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Fibrogenesis in kidney transplantation: potential targets for prevention and therapy

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Abstract

Kidney allograft fibrosis results from a reactive process mediated by humoral and cellular events and the activation of transforming growth factor beta-one (TGF- β 1). It is a process that involves both parenchymal and graft infiltrating cells and can lead to organ failure if injury persists or if the response to injury is excessive. In this review we will address the role of preventive and therapeutic strategies that target kidney allograft fibrogenesis. We conclude that in addition to preventive strategies, therapies based on BMP-7, HGF, CTGF and pirfenidone have shown promising results in preclinical studies. Clinical trials are needed to examine the effect of these therapies on long-term outcomes.

Summary

Kidney allograft fibrosis results from a reactive process mediated by humoral and cellular events and the activation of transforming growth factor beta-one (TGF- β 1). It is a process that involves both parenchymal and graft infiltrating cells and can lead to organ failure if injury persists or if the response to injury is excessive.

In this review we will address the role of preventive and therapeutic strategies that target kidney allograft fibrosis. We will examine these strategies based on their relationship to TGF- β 1, the primary profibrotic cytokine in the kidney. We will thus evaluate the effects of calcineurin-inhibitor minimization, rapamycin, chemokines, oxidative stress and RAS blockade on upstream events. We will also discuss the targeting of downstream molecules including TGF- β 1 and its signaling pathways, pirfenidone, Connective Tissue Growth Factor (CTGF), Vascular Endothelial Growth Factor (VEGF), Hepatocyte Growth Factor (HGF) and Bone morphogenetic Protein-7 (BMP-7).

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Author contribution:

Both Drs. Djamali and Samaniego

- * Participated in the design of the manuscript
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- * Participated in the performance of the research in the field of oxidative stress
- * Participated in data analysis

We conclude that in addition to preventive strategies, therapies based on BMP-7, HGF, CTGF and pirfenidone have shown promising results in preclinical studies. However, most of these emerging tools are still in an experimental phase and clinical trials are needed to examine their long-term effects in kidney transplantation.

Introduction

Fibrosis is the replacement of normal tissue by scar tissue as the result of a reactive or reparative process called fibrogenesis. While self-contained scar tissue may have no effect on long-term outcomes, fibrogenesis will result in organ failure if injury persists or if response to injury is excessive. Protocol biopsies have played an important role demonstrating that fibrosis occurs before renal dysfunction. A protocol biopsy study from the Mayo Clinic demonstrated that fibrosis alone was not a predictor of outcomes while coexistent fibrosis and inflammation (as a marker of ongoing injury) resulted in poor allograft survival (1). In this review, we will address both the mechanisms and therapies of fibrogenesis in kidney transplantation. Rather than providing an extensive list, our discussion will focus on the TGF- β 1 signaling pathway and epithelial-to-mesenchymal transition (EMT) given their respective roles in kidney fibrogenesis (2,3) (Figures 1,2). EMT has indeed been used as a surrogate marker of fibrogenesis in kidney allografts (3,4). It is a profibrotic process (primarily activated by TGF- β 1) in which tubular epithelial cells are progressively transformed into myofibroblasts. EMT includes the loss of cell-cell adhesion molecules and *de novo* expression of mesenchymal markers. These events are followed by tubular basement membrane disruption, cell migration and fibroblast invasion in the interstitium with production of profibrotic molecules including collagen and fibronectin. This topic was recently reviewed in depth (5). Although EMT is increasingly used as a surrogate of allograft fibrosis, this process is not the sole source of interstitial myofibroblasts which may also originate from local resident fibroblasts, pericytes, endothelial cells and bone-marrow derived cells (6).

(1) PREVENTIVE STRATEGIES

A detailed discussion of preventive strategies for acute rejection, infections and ischemia-reperfusion injury is out of the scope of this manuscript. Instead, we will focus on the role of more novel injury pathways including chemokines, oxidative stress, calcineurin-inhibitor minimization and RAS blockade.

(a) Chemokines—Chemokines are a family of small size (8-10 kd) chemotactic cytokines that mediate inflammation. So far, over 50 chemokines and 20 chemokine receptors have been identified.

Role in fibrogenesis: In transplantation, chemokines play a key role in the recruitment and activation of T-cells and monocyte/macrophages.

Experimental and clinical studies: BX-471 a chemokine receptor type 1 (CCR1) antagonist prevented the infiltration of T-cells and macrophages and decreased cell proliferation, myofibroblast activation and collagen deposition in rat kidney allografts (7) (Table 1). Consistent with these findings, CCR1 blockade successfully reduced renal injury and interstitial fibrosis in experimental models of nephrotic syndrome, lupus nephritis and unilateral ureteral obstruction (8-10).

Similarly, Met-RANTES, a chemokine receptor antagonist (CCR5) that blocks the effects of RANTES (regulated upon activation, normal T-cell expressed) improved proteinuria, glomerulosclerosis and tubulointerstitial fibrosis in rat kidney allografts (11). Met-RANTES

therapy also decreased macrophage recruitment, TGF- β 1 and PDGF mRNA levels in the allograft, suggesting an important role for RANTES in the pathogenesis of kidney allograft fibrogenesis (11).

Future directions: Chemokine inhibition strategies are being tested in clinical trials for multiple sclerosis, rheumatoid arthritis and psoriasis. However, the complex regulation of these molecules and their differential expression during acute and chronic rejection will require further preclinical and clinical studies in kidney transplantation (12).

(b) OS (Oxidative Stress)—OS is a term that signifies damage to DNA, proteins, lipids, carbohydrates, cells and tissues caused by reactive oxygen species (ROS). The balance between ROS production and antioxidant defenses defines the degree of OS in a given tissue (13).

Role in fibrogenesis: ROS play an important role in TGF- β 1-induced EMT primarily through activation of Mitogen Activated Protein-Erk Kinase (MEK) pathway and subsequent induction of Smad molecules in tubular epithelial cells (14).

Experimental and clinical studies: OS is increased in kidney allografts and is associated with EMT and chronic tubulointerstitial fibrosis (13) (Figures 1,2, Table 1). Inhibition of Nox enzymes (upstream generators of superoxide anion) with apocynin and diphenyleneiodonium prevented fibrogenesis and downregulated phospho-Smad2 in the Fisher-to-Lewis model (15). Consistent with these results, L-arginine decreased proteinuria and glomerulosclerosis in the same transplant model (16). However vitamin E supplementation alone did not prevent chronic allograft injury (17). In clinical transplantation, intra-operative intravenous injections of recombinant human SOD decreased the incidence of acute and chronic rejection (18) while treatment prior and after reperfusion made no difference in allograft function 48 hours after transplant (19). In aggregate, these studies demonstrate that OS is involved in the pathogenesis of kidney allograft fibrosis with a potential role as a common mechanism of injury (13).

Future directions: Antioxidant strategies may be beneficial in the prevention of fibrogenesis in kidney allografts. However, clinical trials are needed to determine the type, dose and timing of specific antioxidant interventions.

(c) Calcineurin inhibitor minimization strategies and sirolimus—CNIs including cyclosporine A (CsA) and tacrolimus, the cornerstone of maintenance immunosuppression, are also profibrotic agents making it difficult to reconcile effective immunosuppression with successful long-term allograft outcomes.

Since sirolimus (inhibitor of the mammalian target of rapamycin) has both immunosuppressant and antifibrotic properties; it was thought that its use might be beneficial in CNI sparing protocols. Despite the initial enthusiasm, studies have shown no consistent long-term benefit in patient and kidney outcomes in sirolimus-based CNI sparing protocols (20). Moreover, the therapeutic window of sirolimus is narrow with significant adverse effects including anemia, hyperlipidemia, delayed wound healing, peripheral edema and proteinuria thereby adding a significant element of morbidity. The recent ELITE-SYMPHONY trial provides a good example of the limited role of rapamycin in kidney transplantation (21). This multicenter randomized controlled trial of 1645 patients was initiated to assess whether a mycophenolate mofetil-based regimen would permit the administration of lower doses of adjunct immunosuppressive agents (e.g., CsA, tacrolimus, and sirolimus), yet maintaining an acceptable rate of acute rejection and a favorable tolerability profile. The regimen including low-dose tacrolimus resulted in better renal function, allograft survival and acute rejection rates while low-dose sirolimus was associated with worst graft outcomes and adverse event rates

(21). However, no protocol biopsies were performed to analyze the effect of immunosuppression on fibrosis.

Alternative drug-minimizing “tolerogenic” immunosuppressive strategies with Alemtuzumab and Belatacept are on their way in clinical transplantation. Alemtuzumab or Campath 1H is a monoclonal anti-CD52 antibody that was introduced to kidney transplantation in the late nineties with the hope of establishing near tolerance (22). Subsequent pilot studies with the drug alone or in monotherapy with sirolimus demonstrated high rates of acute antibody-mediated rejection leading to abandoning the concept of “Alemtuzumab-induced tolerance” (23,24). Long-term, prospective, randomized studies with Alemtuzumab are still necessary to determine the optimal immunosuppressive regimen with this antibody. Belatacept is an investigational humanized selective costimulation blocker antibody that has recently undergone phase II and III trials in kidney transplantation (25,26). Patients were randomly assigned to receive an intensive or a less-intensive regimen of Belatacept or cyclosporine. All patients received induction therapy with Basiliximab, Mycophenolate Mofetil, and corticosteroids. Final results from phase III trials (BENEFIT and BENEFIT-EXT studies) have not been published yet (26). However, preliminary data suggest that Belatacept therapy is associated with improved one year GFR and metabolic profile despite a greater risk of acute rejection and lymphoproliferative disorders. There is no information on the incidence of allograft fibrosis so far.

In aggregate, long-term studies are needed to determine whether the use of drug minimizing strategies is safe and associated with less allograft fibrosis.

(d) RAS (Renin-Angiotensin-System) blockade—RAS activates TGF- β 1, apoptosis, oxidative stress and atherogenesis in the cardiovascular system and the kidneys (27-29).

Role in fibrogenesis: RAS blockade has antifibrotic and antiproteinuric properties in experimental and clinical studies of kidney disease (30). ACE-inhibitors (ACE-I) and Ang II receptor blockers (ARB) have now become the first line of therapy in patients with chronic kidney disease and hypertension or proteinuria (30).

Experimental and clinical studies: In the Fisher-to-Lewis model of kidney transplantation, losartan prevented allograft fibrogenesis and increased survival compared to calcium channel blockade (31). Consistent with these findings, a large retrospective study of more than 2000 kidney transplant recipients showed that 10 year patient and allograft survival was significantly improved in individuals treated with an ACE-I or an ARB (32). Conversely, a retrospective analysis of 17,000 kidney transplant recipients from the Collaborative Transplant Study (CTS) showed no survival benefit from RAS blockade over 6 years (33). These discrepant findings may be explained by the lack of information on kidney biopsies, incomplete data on proteinuria, length of follow-up and different methods in RAS blockade (ever vs. never in one study compared to continuous use in the other study). However, a meta-analysis of 21 randomized controlled trials (RCT) including 1549 patients showed that RAS blockade was associated with a significant drop in hematocrit (−3.5%), GFR (−5.8 mL/min) and proteinuria (0.47 g/d) over a two year time period (34).

Future directions: In comparison to patients with native kidney disease, there is little information from prospective RCT examining the effects of RAS blockade on long-term outcomes in kidney transplantation. The ongoing Canadian ACE-I trial (ISRCTN-78129473) and American Angiotensin II Blockade for the Prevention of Cortical Interstitial Expansion and Graft Loss in Kidney Transplant Recipients (NCT00067990) studies will provide clinically meaningful evidence about whether RAS blockade will reduce patient mortality and prolong allograft survival in renal transplant recipients.

(2) TREATMENT STRATEGIES

(a) TGF- β 1—The TGF- β superfamily includes the three TGF- β isoforms (TGF- β 1, -2, and -3), activins and bone morphogenetic proteins (BMP) (2). The TGF- β isoforms are widely expressed and act on virtually every cell type in mammals by engaging intracellular signaling cascades of Smad or non-Smad family of proteins. Receptor-activated Smad protein complexes accumulate in the nucleus where they participate directly in transcriptional activation of target genes.

Role in fibrogenesis: TGF- β 1 is a key modulator of glomerulosclerosis, tubulointerstitial fibrosis and EMT in the kidney (2,35,36). It can be activated by antigen-dependent and antigen-independent mechanisms. In turn, TGF- β 1 sets off a cascade of profibrotic molecules through the activation of Smad 2/3 and MEK signaling pathways. This will result in the transcription of genes and activation of molecules involved in matrix accumulation and fibrosis (Figures 1, 2 and Table 1).

Experimental and clinical studies: TGF- β 1 is upregulated in animal and human kidney allografts undergoing chronic rejection and chronic CsA-induced tubulointerstitial fibrosis (37,38). It seems therefore logical that specific anti-TGF- β 1 therapy could result in improved long-term outcomes. Notably, this strategy was recently used in a phase I-II clinical trial for the treatment of systemic sclerosis (NCT00043706). However, and pending the publication of the study results, caution is advised about the blockade of TGF- β 1 in transplantation given the beneficial immunosuppressive properties of this cytokine and its role in the generation of T-regulatory cells (39,40). For example, TGF- β 1 knockout mice die at an early age from multifocal inflammatory disease and evidence suggests that TGF- β 1 in early acute rejection may prevent chronic rejection (39,40).

Future directions: TGF- β 1 is an important regulator of cell proliferation, differentiation, apoptosis, immune response, and extracellular matrix remodeling depending on the physiological context. Clinical trials using anti-TGF- β 1 therapy are needed to determine if the inhibition of this profibrotic molecule will improve long-term outcomes in kidney transplantation. Inhibition of downstream signaling molecules may present an alternative to anti-TGF- β 1 therapy.

(b) Pirfenidone—Pirfenidone (PFD) is an orally active synthetic antifibrotic agent structurally similar to pyridine 2,4-dicarboxylate. It has been used as an anti-TGF- β 1 agent.

Role in fibrogenesis: PFD inhibits TGF- β 1, epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast proliferation (41,42).

Experimental and clinical studies: PFD decreased TGF- β 1 levels and matrix accumulation in experimental models of lung and kidney injury including lung transplantation, remnant kidney model, obstructive kidney disease and chronic CsA nephrotoxicity (41-44) (Figures 1,2 and Table 1). PFD has been used in clinical trials of idiopathic pulmonary fibrosis, multiple sclerosis, diabetic nephropathy (NCT00105391) and focal and segmental glomerulosclerosis (FSGS) (45). In patients with FSGS, treatment was associated with a 25% improvement in the rate of decline of kidney function while adverse effects included sedation, dyspepsia and photosensitive dermatitis (45). The antifibrotic effects of PFD have not been tested in clinical transplantation.

Future directions: Clinical studies are needed to address drug interactions, tolerability and long-term effects of TGF- β 1 downregulation by PFD in kidney transplant recipients.

(c) Smad and Rho inhibition—One may target downstream molecules in the TGF- β 1 signaling pathway as an alternative antifibrotic strategy to inhibiting TGF- β 1. Smad2/3 and small GTPase Ras/Rho molecules are such candidates (46).

Role in fibrogenesis: TGF- β 1 activates Smad 2/3 via phosphorylation through the ALK5 type I receptor. It can also activate Ras/Rho and the downstream MEK pathway via Smad-independent pathways (2,46) (Figures 1, 2).

Experimental and clinical studies: Genetic and chemical inhibition of Smad3 and Rho prevented fibrogenesis in experimental models of kidney injury (47,48). Indeed, Smad3 knockout mice showed resistance to kidney fibrosis in the unilateral ureteral obstruction (UUO) model (47). Likewise, Y-27632 (a Rho-associated coiled-coil forming protein kinase (ROCK) inhibitor) prevented the transcription of fibrosis genes including TGF- β 1, α -SMA and α 1-collagen and matrix deposition in mice undergoing UUO (48). In these studies osteopontin mRNA and angiotensin-II-induced macrophage migration were reduced, suggesting that ROCK inhibition has anti-inflammatory effects (48). Concordant with these studies, Fasudil, a specific Rho kinase inhibitor attenuated myocardial fibrosis and interstitial fibrosis in experimental models of diabetic and obstructive nephropathy (49-51). This drug is being tested in clinical trials of carotid atherosclerosis (NCT00670202).

Future directions: Smad3 and Rho inhibition strategies have anti-inflammatory and antifibrotic effects in experimental studies of kidney injury. However, preclinical and clinical studies are needed to examine the role of these interventions on long-term kidney allograft outcomes.

(d) CTGF (Connective Tissue Growth factor)—CTGF is a 38 kd, heparin binding cysteine-rich protein that can induce cell proliferation, collagen synthesis, chemotaxis and myofibroblast differentiation (52).

Role in fibrogenesis: It may be activated *via* Smad or MEK pathways and in return it can activate TGF- β 1 and inhibit the antifibrotic effects of Bone Morphogenetic Protein 7 (BMP-7) (53).

Experimental and clinical studies: CTGF mRNA and protein levels were increased in a mouse model of kidney transplantation (54). These studies showed that *in vitro*, CTGF induced EMT in mouse tubular epithelial cells. Furthermore, urinary CTGF levels were increased in kidney transplant recipients with chronic allograft fibrosis suggesting that this profibrotic molecule may be used as a surrogate marker of kidney allograft injury (54). Recent intervention studies using CTGF silencing with siRNA decreased allograft fibrosis in the Fisher 344 to Lewis transplant model (55). While a phase 1B study of FG-3019 (a humanized anti-CTGF antibody) is under way to determine the effects of anti-CTGF therapy on disease progression in patients with diabetic nephropathy (NCT00754143), there are no clinical studies addressing the role of this target molecule in kidney transplantation. Meanwhile, it should be noted that CTGF knockout mice suffer from abnormal angiogenesis, osteogenesis and chondrogenesis, reflecting the global role of this growth factor on matrix remodeling in connective tissues (56).

Future directions: Clinical trials are needed to determine the role of CTGF blockade on long-term kidney allograft fibrosis.

(e) VEGF (Vascular Endothelial Growth factor)—VEGF is an important angiogenic factor, constitutively expressed in glomerular podocytes and proximal and distal tubules (57).

It may be induced by hypoxia, oncogenes, TGF- β 1, EGF and PDGF (57). In oncology, greater VEGF expression is associated with poor prognosis in bladder, breast, colorectal and renal cancers and anti-VEGF therapy is being considered for the treatment of these malignancies (58).

Role in fibrogenesis: As a potent angiogenic factor, VEGF is an inducer of proliferation (MEK), permeability (endothelial fenestrations), invasion (matrix metalloproteinases or MMPs) and survival (activation of Akt/PI3K, caspase-inhibition).

Experimental and clinical studies: Loss of glomerular and peritubular capillaries was associated with reductions in renal VEGF expression in a number of experimental models of kidney disease (57). The administration of VEGF stimulated angiogenesis and improved renal function and scarring in experimental models of acute glomerulonephritis suggesting that VEGF therapy may be considered for the prevention or treatment of fibrosis in kidney disease (57). However, treatment accelerated renal injury and fibrosis in diabetic nephropathy indicating that VEGF may have beneficial or deleterious effects depending on the context and availability of endothelial nitric oxide (57). VEGF levels were significantly different in the interstitium and tubules of kidney transplants undergoing acute rejection, chronic fibrogenesis and CsA toxicity (59). In these studies, VEGF expression correlated with TNF- α levels and macrophage infiltration suggesting a proinflammatory role for VEGF. While kidney function in the first 6 months after biopsy was better in patients with marked tubular VEGF expression, interstitial fibrosis and graft loss occurred earlier in these patients (59). In aggregate, these studies demonstrate that VEGF regulation is complex. While this angiogenic factor may act as an antifibrotic agent, it can also result in inflammation, tumor invasion and renal injury depending on the underlying pathobiology.

Future Directions: Despite the number of clinical trials targeting VEGF in malignancies, more studies are needed to understand its regulation and role in native and transplant kidney disease.

(f) Hepatocyte growth factor/scatter factor (HGF/SF)—HGF was originally identified and cloned as a potent mitogen for mature hepatocytes but is now clear that this growth factor acts on various types of cells through its Met receptor tyrosine kinase and elicits pleiotropic effects involved in embryogenesis and tissue repair (60).

Role in fibrogenesis: HGF prevents the initiation and progression of chronic renal fibrosis by inhibiting TGF- β 1 expression, myofibroblast activation and EMT. It can block the nuclear translocation of Smad-2/3 and upregulate the expression of Smad transcriptional corepressors SnoN and TGIF (Figures 1, 2, Table 1) (60, 61).

Experimental and clinical studies: In preclinical transplant studies, treatment with human recombinant HGF (hrHGF) prevented kidney allograft inflammation (decreased TNF- α , MCP-1 and iNOS mRNA levels) and fibrosis (decreased TGF- β 1 mRNA and matrix accumulation) (62). Likewise, human HGF gene therapy (immediately before, or after transplantation) in rats decreased graft infiltration of T-cells and macrophages, and reduced α -SMA and CTGF levels confirming that HGF has both anti-inflammatory and antifibrotic effects (63). HGF may therefore be a good candidate for gene or drug therapy of fibrosis. Clinical trials assessing the safety of intramuscular injection of HGF plasmid to improve limb perfusion have shown encouraging results (64). However, there is a potential risk of cancer, especially in the transplant population, given that c-Met the HGF receptor is a tyrosine kinase receptor involved in the progression of carcinomas and metastasis (65).

Future directions: HGF has demonstrated promising results in preclinical studies and clinical trials of vascular disease. Clinical trials are needed to determine if treatment with recombinant human HGF can prevent kidney allograft fibrosis.

(g) BMP-7 (Bone Morphogenetic Protein-7)—BMP-7 is a member of the TGF β -1 family, but it binds to distinct type I and type II receptors. BMP-7 signals through the ALK3 and ALK6 type I receptors to phosphorylate Smad1, Smad5 and Smad8, whereas TGF- β 1 signals through the ALK5 type I receptor to phosphorylate Smad2 and Smad3 (Figures 1, 2, Table 1) (66).

Role in fibrogenesis: BMP-7 can counterbalance the profibrotic effects of TGF- β 1 via the activation of regulatory Smads 1, 5 and 8 (66-68).

Experimental and clinical studies: Exogenous administration of recombinant human BMP-7 (rhBMP-7) or its transgenic overexpression prevented EMT and fibrogenesis in rodent models of lupus nephritis, UUO and diabetic nephropathy (68). In the kidney allograft, tubulointerstitial fibrosis and EMT were associated with upregulation of intraepithelial phospho-Smad2/3 and concomitant downregulation of phospho-Smad 1/5/8 while BMP-7 increased phospho-Smad 1/5/8 in renal cortical epithelial cells *in vitro* (36). These results suggest that rhBMP-7 or small molecule BMP-7 agonists may prevent fibrosis in kidney transplantation. Clinical trials using these drugs for interbody and posterolateral spinal fusion surgery show that their short-term use may be safe despite the excessive costs (69). It should be also noted that BMP-7 regulation is complex and depends not only on its availability, but also on a balance of agonists and antagonists (68).

Future directions—Clinical trials are needed to determine if recombinant human BMP-7 prevents kidney allograft fibrosis.

CONCLUSIONS

Preventive and treatment strategies targeting the TGF- β 1 signaling pathway are reasonable antifibrotic options in kidney transplantation. More specifically, pirfenidone and therapies targeting BMP-7, HGF and CTGF have shown promising results. However, most of these tools are still in an experimental phase and clinical trials are needed to examine their long-term effects in kidney transplantation.

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Figure 1. Biological pathways involved in allograft fibrosis

Figure 1 is a simplified cartoon representing the biological pathways involved in kidney allograft fibrogenesis. Fibrosis results from antigen-dependent and antigen-independent mechanisms that primarily trigger TGF- β 1 in graft infiltrating and parenchymal cells. These triggers include acute rejection, infections, ischemia-reperfusion injury, oxidative stress and calcineurin inhibitors. Adhesion molecules and chemokines play an important role at this level. In turn, TGF- β 1 switches on the signaling pathways that will result in the activation of myofibroblasts. In theory, the source of interstitial myofibroblasts includes resident fibroblasts, epithelial-to-mesenchymal transition (EMT), bone marrow derived cells, endothelial cells and pericytes. However, as suggested by the “?” more evidence is needed to support the last three mechanisms in kidney transplantation.

Figure 2. Molecules and signaling targets for the treatment of fibrosis in kidney allografts

Figure 2 is a schematic representation of the molecular pathways involved in kidney allograft fibrogenesis with related preventive and therapeutic strategies. TGF- β 1 activates downstream profibrotic signaling pathways that include Smad, small GTPase Ras/Rho and mitogen-activated protein kinase (MEK) pathways. The activation of these pathways will result in a cascade of events culminating in phenotypic changes consistent with epithelial-to-mesenchymal transition (EMT) and fibrosis. During EMT tubular epithelial cells are transformed into myofibroblasts in a stepwise fashion (yellow box). The process is characterized by a loss of cell-cell adhesion and E-cadherin expression (a), *de novo* α -smooth muscle actin (α -SMA) expression and actin reorganization in injured cells (b), tubular basement membrane disruption and cell migration (c) with fibroblast invasion and production of profibrotic molecules including collagen and fibronectin (d). CTGF can induce EMT by inhibiting BMP-7 or activating hypoxia-inducible genes including HIF-1 α and VEGF. VEGF may result in renal injury and fibrosis in the absence of nitric oxide (NO). Pirfenidone (PFD) and HGF can inhibit fibrogenesis by downregulating TGF- β 1 expression. BMP-7 counterbalances the profibrotic effects of TGF- β 1 by activating regulatory Smads 1, 5 and 8.

Table 1

Antifibrotic agents for kidney transplantation

	Animal Studies		Clinical trials*		Limitation
	Transplant	Other	Transplant	Other	
Chemokine blockade					
Antioxidants	+	GN, UUO, IR	–	MS, RA, Psoriasis	Complex regulation
Rapamycin	+	HTN, DN, IR	+	CVD, cancer	Conflicting data
RAS blockade	+	UUO	+	Cancer	A.E.
Anti-TGF-β1	+	CsA, DN, GN, UUO	–	CVD, CKD, HTN	A.E. No long-term data
Pirfenidone	+	CsA	–	Scleroderma	Effects on rejection?
	+	CsA	–	DN, FSGS, IPF, MS	Effects on rejection? Drug interactions?
Smad-3 inhibition	–	UUO	–	–	No data in Tx
ROCK inhibition	–	UUO	–	Atherosclerosis	No data in Tx
Anti-CTGF	+	DN, GN	–	IPF, FSGS, DN	Effects on bone and angiogenesis?
VEGF	–	CsA, DN, GN	–	IHD	Risk of Cancer? Conflicting data
HGF	+	CsA, DN, GN, UUO	–	PVD, LF	Risk of Cancer?
BMP-7	–	DN, GN, UUO	–	OA, fractures, DDD	Complex regulation

For more details regarding clinical trials, please refer to <http://clinicaltrials.gov/>. AE: adverse events, CsA: cyclosporine nephrotoxicity, CVD: cardiovascular disease, DDD: degenerative disc disease, DN: diabetic nephropathy, FSGS: focal and segmental glomerulosclerosis, GN: glomerulonephritis, HTN: hypertension, IHD: ischemic heart disease, IPF: idiopathic pulmonary fibrosis, IR: ischemia reperfusion, LF: liver failure, MS: multiple sclerosis, OA: osteoarthritis, PVD: Peripheral vascular disease, RA: rheumatoid arthritis, Tx: transplant, UUO: unilateral ureteral obstruction