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TGF-β signaling via TAK1 pathway: Role in kidney fibrosis

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Abstract

In progressive kidney diseases, fibrosis represents the common pathway to end-stage kidney failure. Transforming growth factor-β1 (TGF-β1) is a pleiotropic cytokine that has been established as a central mediator of kidney fibrosis. Emerging evidence demonstrates a complex scheme of signaling networks that enable multifunctionality of TGF-β1 actions. Specific targeting of TGF-β signaling pathway is seemingly critical and attractive molecular therapeutic strategy. TGF-β1 signals through the interaction of type I (TβRI) and type II (TβRII) receptors to activate distinct intracellular pathways involving the Smad and the non-Smad. The Smad signaling axis is known as the canonical pathway induced by TGF-β1. Importantly, recent investigations show that TGF-β1 also induces various non-Smad signaling pathways. In this review, we focus on current insights into the mechanism and function of Smad-independent signaling pathway via TGF-β-activated kinase 1 (TAK1) and its role in mediating the profibrotic effects of TGF-β1.

Keywords

Transforming growth factor-β1; intracellular signaling; TGF-β-activated kinase 1; fibrosis; chronic kidney disease

Introduction

Most chronic kidney diseases, regardless of the nature of the initial injury, progress to end-stage renal disease (ESRD) with irreversible loss of tissue and function. Chronic kidney disease (CKD) has become a major public health concern worldwide as the incidence continues to rise and portends high rates of morbidity and mortality.[1] Thus, improved and more effective therapies are critical. The hallmark of progressive CKD is the development of kidney fibrosis that is thought to be the final common mechanism leading to ESRD.[2–4] The pathogenesis of kidney fibrosis is characterized by relentless production and progressive accumulation of extracellular matrix (ECM) proteins, such as collagen and fibronectin, within the kidney which strongly correlates with deterioration of kidney function.[4,5] Transforming growth factor-beta 1 (TGF-β1) has been firmly established as a central mediator of kidney fibrosis associated with progressive kidney diseases.[6,7] Fibrosis represents the final common pathway of tissue injury response that ultimately leads to end-

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stage kidney failure and, therefore, blocking TGF-β signaling pathways is an attractive approach to anti-fibrotic therapy.

TGF-β1 is the prototype member of TGF-β superfamily of multifunctional cytokines that acts as a key regulator of diverse cellular functions such as cellular differentiation, proliferation, apoptosis, and wound healing, and is a potent inducer of ECM synthesis.[8,9] In response to tissue injury, chronically dysregulated expression and actions of TGF-β1 lead to the pathogenesis of kidney fibrosis seen in chronic progressive kidney diseases.[6,10,11] However, more acutely, TGF-β1 promotes wound repair and tissue regeneration, as well as anti-inflammatory effects, and thereby exerts cytoprotective effects to mitigate tissue injury.[11–16] Thus, TGF-β1 plays a dual, seemingly paradoxical, role in tissue injury response. This paradigm suggests that in developing therapeutic interventions, it may be unwise to devise strategies to simply indiscriminately inhibit TGF-β1 actions. Hence, a better understanding of the cellular and molecular mechanisms of TGF-β1 actions will provide the basis that will guide in the development of therapeutic strategies that specifically target its signaling pathway responsible for the deleterious effects of TGF-β1

TGF-β signaling receptors

TGF-β1 actions are mediated through heteromeric interactions of serine/threonine kinases, TGF-β type I (TβRI) and type II (TβRII) receptors, to activate intracellular signaling pathways.[17] Recent investigations have helped to unravel a complex scheme of signaling network downstream of the TGF-β receptors that transduce signals from the cell surface to the nucleus and enable multi-functionality of TGF-β1 in controlling diverse biological functions in a cell-specific and context-specific manner. Initiation of the TGF-β signaling cascade requires ligand binding to TβRII, and in turn, TβRI and TβRII form hetero-tetrameric complexes, by which TβRI is phosphorylated in the cytoplasmic GS domain and activated, which is followed by activation of a number of intracellular signaling mediators of TGF-β1.

Intracellular signaling mediators: The Smad and the non-Smad

The Smads comprise a family of structurally related proteins that represent the human analogues of the *Drosophila* protein MAD (Mothers Against Decapentaplegic) and the *Caenorhabditis elegans* protein SMA (Small body size).[17,18] Receptor-regulated Smads (R-Smads) are recruited and activated by the activated TβRI (Fig. 1). The phosphorylation in the GS domain[19] and L45 loop[20] of TβRI are thought to be crucial for its interaction with R-Smads. Subsequent serine/threonine phosphorylation of the R-Smads, Smad2 and Smad3, leads them to be rapidly dissociated from TβRI and interact to form complexes with common-mediator, Smad4, followed by nuclear translocation where they recognize regulatory Smad binding elements to transcriptionally activate or repress target genes.[21] Both Smad 6 and Smad 7 compete with the R-Smads for binding to the activated receptors and thus function as inhibitory Smads.[22]

During the past decade, important advances in our understanding of TGF-β1-induced signaling have been made and much of the early investigations were focused on studies of Smad signaling which is widely accepted as a canonical pathway induced by TGF-β1.[23] The role of Smads in the context of kidney health and disease is a topic of several previous reviews.[24,25] However, it has become quite evident that the Smad signaling pathway does not explain all of the diverse actions of TGF-β1. In addition to the Smad, a growing body of evidence demonstrates that TGF-β1 also activates various Smad-independent signaling pathways, with or without direct crosstalk with the Smad.[26,27]

A number of noncanonical TGF-β signaling pathways has been identified, including the Rho-like GTPases,[28,29] phosphatidylinositol-3-kinase (PI3K)/AKT,[30–33] and the mitogen-activated protein kinases (MAPKs), namely extracellular signal-regulated kinase (Erk) 1/2,[34,35] c-Jun N-terminal kinase (JNK),[36–38] and p38 MAPK.[39–42] Studies implicate the p38 MAPK signaling pathway in the development of fibrosis in animal models of glomerular and tubulointerstitial injury[43,44] and in human kidney disease.[45] We and others have demonstrated that TGF-β-activated kinase 1 (TAK1) is a major upstream signaling molecule in TGF-β1-induced type I collagen and fibronectin expression through activation of the MAPK kinase (MKK)3-p38 and MKK4-JNK signaling cascades, respectively (Fig. 2).[46–48] Below, we review our current understanding of the molecular mechanisms of Smad-independent signaling pathway via TAK1 and its role in mediating the profibrotic effects of TGF-β1.

TGF-β-activated kinase 1 (TAK1)

TAK1, originally identified as a member of the MAPK kinase kinase (MAP3K) family and known as MAP3K7, is a serine/threonine kinase that is rapidly activated by TGF-β1.[49,50] To date, TAK1 is the only MAP3K family member that has been directly implicated in TGF-β1 signaling. TAK1 can also be activated by various stimuli including environmental stress,[51] proinflammatory cytokines such as tumor necrosis factor (TNF)-α[52] and interleukin (IL)-1,[53] and lipopolysaccharides (LPS).[54] For TAK1 activation, phosphorylation at Thr-187 and Ser-192 in the activation loop of TAK1 is essential.[55,56] Activated TAK1 can transduce signals to several downstream signaling cascades, including the MKK4/7-JNK, MKK3/6-p38 MAPK, and Nuclear Factor-kappa B (NF-kB)-inducing kinase (NIK)-IkB kinase (IKK).[52–54] Recent report has shown that TAK1 is activated by agonists of AMP-activated kinase (AMPK) and ischemia, which in turn activates the LKB1/AMPK pathway, a key energy-sensor pathway.[57] TAK1 is also involved in non-canonical Wnt signaling that functions as a negative feedback mechanism of canonical Wnt signaling.[58] Furthermore, studies indicate that TAK1 can regulate TGF-β-induced activation of Smad signaling by inducing Smad7 expression[59] and also interfering with R-Smad transactivation by direct interaction with the MH2 domain of Smad proteins.[60] In addition to the role of TAK1 in the regulation of Smad function, there is cross-talk between the Smad and downstream targets of TAK1 such as p38 MAPK and ATF2 in regulation of certain TGF-β1 target gene expression.[39,61,62] Collectively, these observations suggest that TAK1 might be the point of convergence in various signaling pathways activated by a variety of stimuli and play a pivotal role in regulating cellular responses.

Molecular mechanism of TAK1 activation

Role of TAK1-binding proteins: (TAB1, 2, 3)

TAK1 is unique among the MAP3K family members in that its activation requires complexing with specific binding partner known as TAK1-binding protein-1, -2, -3 (TAB1, 2, 3).[63–65] The requirement of these TAK1-binding proteins appears to be dependent on the stimuli. Studies in *Tab1*-deficient mouse embryonic fibroblasts reveal that osmotic stress-induced activation of TAK1 is TAB1-dependent, but not in the case of certain cytokines such as TNF-α and IL-1-induced activation of TAK1,[66] whereas TAB1 is essential for TAK1 activity and necessary for TGF-β signal transduction.[67] We have shown that TAB1 is indispensable for TGF-β1-induced TAK1 activation in glomerular mesangial cells.[68] Studies *in vivo* also demonstrate that the TAK1–TAB1 complex is crucial for normal embryonic development and morphogenesis, as genetic inactivation of TAB1 resulted in embryonic lethality and defects in development of major organs including the heart and lung.[67] Moreover, evidence supports cell-type specificity in the involvement of the specific TAK1-binding proteins, such that, in HeLa cells, TNF-α-induced TAK1 activation involved

signaling complex with TAB1 as well as TAB2.[56] TAB3 is a TAB2-related protein which has been shown to play a role in TNF-α and IL-1 signaling pathways.[64,65]

Role of ubiquitin ligase TNF receptor-associated factor 6 (TRAF6)

The molecular events that link the activated TGF-β receptor complex and the intracellular signaling mediators have been well characterized from the standpoint of Smad signaling, whereas events that link the non-Smad signaling molecules are less clearly understood. We know that there are notable differences in the mechanism of Smad2/3 and TAK1 activation. TGF-β1-induced TAK1 activation occurs independent of TβRI kinase activity,[68,69] whereas activation of Smad2/3 involves recruitment and phosphorylation by TβRI and requires kinase activity of TβRI.[19,20] Activated Smad2/3 is then released from the receptor complex to interact with Smad4 to transmit TGF-β1 signals. We have reported that in glomerular mesangial cells, in the absence of ligand stimulation, TAK1 stably associates with TβRI.[68] TGF-β1 stimulation causes rapid dissociation from the receptor complex and in turn activates TAK1. Thus, TGF-β1-induced formation of TβRI-TβRII complex triggers dissociation of TAK1 from TβRI, and subsequently TAK1 is activated through TAB1-mediated autophosphorylation, independent of receptor kinase activity of TβRI.

Although TAB1 is required for TGF-β1-induced TAK1 activation, TAB1 does not interact with TGF-β receptors. On the other hand, TAB2 and another adaptor protein TRAF6 are necessary for the interaction of TAK1 with TβRI and TGF-β1-induced TAK1 activation in glomerular mesangial cells.[68] TRAF6 is a member of a family of RING (really interesting new gene) domain ubiquitin ligases that catalyzes synthesis of polyubiquitin chains linked through Lys-63 of ubiquitin. The highly conserved ubiquitin-binding zinc finger domains in TAB2 preferentially bind Lys-63-linked polyubiquitin chains on TRAF6 and facilitates TGF-β1-induced TAK1 activation.[69–71] TβRI has been found to harbour a consensus binding site for TRAF6 and recent evidence reveals that TRAF6 physically interacts with TβRI and promotes Lys-63-dependent polyubiquitination of TAK1 Lys-34 and subsequent TAK1 activation.[69–72] Thus, TβRI kinase activity is required for activation of the canonical Smad signaling pathway, whereas ubiquitin ligase activity of TRAF6 regulates the activation of TAK1 in a receptor kinase-independent manner. TGF-β1 specifically activates TAK1 through the interaction of TβRI with TRAF6, whereas Smad activation is not dependent on TRAF6.

Molecular mechanism of TAK1 inactivation

Dysregulation and persistent TGF-β1 actions are thought to lead to pathological states. Once TAK1 is activated, efficient down-regulation of TAK1 activity is important to prevent excessive TGF-β1 responses. Cyclic phosphorylation and dephosphorylation of kinases represent a fundamental mechanism responsible for tight regulation of intracellular signaling cascades. TAK1 requires phosphorylation at Thr-187 and Ser-192 within the activation loop of TAK1 for its activation[55,56] and these sites, in turn, serve as substrates for protein phosphatases to inactivate TAK1. Several members of type 2A Ser/Thr protein phosphatase family have been identified as negative regulators of TAK1. Protein phosphatase 2C (PP2C) is capable of binding and dephosphorylating TAK1 in 293 cells under non-stimulated condition.[73,74] More recently, a new Ser/Thr protein phosphatase family member PP6 has been shown to interact with and negatively regulate IL-1-induced TAK1 in 293 cells[75] and TNF-induced TAK1 in fibroblasts.[76] We reported that another type 2A protein phosphatase family member PP2A functions as a negative regulator of TAK1 activation in response to TGF-β1 in glomerular mesangial cells.[77] PP2A associates with TAK1 and TAB1, and Thr-187 in the activation loop of TAK1 is a major dephosphorylation target of PP2A. Our findings in mesangial cells reveal that TAK1 is activated and deactivated very rapidly. This rapid activation and inactivation of TAK1 by TGF-β1 stimulation have been also observed

in cardiac myocytes.[78] These findings suggest that TAK1 activation is tightly regulated and may be controlled through rapid phosphorylation and dephosphorylation, and dysregulation of protein phosphatase-dependent dephosphorylation may cause of prolonged activation of TAK1.

TGF-β/TAK1 signaling and profibrotic response

TGF-β1 is considered the most potent profibrogenic cytokine, and there is increasing evidence implicating a critical role of TAK1 signaling in ECM production and pathogenesis of kidney fibrosis. *In vitro* studies show that TAK1 mediates TGF-β-induced expression of types I and IV collagens and fibronectin in cultured mesangial cells.[46] We have previously reported that TGF-β1-induced activation of the downstream MKK3-p38 MAPK cascade leads to type I collagen expression[40,41] and that TAK1 is a major upstream signaling molecule mediating TGF-β1-induced MKK3 activation and collagen induction in mesangial cells.[47] In fibroblasts, TGF-β-induced fibronectin expression is mediated by TAK1 through MKK4-JNK signaling cascade[48] and TAK1-deficient fibroblasts exhibited reduced profibrotic response to TGF-β1 stimulation.[79] These studies help establish TAK1 as a major regulator of TGF-β signaling and pathogenic mechanisms in renal cellular injury and profibrotic response (Fig. 3).

Several recent investigations have provided *in vivo* evidence that help to corroborate the critical role of TAK1 signaling pathway in tissue injury response and fibrosis. Studies using pharmacologic inhibitors and gene-deficient mice in experimental models of glomerular and tubulointerstitial injury, have shown that activation of MKK3-p38 MAPK[44,45,80–82] and JNK[83–86] pathways promotes renal inflammation and fibrosis. Blockade of MKK3-p38 MAPK or JNK pathways in unilateral ureteral obstruction (UUO) model of renal fibrosis resulted in substantial amelioration and protection against renal inflammation and fibrosis.[44,86,87] Examination of human biopsy tissues have also implicated p38 MAPK[45,88,89] and JNK[84,85] signaling in the development of inflammation and fibrosis in human kidney disease. Both the p38 MAPK and JNK pathways are downstream targets of TAK1 activation. Ma et al.[90] using conditional *tak1* gene deletion in mice and the UUO model of renal fibrosis, reported that TAK1 deletion suppressed interstitial myofibroblast accumulation, collagen deposition, and expression of profibrotic molecules.[90] Taken altogether, these studies establish strong evidence for a pathologic role for TAK1 signaling pathway in the development of inflammatory response and ECM elaboration and the pathogenesis of renal fibrosis.

The profibrotic function of TAK1 signaling is also demonstrated in the heart. Increased expression and activation of TAK1 lead to enhanced p38 MAPK phosphorylation and promote interstitial fibrosis in the myocardium of *Tak1* transgenic mice.[78] Interestingly, hepatocyte-specific deletion of *Tak1* gene in mice resulted in spontaneous hepatocyte death, inflammation, fibrosis, and carcinogenesis, indicating that TAK1 signaling is an essential component for cellular homeostasis in the liver.[91] The seemingly opposite effects suggest that TAK1 signaling is capable of exerting dual functions, like TGF-β1, in tissue/cell type and context dependent manner.

TAK1 signaling in apoptosis and autophagy

TGF-β1 is known to regulate cell survival and cell death, and TAK1 in a similar fashion possesses pro- and anti-apoptotic functions. The TAK1-null phenotype is lethal early in embryonic development, and knockdown of TAK1 expression or inhibition of TAK1 activation augments cell apoptosis induced by TGF-β1 in various cell types *in vitro* and *in vivo*, including the kidney, indicating that TAK1 is required for prevention of apoptosis and plays a role as a cell survival factor.[90,92] Conditional *tak1* gene deletion in mice resulted in a

two-fold increase in the apoptotic response in the obstructed kidney by UUO.[90] In contrast, abrogation of TAK1 activation inhibits TGF-β-induced apoptosis in embryonic fibroblasts, prostate cancer cells, and AML12 liver cells, indicating that TAK1 also acts as a mediator of apoptosis.[69,70]

The role of TGF-β1 as an inducer of autophagy is just beginning to be appreciated, and TAK1 signaling pathway in the regulation of autophagy has been hereto understudied. Autophagy, also known as macro-autophagy (literally, self-eating), is a fundamental cellular homeostatic process by which cells degrade and recycle proteins and remove damaged organelles.[93] In HuH7 human hepatocellular carcinoma cells, MDA-MB-231 breast cancer cells, and primary mouse mesangial cells, TGF-β1 induces accumulation of autophagosomes and conversion of microtubule-associated protein 1 light chain 3 (LC3) to the lapidated form, LC3-II.[33,94] LC3-II serves as a valuable molecular biomarker for the detection and assessment of autophagic activity.[95] TGF-β1 also increases the mRNA expression levels of several autophagy-related genes (ATG), such as Beclin 1, ATG5, ATG7, DAPK (death-associated protein kinase) and LC3 through the non-Smad signaling pathways as well as the Smad pathway.[33,94,96] Recent studies have demonstrated that TAK1-MKK3-p38 signaling axis is critical for the regulation of LC3 expression,[33] whereas NF-kB and JNK are positive regulators of Beclin 1 expression.[97] Moreover, JNK phosphorylation of Bcl-2 results in Beclin 1 activation by promoting the dissociation of Beclin 1 from Bcl-2.[98] Thus, a number of distinct mechanisms are implicated in TAK1-mediated signaling pathways that activate autophagy (Fig. 4).

Autophagy can lead to cell death in response to stress, but it can also act as a protective mechanism for cell survival. It is plausible that the functions of TGF-β1 as both an apoptosis promoter and apoptosis suppressor may relate to its regulation of autophagy. Indeed, it has recently reported that tumor necrosis factor-related apoptosis inducing ligand (TRAIL), which triggers apoptosis preferentially in cancer cells, spares normal untransformed cells from apoptosis by inducing cytoprotective autophagy via TAK1-dependent AMPK activation.[99] AMPK inhibits mammalian target of rapamycin (mTOR), a potent inhibitor of autophagy. We have reported that TGF-β1 induces autophagy through TAK1 and Akt activation, and protects glomerular mesangial cells from undergoing apoptosis during serum deprivation.[33] Although the precise mechanism by which TGF-β1-induced autophagy prevents cell death via apoptosis is not well understood, recent evidence indicates that active caspase-8, a death receptor effector, can be degraded through autophagy.[100]

Implications in anti-TGF therapy

Although there has been a plethora of evidence in preclinical studies that TGF-β blockade diminishes fibrosis in experimental models, limited advances have been made to date in treatment of human disease. A phase I/II randomized, placebo-controlled trial of TGF-β inhibitor therapy using a human anti-TGF-β antibody (Cat-192) in 45 patients with scleroderma was not associated with any clinical benefits in these patients.[101] Recently, the results of the first phase I clinical study using neutralizing anti-TGF-β antibodies for the treatment of kidney disease were reported.[102] Fresolimumab is a human monoclonal antibody that neutralizes all three isoforms of TGF-β. The phase I open-label study designed to assess the safety, tolerability, and pharmacokinetics of single-dose infusion of fresolimumab was conducted in patients with treatment-resistant primary focal segmental glomerulosclerosis (FSGS).[101] The results of this phase I clinical trial indicate that fresolimumab is relatively safe and well tolerated in patient. Larger randomized clinical trial to assess efficacy of this agent is anticipated.

Other anti-fibrotic therapy that is garnering interest is pirfenidone, which may be partially mediated by the inhibition of TGF-β TGF-β promoter activity and TGF-β protein secretion,

and inhibiting TGF-β–induced Smad2 phosphorylation.[103] In early clinical studies, beneficial effects of pirfenidone were noted in a multicenter, double-blind, placebo-controlled, randomized phase III clinical trial of patients with idiopathic pulmonary fibrosis,[104] and pirfenidone has shown promise as a therapy to slow renal function decline in patients with FSGS.[105]

Better anti-fibrotic targets are still needed for an effective therapy in human fibrotic diseases. Excessive TGF-β1 activity leads to fibrotic conditions. A potential candidate is targeting the TAK1 signaling pathway to block TGF-β-induced fibrotic responses. Blockade of TAK1 is a plausible and attractive strategy that targets major proinflammatory, proapoptotic, and profibrotic pathways, such as the p38 MAPK and JNK, in the development of progressive kidney disease. In this endeavor, many formidable challenges are anticipated for future investigations. We are keenly cognizant that we still have a great need to gain increased insights into complex TGF-β signal transduction pathways, and that we have much to discover in this exciting field.

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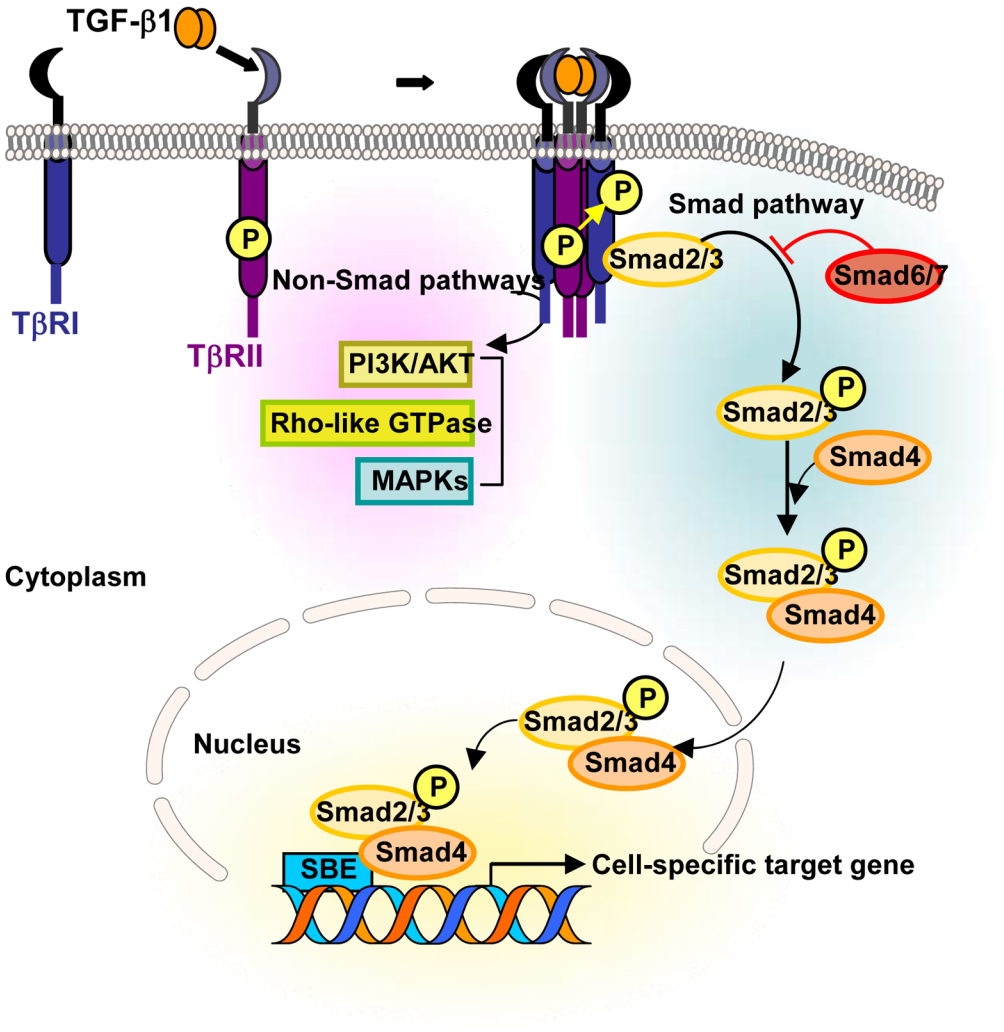

Fig. 1. Overview of TGF-β Signaling

Initiation of the TGF-β signaling cascade occurs upon ligand binding to TβRII and subsequent TβRI-TβRII hetero-tetrameric complex formation, and TβRI is activated through phosphorylated in the cytoplasmic GS domain. This is followed by activation of intracellular signaling mediators of TGF-β1. The canonical Smad pathway involves activation of Smad2/3 through recruitment and phosphorylation by activated TβRI, and requires kinase activity of TβRI. Smad2/3 is then released from the receptor complex to interact with Smad4 to transmit TGF-β1 signals. TGF-β1 also induces various non-Smad signaling pathways, including the PI3K/AKT, Rho-like GTPases, and the MAPKs.

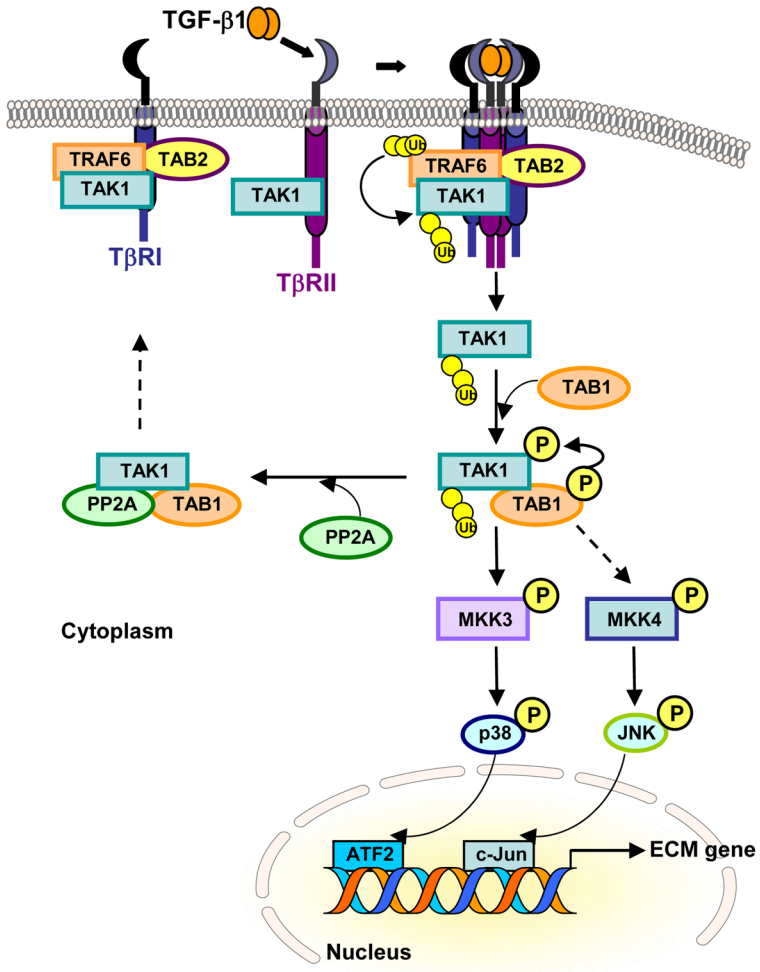

Fig. 2. Schema of TAK1 Signaling pathway

Under unstimulated condition, TAK1 associates with TβRI through complex formation with TAB2 and TRAF6. Upon TGF-β1 stimulation and formation of TβRI-TβRII hetero-tetrameric complexes, autopolyubiqitination of TRAF6 leads to polyubiquitination (Ub) of TAK1, triggering release of TAK1 from the receptor complexes. TAK1 interacts with TAB1, which in turn induces autophosphorylation of TAK1. The activated TAK1 transmits TGF-β1 signal to downstream signaling pathways such as the MKK3-p38 or MKK4-JNK cascade, or is rapidly deactivated by phosphatase PP2A. TGF-β1-induced TAK1 activation occurs independent of TβRI kinase activity.

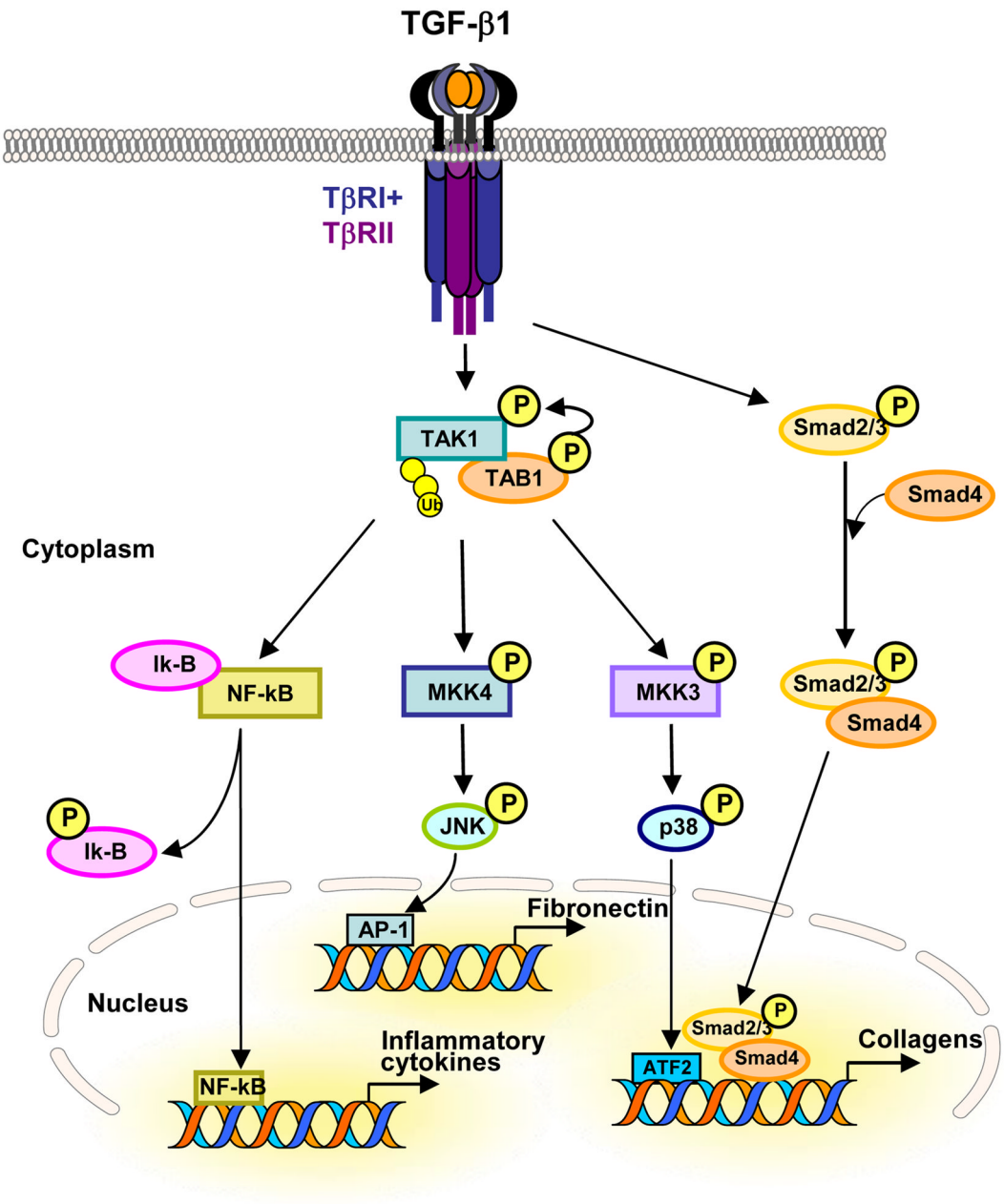

Fig. 3. TGF-β-induced TAK1 signaling and profibrotic response

TGF-β1-induced TAK1 activation triggers downstream signaling pathways such as the MKK4-JNK, or the MKK3-p38 cascade, and promotes degradation of Ik-B, which in turn lead to the activation of transcription factors ATF-2, AP-1 and NF-kB, respectively, to regulate the expression of extracellular matrix proteins, including collagens and fibronectin, and inflammatory cytokines. In addition, TGF-β1-induced Smad activation is also indispensible for TGF-β1-induced type I collagen expression through the crosstalk with TAK1-MKK3-p38 signaling axis.

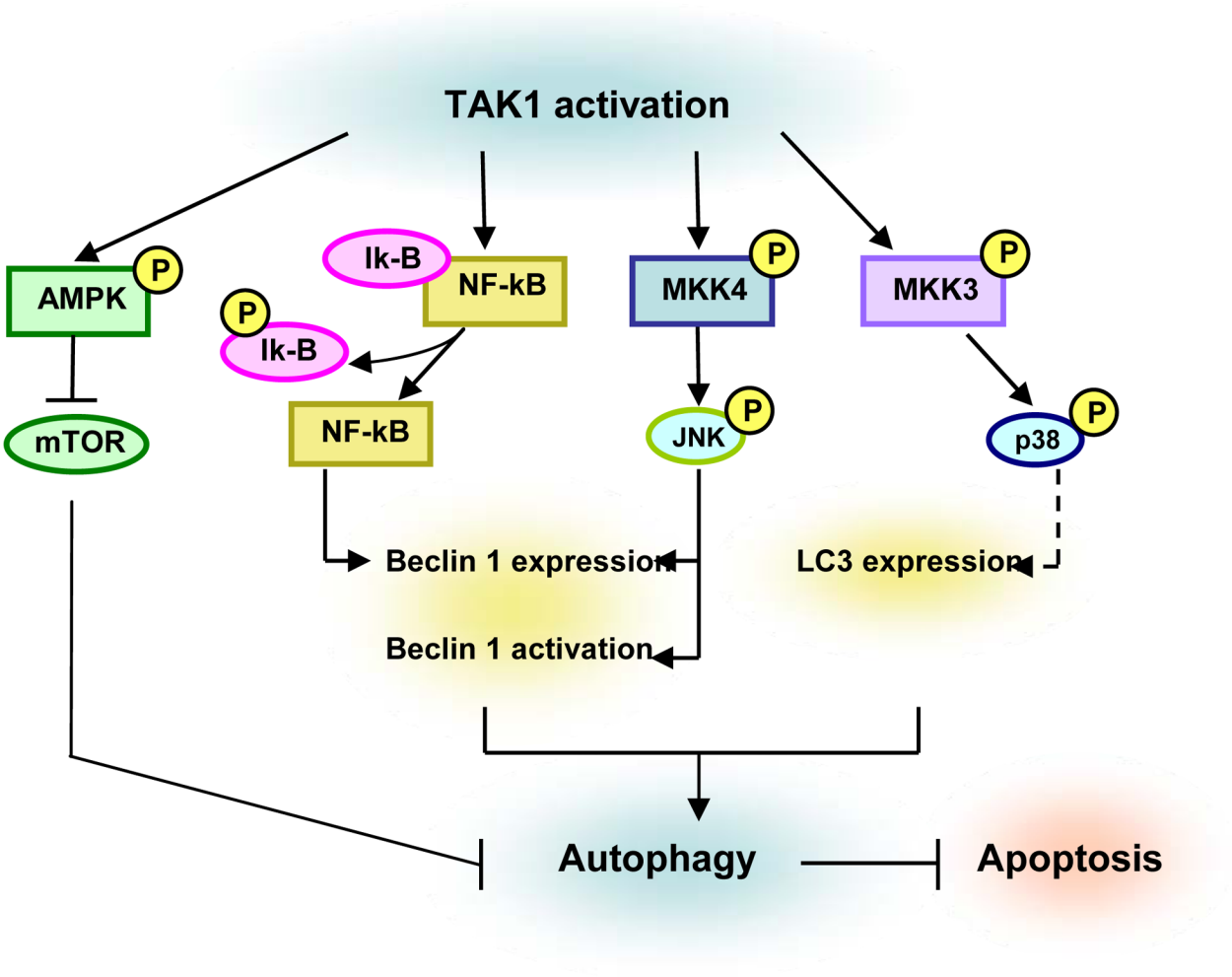

Fig. 4. Signaling pathways implicated in TAK1-mediated autophagy induction

TAK1 activation of MKK3-p38 induces the expression of LC3, whereas JNK activation induces the expression and activation of Beclin 1. NF-kB directly interacts with Beclin 1 promoter region and enhances Beclin 1 transcription. TAK1 activation also leads to the activation of AMPK and inhibits mTOR activity resulting in induction of autophagy. Accumulating evidence indicates that activation of autophagy inhibits apoptosis and can function as a cytoprotective response.