MINIREVIEW

Transforming Growth Factor-β Signal Transduction and Progressive Renal Disease[1]

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Transforming growth factor-β (TGF-β) superfamily members are multifunctional growth factors that play pivotal roles in development and tissue homeostasis. Recent studies have underscored the importance of TGF-β in regulation of cell proliferation and extracellular matrix synthesis and deposition. TGF-β signaling is initiated by ligand binding to a membrane-associated receptor complex that has serine/threonine kinase activity. This receptor complex phosphorylates specific Smad proteins, which then transduce the ligand-activated signal to the nucleus. Smad complexes regulate target gene transcription either by directly binding DNA sequences, or by complexing with other transcription factors or co-activators. There is extensive crosstalk between the TGF-β signaling pathway and other signaling systems, including the mitogen-activated protein kinase pathways. The importance of TGF-β in regulation of cell growth has been emphasized by recent observations that mutations of critical elements of the TGF-β signaling system are associated with tumor progression in patients with many different types of epithelial neoplasms. TGF-β has emerged as a predominant mediator of extracellular matrix production and deposition in progressive renal disease and in other forms of chronic tissue injury. In this overview, recent advances in our understanding of TGF-β signaling, cell cycle regulation by TGF-β, and the role of TGF-β in progressive renal injury are highlighted. Exp Biol Med 227:943–956, 2002.

Key words: transforming growth factor-β; kidney; signaling; extracellular matrix; progressive renal disease

Transforming growth factor-βs (TGF-β) are members of a superfamily of polypeptide growth/differentiation factors identified in a wide variety of organisms ranging from insects to humans (1, 2). TGF-βs play a critical role in regulating many fundamental biological processes such as cell growth, differentiation, development, tissue repair, and apoptosis. Based on structural and biological similarities, the TGF-β superfamily can be subdivided into four major families: the Mullerian inhibitory substance (MIS) family, the inhibin/activin family, the bone morphogenetic protein (BMP) family, and the TGF-β family. MIS can induce regression of the Mullerian duct in male embryos (3). The inhibins and activins were originally iden tified by their ability to regulate hormone secretion in pituitary cells; they can also regulate mammalian erythroid differentiation (4, 5). Activins regulate branching morphogenesis during development of the kidney and other glandular organs (6). BMPs were purified as factors that induce ectopic bone formation and they regulate various early developmental processes in invertebrates and vertebrates (7–12). BMP-7 is essential for normal renal development (13). Mice with homozygous deletions of the BMP-7 gene die of renal failure shortly after birth (14). The kidneys are cystic, with a marked decrease in the number of nephrons (15).

Five distinct members of the TGF-β family have been identified in vertebrates; three of them (TGF-β1, 2, and 3) are expressed in mammals (16). Distinguished initially for their ability to inhibit the growth of most epithelial and hematopoietic cells and to regulate the production of extracellular matrix by mesenchymal cells, these peptides are now known to control a great diversity of developmental processes and to play key roles in acute and chronic inflammation, immunologic reactions, and cell cycle regulation. Abnormalities in TGF-β signaling have been observed in a

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wide variety of disorders, including autoimmune diseases, malignancies, and chronic renal disease (17).

The critical role of TGF-β in growth and development is underscored by studies of mice with isoform-specific targeted deletion of the TGF-β gene (18). TGF-β1 knockout animals have over 50% embryonic lethality associated with defects in early hematopoiesis and vasculature in the yolk sac (19). Live-born mice develop a systemic inflammatory wasting syndrome within 1 week, leading to death after 3–4 weeks of age (18, 20). This syndrome is associated with the development of circulating autoantibodies and enhanced expression of both MHC class 1 and MHC class 2 molecules (19, 21, 22). TGF-β2 knockout mice exhibit perinatal mortality and a wide range of developmental defects involving the heart, lung, musculoskeletal system, kidneys, eye, and inner ear (23, 24). Mice with homozygous deletion of the TGF-β3 gene die within 20 hr of birth. The TGF-β3 knockout phenotype is characterized by cleft palate and delayed pulmonary development (25, 26). The distinct phenotypes of TGF-β1, 2, and 3 knockout mice provide evidence that these TGF-β isoforms play distinctive roles in embryonic growth and development.

Of the TGF-β isoforms, the effects of TGF-β1 on tissue homeostasis and response to injury have been the most fully characterized (1). TGF-β1 activity is regulated at many levels, including transcription, activation of the latent TGF-β1 complex, binding of TGF-β1 to cell surface receptors, and clearance of active TGF-β1 (1). In many cell lines, TGF-β1 is capable of positively regulating its own expression (27). Autoinduction of TGF-β1 transcription appears to be mediated through binding of an AP-1 complex to the TGF-β1 promoter (28). Autoinduction of TGF-β1 may be responsible for the pathologic induction of TGF-β1 that is characteristically associated with fibrosis in the kidney and other organs (29, 30). The TGF-β1 gene encodes a 390-amino acid precursor molecule that contains a signal peptide, the active TGF-β1 molecule, and a latency-associated peptide (31). After removal of the signal peptide, the TGF-β1 gene product is proteolytically cleaved to form mature TGF-β1 and the latency-associated peptide (32). Before secretion, TGF-β1 noncovalently associates with the latency-associated peptide to produce an inactive latent TGF-β1 complex (33, 34). TGF-β1 can be released from the latent complex, and thereby activated by changes in pH, by proteases such as plasmin and cathepsin D, and by thrombospondin (35–37). Once activated, TGF-β1 is capable of binding a cell surface receptor, thereby initiating an intracellular signaling cascade. Recent studies have provided important insights regarding molecular mechanisms of TGF-β signal transduction.

TGF-β Signal Transduction

As with other signaling systems, key elements of TGF-β signaling include binding of the TGF-β ligand to a cell surface receptor, intracellular transduction of a signal from the activated receptor to the nucleus, integration with other signaling pathways, and activation of target genes. Recent developments in these aspects of TGF-β signaling are now considered.

TGF-β Receptors. TGF-β signaling is initiated through binding of activated TGF-β to a heteromeric transmembrane receptor complex consisting of TGF-β receptor type I and type II (38, 39). The unique feature of the type I and type II TGF-β receptors is that they possess serine/threonine kinase activity, as opposed to the tyrosine kinase activity characteristic of membrane-associated protein kinase-linked receptor complexes that are involved in growth factor signaling. Both TGF-β receptor type I and type II are required for signal transduction (38, 40). TGF-β binds the type II receptor, which then recruits and phosphorylates the type I receptor within its cytoplasmic domain (41). The activated type I receptor then phosphorylates cytoplasmic substrates (the Smad proteins, see below), which subsequently form complexes that translocate to the nucleus, thereby regulating transcription of target genes (42).

Binding of TGF-β to the type II receptor may be regulated by a number of cell surface proteoglycans (43). For example, betaglycan, a membrane-anchored proteoglycan, is capable of presenting TGF-β to the kinase subunit of the signaling TGF-β receptor, thereby enhancing cellular responses to TGF-β (44). Cell lines lacking betaglycan have reduced responses to TGF-β, and transfection of these cell lines with betaglycan restores TGF-β responsiveness (45). Endoglin, a dimeric membrane glycoprotein first identified in endothelial cells (46), exhibits a significant degree of sequence homology with betaglycan. Endoglin binds to TGF-β1 and TGF-β3, but not TGF-β2 (47). Endoglin production is increased in rats with renal fibrosis induced by subtotal nephrectomy (48) and in humans with chronic progressive renal disease (49). Decorin, a small molecular weight proteoglycan that associates with extracellular matrix and tissues, is capable of binding and neutralizing TGF-β1 (50). In an acute glomerulonephritis model, injection of recombinant decorin was as effective as anti-TGF-β1 antibody therapy in suppressing TGF-β1-induced matrix accumulation (51). Gene therapy, through transfer of decorin cDNA into rat skeletal muscle, was also effective in preventing fibrosis induced by an experimental rat glomerulonephritis model (52). Although there are no demonstrable abnormalities in kidneys of mice with homozygous deletion of the decorin gene, the severity of interstitial fibrosis induced by unilateral ureteral obstruction is more severe in decorin knockout animals than wild-type controls (53). However, decorin is induced in tubular epithelial cells by high glucose (54), and renal decorin mRNA levels are rapidly increased after induction of experimental diabetes (55). Based on these considerations, it is unlikely that progressive renal disease, at least in diabetes mellitus, is due to a relative deficiency of decorin.

Evidence for the critical role of TGF-β type I and type II receptors in TGF-β signaling and control of cell growth is provided by studies of human neoplasia in which mutations

in both type I and type II TGF-β receptors are observed (56). Reduced expression of the TGF-β type II receptor has been observed in renal cell carcinomas (57).

Increases in TGF-β receptor expression have been described in a variety of experimental renal disease models, including membranous nephropathy (58), adriamycin nephropathy (59), obstructive nephropathy (60), and diabetic nephropathy (61). In spontaneously hypertensive rats, induction of experimental diabetes leads to upregulation of both TGF-β receptor type I and type II (62). Treatment with insulin to normalize blood glucose levels normalizes glomerular TGF-β receptor type I and type II levels (62). TGF-β receptor type II, but not type I, expression is increased in spontaneously hypertensive rats undergoing nephrectomy (62). In an experimental diabetes model, angiotensin-converting enzyme therapy normalized the high glucose-induced TGF-β type II receptor mRNA and protein expression (63, 64). Reduced TGF-β type II receptor expression was associated with prevention of renal hypertrophy and normalization of urine albumin excretion (63). Glomerular immunohistochemical staining for TGF-β type I and type II receptors is observed in renal biopsies obtained from patients with systemic lupus erythematosus, rapidly progressive glomerulonephritis, and, to a lesser extent, membranoproliferative glomerulonephritis (65). No TGF-β type I or type II receptor expression was observed in normal human kidneys or in biopsies obtained from patients with focal-segmental glomerulosclerosis, immunoglobulin A (IgA) nephropathy, or minimal change disease (65).

In mesangial cells, high glucose-induced TGF-β receptor expression promotes increased binding of TGF-β to the receptor complex (66). Vascular smooth muscle cells from atherosclerotic lesions have a higher ratio of TGF-β receptor I to TGF-β receptor II expression compared with normal vascular smooth muscle cells (67). The development of atherosclerotic plaques in humans has been linked to genomic instability in the type II TGF-β receptor gene (68). Although speculative, it is possible that oxidized lipids may promote atherogenesis, at least in part, by inducing somatic mutations in the type II TGF-β receptor gene. The relationship between altered TGF-β receptor expression in renal cells and progressive renal disease awaits clarification.

Smad Proteins. Signals from the activated TGF-β receptor complex are transduced to the nucleus by Smad proteins, a family of transcription factors found in vertebrates, insects, and nematodes (69). To date, the Smads are the only TGF-β receptor substrates with a demonstrated ability to propagate signals. The Smad family consists of receptor-regulated Smads, a common pathway Smad, and inhibitory Smads. Receptor-regulated Smads (R-Smads) (42) are phosphorylated by TGF-β type I receptor. R-Smads include Smad2 and Smad3, which are recognized by TGF-β and activin receptors, and Smads 1, 5, and 8, recognized by BMP receptors. Smad4 is a common pathway Smad (also called cooperating Smad or co-Smad), which is not phosphorylated by the TGF-β type I receptor (70). Inhibitory Smads (anti-Smads) include Smad6 and Smad7, which downregulate TGF-β signaling.

The structure of the Smad family is highly conserved. Smads contain an N-terminal mad homology 1 domain (MH1), which has DNA-binding activity, and a C-terminal MH2 domain, which drives translocation into the nucleus and regulates transcription of target genes (71–73). TGF-β type I receptor-mediated phosphorylation of the C-terminal sequence SSXS appears to relieve these two domains from a mutually inhibitory interaction, leading to R-Smad activation. Co-Smads lack the SSXS sequence and are therefore not phosphorylated by the type I receptor. Their interaction with R-Smads is primarily mediated by the MH2 domain (74, 75). The co-Smads form complexes with R-Smads (76, 77). Although co-Smads are not required for nuclear accumulation of R-Smad containing complexes, they are necessary for the formation of functional transcriptional complexes (70). Both the R-Smads and co-Smads in this complex may participate in DNA binding and recruitment of transcriptional cofactors (Fig. 1). Once within the nucleus, the Smads may function as transcriptional transactivators, with function intrinsic to the MH2 domain; they may form specific associations with nuclear transcription factors such as AP-1 (78) or coactivators such as CBP/p300 (73); or they may directly bind DNA with activity intrinsic to the MH1 domain (79, 80). There is considerable heterogeneity with respect to consensus Smad-binding sites; Smads often bind in association with other transcription factors, including AP-1, Sp1, etc. (17).

The critical role of the Smad signaling pathway in regulation of cell growth has been extensively documented through studies of human cancers. The Smad2 and Smad4 genes have been mapped to chromosome 18q21, a region that is frequently mutated or deleted in pancreatic and colon carcinomas (81, 82). It has been reported that 90% of pancreatic carcinomas show allelic loss of chromosome 18q on which the Smad4 gene is located. The tumor suppressor gene DPC (deleted in pancreatic carcinoma) is identical to Smad4 (70, 75, 83). Smad4 mutations have been identified in 20% of colon carcinomas (84), 10% of lung cancers (85), some breast and ovarian carcinomas (86), and some head and neck squamous cell carcinomas (87, 88). In addition to Smad4, biochemical and functional analysis of Smad2 gene products from colon cancer and lung cancer cells indicate that they harbor loss of function mutations that prevent Smad2 expression or abolish TGF-β-regulated phosphorylation of their MH2 domain (81, 84). Reduced Smad2 and Smad4 expression has been observed in renal cell carcinomas. Reduced Smad2 expression in renal cell carcinoma correlates with a higher tumor grade indicative of a more aggressive tumor (89).

Cultured mesangial cells express Smad2, Smad3, and Smad4. Smad2 and Smad3 are phosphorylated within 5 min of TGF-β1 treatment. TGF-β1-mediated phosphorylation of Smad2/3 promotes association of heteromeric complexes containing Smad2/3 and Smad4 (90). Activation of the

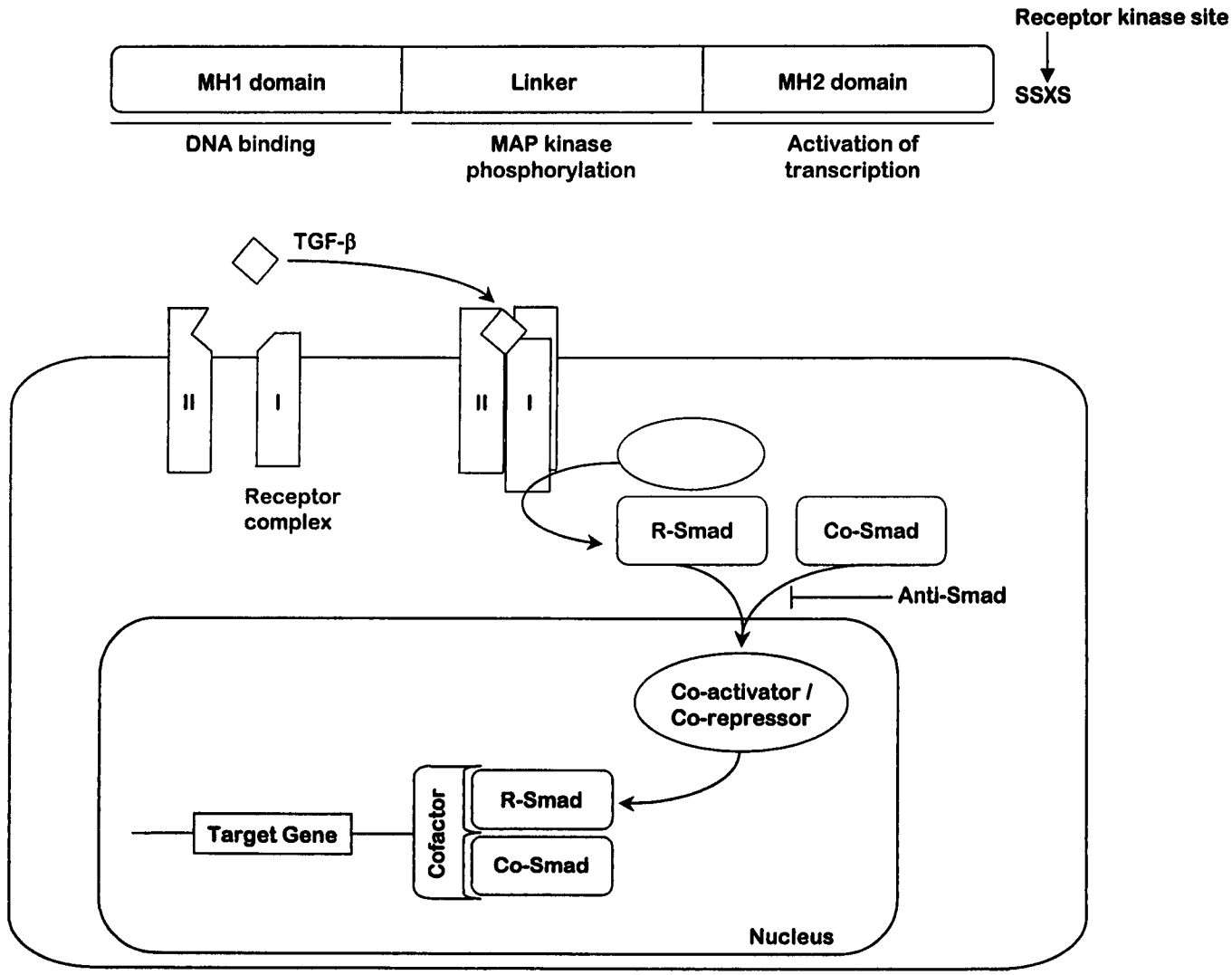

Figure 1. The structure of the Smad family and the TGF-β/Smad signaling pathway. The Smad family contain conserved N-terminal and C-terminal regions known as the MH1 domain and MH2 domain, respectively. The MH1 and MH2 domains are linked by a region that is less well conserved among Smad family. TGF-β induces the association of two type I and two type II serine/threonine kinase receptors, the type II receptor phosphorylates, and activates the type I receptor, which phosphorylates the C-terminal sequence SSXS of R-Smads, and leads to R-Smads activation and accumulation in the nucleus. The co-Smads are required for the formation of functional transcriptional complexes. The Smad complex can recruit co-activators or co-repressors that determine the transcriptional responses.

Smad signaling pathway is associated with increased transcriptional activity of an α2(I) collagen promoter (90). Normal rat glomeruli express high levels of the inhibitory Smads, Smad6 and Smad7 (91). After induction of acute mesangioproliferative glomerulonephritis, increased levels of TGF-β1 expression are associated with decreased expression of Smad6 and Smad7 (91). Additional studies are required to determine whether decreased expression of inhibitory Smads is associated with progressive renal disease in humans.

Crosstalk with Mitogen-Activated Protein Kinase (MAPK) Signaling Pathways. Recent studies have shown that there is extensive crosstalk between the Smad pathway and other signaling pathways. Extracellular receptor-linked kinase (ERK) activation is well recognized as a critical mitogenic signaling pathway that directs proliferation of cells in response to a wide variety of peptide growth factors, including epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). Recent studies have led to the identification of parallel MAPK signaling pathways, including p38 and c-Jun N terminal kinase (JNK) (92). In some cell systems, selective activation of the JNK or p38 pathways may promote apoptosis rather than proliferation (93, 94). TGF-β can directly activate the ERK, p38, and JNK signaling pathways (95, 96). There is considerable crosstalk between the TGF-β-Smad signaling pathway and the MAPK signaling cascades (97–100). Activation of tyrosine kinase receptors for EGF and hepatocyte growth factor (HGF) causes Smad2-dependent gene transcription (101, 102). This effect results from phosphorylation of Smad2, albeit not at the C-terminal serine residues, which are targets for the TGF-β1 receptor kinases. Smad2 can also be activated by MAPKK-1 (MEKK1), an activator of the JNK kinase pathway (103). Phosphorylation of Smad2 by

MEKK1 results in enhanced Smad2-Smad4 interaction, nuclear accumulation, and association of Smad2 with the transcriptional coactivator CREB-binding protein (CBP).

In hypertensive vascular disease and in angiotensin II-mediated hypertrophy of vascular smooth muscle cells, ERK activation leads to induction of TGF-β1 through activation of AP-1 (104). ERK activates AP-1 through induction of c-Fos mRNA via phosphorylation of TCF/ElK-1 (104). A novel MAPKKK termed TGF-β-activating kinase (TAK1) participates in signal transduction of TGF-β1 and has been shown to activate both the p38 pathway (105, 106) and the JNK pathway (96, 107, 108). Crosstalk between TGF-β1 and ERK signaling pathway may be important in progressive renal injury because ERK is upregulated in experimental glomerulonephritis (109). TGF-β1 stimulates acute and chronic activation of ERK in mesangial cells (110).

The well-recognized stimulatory effect of TGF-β on extracellular matrix production may occur, at least in part, through activation of MAPK cascades. The effect of ERK activation (by TGF-β or other factors) on collagen I production may be stimulatory or inhibitory, depending upon cell type (111–116). In vascular smooth muscle cells, angiotensin II promotes collagen production through a TGF-β-dependent pathway (117). Treatment of vascular smooth muscle cells with PD98059, an ERK inhibitor, prevents the angiotensin II-mediated induction of collagen I (116). In mesangial cells, high glucose-stimulated extracellular matrix production is also blocked by PD98059 (118). However, the effect of MAPK pathways on TGF-β-mediated collagen production appears to be cell type-specific. For example, in human fibrosarcoma cells, TGF-β induces fibronectin synthesis through a JNK-dependent, Smad4-independent pathway (119, 120).

Effect of TGF-β

Cell Cycle Regulation. TGF-β inhibits proliferation of renal tubular epithelial cells and glomerular mesangial cells. TGF-β1 most likely inhibits cell growth by regulating the assembly and activity of cyclin-cyclin-dependent kinases (cdk) complexes, which are necessary for cell cycle progression from G_1 to S phase (42, 121, 122). In mammalian cells, cyclin D-cdk4, cyclin D-cdk6, and cyclin E-cdk2 act sequentially during the G_1/S transition and are required for cell cycle progression. Both cyclin D-cdk4/cdk6 and cyclin E-cdk2 phosphorylate the retinoblastoma protein (pRb) at different sites on the molecule (123, 124). Cyclin D-cdk4 and cyclin D-cdk6 are thought to phosphorylate the unphosphorylated pRb, allowing cells to enter the G_1 phase of the cell cycle (123). The hypophosphorylated pRb protein prevents further cell cycle progression, in part through binding to E2F transcription factors. Cells arrested in this phase of the cell cycle may undergo hypertrophy rather than hyperplasia (125, 126). In late G_1 phase of the cell cycle, pRb is inactivated by hyperphosphorylation through activity of cyclin E-cdk2 complexes (123). The hyperphosphorylated pRb does not bind E2F transcription factors, which are then free to activate transcription of genes associated with progression of the cell cycle from G_1 to S phase (127, 128). In renal tubular epithelial cells, TGF-β1 converts a hyperplastic growth response to EGF into a hypertrophic growth response (129). Tubular epithelial cell hypertrophy is associated with arrest of cell cycle progression at the G_1/S interface. As in other cell systems (130), TGF-β1 blocks cell cycle progression by maintaining pRb in its hypophosphorylated state, thus blocking progression through the cell cycle (130, 131). In renal tubular epithelial cells, mesangial cells, and several other cell types, TGF-β inhibits cyclin E-dependent kinase activity without significantly altering cyclin D-cdk4, cyclin D-cdk6, or cyclin E-cdk2 levels (131–135).

Activity of cyclin-cdk complexes is tightly regulated by two families of cdk inhibitory proteins: the INK family, which includes p15, p16, p18, and p19, and the KIP family, which includes p21, p27, and p57 (136). The INK family of cdk inhibitors preferentially binds cdk4 or cdk6, whereas the KIP family blocks activity of a variety of cyclin-cdk complexes, including that of cyclin E-cdk2 (137). TGF-β rapidly induces the synthesis of p15, which binds to cdk4 and cdk6, preventing their interaction with cyclin D (138). TGF-β has also been shown to modulate the activity and expression of p21, p27, and p57, which inhibit the activity of cyclin D-cdk4/cdk6 and cyclin E/A-cdk2 complexes (139–142) (Fig. 2).

Quiescent mesangial cells express high levels of p27. When the mesangial cells are stimulated with PDGF or EGF to proliferate, p27 levels are decreased (143). High glucose, which promotes hypertrophy of mesangial cells in a TGF-β1-dependent fashion, increases both p21 and p27 expression (144). *In vivo*, rat glomeruli express high levels of p27 and low levels of p21. Induction of experimental mesangial proliferative glomerulonephritis is associated with a significant reduction in p27 levels during time periods when mesangial cell proliferation is high (145). Resolution of mesangial cell proliferation is associated with a return to the high baseline levels of p27 and induction of p21 (145). In human crescenteric glomerulonephritis, the cellular crescents express low levels of p27 (146).

TGF-β-mediated induction of p21 may contribute to progressive renal disease. TGF-β promotes hypertrophy in renal cells by inducing p21 levels (147). In experimental diabetic nephropathy, mice with homozygous deletion of the p21 gene do not develop glomerular hypertrophy, despite increased TGF-β levels (147). Following subtotal nephrectomy, $p21^{-/-}$ animals fail to develop progressive renal failure compared with wild-type controls (148). These studies suggest that hypertrophy, induced by TGF-β-mediated stimulation of p21, may contribute to progression of renal injury.

Extracellular Matrix Synthesis. TGF-β promotes the accumulation of extracellular matrix by increasing expression of extracellular matrix genes and by inhibiting the production of proteins responsible for breaking down extra-

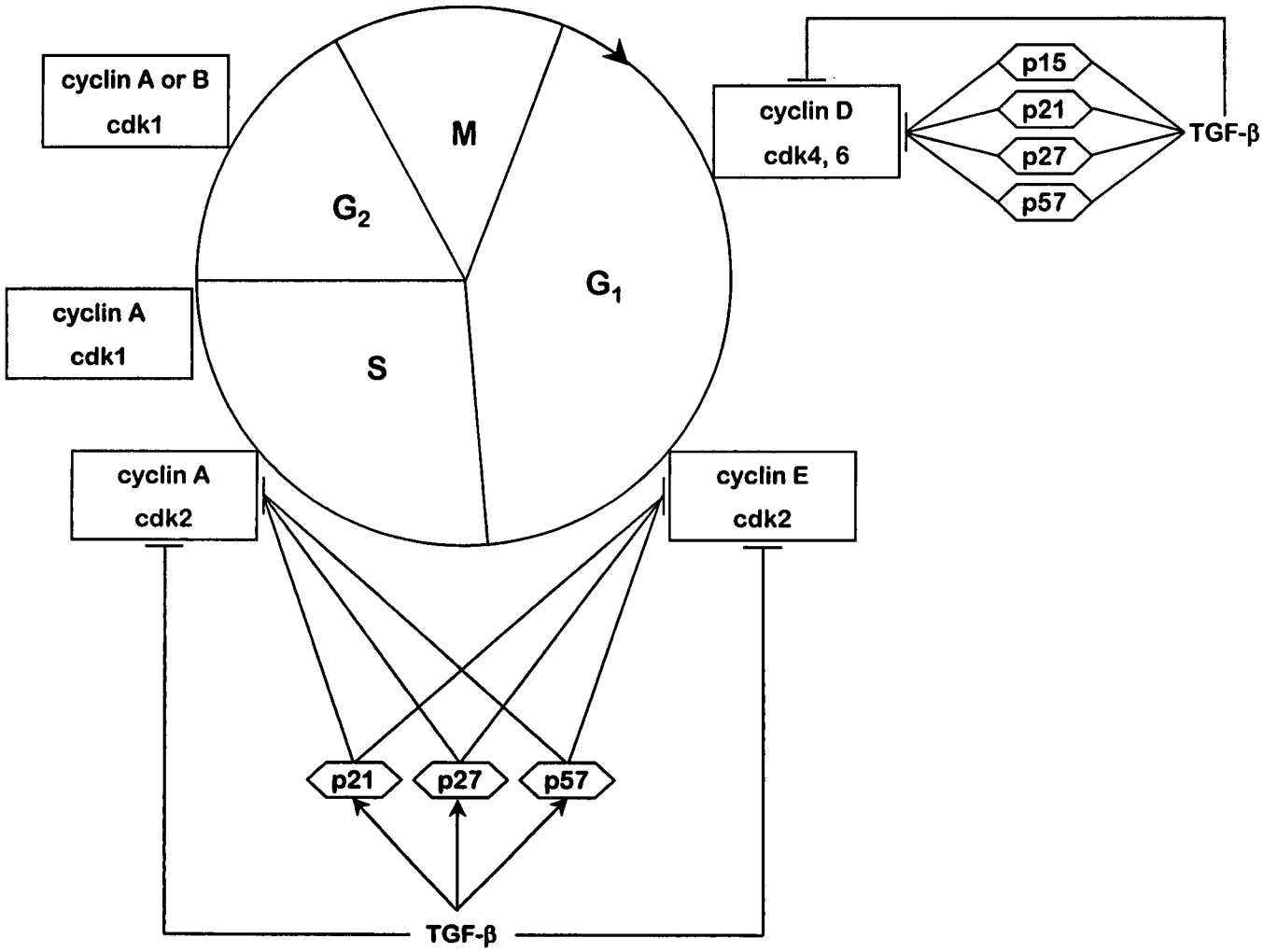

Figure 2. Effect of TGF-β on the cell cycle. Cell cycle progression is controlled by the cyclins and cdks that are specific for each phase of the cell cycle. The cyclin/cdk complexes have kinase activity and phosphorylate retinoblastoma protein (pRb), causing progression from G_1 to S phase. TGF-β inhibits the expression of cyclins and cdks. In addition, TGF-β induces the cdk inhibitors p15, p21, p27, and p57, which bind to the cyclin/cdks, preventing phosphorylation of pRb, thus blocking S phase entry.

cellular matrix. TGF-β increases the synthesis of many extracellular matrix proteins, including collagen types I (149–152), II (153), III (154), IV (155–157), V (158), and VII (159), fibronectin (151, 154, 160), thrombospondin (160), osteopontin (161), tenascin (162), elastin (163), and proteoglycans betaglycan (1) and decorin (50). TGF-β decreases production of proteases that break down extracellular matrix macromolecules, including serine, thio, and metalloproteinases, plasminogen activator (164), stromelysin, and collagenase (165, 166). TGF-β increases the synthesis of inhibitors of metalloproteinases such as plasminogen activator inhibitor-1 (PAI-1) and tissue inhibitors of metalloproteinases (TIMPs) (167–169). Expression of integrins, cell surface receptors for extracellular matrix, are induced by TGF-β. Integrins facilitate the attachment of cells to specific matrix proteins (170). TGF-β is chemotactic for fibroblasts and monocytes, thereby promoting influx of matrix synthesizing cells to sites of tissue injury (171).

Most of the studies related to TGF-β1-induced matrix synthesis have focused on the collagen I genes. A variety of transcription factors have been shown to confer a transcriptional response to TGF-β1, including NF1 (172), Sp1 (152, 173–176), Sp3 (174, 177), and TAE (178). Activation of AP-1 may either stimulate or inhibit collagen I production, depending upon cell type (115, 179, 180). Smad proteins have been shown to be directly involved in transcriptional regulation of collagen I and collagen VII by TGF-β1 (181, 182).

Collagen IV is the major constituent of the glomerular basement membrane (GBM) (156, 157). In a recent study, basal collagen IV mRNA levels in tubular epithelial cells derived from mice with homozygous deletion of the TGF-β1 gene were significantly lower than collagen IV mRNA levels in wild-type cells, suggesting that autocrine production of TGF-β1 plays an important role in regulating basal collagen IV expression (183). Although the collagen IV promoter has been isolated from human (184), mouse (185), and rat (186), the sequence elements within the collagen IV gene that confer a transcriptional response to TGF-β have not yet been defined.

Progressive Renal Disease. Chronic renal disease, regardless of the primary etiology, is characterized by glomerular sclerosis and interstitial fibrosis (29, 187). Tissue fibrosis is the result of excessive accumulation of extracellular matrix that, in the kidney, impairs renal function and finally leads to organ failure. Recent studies have revealed that local production of TGF-β, either by intrinsic renal cells or by infiltrating inflammatory cells, has a key role in pathologic matrix deposition after tissue injury (29). Upregulated TGF-β receptor expression has been observed in experimental glomerulonephritis (58, 59), and may contribute to increased TGF-β signaling in progressive renal disease.

There is now a large body of evidence obtained from experimental and human studies that conclusively demonstrates that TGF-β1 plays a critical role in the development and progression of renal injury (Table I). Border *et al.* (188) used an acute mesangial proliferative glomerulonephritis model in initial studies to demonstrate the role of TGF-β1 in promoting matrix deposition following acute injury. In this model, injection of an ATS leads to a selective mesangiolysis, followed by mesangial cell proliferation and a dramatic increase in matrix production. One week after disease induction, the glomeruli are characterized by overexpression of TGF-β and excessive accumulation of extracellular matrix (189, 190). Anti-TGF-β antibody (188), decorin, a proteoglycan capable of binding and inactivating TGF-β (51), and phosphorothioate-modified TGF-β1 antisense oligonucleotides (191) are all capable of preventing the excessive extracellular matrix deposition after ATS injection. In acute ATS-induced glomerulonephritis, TGF-β production returns to normal within several weeks. However, in a model of chronic progressive glomerulonephritis induced by two injections of ATS 1 week apart, sustained TGF-β overexpression has been identified as a cause of the ongoing matrix accumulation that leads to progressive renal failure (29).

Table I. Animal Models and Human Renal Diseases in which Increased TGF-β Expression Has Been Reported

	References
Animal models	
Antl-GBM glomerulonephritis	226
Immune complex nephritis	227
Acute ATS glomerulonephritis	228
TGF-β transgenic mouse	193
Cyclosporin nephrotoxicity	229
Adriamycin nephropathy	59
Angiotensin-induced nephropathy	207
Anti-oxidant deficiency	230
Chronic rejection	231
Radiation nephropathy	232
Chronic ATS glomerulonephritis	233
Membranous nephropathy	58
Diabetic nephropathy	234
Ureteral obstruction	235
Nephrotoxic nephritis	236
RF/J mice	237
Acute and chronic puromycin-induced nephrosis	238
HIV nephropathy	239
Hypertensive renal injury (Dahl rats)	240
Remnant kidney	241
GH transgenic mouse	242
Aging	243
Protein overload proteinuria	244
Diet hypercholesterolemia	245
Lupus nephritis	246
Polycystic kidney disease	247
Human Disease	
Diabetic nephropathy	248, 249
Glomerulonephritis	249, 250
IgA nephropathy	
Focal and segmental sclerosis	
Crescentic glomerulonephritis	
Lupus nephritis	
HIV nephropathy	251, 252
Allograft rejection	253
Cyclosporine nephropathy	254

Note. ATS, antithymocyte serum; HIV, human deficiency virus; GH, growth hormone.

In another *in vivo* model, hepatic expression of a TGF-β1 transgene under the control of an albumin promoter/enhancer (Alb/TGF-β1) in the liver of transgenic mice results in renal fibrosis in addition to hepatic fibrosis (192). Renal lesions correlated well with increased levels of circulating TGF-β1 and were characterized by progressive mesangial expansion, accumulation of glomerular immune deposits and matrix proteins, and interstitial fibrosis. Glomerular deposits lacked complement and distributed predominantly in subendothelial and mesangial locations. Renal failure with nephrotic syndrome was fatal in one-quarter of severely affected transgenic mice (193). These findings indicate that chronically elevated levels of circulating TGF-β1 induce progressive glomerulosclerosis.

In mesangial cells, recent studies have demonstrated that TGF-β is an essential signaling intermediate for collagen production in response to a variety of injurious stimuli, including angiotensin II, thromboxane A2, reactive oxygen species, and high glucose (194, 195).

Diabetic nephropathy is now recognized as the most common cause of end-stage renal disease in the United States and Europe (196). Recent studies have shown that TGF-β1 plays an essential role in promoting the excessive extracellular matrix deposition characteristic of diabetic nephropathy (197). In cultured mesangial cells and tubular epithelial cells, high glucose or advanced glycosylated end-products (AGE) stimulate extracellular matrix production in a TGF-β1-dependent fashion (198–200). High glucose concentrations directly activate transcription of the TGF-β1 gene in mesangial cells (201). In cultured mesangial cells, reactive oxygen species and protein kinase C activation may be essential intermediates in the TGF-β signaling pathway triggered by high glucose (194). Increased production of TGF-β is an early event following induction of experimental diabetes (202). In experimental diabetes models, treatment with TGF-β antibodies or with TGF-β1 antisense oli-

godeoxynucleotides reduce proteinuria, prevent glomerular hypertrophy, and show less extracellular matrix deposition than untreated diabetic controls (203, 204). Expression of TGF-β is elevated in humans with diabetic nephropathy (205). In patients with type 2 diabetes, increased renal production of TGF-β has been demonstrated (206). These studies provide evidence that the TGF-β signaling system may provide an important therapeutic target for preventing progressive renal disease in patients with diabetes mellitus.

Intrarenal activation of the renin-angiotensin system is a characteristic feature of progressive renal disease arising from many different etiologies. In cultured mesangial cells and proximal tubular epithelial cells, TGF-β is an essential intermediate for angiotensin II stimulation of extracellular matrix synthesis (207, 208). Activation of protein kinase C and production of reactive oxygen species appear to be involved in angiotensin II-mediated induction of TGF-β1 (209–211). TGF-β1-neutralizing antibodies or TGF-β1 antisense oligonucleotides block angiotensin II stimulation of extracellular matrix production (208). In a variety of experimental models, including diabetic nephropathy, subtotal nephrectomy, and adriamycin nephrosis, angiotensin-converting enzyme inhibitors decrease renal TGF-β expression and improve renal function (63, 212, 213). Angiotensin-converting enzyme inhibitors have been shown to preserve renal function in patients with diabetes (214) and they have been advocated as a mainstay for therapy for patients with a variety of nondiabetic renal diseases (215). In patients with IgA nephropathy, angiotensin-converting enzyme inhibitor therapy significantly reduced renal TGF-β1 gene expression (89).

Increased deposition of TGF-β has been identified in a wide variety of human renal diseases. Glomerular immunoreactivity for TGF-β correlates with severity of glomerular proliferative lesions: high levels of expression were observed in patients with proliferative IgA nephropathy, membranoproliferative glomerulonephritis, proliferative lupus nephritis, and rapidly progressive glomerulonephritis; and low levels of expression were observed in normal kidneys or in kidneys of patients with focal-segmental glomerulosclerosis (65). Interstitial expression of TGF-β1 in renal biopsies obtained from patients with a variety of glomerular diseases correlate with extent of interstitial fibrosis, tubular atrophy, and α smooth muscle actin expression (216, 217). In recipients of renal allografts, increased renal expression of TGF-β predicts an increased rate of decline in renal function (218, 219).

Given the critical role of TGF-β in the progression of chronic renal disease, there has been recent interest in determining whether urine TGF-β excretion may serve as a noninvasive means to predict adverse renal outcome in patients with glomerular diseases. Increased urine TGF-β excretion is observed in patients with diabetes and membranous nephropathy (220, 221). Urine TGF-β levels correlate with urine protein excretion. In patients with IgA nephropathy and focal-segmental glomerulosclerosis, urine TGF-β levels correlate with extent of interstitial fibrosis, mesangial matrix increase, and urine protein excretion (222). In patients with crescenteric IgA nephropathy, steroid therapy reduced urine TGF-β excretion (223). Based on this observation, urine TGF-β levels may be a marker of disease activity in patients with glomerulonephritis.

Conclusions and Perspectives

Human and experimental studies have conclusively demonstrated that TGF-β plays a critical role in the progression of renal disease. Increased TGF-β deposition is a feature of many human renal diseases; tissue or urine TGF-β levels may predict the development of progressive renal disease in patients with glomerulonephritis. Antagonists of TGF-β signaling may play an important role in preventing progression of renal injury to end-stage renal disease (224, 225). Angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists have received the most attention as antagonists of TGF-β signaling. However, TGF-β signaling involves extensive crosstalk with other signaling pathways. The complexity of these interactions is only beginning to be defined. It is likely that delineation of these interacting pathways may lead to more specific agents designed to inhibit the fibrogenic pathways triggered by TGF-β.

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