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LEADING ARTICLE

Targeting Focal Adhesion Kinase in Fibrotic Diseases

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Abstract Fibrotic diseases such as idiopathic pulmonary fibrosis or scleroderma (systemic sclerosis) are chronic fibroproliferative disorders for which there are currently no effective treatments. Dysregulated normal tissue repair process is considered to cause a fibrotic response culminating in compromised organ function due to excess extracellular matrix deposition. The mechanisms underlying the pathophysiology of fibrosis are poorly understood. Recent findings suggest that focal adhesion kinase (FAK) plays a key role in development of fibrotic disorders, and it appears to be an attractive target for antifibrotic therapy. Here, we review the emerging role of FAK as a key regulator of fibrotic signaling and its potential as a future therapeutic target to counteract fibrosis.

1 Introduction

Chronic fibrotic disorders, including idiopathic pulmonary fibrosis (IPF) or systemic sclerosis (SSc), contribute towards significant mortality and morbidity worldwide [1, 2]. Unfortunately, these diseases are unresponsive to the majority of currently available pharmacologic therapies [2]. Fibrogenesis is considered as the result of a dysregulated wound healing response where the activation of fibroblasts into alpha-smooth muscle actin (α-SMA)–positive myofibroblasts is an essential step in the evolution of fibrotic disorders. Myofibroblasts are responsible for the production and deposition of the extracellular matrix (ECM) components that are a hallmark of the disease, which leads to the destruction of organ architecture [3, 4]. The profibrotic factor transforming growth factor beta-1 (TGF-β1) has been shown to be an important mediator of tissue fibrosis and can induce myofibroblast formation in vitro and in vivo [5–9]. Recent work during the past 10 years has also focused interest on many other factors that fine tune myofibroblast differentiation, including the ED-A splice variant of fibronectin, endothelial-derived factors such as endothelin-1 (ET-1), the matricellular proteins, which include connective tissue growth factor (CCN2/CTGF) or variations in matrix stiffness [10–13]. These factors influence fibroblast adhesion to the matrix, typically through integrin binding and activation of focal adhesion kinase (FAK). In fact, it is now appreciated that increased adhesive signaling and FAK activation is a hallmark of lesional fibroblasts [14]. To date, the role for FAK in tissue fibrogenesis is just beginning to unravel, although a few studies have recently explored the role of FAK in lung and skin biology. The mechanisms leading to myofibroblast differentiation are not fully understood, and studies on the factors and signaling pathways that govern myofibroblast formation will be necessary for the design of new therapeutic strategies aimed at counteracting fibrotic disorders. In this review, we integrate recent findings regarding the emerging role of FAK in myofibroblast differentiation and fibrotic disorders such as IPF or SSc.

2 Focal Adhesion Kinase (FAK)

FAK is a 125-kDa non-receptor cytoplasmic tyrosine kinase that was firstly identified in 1992 as a highly tyrosine-phosphorylated protein that resides at sites of integrin

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clustering—the so-called focal adhesions [15–17]. Today FAK is well recognized as an important mediator of both integrin and growth-factor signaling [18]. FAK is a major regulator of cell proliferation, differentiation, survival and migration [19]. FAK is a ubiquitously expressed protein that is composed of an N-terminal FERM (protein 4.1, ezrin, radixin and moesin sequence homology) domain, a central kinase domain, 3 proline-rich regions and a C-terminal focal-adhesion targeting (FAT) domain (Fig. 1) [20]. The FERM domain binds to sequences in the cytoplasmic domain of β-integrin subunits targeting FAK to sites of integrin or growth factor receptor clustering and facilitates a signaling linkage from receptor tyrosine kinases (RTK) [21–23]. The FAT region at the C-terminal domain of FAK promotes the colocalization of FAK with integrins at focal contacts [24]. The C-terminal, non-catalytic domain of FAK, termed FRNK (FAK-related-non-kinase), is expressed independently in certain cells and may function as negative endogenous regulator of FAK kinase activity (Fig. 1) [25].

FAK activation is primarily mediated by autophosphorylation of FAK on Y397 that occurs in response to many stimuli, including environmental cues and soluble growth factor signaling through RTK or G protein coupled receptors (GPCRs) [26, 27]. Phosphorylation of FAK on Y397 results in a high affinity binding site for Src (sarcoma) kinase, thereby creating a functional bipartite kinase complex (Fig. 1) [28]. The association of Src with FAK results in the activation of the kinase activity of Src. Now, Src phosphorylates FAK on Y576 and Y577 within the FAK catalytic domain and leads to the full enzymatic activity of FAK [29]. Src can further phosphorylate FAK on Y407, Y861, and Y925 with phosphorylated Y925 acting as a docking site for growth-factor-receptor bound protein 2 (GRB2), which results in the activation of the RTK/Ras guanosine triphosphate (GTP)ase/mitogen-activated protein (MAP) kinase (MAPK) cascade (Fig. 1). Functionally, the FAK/Src complex regulates cell adhesion, motility, and migration, as well as cell growth and survival.

3 Role of FAK in Fibrosis

In the past two decades, extensive research has been performed to investigate the role of FAK during embryonic development and in the pathogenesis of human disease, including the progression of multiple mesenchymal and epithelial malignant tumors, Alzheimer's, rheumatoid arthritis, cardiac hypertrophy, hypertension, and atherosclerosis [30–35]; in addition, its role in fibrotic disorders such as SSc or IPF is now emerging. FAK has attracted special attention for its role in mediating fibrotic responses, and that its inhibition could be of therapeutic importance in counteracting profibrotic mechanisms. Recent in vitro and in vivo studies document the role of FAK in fibrotic signaling in mice and humans, although much remains to be explored regarding the mechanisms by which FAK contributes to fibrogenesis.

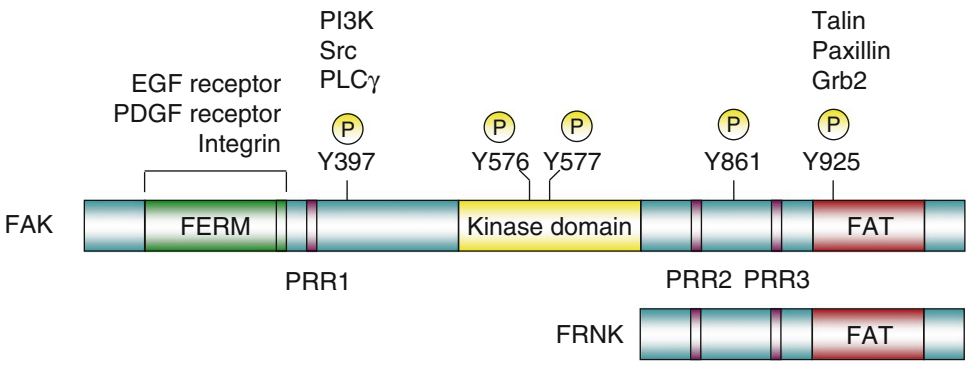

Fig. 1 Focal adhesion kinase (*FAK*) domains and phosphorylation sites. The N-terminal FERM (protein 4.1, ezrin, radixin and moesin sequence homology) domain directs interactions of FAK with epidermal growth factor (*EGF*) and platelet-derived growth factor (*PDGF*) receptors as well as integrins. FAK also contains three proline-rich regions (*PRR1–3*), which bind SH3 domain-containing proteins such as p130Cas (protein 130 kDa Crk-associated substrate), GRAF (GTPase regulator associated with FAK) and ASAP1 (ArfGAP with SH3 domain, ankyrin repeat and PH domain 1). FAK is phosphorylated (*P*) on different tyrosine residues, including Y397, Y407, Y576, Y577, Y861 and Y925. FAK phosphorylation on Y397 results in the binding of SH2 domain-containing proteins including sarcoma (*Src*), phospholipase C gamma (*PLCγ*) and phosphatidylinositol 3-kinase (*PI3 K*) among other proteins. Phosphorylation of Y576 and Y577 within the kinase domain drives FAK to full kinase activity. FAK phosphorylation at Y925 creates a binding site for GRB2 adaptor protein leading to mitogen-activated protein kinase (*MAPK*) cascade activation. The C-terminal domain encompasses the focal-adhesion targeting (*FAT*) domain that targets FAK to focal adhesions by associating with proteins such as talin and paxillin. The FAT domain also links FAK to the activation of Ras homolog gene family (*Rho*) GTPases by binding to p190 Rho guanine nucleotide exchange factor (*RhoGEF*). FAK-related non-kinase (*FRNK*) is autonomously expressed and has an identical sequence to the C-terminal domain of FAK. *GTP* guanosine triphosphate

3.1 Role of FAK in Myofibroblast Differentiation

FAK plays a central role in mediating adhesive signaling through integrin activation but also participates in transduction pathways activated by growth factors via GPCRs and RTKs (Fig. 2). Emerging in vitro data has focused interest on the regulatory mechanisms by which FAK contributes to myofibroblast formation in fibrotic diseases.

Myofibroblasts are pathogenic in pulmonary fibrotic disease due to their excessive production of ECM within the lung that typically results in respiratory failure. TGF-β1 is a well known inducer of myofibroblast differentiation in the lung, but the molecular mechanisms for this effect remain obscure. Recent data demonstrated that TGF-β1-induced myofibroblast differentiation of lung fibroblasts is dependent on adhesion-mediated signaling through FAK activation [36]. In this regard, pharmacologic inhibition of FAK or overexpression of kinase-deficient FAK inhibits TGF-β1-induced α-SMA expression [36, 37]. Recent molecular studies showed that JNK (c-Jun N-terminal kinase) and TAK1 (TGF-β-activated kinase 1) kinase operate downstream of FAK/Src in mediating fibrogenic responses upon TGF-β1 stimulation [38, 39]. Proliferation and expansion of interstitial fibroblasts are predominant features of progressive chronic kidney diseases. Thus, it has been shown that MAP-kinase activity necessary for TGF-β1-stimulated type I collagen expression requires FAK activity in human kidney mesangial cells [40]. In rat hepatic stellate cells (HSC), specific shRNA (short hairpin RNA) targeting of FAK attenuated ECM synthesis and

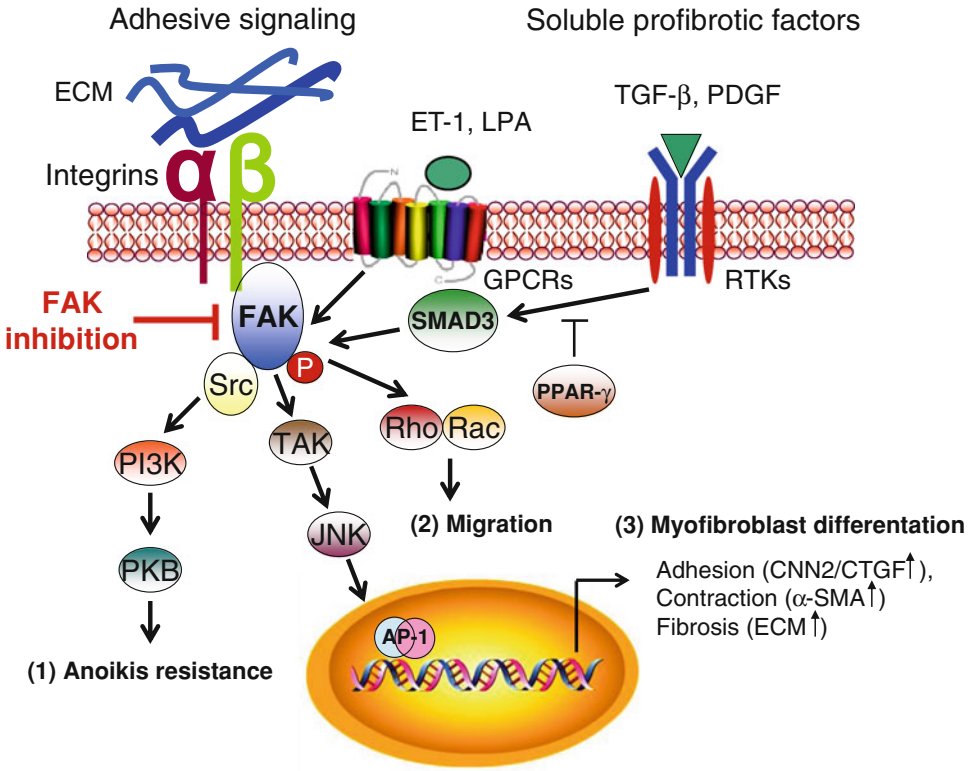

Fig. 2 Focal adhesion kinase (*FAK*) integrates growth factor and integrin signals to promote myofibroblast formation and fibrosis. FAK has been shown to be important in transducing mechanical stimuli to downstream biochemical pathways that lead to fibroblast activation. FAK is also involved in transducing signals from growth factors such as transforming growth factor beta-1 (*TGF-β1*) or platelet-derived growth factor (*PDGF*) through their specific receptor tyrosine kinases (*RTKs*). Several bioactive peptides, including endothelin, lysophosphatidic acid (*LPA*) and Ang II (angiotensin II), also activate FAK via their specific cell-surface G protein coupled receptor (*GPCR*). Taken together, these factors regulate anoikis resistance phenotype, cell migration, and myofibroblast differentiation in a FAK-dependent manner. TGF-β1 and endothelin-1 (*ET-1*) cause FAK activation leading to TGF-β-activated kinase 1 (*TAK1*) and c-Jun N-terminal kinase (*JNK*) phosphorylation to ultimately promote fibroblast activation. Therefore, migration and survival of alpha-smooth muscle actin (*α-SMA*)-positive myofibroblast-producing collagen type I are key steps for the development of fibrosis. Peroxisome proliferator-activated receptor-γ (*PPAR-γ*) ligands inhibit TGF-β1-induced myofibroblast differentiation by targeting the FAK pathway. Thus, FAK functions as a common target of multiple factors including environmental cues and soluble profibrotic factors. In summary, FAK is a central mediator of fibrogenesis and FAK inhibition may represent a useful therapeutic tool in the treatment of fibrotic diseases. *AP-1* Activator protein 1, *CCN2/CTGF* connective tissue growth factor, *ECM* extracellular matrix, *P* phosphate group, *PI3 K* phosphatidylinositol 3-kinase, *PKB* protein kinase B, *Rac* Ras-related C3 botulinum toxin substrate, *Rho* Ras homolog gene family, *SMAD3* Smad family member 3: the SMAD proteins are homologs of both the *Drosophila* protein, mothers against decapentaplegic (*MAD*) and the *Caenorhabditis elegans* protein SMA (from gene sma for small body size), *Src* sarcoma, ↑ indicates increase

promoted ECM degradation, making FAK a potential target for novel anti-fibrosis therapies in hepatic fibrosis [41]. Importantly, FAK has also been implicated in TGF-β1-induced renal tubular epithelial-to-mesenchymal transition (EMT) [42]. In contrast to this line of thinking, another study shows that FAK was not necessary for TGF-β1-mediated myofibroblast differentiation, but FAK was necessary for fibroblast growth factor and heparin (FGF/h)-mediated inhibition of myofibroblast differentiation [43].

ET-1 is widely accepted as one of the main profibrotic factors that cause fibroblast activation [44]. More recently, the present authors have shown that β1-integrin/FAK signaling directs ET-1-induced myofibroblast differentiation [45].

Recent experiments suggest that some endogenous mechanisms operate to downregulate FAK activation in myofibroblasts. Firstly, the non-catalytic FAK-related non-kinase FRNK has been shown to abrogate TGF-β1-induced myofibroblast differentiation in vitro and in vivo [46]. Prostaglandin E2 (PGE2) was also identified to have the potential to limit TGF-β1-induced myofibroblast differentiation by inhibiting FAK activation [47]. Similarly, peroxisome proliferator-activated receptor-γ (PPAR-γ), a key anti-fibrotic factor [48], when activated by its ligands, inhibits TGF-β1-induced profibrotic mechanism by targeting the FAK pathway [49].

3.2 Role of FAK in Cell Survival

Myofibroblast apoptosis is critical for the normal resolution of a wound healing program and impaired myofibroblast apoptosis is associated with tissue fibrosis [50]. FAK deactivation has been associated with apoptosis induced by loss of adhesion (termed 'anoikis') [51, 52]. TGF-β1 functions as an anti-apoptotic signal for fibroblasts/myofibroblasts during tissue fibrogenesis. Recently, it has been demonstrated that TGF-β1/SMAD3[1]/FAK signaling promotes anoikis-resistant myofibroblast phenotype [53]. Recent findings support the notion that TGF-β1 promotes an antiapoptotic phenotype in myofibroblasts by the activation of the FAK pathway and the prosurvival protein kinase B/Akt (PKB/Akt) pathway [37]. In this regard, FAK-mediated PKB/Akt activation in fibroblasts is under phosphatidylinositol 3-kinase (PI3K) control [51, 54]. Thus, the pathological FAK/PI3K/Akt signaling pathway enhances proliferation and survival of primary lung fibroblasts [51, 55]. In contrast, it has been recently shown that TGF-β1 co-ordinately and independently activates the FAK and AKT signaling pathways to confer an anoikis-resistant phenotype to myofibroblasts [53]. In a sequential model, PGE2 diminishes TGF-β1-induced phosphorylation of FAK and, in turn, limits activation of PKB/Akt pathway [47]. Moreover, another in vitro study defines a novel mechanism by which ET-1 promotes myofibroblast resistance to apoptosis through FAK-dependent upregulation of survivin [56].

3.3 Role of FAK in Cell Migration

Fibroblast and myofibroblast migration plays an important role in the normal wound healing process; however, increased cell migration may contribute to the accumulation of myofibroblast in the fibrotic lesions, thus contributing to the development of fibrosis [57]. FAK can regulate cell motility by influencing the cytoskeleton organization, structures of focal adhesions and membrane protrusions [18]. To note, FAK-deficient fibroblasts show significantly decreased cell migration, and re-expression of FAK in those cells restores the cell migration [58]. Expression of the mutated Y397F FAK effectively inhibits FAK-mediated cell migration. Previous studies have shown that FAK is activated in response to diverse pro-migratory stimuli including lysophosphatidic acid (LPA) and platelet-derived growth factor (PDGF) [59, 60]. In fact, LPA and PDGF-mediated myofibroblast migration through FAK activation contribute to the accumulation of hepatic myofibroblast and hence to the development of hepatic fibrosis [61]. Interestingly, endogenous FRNK expression is inversely correlated with FAK activation and cell migration rate in lung fibroblasts. Accordingly, FRNK overexpression abrogates cell migration, and blocks the activation of FAK in lung fibroblasts [46].

3.4 FAK in Human Fibrotic Disorders

Recent evidence clearly shows that fibrotic cells often display persistent FAK activation and enhanced adhesion capacity, but regulatory mechanisms for this effect remain elusive. Lesional skin fibroblasts taken from scleroderma patients display increased FAK expression and activity when compared with normal human skin fibroblasts [14]. TGF-β has been shown to activate FAK in human skin fibroblasts from control donors and SSc patients. Accordingly, pharmacologic or genetic FAK inhibition results in the downregulation of TGF-β-induced pro-fibrotic genes including collagen type I and α-SMA expression in fibroblasts [14]. In addition to the research on the skin, studies performed on pulmonary fibrosis provide further evidence of the role of FAK in fibrosis. We have recently shown that FAK expression and activity were upregulated in fibroblast foci and remodeled vessels from lung fibrosis patients [45]. Treatment of lung myofibroblasts with FAK inhibitors in these cells has been shown to abrogate the ability of TGF-

[1] Smad family member 3: the SMAD proteins are homologs of both the *Drosophila* protein, mothers against decapentaplegic (MAD) and the *Caenorhabditis elegans* protein SMA (from gene sma for small body size).

β1 or ET-1 to induce myofibroblast differentiation and collagen deposition [36, 45]. Moreover, previous data demonstrate that lung fibroblasts from IPF patients have increased cell migration and FAK phosphorylation when compared with normal human lung fibroblasts [62]. FRNK overexpression abrogated the increased cell migration, and the increased FAK activation, in IPF lung fibroblasts [62]. In conclusion, data from patients showed that persistently activated adhesion and adhesive signaling, including FAK activation, is a hallmark of fibrotic cells.

3.5 FAK in Animal Models of Fibrosis

Knockout of the *FAK* gene in mice is lethal [58]. Studies performed with conditional cell-specific inactivation of the *FAK* gene in mice have provided useful insights into the role of FAK in fibrosis in vivo. In normal mouse skin, low levels of FAK phosphorylation are detected. However, it has been reported that FAK is activated after cutaneous injury and skin scarring [63]. Previous studies using keratinocyte-specific FAK knockout mice reported no wound healing phenotype [64]. Alternatively, fibroblast-specific FAK conditional knockout mice subjected to a hypertrophic scar-like mouse model of cutaneous scarring demonstrated less fibrogenesis when compared with wild-type mice [63]. The present authors and others have recently showed that FAK expression and activity are up-regulated in fibrotic foci in animal models of fibrosis, suggesting a role for FAK in vivo to promote lung fibrosis [37, 45]. Thus we have demonstrated that pharmacologic or genetic inactivation of FAK resulted in marked attenuation of lung fibrosis in a mouse model [45]. Similarly, in vivo administration of AG1879, a dual protein kinase inhibitor of PKB/Akt and FAK, could inhibit lung fibrogenesis in vivo [37]. Additionally, FRNK knockout mice displayed increased fibrogenesis in response to a pulmonary fibrotic stimulus in vivo, as compared with wild type mice, suggesting that FRNK is an endogenous inhibitor of FAK signaling in vivo [46].

4 Therapeutic Potential of FAK Inhibition in Fibrotic Disorders

Fibrotic diseases are largely initiated by chronic inflammatory processes. Thus, early studies focused on the effects of corticosteroids with or without immunosuppressive drugs, because of their known anti-inflammatory effects. Unfortunately, a large number of trials have shown little or no effect of these drugs on the progression of fibrosis. To date, it is widely speculated that the key effector cell in fibrogenesis is the myofibroblast. Given the pivotal role of FAK in fibroblast activation, drugs targeting FAK actions are thought to be beneficial in counteracting fibrosis.

A variety of in vitro and in vivo studies using murine models of fibrotic diseases suggest that FAK inhibitors

Table 1 In vitro focal adhesion kinase (FAK) inhibition in fibrotic research

Drug/strategy	Cell type	In vitro effect	References
Pharmacologic inhibition			
PP2	Human fetal lung fibroblast	Prevented TGF-β1-induced α-SMA expression	[36]
AG1879	Mouse lung fibroblast	Reduced α-SMA expression and survival in lung fibroblasts derived from bleomycin-treated lungs	[37]
PF-562,271	Mouse embryonic fibroblast	Abrogated ET-1-induced profibrotic gene expression	[45]
PF-573,228	Human skin fibroblast	Dismissed collagen production and survival	[63]
Genetic inhibition			
FAK −/−	Mouse embryonic fibroblast	Unable to induce profibrotic gene expression upon TGF-β1 or ET-1 stimulation	[39, 45]
FAK shRNA	Hepatic stellate cell	Inhibited the expression of collagen I mRNA	[41]
FAK siRNA	Renal tubular epithelial cell	Abolished TGF-β1-induced renal tubular EMT	[42]
	Human Tenon's fibroblast	Prevented TGF-β1-induced expression of α-SMA	[74]
	Neonatal rat cardiac fibroblast	Impaired stretch-induced fibroblast activation	[75]
	NIH3T3 cell	Attenuated force-mediated α-SMA expression	[76]
FAK mutant	Human skin fibroblasts from scleroderma patients	Y397F point mutants inhibits α-SMA overexpression	[14]
	Human kidney mesangial cell	Y397F point mutants inhibits TGF-β-induced collagen expression	[40]
FRNK overexpression	Human lung fibroblast from IPF patients	Abrogated increased cell migration	[46]

α-SMA alpha-smooth muscle actin, *EMT* epithelial-to-mesenchymal transition, *ET-1* endothelin-1, *FRNK* FAK-related-non-kinase, *IPF* idiopathic pulmonary fibrosis, *shRNA* short hairpin RNA, *siRNA* small interfering RNA, *TGF-β1* transforming growth factor beta-1

Table 2 In vivo focal adhesion kinase (FAK) inhibition in fibrotic research

Drug/strategy	In vivo mouse model	In vivo effect	References
Pharmacologic inhibition			
PF-562,271	Bleomycin-induced lung fibrosis	Prevented lung fibrogenesis with non anti-inflammatory effect	[45]
PF-573,228	HTS-like model of cutaneous scarring	Less scar formation and reduced fibroblast proliferation	[63]
AG1879	Bleomycin-induced lung fibrosis	Prevented lung fibrogenesis with non anti-inflammatory effect	[37]
Genetic inhibition			
Keratinocyte-specific FAK knockout mice	6 mm full-thickness skin wound healing model	No wound healing phenotype	[64]
Fibroblast-specific FAK conditional knockout mice	HTS-like mouse model of cutaneous scarring	Less scarring	[63]
Myocyte-specific FAK knockout mice	Pressure overload–induced cardiac hypertrophy	Prevented cardiac hypertrophic and fibrosis	[77]
Cardiomyocyte-specific FAK knockout mice	Murine cardiac hypertrophy model induced by Ang II infusion	Increased hypertrophy and cardiac fibrosis	[78]
FRNK knockout mice	Bleomycin-induced lung fibrosis	Increased fibrogenesis and myofibroblast formation	[46]
siRNA FAK	Bleomycin-induced lung fibrosis	Prevented fibrogenesis with non anti-inflammatory effect	[45]
	Pressure overload–induced cardiac hypertrophy	Prevented cardiac hypertrophic and fibrosis	[79]

FRNK FAK-related-non-kinase, *HTS* hypertrophic scar, *siRNA* small interfering RNA

exhibit potent antifibrotic effects (Tables 1, 2). In recent years, several orally bioavailable adenosine triphosphate (ATP)-competitive FAK inhibitors have been developed by pharmaceutical companies and have entered into early human clinical trials [65, 66]. One of the first clinically available specific FAK inhibitors was PF-562,271, which inhibited FAK phosphorylation in vivo in a dose-dependent fashion in several human subcutaneous xenograft models [67]. Recently, the present authors showed that PF-562,271 also prevented bleomycin-induced lung fibrosis in a mouse model [45]. The phase I study using PF-562,271 was performed in patients with head and neck, prostatic, and pancreatic cancer (NCT00666926, http://clinicaltrials.gov/). Clinically, PF-562,271 prolonged disease stabilization in a subgroup of patients. Due to the low toxicity of this drug, combination therapies with blocking antibodies or antagonists/inhibitors of profibrotic factor receptors seem possible. To note, PF-04554878 (NCT00787033, http://clinicaltrials.gov/) and GSK2256098 (NCT00996671 and NCT01138033, http://clinicaltrials.gov/) are being evaluated in phase I clinical trials in healthy volunteers and cancer patients. No final results have been reported so far.

The utilization of FAK inhibitors, initially developed as anticancer drugs, may have several rationales in fibrotic conditions, e.g., targeting of separate profibrotic factor signaling such as TGF-β1, ET-1, and cell microenvironment signals; or inhibiting different pathologic processes such as myofibroblast differentiation, fibroblast migration, and fibroblast resistance to anoikis. Thus, the possible indication for FAK inhibitors may lie in the prevention of the formation, invasion, and/or recruitment of collagen-producing cells, independent of their tissue/cell origin. However, to our knowledge, there are no clinical studies that have reported the effects of FAK inhibitors in any fibrotic diseases. Thus, FAK inhibitors should be considered for clinical trials within the next few years in order to elucidate whether inhibition of the FAK pathway is efficacious for the treatment of human fibrotic disorders. Our preclinical results suggest that PF-562,271, which has thus far been well tolerated in healthy volunteers and cancer patients, may offer therapeutic power for the treatment of fibrosis-related diseases.

5 Combination Regimens with Other Targeted Therapies

Since FAK interacts with other signaling molecules and pathways (Figs. 1, 2), there are potential promising combinatory treatment options of FAK inhibitors and other drugs. Recent in vitro studies identified novel non-canonical smad TGF-β1 targets including FAK that are activated by TGF-β1 in fibroblasts [36, 39, 46, 47, 53]. Interestingly, FAK regulates the formation of a tripartite membrane

signaling complex in tumor cells that includes both TGF-β1 receptors and integrins [68]. Thus, combined FAK and TGF-β1 signaling inhibition may therefore have a biologic rationale. In fact, GC1008, which is a monoclonal antibody against TGF-β1 (NCT00356460, http://clinicaltrials.gov/), and PF-03446962, an antibody against one class I TGF-β receptor (NCT00557856, http://clinicaltrials.gov/), are in phase I testing. All together, these findings suggest that combined inhibition of TGF-β signaling and FAK, even in dose-sparing protocols, might be effective treatments in fibrogenesis. Reciprocal TGF-β-integrin signaling is normally implicated in a variety of pathologic processes including fibrogenesis [69, 70]. Accumulating evidence indicates that crosstalk between integrins and TGF-β signaling results in FAK activation [68]. Importantly, β1 integrin has been implicated in fibrosis and PF-04605412, a monoclonal antibody against α5β1 integrin, is being evaluated in phase I clinical trials in healthy volunteers and cancer patients (NCT009152783, http://clinicaltrials.gov/) [48, 70, 71]. Thus, combinatorial targeting of FAK and integrins may have a rationale to counteract human fibrogenesis.

Inhibition of intracellular pro-survival signal pathways may enhance the efficacy of FAK blockade. In this regard, due to its mutual activation, it is well known to have a synergistic effect of FAK and Src inhibition in cancer cells to promote cell apoptosis [72, 73]. Several small molecule inhibitors of Src including dasatinib are currently being investigated in clinical trials (NCT009152783, http://clinicaltrials.gov/). Recent data have described independent effects of the FAK and PKB/Akt pathway to trigger cell anoikis resistance [53]. Thus, combinatorial FAK and PKB/Akt treatments may have a rationale to induce myofibroblasts apoptosis. To note, Akt oral inhibitors are being evaluated in phase I clinical trials in healthy volunteers and cancer patients (NCT01266954, http://clinicaltrials.gov/).

6 Conclusion

Currently, there is no appropriate therapy available to counteract fibrotic disorders. The complexity of pathways operating to modulate fibroblast activation is only now becoming apparent. It is now known that the microenvironment plays an active role in fibroblast activation, hence in wound healing and fibrogenesis. Within this environment, fibroblasts respond to a host of signals including profibrotic growth factors such as TGFβ, ET-1, and CTGF and chemotactic factors such as LPA and PDGF, as well as signals from the extracellular matrix (Fig. 2). Targeting the pathways that mediate many of these signals has been a major goal in the effort to develop antifibrotic therapeutics. We emphasize that FAK is an attractive target for the therapy of fibrosis and needs further investigation.

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