial-to-mesenchymal transition (EMT), among other sources depending on the tissue in question [5] (Figure 1). Ideally, at the resolution of the wound healing process, myofibroblasts undergo apoptosis to clear the wound site. However, under pathological conditions, myofibroblasts are retained at the site of the wound, where they partake in overproduction of collagen and deposition of a fibrotic scar [2, 6]. Many cytokines and growth factors have been interrogated for their roles in activation and resolution of myofibroblasts in the wound site, and in some cases investigated for their ability to modulate the fibrotic response. One of the most well-studied and promising of these is the cytokine known as fibroblast growth factor 2 (FGF2).

FGF2 signaling

FGF2, also known as basic fibroblast growth factor (bFGF), is one of the family of mammalian fibroblast growth factors consisting of at least 23 members (22 identified in humans to date). Fibroblast growth factors have been implicated in a wide array of cellular and organismal processes including embryonic development, pathogenesis, oncogenesis, and tissue repair, among many others. At the cellular level, the roles of fibroblast growth factors

prove every bit as myriad, where they contribute to processes as diverse as differentiation, metabolism, self-renewal, and proliferation, among others [7].

FGF2 is encoded by the *FGF2* gene, which is located on chromosome 4 in humans [8]. *FGF2* codes for a single transcript, which is translated into 5 different protein isoforms by the use of alternative, in-frame start codons. The shortest isoform, translation of which is initiated by a canonical 5'-AUG-3' start codon, is known as the low molecular weight or 18kDa FGF2 isoform. Each of the other isoforms (22kDa, 22.5kDa, 24kDa, and 34kDa, known as the high molecular weight isoforms) is a co-linear, N-terminal extension of the low molecular weight 18kDa FGF2 isoform, with translation initiated at one of four non-canonical 5'-CUG-3' start codons [9]. While the high molecular weight FGF2 isoforms have attracted significant attention from researchers in recent years, the overwhelming majority of the data, both clinical and mechanistic, regarding the antifibrotic effects of FGF2 in particular have been garnered from experimentation with low molecular weight FGF2. Thus, from here onward, "FGF2" will refer to the low molecular weight 18kDa isoform of FGF2 unless explicitly stated otherwise.

From cells that produce it, FGF2 is secreted via an unconventional mechanism that is not entirely understood, but that appears to act via direct translocation across the plasma membrane, rather than via vesicle-mediated exocytosis as is typical with most other secreted proteins [10]. As FGF2 is generally secreted, it thus acts predominantly in an autocrine or paracrine manner via its cell surface receptors, the fibroblast growth factor receptors (FGFRs). There are four genes encoding FGFRs, *FGFR1*, *FGFR2*, *FGFR3*, and *FGFR4*. Each of these genes encodes for an FGF-specific receptor tyrosine kinase. With the exception of *FGFR4*, each of the *FGFR* genes contains two receptor isoforms, denoted *FGFRx* IIIb or IIIc, where "x" refers to the gene from which the transcript is transcribed, and "IIIb" or "IIIc" refer to inclusion of mutually exclusive exons (exon IIIb or exon IIIc) in the particular transcript variant of the receptor [11]. Alongside the diversity present in the family of FGF ligands, this variable inclusion of alternate exons in FGFRs imparts an additional degree of receptor-ligand specificity to FGF signaling. While FGF1, the prototypic member of the FGF family, is able to bind and activate all of the FGF receptor variants, FGF2 is unable to activate FGFR2 IIIb and FGFR3 IIIb, accounting at least in part for the differences in action of these two growth factors under certain contexts [12]. When FGF2 comes into contact with its receptor, an interaction mediated by heparin sulfate proteoglycans on the cell surface [13–15], it induces structural changes in FGFR dimers leading to increased intracellular receptor tyrosine kinase activity [16]. Intracellular adaptor and effector proteins are recruited to transduce the growth factor signal, leading to stimulation of myriad pathways, most notably the mitogen-activated protein kinases (MAPKs) [17–20], but also other pathways such as Akt/mTOR [21–23], STAT [24–26], and PLC γ [27–30] (Figure 2). The myriad combinations of variant-specific and isoform-specific FGF/FGFR interactions, coupled with a variety of downstream signal transduction pathways, is responsible for the pleiotropy of FGF2 signaling specifically, and of fibroblast growth factor signaling more generally.

Of all of the fibroblast growth factors, FGF2 is one of the most well-studied members, particularly for its roles in osteogenesis [31–34], stem cell self-renewal [23, 35–38], and

tissue repair. Specifically, FGF2 has been recognized as a potent antifibrotic agent when applied to wounds of both non-human animals [39–42] and humans [43–47]. Though the role of FGF2 as an efficacious anti-fibrotic is clear, the mechanisms underlying its therapeutic effects are not entirely understood. Here we will review evidence regarding the mechanisms by which pharmacologic FGF2 acts as an antifibrotic agent, particularly through its actions on myofibroblasts and on cells that have the potential to become them, as myofibroblast activity is an integral paradigm underlying fibrotic pathology, and a paradigm in which FGF2 has been directly implicated.

Evidence for FGF2 as an antagonist of myofibroblast differentiation/activity

In dermal fibroblasts, there are a substantial number of reports implicating FGF2 in the inhibition of myofibroblast differentiation or attenuation of myofibroblast phenotypes or presence in the wound site.

FGF2 promotes myofibroblast apoptosis in vivo

In a rat skin wound model, FGF2 promoted wound healing and acceleration of wound closure concurrent with an alleviation in scar formation. FGF2-treated animals demonstrated reduced accumulation of types I and III collagen in the wound relative to control animals, and FGF2-treated rat skin stained positive by the apoptotic TUNEL assay in α -SMA⁺ cells (a common marker indicative of myofibroblasts) in FGF2-treated but not untreated rats, likely indicating the existence of FGF2-mediated apoptosis in wound myofibroblasts *in vivo* [42]. Concordantly, other reports detailed that administration of FGF2 to rat incisional wounds resulted in an increase in apoptosis of granulation tissue fibroblasts, and a subsequent decrease in tissue fibrosis [41, 48]. Interestingly, since most of the reports of FGF2-induced promotion of myofibroblast apoptosis have been performed *in vivo*, and based on reports in which FGF2 fails to induce myofibroblast apoptosis *in vitro* [49], it is likely that FGF2-mediated induction of apoptosis in myofibroblasts occurs at least in part cell-nonautonomously, via an indirect pathway involving other cell types. Taken together, evidence from these reports suggests that FGF2 has the ability to reduce the presence of aberrant myofibroblasts in the wound site through promotion of myofibroblast apoptosis.

FGF2 antagonizes activation and TGF- β signaling in fibroblasts

Extracellular TGF- β ligands transmit their biological signal via activation of transmembrane receptors on the cell surface, leading canonically to activation, via C-terminal phosphorylation, of intracellular signaling molecules known as SMADs. Receptor-activated SMAD2 and SMAD3 then associate with the co-SMAD, SMAD4, and the complex translocates to the nucleus. The nuclear SMAD complex associates with other adaptor proteins to enhance its activity and binds SMAD-binding elements (SBEs), sequence-specific elements located in the promoters of TGF β -sensitive genes (Figure 3). TGF- β signaling is known to be the primary chemical mediator of fibroblast activation, as well as an essential player in diverse fibrotic disease states. Thus, it is unsurprising that antagonism between FGF2 and TGF- β , and the effects of this antagonism on cellular phenotypes, is a recurring theme in much of the literature on the antifibrotic effects of FGF2.

In corneal fibroblasts, FGF2 inhibited fibroblast activation, as assessed by a reduction in the number of α -SMA⁺ cells, while promoting fibroblast proliferation and migration, processes that tend to be inhibited by TGF- β and impaired in myofibroblasts [50]. In an *in vitro* wound model, primary rabbit corneal fibroblasts displayed decreased TGF- β 1 transcript in response to FGF2, implicating potential regulation by FGF2 of TGF- β at the transcript level [51]. Another report demonstrated that FGF2 also had the potential to reverse fibroblast activation while simultaneously downregulating protein expression of TGF- β RI/II, potentially inhibiting TGF- β at the level of regulation of the receptor [52]. Evidence for the downregulation of TGF- β receptor expression by FGF2 has also been reported in human skin-derived multipotent progenitor cells [53]. Exogenous FGF2 has been shown to downregulate protein expression of both connective tissue growth factor (CTGF) isoforms, expression of which is upregulated upon stimulation with TGF- β [54]. Thus, the effect on CTGF signaling by FGF2 may be a direct consequence of its antagonism of TGF- β signaling. CTGF is an important profibrotic mediator with demonstrated roles in various organ fibroses including not only that of the cornea [55, 56] but also the heart [57–59], the liver [60], and the skin, among others [61–64]. Thus, a decrease in CTGF expression would be expected to result in improved healing outcomes. FGF2 attenuated TGF- β -mediated induction of α -SMA and contractile function in human cardiac myofibroblasts, as well as deposition of collagen and expression of TIMP1/2, resulting in a less myofibroblastic phenotype [49, 65]. Other evidence for antagonism between FGF2 and TGF- β signaling in cardiac fibroblasts includes the finding that overexpression of kinase-dead TGF- β RII resulted in an increase in proliferation, in a manner dependent on active FGFR1, and that this increase in proliferation was potentiated substantially by addition of exogenous FGF2, suggesting that TGF- β signaling inhibits FGF2/FGFR1-mediated mitogenesis in cardiac fibroblasts [66]. Abadalla et al. detailed that, in mouse embryonic fibroblasts, FGF2 attenuated TGF- β -mediated activation of Akt and subsequent increase in α -SMA protein via downregulation of serum response factor (SRF), a myofibroblastic transcription factor. This antagonism of Akt activation and α -SMA expression was ablated by addition of rapamycin, implicating mTOR in this FGF2-mediated effect [67]. Exogenous FGF2 dose-dependently inhibited synthesis of type I collagen and total collagen more generally in both primary healthy and keloid fibroblasts, processes known to be driven by TGF- β signaling, further suggesting a degree of generality to the inhibitory effect of FGF2 on collagen synthesis in fibroblasts [68]. Our lab recently demonstrated that FGF2 was able to inhibit TGF- β 1-mediated activation of several lines of primary adult and neonatal human dermal fibroblasts, and that this inhibition could be abrogated by treatment with small molecule inhibitors of ERK and JNK but not of p38. We also demonstrated that treatment with ERK or JNK inhibitor in the absence of exogenous FGF2 was sufficient to induce fibroblast activation, potentially implicating basal activation of these pathways in the promotion of myofibroblast phenotypes, or implicating well-established cross-talk between MAPK and TGF- β signaling more generally in this process [69]. Taken together, these data suggest that FGF2 has the ability to prevent or reverse fibroblast activation via various cellular pathways and mechanisms that are likely context-dependent.

Recent evidence suggests that some antifibrotic effects of FGF2 may proceed via microRNA-mediated mechanisms. MicroRNAs (miRNAs) are non-coding RNAs that have

the ability to regulate gene expression in a sequence-specific manner [70], and have been implicated in various disease states including but not limited to fibrosis [71–75]. In HUVECs, FGF2 induced Ras-dependent expression of miR-20a, for which putative sequence-specific binding sites exist in the 3'UTR of several TGF- β -related genes including the genes encoding TGF- β RI, TGF- β RII, and SMADs 2, 3, and 4. Induction of miR-20a by FGF2 also depended on the activity of ERK, PI3K, and JNK, but not PLC γ nor p38. Knockdown of miR20a attenuated the ability of FGF2 to inhibit TGF- β -mediated endothelial to mesenchymal transition (EndMT) as evidenced by rescue of SMAD2/3 activation, rescue of SM22 α , TGF β -RI, and TGF β -RII expression, and downregulation of VE-cadherin [76]. In mouse lens fiber cells, FGF2 has been determined to have a whole host of miRNA target genes, including genes encoding miRNAs that are involved in TGF β , MAPK, integrin, and Wnt signaling pathways, among others, each of which has been implicated in fibrotic pathology [77]. This suggests that miRNAs may serve as possible mediators through which FGF2 exerts its antifibrotic effects. Taken together, these reports suggest that FGF2 may inhibit myofibroblast formation at the wound site via miRNA-mediated mechanisms that target major cellular pathways that have been implicated in fibrosis.

FGF2-mediated downregulation of integrin α 11 may be partially responsible for myofibroblast antagonism

Integrin α 11 has been demonstrated to be upregulated in cells under mechanical strain [78], and it has been determined to be causally involved in the differentiation of fibroblasts into myofibroblasts in multiple pathogenic disease states [79–81]. Integrin α 11 is one of the integrin binding partners of integrin β 1, and the α 11 β 1 heterodimer is the dominant collagen receptor in embryonic fibroblasts. Integrin α 11 is induced by canonical TGF- β 1/SMAD signaling in primary fibroblasts, and this property of TGF- β responsiveness has been mapped to a SMAD2/3 binding site within the integrin α 11 promoter [82, 83]. Our lab recently reported that administration of exogenous FGF2 to adult human dermal fibroblasts results in substantial downregulation of basal integrin α 11 expression, as well as inhibition of TGF- β -mediated integrin α 11 expression [84]. In addition to attachment to type I collagen, α 11 β 1 also promotes FAK and AKT signaling and results in pro-survival phenotypes in human mesenchymal stem cells [85]. It has also been determined that glycated collagen, such as that found in the cardiac interstitium of diabetic patients, activates TGF- β 2/SMAD3-mediated activation of integrin α 11, contributing to fibroblast activation and subsequent cardiac fibrosis [83]. Further evidence implicating integrin α 11 signaling in fibrotic pathology comes from observations that abrogation of integrin β 1 signaling (the heterodimeric partner of integrin α 11 which is also upregulated under fibrotic conditions) is sufficient to block or reverse fibrotic phenotypes and gene expression paradigms in myofibroblastic activated hepatic stellate cells *in vitro* [86]. Taken together, these data suggest that FGF2 may lessen the fibrotic pathophysiological response by antagonism of fibroblast activation via downregulation of integrin α 11 signaling.

FGF2 antagonizes contractile phenotypes and myofibroblast differentiation of non-fibroblast progenitors

As mentioned earlier, several other cell types, aside from resident fibroblasts, can differentiate to form myofibroblasts at the wound site and thus may contribute to the fibroproliferative response *in vivo* (Figure 1). Recent evidence has demonstrated the ability of FGF2 to antagonize myofibroblast differentiation from some of these progenitor populations as well.

Culture of retinal pericytes in the presence of exogenous FGF2 maintained a highly proliferative, non-contractile phenotype devoid of α -SMA expression, whereas removal of FGF2 or culture in the presence of TGF- β 1 induced withdrawal from the cell cycle and promoted a contractile phenotype [87]. Li et al. demonstrated that seeding of FGF2-producing, bone marrow-derived mesenchymal stem cells into a graphene scaffold and implantation into a dorsal excision wound model resulted in faster healing and a decrease in expression of fibrosis-associated genes encoding α -SMA and TGF- β 1, and an increase in expression of the gene encoding the transforming growth factor family member TGF- β 3 [88]. TGF- β 3 has demonstrated antifibrotic activity *in vivo* [89], and elevated TGF- β 3/TGF- β 1 ratios have been associated with scarless healing phenotypes including those of oral mucosa [90] and those induced by tattooing [91]. In human valvular interstitial cells, which are of mesenchymal origin and are largely myofibroblastic in phenotype, addition of exogenous FGF2 reduced cell area, contractile activity, and a host of myofibroblast markers and deposition of ECM proteins [92]. Valvular interstitial cells demonstrate a classical activation response to TGF- β , and it has been reported that FGF2 has the ability to impede this TGF- β response in an ERK-dependent manner, likely via a decrease in SMAD-driven promoter activity through inhibition of SMAD3 nuclear accumulation [93]. Though the mechanisms underlying the inhibition of nuclear translocation of SMAD3 by FGF2 are not entirely clear, it may be the case that MAPK signaling induced by FGF2 regulates the phosphorylation status of the linker region of SMAD signal transducers, the effects of which are context-dependent and not entirely understood, but are known to affect SMAD localization and, intriguingly, extracellular matrix synthesis in various contexts [94–96]. In vascular smooth muscle cells, FGF2 promotes proliferation, which is associated with a synthetic, non-contractile phenotype compared to a contractile phenotype, analogous to the phenotypic switch between more highly proliferative, less contractile fibroblasts and less proliferative, more contractile and matrix-synthetic myofibroblasts [97]. In primary human aortic smooth muscle cells, knockdown of FGFR1 or of the adaptor protein FRS2 α potentiated TGF- β signaling and induced a contractile phenotype. In contrast, addition of exogenous FGF2 or overexpression of constitutively active FGFR1 each maintained smooth muscle cells in a proliferative, noncontractile state, suggesting that signaling downstream of FGFR1 is responsible, at least in part, for the antagonism of contractile phenotype in these cells demonstrated upon addition of exogenous FGF2, and may provide hints to the identity of the pathways underlying the anti-contractile properties witnessed in other cell types such as valvular interstitial cells or fibroblasts [98]. Similarly, in human airway smooth muscle cells, exogenous FGF2 demonstrated ERK-dependent attenuation of TGF- β -induced contractile activity and expression of smooth muscle/myofibroblast-associated contractile

proteins α -SMA, calponin and SM22 α . This antagonism of contractile activity may be independent of direct effects on canonical TGF- β signaling, or at least may be downstream of canonical SMAD phosphorylation, as exogenous FGF2 did not affect the levels of p-SMAD2/3 induced by TGF- β . FGF2 was able to both inhibit *de novo* TGF- β -mediated contractile protein expression and to reverse existing contractile protein expression, when added concurrently with, or 24 hours after, TGF- β respectively. This effect was demonstrated even in the presence of TGF- β RI inhibitor, suggesting that the anti-contractile effect of FGF2 is unlikely to be time sensitive, at least in this context, and is independent of TGF- β RI activity [99]. Taken together these data suggest that FGF2 can inhibit contractile activity and myofibroblast phenotypes in cell types other than fibroblasts, likely at least in part by interference with signal transduction via TGF- β and SMADs.

Evidence also exists that FGF2 has the ability to suppress acquisition of mesenchymal markers and myofibroblastic phenotypes in other cell types. In primary mouse lens epithelial cells, FGF2 antagonized TGF- β 2-mediated epithelial-to-mesenchymal transition (EMT), a form of transdifferentiation involved in the establishment of a fibrotic eye disease known as posterior capsule opacification. FGF2-treated lens epithelial cells demonstrated diminished presence of stress fibers and α -SMA expression, a process reversed by ERK inhibition [100]. In lung epithelial cells, FGF1 (the closest family member to FGF2) contributed to the maintenance of epithelial morphology and phenotypes in contrast to TGF- β 1 treatment, as assessed by an increase in expression of E-Cadherin and decrease in expression of α -SMA, in a manner also dependent on ERK [101]. Recently, Wang et al. reported that FGF2 attenuated the differentiation of rat epidermal stem cells into myofibroblasts, as assessed by downregulation of Col I, Col III, and α -SMA, in a manner dependent on both FGFR activation and Notch/Jagged signaling. *In vivo*, pharmacologic application of FGF2 to a rabbit ear wound model resulted in accelerated wound closure and better scar quality as determined by histological analysis. FGF2 treatment resulted in a greater proportion of proliferative epidermal cells and a lesser proportion of proliferative mesenchymal (α -SMA⁺) cells, suggesting that FGF2 may promote retention of epidermal stem cells in their progenitor state *in vitro* by antagonizing differentiation to myofibroblasts or myofibroblast-like cells [102]. Similarly, in mouse lymphatic endothelial cells, FGF2 suppressed mesenchymal phenotypes and preserved endothelial phenotypes, while depletion of FGF2 or addition of TGF- β resulted in promotion of the endothelial-to-mesenchymal transition (EndMT). Overexpression of H-Ras rescued the EndMT-suppressing effects of FGF2 depletion via inhibition of TGF β -mediated SMAD2 activation, implicating FGF2/FGFR/Ras in TGF β signaling and maintenance of endothelial cellular identity. Knockdown of endogenous Ras reduced endothelial markers, suggesting that baseline Ras activity maintains endothelial and suppresses mesenchymal phenotypes in endothelial cells [103]. Taken together, these data suggest that FGF2 is able to prevent or reverse transdifferentiation to myofibroblasts from precursors such as endothelial and epithelial cells.

FGF2 suppresses pro-fibrotic gene expression

In addition to its ability to inhibit or antagonize differentiation of progenitor cells to myofibroblasts, FGF2 is also able to shift the gene expression profiles, particularly of fibroblasts, to a paradigm less supportive of fibrosis.

In human dermal fibroblasts derived from both healthy and fibrotic human skin, exogenous administration of recombinant human FGF2 resulted in a shift in gene expression to a more anti-fibrotic signature. FGF2-treated fibroblasts demonstrated decreased expression of transcripts encoding collagen I, collagen III, α -SMA, and the interstitial collagenase MMP-1 [104]. MMP-1 has also been demonstrated to be sensitive to FGF2 stimulation in varied cell types including osteoblasts and osteoblast-enriched calvariae [105–107] and smooth muscle cells [108], alongside additional reports in fibroblasts [109, 110]. MMP-1 degrades interstitial collagen fibrils, including those of type I collagen, which is deposited in large quantities during the process of fibrosis. Thus it is likely that MMP-1 expression induced by FGF2 and subsequent degradation of fibrotic type I collagen mediates, in part, the antifibrotic effect of pharmacologic FGF2.

There are several reports of FGF2 regulating expression of other cytokines that are involved in fibrosis. In adipose-derived stem cells which, like fibroblasts, are derived from mesenchymal progenitors, FGF2 upregulated expression of the antifibrotic cytokine HGF in a manner dependent on JNK activity. *In vivo* inhibition of JNK, HGF, or FGF-2 in a mouse model of adipose tissue ischemia-reperfusion injury resulted in a significant increase in the severity of fibrosis as assessed by fibrotic area. Coupled with the *in vitro* data, it appears that one of the mechanisms by which FGF2 inhibits fibrosis *in vivo* may be via JNK-dependent activation of HGF [111]. Other reports have indicated that FGF2 upregulates HGF expression in fibroblasts as well, suggesting a degree of generality to this relationship [104, 110]. HGF has recently been implicated in the scarless wound healing phenotype of human oral mucosa fibroblasts, as knockdown of HGF was sufficient to sensitize oral mucosa fibroblasts to activation by TGF- β [112]. HGF has also been demonstrated to have anti-fibrotic activity not only in the liver, as might be expected from its name [113, 114], but also in the lungs [115], the heart [116], and the skin [117, 118], thus potentially implicating the actions of HGF in the anti-fibrotic activity of FGF2 as well. Another report demonstrated a shift, similar to those that have been reported previously, in gene expression profile of human hypertrophic scar fibroblasts upon administration of exogenous FGF2, but also demonstrated additional changes in gene expression, including a decrease in the transcripts encoding the ECM protein fibronectin, the collagen maturation enzymes lysyl hydroxylase and prolyl hydroxylase, the collagenase inhibitor TIMP1, and the pro-fibrotic matricellular protein CTGF. FGF2 also induced gene expression associated with a reduction in canonical TGF- β signaling via a decrease in expression of the TGF- β signal transducer SMAD2 and an increase in the TGF- β signal inhibitor SMAD7 [42] (Figure 3). Our lab demonstrated, in a transcriptome-wide analysis, a similar shift in the gene expression of FGF2-treated adult primary human dermal fibroblasts to a less fibrotic profile. This shift included dysregulation of a substantial number of genes involved in inflammation, cytokine signaling, extracellular matrix deposition, and cytoskeletal activity, processes intimately involved in the development and maintenance of fibrosis at the cellular and tissue levels [110]. Specifically, exogenous FGF2 resulted in a substantial downregulation of expression of the pro-fibrotic cytokine interleukin 6 (IL-6), a finding that has also been described elsewhere, though this activity appears to be context-dependent and/or cell type-specific [119–122]. Due to the data demonstrating that FGF2 can negatively regulate IL-6 and the well-established links between interleukin-6 in fibrosis, further discussion of this relationship is warranted.

Interleukin 6 is a fibrotic mediator and correlates with severity of fibrotic phenotypes

IL-6 is a critical mediator of fibrosis, and a pleiotropic cytokine that signals via a heterodimeric receptor construct consisting of IL-6R and gp130. Upon stimulation, IL-6R results in activation of the JAK/STAT pathways, through STAT3 specifically [123]. IL-6 signaling has been demonstrated to be sufficient to induce scarring in an otherwise non-scarring fetal skin graft wound model. Indeed, the scarless wound healing response of fetal skin has been associated in part with the diminished production of IL-6 in response to stimulation by PDGF [124]. The fibrotic molecular targets of IL-6 are myriad. In human dermal fibroblasts, treatment with exogenous IL-6 induces production of collagen and glycosaminoglycans in a dose-dependent manner [125]. In dermal fibroblasts from IL-6 knockout mice, exogenous recombinant IL-6 modulates MMP and TIMP expression, dysregulating ECM remodeling and composition [126], induces expression of TGF- β 1 [127], and upregulates the myofibroblast-associated contractile protein α -SMA [128]. IL-6/STAT3 signaling was implicated in the pathology of hypertrophic scar fibroblasts as an inducer of expression of *Col1A2* and *Fn*, as inhibition of STAT3 was sufficient to attenuate expression of these ECM genes, suggesting that IL-6 signaling may be overactive in hypertrophic scar fibroblasts relative to fibroblasts constituting non-pathological dermis [129]. In hepatic stellate cells, which are recognized as a major source of pathological myofibroblasts in liver fibrosis [130], IL-6 induces stellate cell activation into myofibroblast-like cells that express elevated levels of type I collagen and α -SMA, in a manner dependent on both STAT3 and MAPK activity [131, 132]. Thus, the roles of IL-6 in fibrotic pathology are well-established.

Unsurprisingly, treatments that exacerbate or attenuate fibrotic phenotypes also tend to induce or antagonize IL-6 expression, respectively. Keloid fibroblasts, which are pathologically similar to fibroblasts derived from hypertrophic scar, demonstrated decreased IL-6 expression alongside decreased p-STAT3, p-SMAD3, and types I and III collagen in response to sulforaphane, which also induced cell cycle arrest and apoptosis [133]. Pretreatment of mice with the superoxide dismutase mimetic manganese (III)tetrakis (4-benzoic acid) porphyrin (MnTBAP) resulted in attenuation of a subsequent fibrotic response upon administration with bleomycin, which was associated with a dampening of bleomycin-induced upregulation of IL-6, as well as of collagen, the profibrotic cytokine IL-8, vascular endothelial growth factor (VEGF), and several chemokine C-X-C motif ligand (CXCL) family members [134]. Administration of pirfenidone, a small molecule antifibrotic agent, attenuated TGF- β -mediated secretion of IL-6 in mouse pulmonary fibroblasts. Pirfenidone also potently attenuated the fibrotic response to bleomycin *in vivo* in the mouse lung, alongside an associated decrease in IL-6 measured in the bronchoalveolar lavage fluid [135]. Knockout of IL-10 is sufficient to induce scarring in fetal mouse wound models [136], likely via an increase in IL-6-mediated monocyte activation [137], as IL-10 has been shown to inhibit transcription of interleukin-6 [138]. IL-6 is a known target gene of the pro-fibrotic cytokine TGF- β in various cell types including fibroblasts [139, 140], and IL-6 has the ability to induce or synergize with TGF- β signaling in certain contexts [141–143]. Co-culture of human hypertrophic scar fibroblasts with adipose-derived stem cells attenuated their myofibroblastic properties as indicated by decreased expression of TGF- β 1 and decreased SMAD2/3 activation, decreased α -SMA expression and contractile ability,

decreased expression of fibrotic ECM components including collagen I and fibronectin, and decreased expression of IL-6 and activation of STAT3 [144]. Taken together these data suggest that the robust attenuation of pro-fibrotic gene expression patterns in fibroblasts mediated by FGF2 may stem in part from downregulation of IL-6, in a manner that antagonizes fibrotic cellular processes at the wound site.

Conclusion

There is ample evidence that exogenous FGF2 has the potential to attenuate fibrotic phenotypes and drive more desirable, regenerative resolution of wound healing in damaged tissue, characterized by lesser severity of scar deposition. However, the mechanisms by which FGF2 ameliorates fibrosis are not entirely understood. In this review we have described several proposed mechanisms through which administration of pharmacologic FGF2 may result in better wound healing outcomes overall. We have presented evidence that FGF2 promotes myofibroblast apoptosis *in vivo*, antagonizes pro-fibrotic TGF- β signaling, inhibits fibroblast activation and prevents transdifferentiation of non-fibroblasts into myofibroblasts, and promotes a less fibrotic gene expression paradigm. With a better understanding of the mechanisms by which FGF2 antagonizes fibrotic processes *in vivo*, and a more intimate understanding of the molecular pathways involved in FGF2-mediated antagonism of these processes, we hope to gain insight into the basic biology of the wound resolution process, as well as into possible ventures that may yield much-needed molecular antifibrotic therapies.

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Biographies

David Dolivo received a B.S. in Biochemistry from the University of Illinois at Chicago in 2012. In 2013 he was awarded an IGERT fellowship to pursue his Ph.D. in Biology and

Biotechnology at the Worcester Polytechnic Institute in Worcester, Massachusetts, where he currently resides as he completes his thesis research in the laboratory of Professor Tanja Dominko. David is focused on the cellular and molecular mechanisms of fibrosis and the contributions of FGFR and MAPK signaling to fibroproliferative disease phenotypes.

Sara Larson is currently working on completing her bachelors in Biology/Biotechnology at Worcester Polytechnic Institute and is planning to pursue a Ph.D. in the coming years. Her senior thesis and current research focuses on the cell biology of fibroblast activation.

Tanja Dominko received a Ph.D. in Endocrinology-Reproductive Physiology from the University of Wisconsin, Madison. Her postdoctoral studies at The Oregon Regional Primate Research Center in Portland, OR focused on assistive reproductive technologies for disease modeling in non-human primates. She is an Associate Professor at Worcester Polytechnic Institute in Worcester, Massachusetts where her laboratory is investigating molecular mechanisms involved in low oxygen-mediated FGF2 signaling during cellular reprogramming and fibroblast phenotype switching. This research is contributing to new strategies for regenerative medicine and wound healing.

Highlights

- FGF2 acts potently as an antifibrotic in non-human animal and human models.
- FGF2 promotes the apoptosis of myofibroblasts *in vivo*.
- FGF2 inhibits TGF- β signaling in fibroblasts and other myofibroblast progenitors.
- FGF2 promotes a gene expression paradigm reminiscent of regenerative healing.

Figure 1. Sources of wound myofibroblasts

Depending on the tissue, wound fibroblasts can be derived from various sources. Chemical signals in the wound site promote the migration of fibroblasts from neighboring tissues into the wound site. Additionally, mesenchymal progenitor cells from neighboring tissues, such as the bone marrow, can migrate to the wound site and differentiate into fibroblasts in order to take part in the wound healing process. Transdifferentiation can also contribute to the presence of fibroblasts at the wound site. For example, epithelial cells can undergo the epithelial-to-mesenchymal transition (EMT) whereby they gain enhanced motility characteristic of fibroblasts and can migrate to the wound site as well. In all of these cases, fibroblasts at the wound site can become activated to become myofibroblasts by chemical and physical factors, most notably transforming growth factor beta (TGF- β) signaling, where they contribute to wound contraction and deposition of extracellular matrix. Myofibroblasts can be characterized phenotypically by their large spread area and large, “supermature” focal adhesions, cytoplasmic stress fibers containing the actin isoform smooth muscle alpha actin (α -SMA), enhanced contractile activity, and high degree of synthesis and deposition of extracellular matrix molecules including collagen I and ED-A fibronectin.

Figure 2. FGF2/FGFR signaling

Schematic overview of FGF2/FGFR signaling. The extracellular FGF2 ligand binds its receptor, FGFR, in conjunction with cell surface heparin sulfate proteoglycans (HSPGs). This causes receptor dimerization and activation of intracellular tyrosine kinase (TK) domains. These tyrosine kinase domains can directly phosphorylate target signaling proteins such as JAK, leading to activation of JAK/STAT signaling, or PLC γ . Alternatively, the tyrosine kinase domains can phosphorylate adaptor proteins like FRS2 α , leading to activation of myriad downstream pathways. Adaptor protein-mediated signal transduction through PI3K leads to activation of AKT and, subsequently, mTOR. In parallel, activation of RAS transduces a signal that activates mitogen-activated protein kinases (MAPKs). RAS can activate RAF, which leads to phosphorylation and activation of ERK and subsequent ERK signaling targets, or RAC, which leads to activation of the MAPKs p38 and JNK. The diversity of FGF2/FGFR signal effectors accounts for much of the complexity and the pleiotropy of FGF2 signaling.

Figure 3. TGFβ signaling

Schematic overview of canonical TGF-β/SMAD signaling. The extracellular TGF-β ligand binds to the TGF-βRII receptor, which subsequently phosphorylates TGF-βRI and propagates the growth factor signal. In the canonical signaling pathway, TGF-βRI phosphorylates serine residues in the C-terminal, MH2 region of SMAD2 and SMAD3. When phosphorylated, these residues are able to associate with SMAD4 and translocate to the nucleus, where the SMAD complex associates with other protein co-factors (not shown) at SMAD binding elements (SBEs) located in the promoters of particular genes, driving expression of these genes. The inhibitory protein SMAD7 inhibits the interaction between TGF-βRI and SMAD2, thus preventing SMAD2 phosphorylation and subsequent association with SMAD4, which is necessary for nuclear translocation and promotion of further TGF-β-mediated signal transduction.