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Renal Interstitial Fibrosis: Mechanisms and Evaluation In: Current Opinion in Nephrology and Hypertension

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Abstract

Purpose of Review—Tubulointerstitial injury in the kidney is complex, involving a number of independent and overlapping cellular and molecular pathways, with renal interstitial fibrosis and tubular atrophy (IF/TA) as the final common pathway. Furthermore, there are multiple ways to assess IFTA.

Recent findings—Cells involved include tubular epithelial cells, fibroblasts, fibrocytes, myofibroblasts, monocyte/macrophages, and mast cells with complex and still incompletely characterized cell-molecular interactions. Molecular mediators involved are numerous and involve pathways such as transforming growth factor (TGF-**β**), bone morphogenic protein (BMP), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF). Recent genomic approaches have shed insight into some of these cellular and molecular pathways. Pathologic evaluation of IFTA is central in assessing the severity of chronic disease; however, there are a variety of methods used to assess IFTA. Most assessment of IFTA relies on pathologist assessment of special stains such as trichrome, Sirius Red, and collagen III immunohistochemistry. Visual pathologist assessment can be prone to inter- and interobserver variability, but some methods employ computerized morphometery, without a clear consensus as to the best method.

Summary—IFTA results from on orchestration of cell types and molecular pathways. Opinions vary on the optimal qualitative and quantitative assessment of IFTA.

Keywords

kidney/renal fibrosis; epithelial/mesenchymal transition; myofibroblast; morphometry

Introduction

Interstitial extracellular matrix (ECM) accumulation, common to many chronic kidney diseases, contributes to functional loss. Kidney interstitial fibrosis (IF) can be defined as the accumulation of collagen and related molecules in the interstitium. Interstitial collagen is normally present in the kidney, particularly type I and III, which serve as structural scaffolding.[1, 2] This review addresses mechanisms by which IF arises, shown through animal experimentation and analysis of human kidneys. In addition, approaches to assess IF are considered.

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IF patterns differ and probably do not have identical causes or consequences. Broad scars with tubular loss are the sequelae of severe focal injury and parenchymal destruction, such as in pyelonephritis and infarcts.[3] This pattern is no doubt a “wound healing” response to repair integrity loss and prima facie beneficial to renal function. In contrast, a second pattern (far more common in renal biopsies) is diffuse or patchy fine IF, surrounding either atrophic or normal tubules and associated with either diffuse or focal disease of glomeruli, tubules, or vessels.[4, 5] Many studies show a reciprocal correlation between kidney function and the IF extent.[6–14]

Tubular atrophy (TA) is defined as loss of specialized transport and metabolic capacity and typically manifested by small tubules with cells with pale cytoplasm or dilated, thin tubules. TA is usually associated with IF (often abbreviated IFTA); but probably has distinct mechanisms related to blood flow, glomerular filtration rate (GFR) or tubular continuity loss. However, IF and TA are separable, as shown by the profound TA in renal artery stenosis, which characteristically has little or no fibrosis (or inflammation).[3]

Cellular and Molecular Mediators

IFTA results from an orchestration of multiple cell types, as detailed below.

Fibroblasts and Myofibroblasts

Fibroblasts constitute a large proportion of renal interstitial cells and are the major cell maintaining constituent ECM, which can be considered the kidney “skeleton”. Fibroblasts lack a cell type-specific marker, making their study difficult.[1] Fibroblasts are distinguished from other interstitial cells by their abundance of rough endoplasmic reticulum, prominent F-actin cytoskeleton, and by ecto-5′-nucleotidase expression in their plasma membrane. Fibroblasts interact with other cells, such as dendritic cells, through cell processes.[15] Fibroblasts may acquire a myofibroblastic phenotype under paracrine signals after attaching to injured tubular basement membranes (TBM),[16–19] eventually producing collagen type III.[20] This hypothesized phenomenon is likely a crucial event in IF.[1, 21, 22]

Myofibroblasts express smooth muscle actin (SMA), contain microfilaments with focal densities (stress fibers), peripheral myofilament condensations known as fibronexi connecting actin microfilaments with extracellular fibronectin, round nuclei, and frequent attachments to basement membranes.[15, 23, 24] Myofibroblasts also contain vimentin, fibronectin with the splice variant containing ectodomain A [15, 18], and S100A4 [also known as FSP-1];[15, 20] S100A4, once considered myofibroblast specific, also colocalizes with some leukocytes.[15, 20] Interstitial myofibroblasts have multiple potential origins with candidates being fibroblasts, pericytes, perivascular cells.[20, 25–30], and endothelial cells.[31, 32]

Fibrocytes

Fibrocytes, thought to be distinct from fibroblasts, are spindle-shaped, ECM-producing cells derived from peripheral blood leukocytes.[33] Both hematopoietic (e.g., CD45) and stromal cell (e.g., type I collagen) markers can be detected on fibrocytes; and furthermore, these cells also express chemokine receptors. Fibrocytes are found in injured kidneys, possibly through in situ differentiation or infiltration through chemokine gradients.[34–36] T-helper-2-type (T_H2) cytokines appear to be profibrotic, inducing differentiation of human fibrocytes; and in contrast, T-helper-1-type (T_H1) cytokines can inhibit differentiation of fibrocytes.[34, 37, 38] Fibrocytes may be affected by drugs such as cyclosporine which induces type I collagen, possibly explaining IFTA attributed to chronic calcineurin inhibitor toxicity.[34] Fibrocytes are present in systemic nephrogenic fibrosis, related to gadolinium administration.[39]

Extracellular matrix

Multiple ECM components besides collagen have a crucial role in IF.[40–44] Tissue transglutaminase (tTG) expression is increased in animal and human renal disease models, correlating with IF severity; and tTG crosslinks proteins, stabilizing ECM and conferring resistance to protease degradation.[45–47] Matrix metalloproteinase (MMP) enzymes are comprised of proteolytic enzymes that can degrade all matrix protein components.[48] Tissue plasminogen activator (tPA), although proteolytic, increases IF development by inducing MMP-9 gene expression, leading to TBM disruption and EMP promotion.[49] Mice without the MMP-9 gene have less IFTA in obstructive nephropathy.[48] Plasmin, a serine protease, can activate MMPs, leading to ECM degradation and reduced IF.[50–52] Decreased laminin, a component of glomerular and PTC basement membranes, leads to more IFTA.[53, 54] ECM production is also affected by the renin/angiotensin system.[55–62]

Tubular Epithelial Cells

Tubular epithelial cells (TECs) are postulated to contribute to increased ECM through the process of epithelial-to-mesenchymal transition (EMT), defined as the stepwise loss of epithelial markers, such as E-cadherin, and the acquisition of mesenchymal markers, such as vimentin and SMA.[1, 63, 64] As an ultimate step in EMT, cells acquire increased motility and traverse basement membranes into the interstitium.[65] Convincing experimental evidence for epithelial cell migration into the interstitium has been acquired in IF in mouse kidneys, where the origin of the cells can be followed with indelible genetic markers.[2, 65] A similar in situ change in tubular epithelial phenotype occurs in humans, but emigration of TECs into the interstitium has not been demonstrated. Therefore, many question the migration feature of EMT.[66–68] A recent Banff Conference symposium on EMT concluded that the in situ epithelial response exists but needs a name that does not imply emigration of tubular cells to the interstitium,[69] which we will here term “epithelial mesenchymal phenotype” (EMP).

TECs clearly undergo marked phenotypic changes in acute injury[70] and appear to provide key signals to provide IF.[1] Intratubular stretch, fluid shear stress, and biomechanical forces modify intracellular signaling and gene expression, contributing to IF.[16] EMP is supported by the finding of increased intermediate filaments (e.g., vimentin and nestin) in injured tubular epithelium,[71, 72] an association with increased collagen type I and III expression in TECs,[73] and altered E-Cadherin expression.[74] Transcription factors such as the zinc-finger transcription factor snail homolog 1 (Snai1)[75], which interacts with the notch signaling pathway[76], appear to be important to EMP. An important component in the regulation of genes in EMP and IF may include micro(mi)RNAs[77–86], some antifibrotic[87] and others profibrotic.[88] Autophagy, the process whereby cells undergo “self digestion”,[1, 89, 90] and endoplasmic reticulum stress are important to IFTA.[91–97] TECs may undergo damage through the increased action of lipids in a “lipid nephrotoxicity” process, mediated in part by peroxisome proliferator-activated receptor (PPAR) expression.[98–104]

VEGF-A overexpression in tubular cells can result in increased serum VEGF levels, leading to increased capillary number and size, type IV collagen deposition, and fibroblast and myofibroblast numbers.[105] However, other studies show that VEGF administration may decrease IF.[106] Hypoxia promotes fibrosis through multiple mediators including hypoxia-induced factor-1α (HIF-1α).[107–110]

Epigenetic modification through methyltransferase Dnmt1 hypermethylation of the Ras oncogene inhibitor RASAL1 decreases IF.[1, 111, 112] Another chromatin structure

modifier, histone deacetylase (HDAC), modulates proinflammatory and fibrotic changes in tubulointerstitial injury,[113, 114] and histone methylation may also be important in fibrotic gene expression.[115] Growth arrest may lead to renal injury through increased fibrosis, characterized by an increased proportion of TECs in phase G2/M, which gives the cells a profibrotic phenotype, in large part mediated by JNK signaling. Pharmacologic induction of growth arrest can promote fibrosis.[116]

Inflammatory cells

Numerous inflammatory cell types contribute to IFTA, as discussed below.

Lymphocytes—Lymphocytes appear to have important roles in the genesis of IFTA.[117–119] CD4+ T cells are considered particularly crucial to this process, since CD4+ but not CD8+ lymphocyte reconstitution increased IF in RAG knockout mice and CD4+ depletion decreased IF.[117] A recent microarray analysis of renal allografts showed increased T-cell and natural killer gene sets in IFTA development.[120] High T cell and macrophage but not B cell infiltration is associated with low IL-10 expression, which conferred susceptibility to IFTA.[121]

Monocyte/Macrophages—Monocyte/macrophages are heterogeneous, and some are profibrotic,[122, 123] particularly CD11b+ cells.[124, 125] Galectin-3 (Gal-3) is a profibrotic mediator comprised of a β-galactosidase-binding lectin released from macrophages.[125] Gal-3 may protect renal tubules from chronic injury by enhancing ECM remodeling and attenuating fibrosis.[126] The macrophage growth factor, CSF-1, is released by renal tubular cells, leading to repair and reduced IF.[127] Models constructed to investigate the role of macrophages in IF include: adriamycin-induced nephropathy,[128] cyclophosphamide depletion of macrophages,[129] and adoptive transfer of bone marrow-derived macrophages.[129, 130] The role of macrophages in IF is clearly complex, since some subsets of bone marrow derived monocytes may actually attenuate fibrosis.[130]

Dendritic cells—Dendritic cells are present in substantial numbers in the renal interstitium,[15] and recent studies have shown their importance in IF. Dendritic cell depletion through injection of diphtheria toxin in transgenic mice with a CD11c/diptheria toxin receptor may ameliorate IF.[131, 132] Other studies show that dendritic cells act indirectly, activating T cells to produce fibrosis.[119]

Mast cells—Mast cells are a component of the primary innate immune system and are typically infrequent in normal kidneys, often congregating around vessels and epithelium. Increased mast cell numbers have been associated with profibrotic roles.[133–137], inversely correlating with renal function.[135, 137, 138] Mast cell deficiency has been associated with decreased fibrosis.[135] Mast cells have also been associated with antifibrotic actions.[139]

Endothelial cells, Peritubular Capillaries, and Vascular Supply

Experimental evidence supports the view that endothelial cells contribute to interstitial fibroblasts, possibly through a process of endothelial-to-mesenchymal transition.[21, 29, 31, 33, 140–143] PTCs decrease with time in allografts and are inversely related to renal function; decreased PTC density at 3 months predicts later loss of function at one year.[144]

Kidney lymphatic vessels are important in facilitating inflammatory cell emigration. IF is associated with increased lymphangiogenesis, partly driven by VEGF-C.[145] Angiogenesis and inflammation inhibition with sirolimus can prevent IF.[146] Newly formed lymphatics may be found close to glomeruli with tuft adhesions,[145] perhaps participating in the

misdirection of urine filtrates in these areas.[147] Other studies have found a connection between lymphangiogenesis, tissue remodeling, and differential proteoglycan expression. [148] Lymphangiogenesis occurs as early as 72 hours after transplantation and tends to correlate with inflammation.[149]

Molecular Mediators and Signaling Pathways(Table 1)

Transforming growth factor-β (TGF β) is regarded as a central mediator of IF.[1, 45, 150–155] TGF-**β** upregulation occurs in nearly every chronic kidney disease [both human and animal]. TGF-**β,** possibly one of the most widely studied regulators of ECM production, is produced in a latent form, TGF-**β1**, bound to latency-associated peptide (LAP), which inhibits TGF-**β** receptor binding. Latent TGF-**β** binding protein (LTBP) binds this complex and inhibits binding to the ECM.[156, 157]

TGF-**β** acts through Smad for downstream signaling,[158–160] which is amplified if Smad antagonists [e.g., SnoN and Ski corepressors] are lost.[161–163] Resulting fibrogenic signals stimulate fibroblasts, presumably initiating tubular EMP. TGF-**β** also works through the jagged/notch pathway, which may be inhibited to decrease fibrosis,[164–166] and Crim1, which binds and regulates TGF**β**-2, VEGF, and PDGF-**β**, to decrease fibrosis. [167]

Bone morphogenic protein (BMP), particularly BMP-7, acts as a natural TGF-**β** antagonist; and due to this, BMP may have renoprotective effects and may possibly reverse IF.[161, 168–170] Sclerostin domain-containing protein 1 (also known as uterine sensitization-associated gene 1 [USAG-1]) is an endogenous inhibitor of BMP-7[1, 45, 171, 172]. Inhibiting the circulating proteolytic enzyme BMP1–3 [a tolloid-like proteinase] reduces IF. [173, 174]

Toll-like receptors (TLRs) participate in IFTA.[175, 176] TLR4 modulates IFTA susceptibility through the BAMBI (BMP and Activin Membrane Bound Inhibitor), a negative regulator of TGF-**β**, attenuating tubular injury but promoting IF;[176] however, other studies fail to show TLR influence.[177, 178]

TGF-**β** activation blockade can be accomplished with decorin antisense TGF-**β** expression inhibition, neutralizing TGF-**β** antibodies and soluble TGF-**β** receptors.[179] Small molecule inhibitors to TGF-**β** are being developed.[180] However, it is difficult to ascertain whether TGF-**β** inhibition will be universally useful in inhibiting fibrosis. TGF-**β** also has anti-inflammatory properties; and there is concern that TGF-**β** inhibition could lead to increased inflammation and thus fibrosis.[180] TGF-**β** action may also be mediated by reactive oxygen species and oxidative damage.[181]

The TGF-β-inducible integrin **α**V**β**6 appears to be restricted to epithelial cells where it is normally expressed in renal tubules at low levels and elevated during injury, development, and neoplasia. This integrin appears to have a role in increasing IF and inflammation.[182] Integrin-linked kinases have associated with increased IFTA, corresponding with increases in collagen IV and TGF-**β**.[183]

Hepatocyte growth factor (HGF) is considered to be an antifibrotic factor with effects opposite TGF-**β**, blocking Smad2/3 nuclear translocation in interstitial fibroblasts, inhibiting tubular EMP. Administration of HGF or its gene can decrease IF observed in animal models. [184–186] However, long-term proteinuria has been demonstrated with HGF administration. [187]

Platelet-Derived Growth Factor (PDGF), comprising four isoforms (PDGF-A,-B,-C, and -D) and two receptor chains (PDGFR-α and -β), appears to have an important role in IF.[23,

188] PDGF-C,-α, and -CC are noteworthy contributors to the renal cortical interstitium.[1, 23, 189] Data indicates that PDGF-CC directly induces fibroblast proliferation and enhances leukocyte infiltration;[189] but some studies have only demonstrated PDGF-CC in the peritubular capillary (PTC) endothelium.[190]

The janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway appears to be important in IFTA development, and STAT inhibition may be useful therapeutically.[191] Fibrinogen acts as a fibroblast mitogen, promoting IFTA.[192]

Complement inhibition or lack of complement components appears to be antifibrotic.[193, 194] Proteomic data indicates contributions from the alternative rather than the classic complement pathway to IFTA.[195]

Evaluation of Interstitial Fibrosis

IF extent is predictive of renal allograft outcome and may be considered a surrogate marker.[196–200] Several applications require accurate IF measurement (Table 2)[6–14, 24, 169, 200–211] including research focused on therapeutic inhibition of IF and comparison of renal allograft protocol biopsies.[45, 180, 212, 213] Visual assessment of trichrome-stained slides is often standard institutional practice,[214] but studies have shown this approach may have poor reproducibility.[215, 216] Several morphometry techniques are used to assess IF (Figure 3), including morphometry of slides stained with trichrome;[10, 202] Sirius Red, specific for collagen types I and III under polarized light;[24, 206, 217] and collagen immunohistochemistry, particularly type III collagen.[12, 218, 219] Computer-assisted morphometry has shown utility in the analysis of studies employing trichrome, Sirius Red, and collagen III immunohistochemistry; and analysis in some of these studies have shown correlation with GFR.[6, 8, 11, 12, 24, 169, 202, 206, 217–222] (Table 2)

There are intrinsic limitations in the measurement of IF, some of which are due to sampling. For example, one study estimated that repeat biopsies show a decrease in the measured level of fibrosis, presumably due to sampling, in 12% of cases.[223] In addition, not all fibrosis is “equal” or the “same” in quality and thus aggregate quantity. For example, “active” or “young” IF may have greater potential for remodeling. Broad scars may have different consequences than diffuse, fine IF. Inflammation in areas of IF has also been noted in several studies to be an adverse risk factor for progression of renal disease.[18, 200, 223–227]

Overall, there is no consensus regarding the best way to assess IF. Efforts to reach a consensus or at least provide recommendations are currently underway under the auspices of the Banff Conference of Allograft Pathology.[228]

Conclusion

Molecular mechanisms leading to IFTA are complex and typically interrelated with the primary processes leading to renal injury. Further elucidation of these mechanisms could lead to targeted inhibitors to alleviate terminal scarring. Furthermore, there are number of ways to assess fibrosis; and efforts are underway to improve these methods.

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Abbreviations

ACE	angiotensin converting enzyme
AT1/2R[B]	angiotensin type 1/2 receptor [blockade]
BMP	Bone morphogenic protein
CAV1	caveolin-1
CTI	chronic tubulointerstitial injury
CYP	cytochrome P450
ECM	extracellular matrix
EMP	epithelial to mesenchymal phenotype
EMT	epithelial to mesenchymal transition
Gal-3	Galectin-3
GFP	green fluorescent protein
GFR	glomerular filtration rate
HGF	hepatocyte growth factor
HIF	hypoxia-induced factor
IF/TA	interstitial fibrosis/tubular atrophy
ILK	integrin-linked kinase
JAK/STAT	janus kinase/signal transducer and activator of transcription
LTBP	latent TGF-β binding protein
miRNAs	microRNA
MMP	matrix metalloproteinase
PPAR	peroxisome proliferator-activated receptor
PDGF	platelet-derived growth factor
PTC	peritubular capillary
RAG	recombinase activator gene
SMA	smooth muscle actin
tPA	tissue plasminogen activator
TEC	tubular epithelial cell
TGF	Transforming growth factor
TLR	Toll-like receptor
UUO	unilateral ureteral obstruction
USAG-1	uterine sensitization-associated gene 1 [also known as sclerostin domain-containing protein 1]
VEGF	vascular endothelial growth factor

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(*) for special interest,

(**) for outstanding interest

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Bullet Point Summary

- Interstitial fibrosis and tubular atrophy formation results from a complex cellular and molecular milieu participating in extracellular matrix formation.
- Molecular mediators involved are numerous and involve pathways such as transforming growth factor (TGF-β), bone morphogenic protein (BMP), platelet-derived growth factor (PDGF), and hepatocyte growth factor (HGF); and important cells include epithelial cells, fibroblasts, myofibroblasts, fibrocytes, endothelial cells, lymphocytes, monocyte/macrophages, dendritic cells, and mast cells.
- Epithelial and endothelial cells may undergo transitional to mesenchymal cells; however, this research may simply indicate transition to a phenotype rather than an actual cell type transition.
- Recent genomic approaches have revealed the interplay of molecular and cellular factors, including the role of lymphocytes, in fibrosis formation
- The assessment of fibrosis involves a number of visual and morphometric methods, many of which correlate with renal function; but there is no clear consensus regarding the best method.

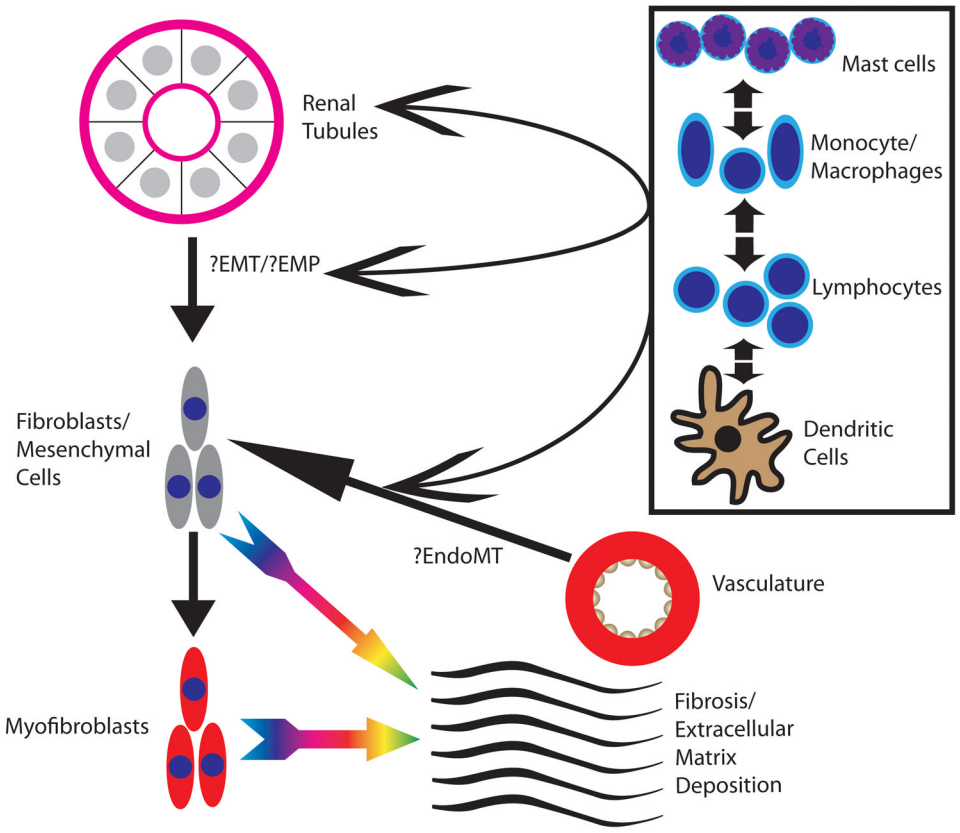

Figure 1. Cellular mediators of fibrosis

Cells involved in fibrosis include the renal tubules, the renal vasculature, and inflammatory cells, including lymphocytes, monocyte/macrophages, mast cells, and dendritic cells. The renal tubules at least undergo changes that impart them with a epithelial-mesenchymal phenotype (EMP) and are possibly involved in a process of epithelial-mesenchymal transition (EMT). The endothelium is possibly involved in a process of endothelial-mesenchymal transition (EndoMT). Evidence shows that the inflammatory cells participate in both the process of EMT/EMP and EndoMT. Fibroblasts/mesenchymal cells mediate the production of fibrosis and extracellular matrix (ECM) deposition and also may undergo a transition to a myofibroblastic phenotype, further leading to the production of fibrosis and ECM deposition.

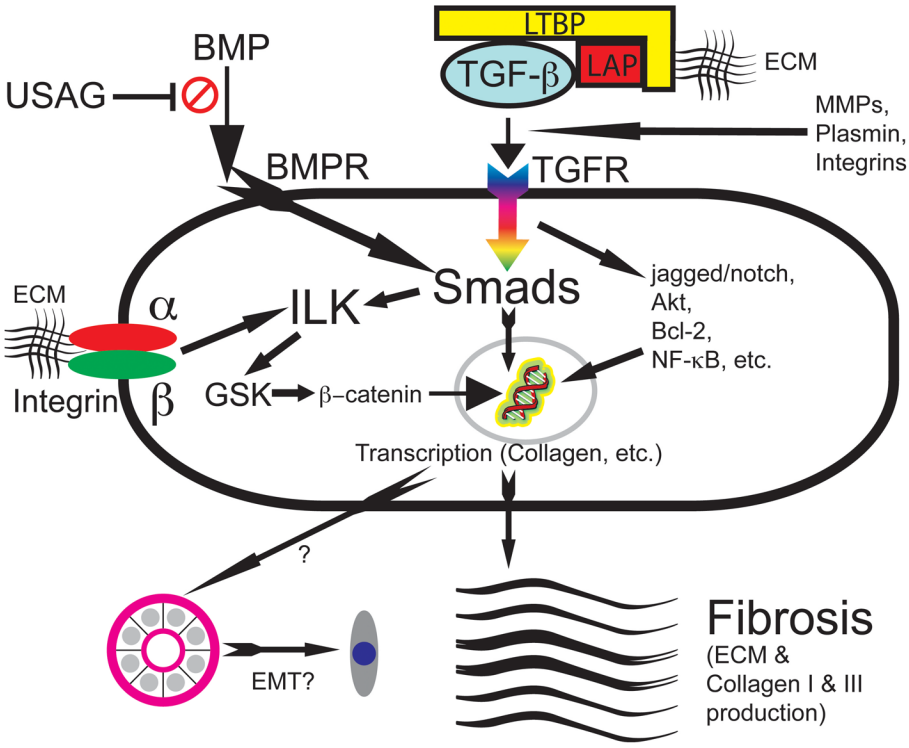

Figure 2. Important molecular mediators of fibrosis

Transforming growth factor (TGF-β) is released through interactions with the extracellular matrix (ECM) and matrix metalloproteinases (MMPs), plasmin, and integrin; and when released from inhibition by latent TGF-β binding protein (LTBP) and latency-associated peptide (LAP), TGF-β binds the transforming growth factor receptor (TGFR), activating intracellular signals such as the Smad, jagged/notch, Akt, Bcl-2, and NF-κB pathways. These lead to nuclear transcription, ultimately culminating in collagen and ECM production and possibly leading to epithelial to mesenchymal transition (EMT). Smads also act on the integrin-linked kinase (ILK), which acts through glycogen synthase kinase (GSK) to produce β-catenin, which traverses into the nucleus to also induce transcription. The integrins (typically with α and β components [e.g., α5β6 integrin]) also act through ILK in a similar manner. Bone morphogenic protein (BMP), when binding to the BMP receptor (BMPR) also works through Smad, a process inhibited by sclerostin domain-containing protein 1 (also known as uterine sensitization-associated gene 1 [USAG-1]).[Figure adapted from [1, 229, 230].]

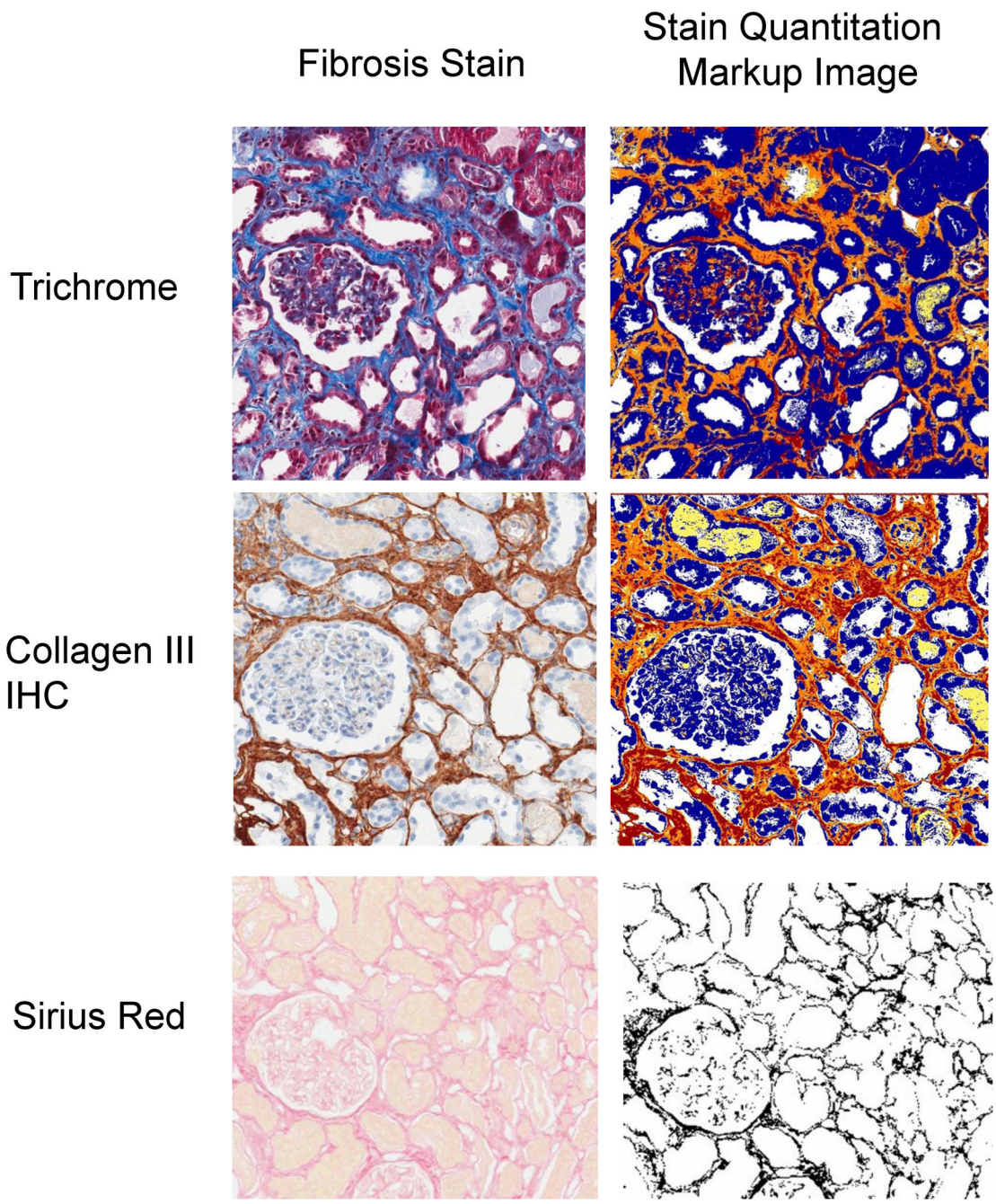

Figure 3. Fibrosis morphometry

Stains used to assess fibrosis are shown, including: Trichrome, Collagen III immunohistochemistry, and Sirius Red [on the left] with their corresponding quantitation markup images shown [on right].

Table 1

Mediators of Interstitial Fibrosis and Selected Interaction Partners

Mediator	Primary role	Interaction
Fibrinogen	Profibrotic	Acts as a fibroblast mitogen
G2/M arrest	Profibrotic	Increase of cells in the G2/M phase gives the cells a profibrotic phenotype
Galectin-3	Profibrotic	Released from macrophages
Integrins	Profibrotic	TGF-β-inducible integrins (e.g. αVβ6) act through integrin-linked kinases to produce collagen
Jagged/notch	Profibrotic	Downstream of TGF-β; may be inhibited to decrease fibrosis
JAK/STAT	Profibrotic	May be a useful therapeutic target to decrease IF
MMP	Profibrotic	Can degrade ECM but also disrupts basement membranes
PDGF	Profibrotic	Induces fibroblast proliferation and leukocyte infiltration; inhibited by Crim1
Smad	Profibrotic	Downstream of TGF-β
TGF-β	Profibrotic	Downstream: Smad, jagged/notch; Smad antagonist corepressors such as SnoN and Ski; latency-associated peptide (LAP) binds TGF-β1, inhibiting binding to the TGF-β receptor; latent TGF-β binding protein (LTBP) binds the complex and inhibits binding to the ECM; Inhibited by Crim1
TLRs	Profibrotic	Acts through BAMBI (BMP and Activin Membrane Bound Inhibitor) [negative regulator of TGF-β] to attenuate tubular injury but promote IF
tPA	Profibrotic	Induces MMP-9 gene expression, leading to TBM disruption and increased IF
tTG	Profibrotic	Crosslinks proteins, Stabilizes ECM
VEGF	Profibrotic	Increased capillaries and lymphatics, fibroblasts/myofibroblasts, and collagen deposition; Inhibited by Crim1
miRNA	Antifibrotic/Profibrotic	Some are antifibrotic and some are profibrotic; some may be therapeutic targets
BMP	Antifibrotic	Antagonist of TGF-β; inhibited by sclerostin domain-containing protein 1 (also known as uterine sensitization-associated gene 1 [USAG-1])
Crim1	Antifibrotic	Binds TGF-β, VEGF, and PDGF-â to decrease fibrosis
CSF-1	Antifibrotic	Macrophage growth factor released by renal tubular cells leads to repair and reduced IF
HGF	Antifibrotic	blocks Smad (e.g., Smad 2/3)
Plasmin	Antifibrotic	Activates MMPs, leading to matrix degradation and reduced IF
RASAL1	Antifibrotic	Ras oncogene inhibitor hypermethylated by methyltransferase Dnmt1 decreases IF

BMP: Bone morphogenic protein, ECM: extracellular matrix, HGF: hepatocyte growth factor, IF: interstitial fibrosis, JAK/STAT: janus kinase/signal transducer and activator of transcription, miRNAs: microRNA, MMP: Matrix metalloproteinase, PPAR: peroxisome proliferator-activated receptor, TBM: tubular basement membrane, tPA: tissue plasminogen activator, TGF: Transforming growth factor, TLRs: Toll-like receptors, tTG: tissue transglutaminase, VEGF: vascular endothelial growth factor

Table 2

Interstitial Fibrosis Evaluation

Method	Description	Measure	Ref.(s)
VA; "routine"	IF, inflammation, and glomerulopathy correlated with poor allograft outcome	By multivariate analysis, IF and inflammation lead to poorer survival (HR = 8.5, p < 0.0001); IF alone had less effect (HR = 4.8, p = not significant)	[200]
IA; TC (Masson), SR, and SMA IHC	IA and semi-quantitative assessments (VA) were performed on late allograft biopsies	IF by VA predicted Banff '97 ci scores (p < 0.0001) and correlated with GFR, Cr, and urine total protein (r = − 0.48, p = 0.0007; r = 0.46, p = 0.0009; r= 0.51, 0.0009, respectively). Of IA methods, only SR-nonpolarized score correlated with GFR and urine total protein (r = −0.29, p = 0.05; r = 0.29, p = 0.05, respectively)	[14]
IA; TC (Masson)	IF IA correlates with serum Cr in IgA nephropathy and MPGN	IF occupied > 10% of the interstitium in all 10 cases and > 20% in 6 and IF IA correlated with serum Cr	[201]
IA; TC (Masson)	IA of IF in patients receiving cyclosporine	IF grade by IA correlated with worsened Cr clearance between 1 and 3 years	[10]
IA; TC (Light Green)	IF IA in patients randomized to cyclosporine or conversion to sirolimus	No difference in groups with respect to fibrosis but GFR improved significantly in the conversion group	[202]
IA; TC (Light green)	Quantitative IF in sequential renal biopsies	IF evolution correlated with eGFR	[11]
IA; SR and collagen	Renal IF correlates with presence of TGF-β, decorin, SMA, and interstitial collagens	In all samples with IF, TGF-β up-regulation was observed in combination with reduced decorin expression	[169, 203– 205]
IA; SR	SR IA predicted long-term renal allograft function	Cortical IF correlated with time to graft failure (r = 0.64, P < 0.001) at 6 months post transplant	[24]
IA; SR	SR IA predicted long-term renal allograft function	Positive correlation (r = 0.62, P<0.001) between SR fibrosis and decreased GFR	[7]
IA; SR	SR IA corresponded to light microscopic semiquantitative measurements (r = 0.439, P = 0.0003 overall and r = 0.704, P < 0.0001 for just baseline specimens) in kidney allografts	Semiquantitative methods correlated best with long-term graft function (serum Cr at 8 – 10 years (P = 0.010) and late graft loss (P = 0.0445)	[206]
IA; SR	IF in non-heart-beating donor kidneys and conventional heart-beating donor kidneys	No significant difference in IF between the two groups	[207]
IA; SR	IF scoring predicts survival in lupus nephritis	Fibrillary collagen was predictive of Cr doubling (*P* = 0.01) and relapse (P = 0.06)	[208]
IA; SR	IA-based application (Fibrosis HR) for IF and glomerular morphometry	Intra- and interoperator variability was present in manual segmentation of IF, mesangial matrix, and glomerular areas but interactive identification didn't have this variability	[209]
IA; SR	IF measurements using digital imaging coupled with point counting correlated with GFR	Direct relationship between interstitial volume fraction and renal function (r^2 = 0.54)	[8]
IA; SR	SR IF measurement combined with ultrasound measurements of renal artery resistance index helped predict "chronic allograft nephropathy"	Positive correlation (r = 0.62, P <0.001) between picroSR-stained cortical fractional IF volume and decreased GFR	[9]
IA; CIII IHC	IF measurements by a semiautomatic system correlate with GFR in protocol renal transplant biopsy specimens	Area fraction of collagen III IHC of > 40% @ 6 months associated with decreased GFR @ 24 months compared with ≤ 40% (r=−0.32, P=0.03)	[12]
IA; CIII IHC	IF measurements by a semiautomatic system correlate with GFR in protocol renal transplant biopsies	GFR correlated negatively with interstitial volume fraction @ 6 months (P = 0.05)	[13]

Method	Description	Measure	Ref.(s)
IA and VA; TC (Masson)	Cyclosporine (CsA) therapy effects on fibrosis IA	IF measured by IA was significantly higher in the CsA group only in renal allografts 6 months posttransplant ($P < 0.04$)	[210, 211]
IA and VA; CII IHC, TC, and SR	Comparison of CII IHC, TC, and SR IA	Collagen III IHC and VA of TC-stained slides correlated best with each other and with GFR	[6]

CIII: Collagen III, Cr: creatinine, eGFR: estimated GFR, GFR: glomerular filtration rate, IHC: immunohistochemistry, IF: interstitial fibrosis, IA: Image analysis, MPGN: membranoproliferative glomerulonephritis, SMA: smooth muscle actin, SR: Sirius red, Ref(s): References, TC: Trichrome, TGF-β: transforming growth factor, VA: visual analysis.