TGF-β Signaling in Renal Disease

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Abstract. Since discovery over a decade ago of a role for the cytokine TGF-β as key mediator of glomerular and tubulointerstitial pathobiology in chronic kidney diseases, studies of TGF-β signaling in the kidney have focused on the molecular biology of fibrogenesis. In recent years, glomerular and tubular epithelial cell apoptosis and cellular transdifferentiation have been proposed as putative primary pathomechanisms that may underlie progression of renal disease. This review describes evidence in support of nonlinear models and functional roles of TGF-β signaling in mediating apoptosis and epithelial-to-mesenchymal transdifferentiation (EMT) in chronic progressive renal disease. Emphasis is placed on cell context-dependent models of TGF-β signaling providing a conceptual framework to consolidate seemingly distinct pathomechanisms of progression of glomerular and tubulointerstitial disease.

The progression of chronic renal diseases is an increasingly common condition, often leading to complete destruction of functional kidney tissue and dependency of affected individuals on life-long treatments with dialysis or renal allograft transplantation (1). Pioneering studies of Border et al. (2–4), Ziyadeh and Sharma (5,6), and many others (reviewed in reference 7) indicate a central role for TGF-β and its downstream signaling cascades in activating cellular pathomechanisms that underlie the progression of renal diseases. In general terms, the TGF-β superfamily consists of secreted peptides, of which the three TGF-β isoforms (TGF-β1, -β2, and -β3), activins, and bone morphogenetic proteins (BMP) are best known in mammalian development, homeostasis, and pathobiology. The TGF-β isoforms are widely expressed and act on virtually every cell type in mammals by engaging a ubiquitous intracellular signaling cascade of SMAD family proteins through ligand-induced activation of heteromeric transmembrane TGF-β receptor kinases. Receptor-activated Smad protein complexes accumulate in the nucleus, where they participate directly in transcriptional activation of target genes. Figure 1 shows the basic TGF-β/Smad signaling axis.

Although this seemingly simple linear model has emerged in recent years as a central view of the TGF-β/Smad signaling axis (8), it has become clear that it is insufficient to explain the extensive and context-dependent multifunctionality of TGF-β that is a hallmark of these peptides (9,10). Thus, TGF-β are considered important regulators of cell proliferation, differentiation, apoptosis, immune response, and extracellular matrix remodeling, depending on physiological context. In light of this multifunctionality, it is not surprising that an astonishing array of different cytoplasmic and nuclear proteins and mechanisms have been discovered during recent years, which are exerting direct or indirect agonist or antagonist transmodulation on the central TGF-β/Smad signaling axis. In addition, it is increasingly apparent that TGF-β receptors can activate Smad-independent signaling mechanisms, although it remains unclear how non-Smad pathways are connected to TGF-β receptors at a molecular level (11). A comprehensive overview of the emerging complexity of TGF-β/Smad signaling transmodulators is shown in Figure 2, which is extensively annotated in the corresponding figure legend. Discussion of molecular details of individual TGF-β/Smad transmodulator action is clearly beyond the scope and intent of this review. The interested reader is referred to outstanding general reviews, emphasizing molecular details of the emerging complexity and context-dependence of TGF-β/Smad signaling (see references 12–15). A major focus of the present review is to evaluate critically the TGF-β/Smad signaling system in the context of renal disease progression, not as a monolithic, linear inducer of fibrogenesis, but rather as a segment of dynamically regulated, versatile signaling networks.

Reevaluating Paradigms of Renal Disease Progression

Excessive renal fibrogenesis, the process leading to tissue fibrosis, is considered a dominant pathomechanism induced by TGF-β in the kidney, largely on the basis of the observation that glomerular and tubulointerstitial scarring are universal outcomes of renal disease progression. In part, the current scientific focus on renal fibrogenesis may be attributable to the availability of molecular targets, i.e., extracellular matrix components, and assays that allow renal researchers to monitor fibrotic reactions easily at a molecular and whole organ level. In contrast, it has been more challenging to examine cellular pathomechanisms, such as apoptosis, proliferation, and transdifferentiation, largely because chronic progressive kidney dis-
ease in humans frequently is a protracted, focal, and/or seg-
mental process, making it difficult to detect significant single

cell alterations. However, along with readily apparent renal
scarring, a striking phenotype of chronic progressive kidney
disease is the disappearance of differentiated glomerular, tu-
bular, and vascular cells that constitute the normal nephron
(16). Thus, tubular cell atrophy and dilatation are invariably
associated with progressive nephron loss and onset of tubulo-
interstitial fibrosis, a robust histopathologic predictor of
chronic progressive renal disease (17,18). In addition, loss of
peritubular capillaries is noted in progressive renal disease in
humans (18). Indeed, a chronic hypoxia hypothesis has been
proposed, stating that abnormal post-glomerular hypertension
and vasoconstriction reduce peritubular capillary blood flow
and cause rarification of peritubular capillaries, resulting in
local hypoxia and tubular atrophy (19). Other reports suggest
that tubular atrophy and interstitial fibrosis may be induced by
increased protein content of ultrafiltrate, giving rise to an
inflammatory reaction and interstitial fibrosis (20,21). Upregu-
lation of TGF-ß is consistently associated with these post-
glomerular events, irrespective of the proposed model of
pathogenesis (22–24).

Sclerosing glomeruli are characterized by progressive deple-
tion of podocytes (16,25) and striking loss of glomerular cap-

Figure 1. A simplified cartoon of the SMAD protein family and the basic TGF-ß/Smad signaling axis. TGF-ß ligand-binding with type II
receptor (RII) serine/threonine kinases (constitutively active) induces recruitment of type I receptor (RI) kinases into a heteromeric
ligand-receptor complex, leading to activation of RI kinase. The activated RI then signals to SMAD family members by phosphorylation of
two COOH-terminal serine residues, which are conserved in the receptor-regulated subgroup of Smads, R-Smads (Smad1, 2, 3, 5, and 8).
Smad1, 5, and 8 are substrates of bone morphogenetic proteins (BMP) receptor kinases. Smad2 and 3 are substrates of TGF-ß and activin
receptor kinases. Phospho-Smads interact with cytoplasmic common-partner Smad (Co-Smad), Smad4, which lacks COOH-terminal serines
and is not phosphorylated. R-Smad/Co-Smad complexes translocate to the nucleus and bind to specific DNA sequence motifs in target genes
to participate in transcriptional activation. R-Smads and Co-Smads are characterized by two major domains of homology, MH1 and MH2,
which are connected by a variable linker region. A subfamily of inhibitory Smads (I-Smads) lacks MH1 domains and consists of Smad6 and
Smad7, which inhibit TGF-ß/Smad signaling.
Figure 2. Mediator and modifier steps regulating the TGF-β/Smad signaling axis. Green arrows indicate agonist, and red arrows/lines indicate antagonist function of the depicted steps, respectively. Upon activation of latent TGF-β complexes and release of TGF-β ligand in the extracellular space, ligand binding induces heteromorphic TGF-β receptor complexes and type I receptor kinase activity. Recruitment and C-terminal phosphorylation of cytoplasmic R-Smads, Smad2 and Smad3, by activated TβRI is regulated by cytoskeletal proteins and multiple proteins with scaffold, adaptor, anchoring, and/or chaperone function (pink boxes). Green lettering and (+) indicate positive regulation. Red lettering and (−) indicate negative regulation of TGF-β/Smad signaling. Activated R-Smads and cytoplasmic Co-Smad Smad4 form heteromorphic complexes that translocate to the nucleus via nuclear transporters (yellow box). Nuclear Smad complexes bind to consensus Smad3/4 DNA binding elements (SBE) (beige box) in TGF-β/Smad immediate-early target genes. Example SBE core sequences and their respective positions in select TGF-β target gene promoters are shown (beige box). Smads regulate target gene transcription together with nuclear transcription factors (brown box), co-activators (grey box), and/or co-repressors (olive box). A list of gene symbols for new immediate-early TGF-β target genes, identified by microarray screens (11), is shown (green box). Gene symbols can be searched in NCBI Unigene (http://www.ncbi.nlm.nih.gov/UniGene/). Gene activation may be limited and/or terminated by targeting of nuclear R-Smads for proteasome degradation via E2 or E3 enzyme complexes (light blue box). Cytokine and TGF-β–inducible I-Smads inhibit the TGF-β/Smad signaling axis by competitive interaction with R-Smads and TβR1, and/or by recruitment of Smurf ubiquitin-ligases (light blue boxes) to activated TβR complexes, leading to its removal via proteasomal degradation. Receptor tyrosine kinase–activated MAPK Erk may phosphorylate motifs in the linker regions of R-Smads to prevent nuclear translocation of activated Smad complexes and inhibit the TGF-β/Smad signaling axis. Extracellular modulators: TSP1, thrombospondin 1; LAP, latency-associated peptide; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; IL-1β, interleukin 1β; HGF, hepatocyte growth factor; EGF, epidermal growth factor. Cross-regulating signal transducers: Stat1, signal transducer and activator of transcription 1; NF-κB, nuclear factor κB; Erk, extracellular signal-regulated kinase. Chaperones/Adaptors: SARA, Smad anchor for receptor activation; Hrs/Hgs, hepatic growth factor-regulated tyrosine kinase substrate; Cav-1, caveolin-1; Dab-2, disabled-2; SNX, sorting nexin; TRAP, serine-threonine kinase receptor-associated protein; TRAP-1, TGF-β receptor-associated protein-1. Nuclear transporters: Crm1, chromosome region maintenance 1; Ubiquitin ligases: Ubc3, ubiquitin conjugating enzyme 3; UbcH5b/c, ubiquitin conjugating enzyme H5b/c; ROC1-SCF(Fbw1a): ROC1, Skp1, Cul1, and Fbw1a complex. Transcription factors: AR, androgen receptor; ATR-2, activating transcription factor-2; BF-1, brain factor-1; E1A, early region 1A; ER, estrogen receptor; Evi-1, ectopic viral integration site-1; FAST/FoxH1, forkhead activin signal transducer; Gli3, glioblastoma gene product 3; GR, glucocorticoid receptor; HNF4, hepatocyte nuclear factor 4; LEF/TCF, lymphoid enhancer factor/T-cell factor; ME2, myocyte enhancer-binding factor 2; Menin, multiple endocrine neoplasia-type 1 tumor suppressor protein; Miz1, Myc interacting zinc finger protein 1; OAZ, Olf-1/EBF associated zinc-finger; PEBP2/CBF/A/AML, polyoma-virus-enhancer-binding protein/core-binding factor A/acute myeloid leukemia; SNIP1, Smad nuclear interacting protein 1; TFE3, transcription factor mu E3; Sp1, specificity factor 1; Sp3, specificity factor 3; VDR, vitamin D receptor. Co-Activators: MSG1, melanocyte-specific gene 1; P/CAF, p300/CBP-associated factor. Co-Repressors: SnoN, ski-related novel gene; TGF, TGF-β-interacting factor; HDACs, histone deacetylases; Ski, Sloan-Kettering Institute proto-oncogene.
illaries associated with depletion of endothelial cells (26–28). Kriz et al. (25) proposed that podocyte depletion and/or detachment lead to subsequent synchie formation and tuft adhesions as initial lesions of glomerular injury, consistent with the frequently observed segmental nature of early glomerular disease. Their concept of podocyte depletion as an initiating lesion in glomerulosclerosis is further supported by observations in humans with diabetes. Podocyte numbers are reduced in otherwise normal appearing glomeruli and predict long-term urinary albumin excretion (29). Together, these phenotypic observations suggest that segmental loss of differentiated podocytes and tubular and microvascular endothelial cells may be initial and irreversible lesions in progressive nephron loss. Thus, we propose that a comprehensive model of progression of renal disease must provide pathomechanisms to account for progressive loss of differentiated renal cells.

The common fibrocentric paradigm hypothesizes that fibrosis is the primary pathomechanism mediating renal disease progression and that the primary pathogenetic role of TGF-ß signaling lies in promotion of fibrogenesis (30). However, in the context of the aforementioned observations, this paradigm is apparently insufficient in that it fails to provide molecular and cellular links to explain the loss of differentiated renal cells. There is no direct experimental evidence to support the central tenet of the fibrocentric paradigm, namely that expanded and/or altered extracellular matrix leads to demise of differentiated renal cells and ultimately organ dysfunction. Interestingly, recent analyses of a classical condition of excessive scarring, systemic sclerosis, suggest that autoantibody-mediated endothelial cell apoptosis is a primary pathomechanism leading to secondary sclerotic skin and organ destruction (31,32). Other conditions associated with excessive scarring, such as posttraumatic hypertrophic scars and desmoplastic tumors, are characterized by fibroplasia and increased vascularization, indicating that extracellular matrix expansion per se is not sufficient to induce atrophy and/or depletion of resident cells (33,34). If renal fibrosis is not causing nephron loss, what is the evidence for alternative pathomechanisms mediated by TGF-ß? Interestingly, in contrast with renal research, studies of TGF-ß/Smad signaling in non-renal diseases commonly examine cellular responses other than fibrogenesis, such as cell cycle control, apoptosis, and differentiation. Can nephron loss thus be explained in part on the basis of apoptosis and/or transdifferentiation induced by TGF-ß signaling?

TGF-ß and Tubular Atrophy

There is indeed increasing evidence indicating a role for apoptosis in tubular epithelial cells, causing tubular atrophy in various experimental models and human forms of progressive renal disease, including chronic obstructive nephropathy (CON), cyclosporine-induced allograft nephropathy, diabetic kidneys, and others (35–37). In these studies, TGF-ß1 expression was associated with apoptotic tubular cells. Angiotensin II blockade, or inhibition of TGF-ß using anti-TGF-ß antibodies, reduced tubular epithelial apoptosis and reduced the extent of tubular atrophy in models of CON and diabetic kidneys (38,39). Interestingly, a detailed analysis from our group of tubulointerstitial lesions in TGF-ß1 transgenic mice, a model of progressive glomerulosclerosis and tubulointerstitial fibrosis (40,41), indicates that increased tubular cell apoptosis precedes manifestations of tubular atrophy, tubular dilatation, and perivascular inflammation [Wenjun Ju, Markus Bitzer, and Erwin Böttinger; personal communication, August 2002]. Work by Neilson and colleagues and others (42–44) suggests that tubular epithelial cells may transdifferentiate to acquire (myo)fibroblast phenotypes associated with interstitial fibrosis in experimental models and human renal biopsies (45). Because TGF-ß is a well-known inducer of epithelial-to-mesenchymal transdifferentiation in several organs (11,46,47), it is perhaps not surprising that recent reports also implicate a direct role for TGF-ß in mediating EMT of renal tubular epithelial cells in vivo and in vitro (48,49).

TGF-ß and Podocyte Depletion

As described before, glomerulosclerosis in animal models and humans is characterized in part by depletion of visceral epithelial cells (podocytes) (25,50). Mechanical detachment of podocytes from glomerular basement membranes (GBM) and loss in urinary space, possibly due to altered cell adhesion and/or increased mechanical stress of injured podocytes, has been proposed as a potential mechanism. In addition, it is thought that podocytes are unable to proliferate and to replace the “lost” podocytes in most forms of glomerular injury, leading to a state of relative podocyte insufficiency (25,51,52). We have shown that TGF-ß and Smad7 synergize to induce apoptosis in podocytes in vitro (53). Our in vivo studies indicate that time of peak podocyte apoptosis coincides with expression of TGF-ß1 and Smad7, and with the onset of albuminuria, but precedes mesangial expansion in a TGF-ß1 transgenic model of progressive glomerulosclerosis (53). Recent results from our lab, obtained in CD2AP knockout mice, a new murine model of focal segmental glomerulosclerosis (54), are similar to and confirm these observations in a second, independent experimental model of chronic progressive glomerulosclerosis [Mario Schiffer, Andrey Shaw, and Erwin Böttinger; personal communication, August 2002].

TGF-ß and Loss of Capillary Endothelial Cells

Progressive renal disease is characterized in part by a progressive loss of the glomerular and peritubular microvasculature (27). Loss of glomerular capillaries is associated with increased apoptosis of glomerular endothelial cells and correlates with the development of glomerulosclerosis in rodent models of progressive anti-GBM disease, in remnant kidney models, and in aging kidney (28,55,56). Similarly, endothelial cell (EC) apoptosis may underlie the loss of peritubular capillaries characteristically associated with tubulointerstitial fibrosis and tubular atrophy (57). Several reports demonstrate that TGF-ß and thrombospondin 1 (TSP-1), a putative activator of extracellular TGF-ß, induce apoptosis in microvascular endothelial cells, including glomerular endothelial cells (58,59). In addition, loss of paracrine signaling of angiogenic survival factor vascular endothelial growth factor (VEGF), acting on endothelial cells, enhances EC susceptibility to undergo apo-
poptosis and causes EC growth arrest (27). Loss of expression of VEGF in glomerular diseases and progressive renal disease has been described (27). VEGF is constitutively and selectively expressed in podocytes and tubular epithelial cells in normal kidneys (60,61); therefore, TGF-β–induced depletion of podocytes and/or tubular epithelial cells may be causing the decreased VEGF expression. TGF-β may thus indirectly promote EC apoptosis by depleting VEGF-producing cell types in progressive renal disease.

Revised Model of TGF-β-Mediated Pathomechanisms of Progressive Nephron Loss

On the basis of these observations, we propose a substantially revised model, including new functional roles and multiple pathogenetic endpoints, of TGF-β signaling in renal disease progression (Figure 3). Whereas interstitial and mesangial matrix expansion may result from direct activation of “fibrogenic” signaling networks by TGF-β in mesangial cells and interstitial (myo)fibroblasts, additional primary pathomechanisms mediated by TGF-β signaling should include apoptosis and EMT. Thus, TGF-β signaling may initiate pro-apoptotic effectors and/or EMT in tubular epithelial cells, resulting in tubular degeneration and tubular atrophy (Figure 3). In addition, TGF-β–induced EMT may convert tubular epithelial cells into activated (myo)fibroblasts, which may be responsible for increased deposition of interstitial matrix in response to TGF-β–Smad signaling (Figure 3). Decline of tubular VEGF expression as a result of tubular degeneration/atrophy may indirectly contribute to decreased EC survival in peritubular capillaries and peritubular capillary loss (Figure 3). Thus, nonlinear, response-specific TGF-β signaling networks may lead to tubulointerstitial fibrosis by inducing multiple pathogenic processes in tubular and microvascular cells. In addition, we propose that podocyte and endothelial cell apoptosis are directly induced by TGF-β signaling in glomeruli exposed to various forms of injury (Figure 3). Apoptosis of podocytes may lead to depletion of podocytes and formation of synchiae between bare GBM and Bowman’s capsule. This results in segmental tuft adhesions characteristic of early lesions in progressive glomerulosclerosis (Figure 3). Concomitant loss of glomerular capillaries may be a result of decreased EC survival caused by direct, pro-apoptotic signaling networks of TGF-β in EC and by a decrease of VEGF associated with progressive podocyte depletion (Figure 3).

This model of multiple pathogenetic events mediated by TGF-β further implies engagement of distinct signaling modules/networks for the TGF-β/Smad segment to specify pro-apoptotic signals in podocytes, tubular epithelial cells, and/or endothelial cells, as opposed to EMT signaling cascades in tubular epithelial cells or pro-fibrotic signals in mesenchymal myofibroblasts and/or mesangial cells. The proposed shift of functional endpoints of TGF-β/Smad signaling from fibrosis to apoptosis and EMT as putative primary pathomechanisms for progressive neplon loss has important implications for future experimental approaches and selection of therapeutic targets in progression of renal disease. In the remaining sections, we review recent advances in our understanding of distinct, response-specific signaling networks activated by TGF-β/Smad to mediate EMT and apoptosis.

TGF-β Signaling Networks in Epithelial-to-Mesenchymal Transdifferentiation

Activation of TGF-β signaling is sufficient to induce EMT in cultured epithelial cells, including a non-transformed mouse mammary cell line (NMuMG) and human keratinocytes (HaCat) (11,46,62,63). A role for EMT in tubular atrophy and appearance of myofibroblasts in renal disease was first proposed several years ago (64). However, evidence for TGF-β as a mediator of renal tubular EMT has only recently been reported (49,65). For example, advanced glycation end products (AGE) were found to induce EMT in vitro and in diabetic rats through activation of TGF-β signaling, indicating an important role for this TGF-β–induced response in progression of diabetic nephropathy (49). On the basis of recent studies of signaling pathways activated by TGF-β to induce EMT in various types of epithelial cells, a model of this response-specific TGF-β signaling network is emerging (Figure 4). EMT is a coordinated cellular response that involves several distinct processes, including disruption and disassembly of desmosomes and E-cadherin adherens junctions, remodeling of actin cytoskeleton and stress fiber formation, alteration of cell-matrix adhesion, and increase in cell motility (Figure 4). To date, Smads have been implicated in some aspects of TGF-β–induced EMT. For example, overexpression of Smad2, Smad3, and Smad4 in NMuMG murine mammary gland epithelial cells induces stress fiber formation (46). In addition, TGF-β induces Net1A, a RhoA-specific guanine exchange factor, in a Smad-dependent manner (11,66). Net1A is required for TGF-β–induced F-actin remodeling (66). This is consistent with observations that TGF-β can rapidly induce the activation of the small GTPase RhoA, a regulator of actin cytoskeleton and adhesion junctions in NMuMG cells (67). Inhibitors of Rho kinase can block F-actin remodeling and relocalization of E-cadherin in adherens junctions induced by TGF-β (67). RhoA and phosphatidylinositol 3-kinase (PI3K), signaling through the serine-threonine kinase p160ROCK and Akt/PKB, are both required for...
TGF-β–induced disassembly of cell-cell junctions and F-actin remodeling (47,67). TGF-β induces Smad-interacting zinc finger protein SIP1 (68) and SLUG (11), transcriptional repressors of E-cadherin adhesions junction disassembly, cell motility, F-actin remodeling (stress fiber formation), and activation of transitional progenitor cell factors. TGF-β receptor activated pathways/mediators implicated in EMT include Smads, MEK/ERK, Net1A, RhoA, PI3K, PKB/Akt, p160 ROCK, and hypothetical mediators (+/− Other mediators) (shaded boxes). Genes induced by TGF-β within 4 h (11) are denoted by Unigene Symbols colored in red (searchable at http://www.ncbi.nlm.nih.gov/UniGene/). Genes and pathways are aligned according to time of peak activation. Solid line arrows indicate pathways, programs and cellular responses suggested in reference 11. Broken line arrows show mediators and dependent responses demonstrated in other reports. Dotted line arrows show putative pathways proposed in reference 11.

TGF-β–induced disassembly of cell-cell junctions and F-actin remodeling (47,67). TGF-β induces Smad-interacting zinc finger protein SIP1 (68) and SLUG (11), transcriptional repressors of E-cadherin capable of mediating disassembly of cell adherens junctions in tubular epithelial MDCK cells. We reported a microarray-based screen of transcriptional profiles of TGF-β–induced EMT in human HaCat keratinocytes (11). These studies suggest a rapid (within 4 h) and dynamic change in expression of approximately 4000 transcripts, including factors with roles in mesenchymal progenitor cell types, and Wnt and Notch signaling. Our recent results demonstrate that functional inactivation of γ-secretase, an activator of Notch receptor, completely prevents EMT induced by TGF-β, indicating a role for Notch signaling components downstream of TGF-β [Jiri Zavadil, Lukas Cermak, and Erwin Böttinger; personal communication, August 2002]. In contrast, chemical inhibition of mitogen-activated protein kinase Erk (MAPK Erk) blocks selectively TGF-β–mediated regulation of genes with functional roles in cell-matrix interactions, cell motility, and endocytosis (11). This is consistent with functional inhibition of TGF-β–induced disassembly of adherens junctions and cell motility in the presence of MEK/ERK inhibitor (11).

Signaling networks underlying EMT in many tissues, including kidney, is a young field. Most studies of this cellular response are conducted in the context of embryonic development and tumor invasiveness and metastasis formation. The described knowledge of TGF-β signaling mediating EMT has been obtained largely in these contexts. However, a detailed
understanding of the molecular signaling mechanisms that mediate tubular epithelial cell EMT in nephrons exposed to pathogenic stimuli will likely be essential for development of therapies that can prevent renal disease progression.

**TGF-β Signaling Networks in Apoptosis**

It has been difficult to establish apoptosis as a candidate pathomechanism mediated by TGF-β in progression of renal disease, because detection and reliable quantitation of apoptotic cells in chronic progressive renal injury is complicated by several factors. First, the half-life of apoptotic cells is only a few hours, limiting the window of detection. In contrast with extracellular matrix components, cellular remnants of apoptosis are not accumulating in tissue but are often removed by phagocytosis. In addition, detached apoptotic podocytes and tubular epithelial cells may be flushed away by urine. As a consequence, a low percentage of apoptotic cells detectable in a tissue section may be associated with a significant loss of cell mass (69,70). Interestingly, glomerular cell apoptosis is markedly increased in renal biopsy specimen from patients with IgA nephropathy, with the greatest number, up to one apoptotic cell per cross section, found in lesions described as “predominantly sclerosing” (71,72). The number of apoptotic cells per glomerular cross-section correlates with semiquantitative “sclerosis” scores in IgA nephropathy and lupus nephritis (73,74).

TGF-β can induce apoptosis in many cell types, including renal cells (reviewed in references 53,75,76). A review of apoptotic signaling networks of TGF-β is best guided by organization of apoptotic signaling in three phases (Figure 5). An Initiation phase is triggered by stress signals and/or specific factors (including TGF-β) acting through a subset of receptors. During Initiation and Integration phase, cells balance signals from several signaling pathways (pro- and anti-apoptotic) to determine whether the Execution phase of cell death should be

![Figure 5. Signaling networks mediating TGF-β–induced apoptosis. Apoptosis signaling is comprised of three phases: Initiation, Integration, and Execution. TGF-β receptor activation (RI/RII) leads to activation of Smad3. Smad3 is required for upregulation of pro-apoptotic signaling mediators death-associated protein kinase (DAPK), TGF-β–induced early gene (TIEG), TGF-β–stimulated clone 22 (TSC22), and Smad7. Smad7 inhibits anti-apoptotic signaling by NF-κB, and may be responsive to TGF-β–independent signals from pro-apoptotic factors. Smad7 also activates pro-apoptotic JNK. TGF-β–induced activation of pro-apoptotic MAPK p38 and JNK may be mediated by MAPK cascades via hematopoietic progenitor kinase 1 (HPK1), TGF-β–activated kinase 1 (TAK1), and TAK1 activator (TAB1). Daxx interacts with RII and activates JNK. TGF-β induces apoptosis in murine podocytes through activation of p38 and induction of pro-apoptotic protein Bad, leading to activation of caspases. TGF-β stimulates nuclear translocation of pro-apoptotic ARTS from mitochondria.](image)
activated. Critical regulators of the execution phase are the Bcl2 family of proteins, which include anti-apoptotic (e.g., Bcl-2, Bcl-X<sub>L</sub>) and pro-apoptotic (e.g., Bax, Bad, and Bid) members. Relative expression levels of Bcl proteins can trigger the irreversible execution phase by regulating release of cytochrome c from the outer mitochondrial membrane, leading to activation of effector protease cascades of the caspase family.

There is growing evidence that Smad3 is an important signaling anchor for apoptotic networks. Thus, TGF-β–induced apoptosis is enhanced by overexpression of R-Smads in some cells (77). In addition, a number of target genes of Smad3 appear to mediate TGF-β–induced apoptosis. Smad3 is required for transcriptional activation of death-associated protein kinase (DAPK), which is essential for TGF-β–induced apoptosis in human hepatoma cells and Burkitt Lymphoma cells (78). We also found Smad3-dependent regulation of DAPK in murine podocytes (Markus Bitzer and Erwin Böttinger; personal communication). In addition, activation of several target genes of TGF-β, including TGF-β–induced early gene (TIEG) in mink lung epithelial and Hep3B cells, and TGF-β–stimulated clone 22 (TSC-22) in gastric carcinoma cells (79–81) have been implicated in apoptosis. Because TGF-β activates transcription of these genes, it is likely that Smad3 is required for these signals. In addition, TGF-β–induced activation of pro-apoptotic Bcl member Bad in FaO rat hepatoma cells is mediated by Smad3 (82). Daxx is a Fas-receptor–associated protein that can interact directly with TGF-β type II receptor. Daxx activates the JNK pathway and mediates apoptosis induced by Fas, but dominant negative mutant Daxx or functional inactivation with Daxx antisense oligonucleotides also inhibit TGF-β–induced apoptosis in B cells and mouse hepatocytes (83). Thus, Daxx may mediate a direct biochemical connection between the TGF-β receptors and the apoptotic signaling network.

Several reports implicate MAPK pathways in apoptotic signaling by TGF-β. Activation of TGF-β–activated kinase-1 (TAK-1), a protein of the MAP kinase kinase kinase family, activates p38 and JNK signaling in TGF-β family–induced apoptosis (84). In addition, the upstream activator of TAK1, TAB1, may link pro-apoptotic MAPK to TGF-β receptors through receptor-mediated activation of hematopoietic progenitor kinase 1 (HPK1) (85,86). p38 signaling is required for TGF-β–induced apoptosis in murine podocytes (53). It is unclear how TGF-β receptors signal to MAPK pathways or HPK1 to induce apoptosis. There is also evidence that Smad7 may induce apoptosis by activation of JNK (87). Indeed, the I-Smad7 has been implicated in apoptosis signaling by TGF-β and TGF-β–independent pathways. TGF-β–stimulated expression of Smad7 is required for induction of apoptosis via p38 activation in prostatic carcinoma cells (88), indicating that Smad7 may function downstream of TGF-β in apoptosis signaling networks. However, ectopic expression of Smad7 can induce apoptosis independently of TGF-β pathways in murine podocytes (53) and distal tubular epithelial MDCK cells (89). Both reports indicate that Smad7 inhibits nuclear translocation and transcriptional activator function of NF-κB, a major activator of transcription of genes with anti-apoptotic functions (53,89). Because Smad7 is regulated by TGF-β in a Smad3-dependent manner (90), and by other extracellular stress and receptor signals (91–93), it may have an important role in enhancing TGF-β–dependent pro-apoptotic signaling, as well as inhibition of anti-apoptotic pathways independent of TGF-β (93). A recent report by Larisch et al. (94) identifies a septin family protein, apoptosis-related protein in the TGF-β signaling pathway (ARTS), which is required for TGF-β–induced apoptosis in rat prostate cells. ARTS is normally localized to mitochondria and translocates to the nucleus when TGF-β induces apoptosis. Together, these TGF-β–regulated signaling mediators of apoptosis participate in the Integration and Execution phases of apoptotic signaling networks by stimulating expression of pro-apoptotic members and by suppressing expression of anti-apoptotic members of the Bcl protein family, respectively (95–97).

**Concluding Remarks**

In this review, we attempt to provide a rationale for a revised model of TGF-β signaling in progressive renal disease. The role of TGF-β in renal fibrosis is widely accepted and targets of fibrogenic signaling have been discussed (98,99); we have therefore focused on TGF-β–mediated apoptosis and EMT. Apoptosis and EMT are considered primary cellular pathways mediated by response-specific TGF-β signaling networks in various forms of tissue injury. We propose that these mechanisms may cause progressive loss of differentiated renal cells, a hallmark of chronic progression of renal disease. TGF-β–induced apoptosis is likely to have a pathogenetic role in podocyte depletion and glomerulosclerosis, tubular degeneration/atrophy, and loss of glomerular and peritubular capillaries. In addition, EMT induced by TGF-β may contribute to tubular atrophy and generation of interstitial myofibroblasts, leading to concomitant tubulointerstitial fibrosis. The revised model of TGF-β signaling in renal disease is intended to stimulate new perspectives on selection of scientific and therapeutic approaches to understand and prevent chronic progression of renal disease.

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