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Abstract

The balance between production and degradation of type I collagen plays a critical role in the development and maintenance of organ and tissue integrity. It also represents the most crucial element governing the process of tissue repair. The synthesis of type I collagen gene is highly regulated by different cytokines at transcriptional level. Especially, TGF-β, a key player in the physiopathology of tissue repair, enhances type I collagen gene expression. In contrast, TNF-α whose matrix-remodeling function is opposite to that of TGF-β, reduces type I collagen gene expression. This review focuses on transcriptional regulation of type I collagen by TGF-β and TNF-α, and on the molecular mechanisms that control the antagonistic activity of TNF-α against TGF-β-driven type I collagen gene expression;

Keywords: Type I collagen, TGF-β, TNF-α, Fibrosis

1. Introduction

Fibrosis is a complex tissue disease whose predominant characteristic is the excessive deposition of extracellular matrix (ECM) components, especially collagens the major fibrous proteins in ECM [1]. Collagen deposition can take place in various internal organs, including lungs liver, kidney and skin. In most cases, fibrosis is a reactive process involving different pathophysiological events such as the attraction of blood-borne cells (e.g., leukocytes, platelets, activated lymphocytes), the alteration of microvascular cells (interrupting non-thrombogenic, permeability regulating, and cell-adhesive functions of the microcirculation), and the activation of resident mesenchymal cells (fibroblasts, endothelial cells, pericytes) leading to excessive collagen deposition. Thus, the net accumulation of collagen in tissue fibrosis is a result of an imbalance between the factors leading to enhanced production and deposition, or impaired degradation and removal of collagen. Contributing to the metabolic modulation are cytokines and growth factors, a group of diverse molecules derived from blood cells, such as platelets, or elaborated locally by mesenchymal and epithelial cells [2].

To date about 20 types of collagens have been identified. The molecular nature and functions of several collagens have been characterized [3]. Type I collagen, the major component of ECM is composed of glycine- and proline rich two-α1(I) and one-α2(I) chains which are the products of two genes, COL1A1 and COL1A2. The pro-α1(I) and pro-α2(I) polypeptide chains are synthesized by fibroblasts, osteoblasts, or odontoblasts and enter into endoplasmic reticulum where specific proline and lysine residues are hydroxylated to form hydroxyproline and hydroxylysine, respectively, which help the pro-α chains to combine with other chains by hydrogen bonds and form the triple helix procollagen structure. Procollagens are secreted by the fibroblasts through the Golgi apparatus in the extracellular space where the N-terminal and C-terminal propeptides are cleaved by specific proteases. The mature processed collagen molecules aggregates to form larger collagen fibrils and help to form the

ECM with other components [4]. Therefore, normal structural and functional type I collagen production and deposition in ECM to make normal physiological connective tissue needs regulation at several steps. Abnormality in any step may cause hyper-synthesis and accumulation of collagen in ECM, which in turn causes different diseases in humans such as fibrosis. Several studies have shown that the exaggerated tissue deposition of type I collagen in this type of disease is largely due to an increase in the rate of transcription of the corresponding genes [1, 2, 5]. Therefore, it is very useful to understand the molecular mechanisms that cause the upregulation of type I collagen gene expression to better understand the fibrotic disease or other collagen related diseases. To date, numerous efforts have been made to identify the factors and the signal transduction pathways that are involved in transcription of type I collagen gene expression. Identification of the regulatory elements within the promoters of this gene and of the transcription factors that bind to these elements is, therefore, crucial for understanding the mechanisms involved in the development of tissue fibrosis.

2. Transcriptional regulation of Type I collagen genes by TGF-β

Transforming growth factor β (TGF-β) is a key regulator of ECM assembly and remodeling. This action is exerted through two complementary pathways, one that reduces matrix degradation and the other that stimulates matrix accumulation. In simplified terms, TGF-β inhibits the synthesis of extracellular proteinases while upregulating the production of their inhibitors and that of structural ECM components. The combined action of TGF-β on the genes implicated in the formation and degradation of the ECM is mostly exerted at the transcriptional level through well-defined intracellular pathways [6]. Thefore, in the context of fibrotic diseases, it appears crucial to understand the molecular mechanisms that cause the transcriptional response of type I collagen genes to TGF-β.

2.1. The TGF-β signal transduction machinery (Figure 1)

The TGF-βs signal via serine/threonine kinase transmembrane receptors that phosphorylate cytoplasmic mediators of the Smad family [7, 8]. The ligand-specific Smad1, Smad2, Smad3 and Smad5, interact directly with, and are phosphorylated by, activated TGF-β receptors type I [8, 9 10]. Smad1 and Smad5 are specific for BMPs, whereas Smad2 and Smad3 can be activated by both TGF-β and activin receptors. They all consist of two conserved Mad-homology (MH) domains that form globular structures separated by a linker region [11]. The N-terminal MH1 domain has DNA-binding activity whereas the C-terminal MH2 domain has protein-binding properties. Phosphorylation of R-Smads by type I receptors occurs principally on two serine residues within a conserved -SS(M/V)S- motif at their C-terminus [9, 10]. Receptor-activated Smads are kept in the cytoplasm in the basal state bound to the protein SARA (Smad anchor for receptor activation) [12]. Upon phosphorylation at their SSXS carboxy-terminal motif, they are released from SARA and form heteromeric complexes with Smad4, a common mediator for all Smad pathways. The resulting Smad heterocomplexes are then translocated into the nucleus by a mechanism involving the cytoplasmic protein importin [13, 14], where they activate target genes, binding DNA either directly or in association with other transcription factors [9, 10]. Members of the third group of Smads, the inhibitory Smads Smad6 and Smad7, prevent phosphorylation and/or nuclear translocation of receptor-associated Smads, and recruit E3-type ubiquitin ligases to the receptors complexes, ultimately leading to their degradation [15, 16]. Following target gene transcription, Smad complexes are released from the chromatin and undergo ubiquitination, followed by proteasomal degradation [17].

Several cross-signaling mechanisms have been described that implicate Smad proteins. For example, Smad3 interacts with the vitamin D receptor to mediate the additive effect of

TGF-β on vitamin D3-induced transcription [18]. Smad3 also interacts with TFE3 and Sp1 to activate transcription from the *PAI-1* and *p21*^{cik} promoters, respectively [19, 20]. Smad/AP-1 interactions have also been reported, which may lead to either additive [21, 22, 23], or antagonistic [23, 24] activities on gene transactivation. The outcome is dependent on the structure of target promoters, as not only the presence of AP-1- and/or Smad-specific *cis*-elements, but also their respective positions, may influence the transcriptional outcome response [22, 23]. Another possibility for Smads to interfere with other transcription factors occurs through direct interactions with transcriptional co-activators such as CBP/p300. The latter proteins modify transcription either by altering chromatin structure so that the underlying DNA sequences are exposed to the transcriptional apparatus [25], or by directly recruiting the RNA polymerase II holoenzyme to the promoter [26]. Smad.CBP/p300 interaction is required for transcriptional activation of several TGF-β dependent promoters [27, 28, 29, 30, 31]. Several proteins including the viral oncoprotein E1A [32], the proto-oncogenes c-Ski and SnoN [33, 34], TGIF [35], Snip-1 [36], SIP1 [37], and c-Jun [23, 24] compete for R-Smad/Smad4 binding to CBP or p300, thereby interfering with Smad-dependent gene expression.

TGF-β also initiates other signaling pathways, such as a variety of MAP kinase pathways that may be required for both Smad-dependent and Smad-independent transcriptional responses to TGF-β [38, 39, 40]. The particular combinations of MAP kinases activated by TGF-β are cell type dependent. For example, in mink lung epithelial cells, TGF-β-induced activation of the Jun-N terminal kinase (JNK) mediates Smad3 phosphorylation, which is required for the transcriptional activation of Smad3-dependent responses [41], while in rat articular chondrocytes, TGF-β induces activation of ERK1/2, but not p38 or JNK MAP kinases [42]. Furthermore, TGF-β-induced activation of fibronectin in a variety of epithelial cell lines requires activation of JNK and is independent of Smad signaling [43]. The precise

mechanisms by which MAP kinases are activated in response to TGF-β is rather poorly understood. TGF-β-induced JNK signaling may involve Rho-like guanosine triphosphatases [44, 45], but linkage to the TGF-β receptor complex is unclear. Some insights into upstream links of MAP kinase pathways to TβRI have been provided with the identification of TAK1 and TAB1, a novel MAP kinase kinase kinase [46, 47] and its activator, which have been shown to be important for TGF-β signaling through p38 MAP kinase. However, direct linkage between the TAB/TAK1 cascade and TGF-β receptors has not been demonstrated [48]. Downstream, components of MAP kinase-signaling pathways may also interact with the R-Smad/Smad4 complex in the nucleus, providing an additional level of transcriptional crosstalk between these pathways [49].

2.2. Transcriptional regulation of COL1A1 by TGF-β

The human pro-*COL1A1* gene is located in 17q21.31-22.05 [50]. The regulatory regions within the promoter contain several repressor and enhancer element, and several transcription factors interact with these upstream regulatory elements to control basal, cell-type-specific, and cytokine-modulated gene expression [2, 5].

Using a series of deletion constructs containing portions of the 5'-flanking region of human α1(I) procollagen gene (*COL1A1*) promoter (with end point from -5,3 kb to -84 base pairs) transiently transfected into NIH/3T3 cells, Jimenez et al. demonstrated that TGF-β responsive sequences are located between -174 and -84 bp from the transcription start site [51]. This region contains a binding site for Sp1 at -87 bp and an element with complete identity to the 3' portion of the canonical NF-1-binding motif, located in reverse orientation at -95 bp. These overlapping potential binding sites have been shown to be important in the basal expression of the mouse and the rat *COL1A1* genes. In addition, a sequence similar to the AP-1 binding site is located in reverse orientation at –165 bp, and an Sp1-like sequence at

–148. Using EMSA experiments, this group demonstrated that TGF-β caused marked stimulation of the DNA binding activity of nuclear factor interacting with an Sp1-like binding site located within a region encompassing -164 to -142 bp of the promoter, suggesting that the Sp1-like sequence and not the NF-1-like sequence identified as the TGF-β responsive element in the rat *COL1A1* gene promoter, is involved in TGF-β-induced transcriptional activation of the human *COL1A1* gene [51]. With respect to this work, Gaidarova and Jimenez demonstrated that two DNA intercalators, the mithoxantrone and WP631, known to prevent Sp1-DNA binding, inhibit TGF-β-stimulated *COL1A1* expression in human adult dermal fibroblasts [52]. In contrast, an AP-1 binding site located at the +598 to +604 in the first intron of a human promoter-enhancer construct has been shown to be involved in TGF-β modulation of the *COL1A1* gene in Ito cells [53]. Different cell types used in these studies may account for the observed differences.

2.3. Transcriptional regulation of COL1A2 by TGF-β

Early observations demonstrated that a segment of the mouse *COL1A2* promoter between –350 and –300, overlapping a nuclear factor 1 (NF1) binding-site, is needed for TGF-β stimulation in NIH 3T3 cells [54]. Regarding, the human *COL1A2* promoter, original works demonstrated that a 135 bp region of the promoter within 330 bp of the transcription start site could confer responsiveness to TGF-β [55]. This rather large region was shown to contain two smaller regions, the first referred as box A, spanning nucleotides –330 to –286 and containing two distinct nuclear binding sites called 5A and 3A, the other referred to as box B, from nucleotides –271 to –255. The upstream element, box A, was shown to bind Sp1 and to confer high basal promoter activity [55]. Box B harbors both a putative AP-1 binding element and a non-canonical NF-κB binding site [55, 56].

Using a series of 5'deletion *COL1A2* promoter/chloramphenicol acetyltransferase (CAT) reporter gene constructs, Chung et al. identified the region located between residues -265 and -241, as critical for TGF-β response [57]. Specifically, they demonstrated that this 25-base pair region mediates the up-regulatory effect of TGF-β on *COL1A2* promoter activity and allows antagonistic activity of TNF-α on the TGF-β effect. Transient cell transfection experiments with plasmid constructs in which the potential AP-1-binding site located within this short region of promoter was modified by site directed mutagenesis indicated that this element plays a significant role in the basal activity of the promoter. Furthermore, this sequence is essential for TGF-β response and does not require the presence of the three Sp-1-binding sites located further upstream, between nucleotides -273 and -304. In addition, overexpression of c-jun in co-transfection experiments with *COL1A2* promoter/CAT constructs blocks the TGF-β response, further implicating AP-1 in the regulation of *COL1A2* gene expression.

Contrasting with this work, Inagaki et al., reported that TGF-β stimulates transcription of the human *COL1A2* promoter by increasing the affinity of an Spl-containing protein complex for its cognate DNA-binding site [55, 58].

Chen et al., demonstrated that Smad3 and Smad4 are required for the TGF-β induced COL1A2 transcription in human skin fibroblasts [59, 60]. Using transfections with a series of 5' deletions of the human *COL1A2* promoter, these authors identified a functional Smad-binding element of the *COL1A2* promoter harboring a CAGACA consensus sequence between -353 and -148 bp that is both necessary and sufficient for stimulation by TGF-β, and demonstrate that interaction of this Smad-binding element with endogenous Smads is required for the full TGF-β response in fibroblast [60]. Cooperation between Sp1 and Smad proteins was reported, but the cis-element involved in this phenomenon were not identified precisely [61]. Smad.Sp1 interactions have been described previously, and play a role in the activation

of the p21 promoter by TGF-β [20]. In the latter example, Smad proteins do not bind DNA diectly but rather act as transcription factors through their interaction with DNA-bound Sp1 complexes. In the context of the human COL1A2 promoter, using EMSA experiments, Poncelet and Schnaper observed that Sp1 and Smads form complexes with the -283/-250 promoter sequence harboring both Sp1 binding elements and a CAGACA consensus sequence [62]. Ghosh et al., demonstrated that the ubiquitous transcriptional coactivators p300 and CREB-binding protein (CBP) enhanced basal as well as TGF-β- or Smad3-induced COL1A2 promoter activity, and stimulated the expression of endogenous Type I collagen. The effect of p300 on COL1A2 transcription appeared to be due, in part, to its intrinsic acetyltransferase activity, as stimulation induced by a histone acetyltransferase-deficient mutant p300 was substantially reduced. Transactivation of *COL1A2* by p300 involved the Smad signaling pathway, as Smad4-deficient cells failed to respond to p300, and stimulation was rescued by overexpression of Smad4 [63].

3. Transcriptionnal regulation of type I collagen genes by TNF-α

Tumor necrosis alpha (TNF-α), released by activated macrophages, plays a key role in inflammatory disorders such as rheumatoid arthritis ans osteoarthritis [64]. ECM degradation is the hallmark of these conditions and an important component in morphogenesis, organogenesis, and tissue remodeling, as well as in wound healing and tissue repair. TNF-α reduces ECM deposition either by inducing the production of stromal collagenases or by inhibiting the synthesis of structural components such as the type I collagen, the major structural component of connective tissue. TNF-α also counteracts TGF-β stimulation of type I collagen gene expression [65, 66, 67]. This finding reflects the functionally antagonistic nature of these cytokines and represents a useful paradigm to study the complex cellular signals that regulate ECM formation and remodeling.

3.1. The TNF-α signal transduction machinery (Figure 2)

TNF-α exerts its functions by interaction with the death domain-containing TNF-receptor 1 (TNF-R1) and the non-death domain-containing TNF-receptor 2 (TNF-R2), both members of a receptor family complementary to the TNF ligand family. Several proteins that interact directly or indirectly with the cytoplasmic domains of TNF-R1 and TNF-R2 have been identified in the recent years giving ideas how these receptors are connected to the signaling cascades leading to activation of NF-κB and JNK. Of special interest are TNF receptor-associated factor (TRAF)-1 and -2, which defines a novel group of adaptor proteins involved in signal transduction by most members of the TNF receptor family, of IL-1 receptor and IL-17 receptor as well as some members of the TOLL-like receptor family. TRAF2 is currently the best-characterized TRAF family member, having a key role in mediating TNF-R1-induced activation of NF-κB and JNK [68, 69, 70].

NF-κB proteins are a family of cytoplasmic hetero-dimeric transcription factors that are inactive due to their association with an inhibitory protein, termed IκB. Upon phosphorylation by the IKK complex, which consists of two highly homologous kinases, IKKα and IKKβ, and the non-catalytic adaptor NEMO/IKKγ. Upon phosphorylation by IKKα and IKKβ, IκB becomes ubiquitinated and degraded by proteosome complexes. Once free, unbound NF-κB dimerizes and becomes an active transcription factor that translocates to the nucleus and regulates transcription of NF-κB-responsive genes. According to this model, TNF-RI activation may stimulate RIP kinase activity, which in turn, activates IKKα and IKKβ. This could lead to IκB phsophorylation and degradation, resulting in the activation of NF-κB [68, 69, 70].

JNK is typically activated by a variety of physical and chemical stress but also by cytokines like TNF-α. JNK can be triggered by two members of the MAPK kinases (MKK),

MKK4 and MKK7 which are targets of a variety of MAP3Ks, among these are the apoptosis signal-regulating kinase (ASK)-1, the TGF-ß-activating kinase (TAK)-1 and the MEK kinase (MEKK)-1. All of these kinases have been described to interact with TRAF2. Accordingly, the pathway leading from TNF-R1 to JNK might include TRADD, TRAF2, and a MAP3K, initiating the kinase cascade leading to JNK. Much less is known about the signaling pathways leading to the TNF-induced activation of another MAPK, p38. The currently known four isoforms of p38, p38 to p38, are all activated by MKK6 and MKK3. In addition, MKK4 can also activate certain isoforms of p38. A targeted disruption of the MKK3 gene causes a selective defect in the response of fibroblasts to TNF-, including reduced p38 MAP kinase activation, suggesting that MKK3 is utilized by TNF receptor signaling [68, 69, 70].

3.2. Transcriptional regulation of COL1A1 by TNF-

In human dermal fibroblasts, using a series of deletion constructs or various substitution mutations of the human *COL1A1* 5'-flanking promoter region, Mori et al., observed that TNF- suppress promoter activity through a proximal short promoter elements containing only 107 bp [71]. EMSA experiments and functional analysis with short substitution mutations between -101 and -97 bp or between -46 and -38 bp suggest that TNF- suppressed *COL1A1* promoter activity through these elements located between -101 and -97 bp and between -46 and -38 bp of the COL1A1 promoter, and that the suppression involved interactions between DNA and yet unidentified protein [71].

In rat hepatic stellate cells, a functional TNF--responsive-element (TRE) has been identified in the -378 to -345 region of the rat *COL1A1* promoter [72]. The authors demonstrated that TNF- induces nuclear translocation and binding of transcriptional complexes containing p20C/EBP, p35C/EBP, and C/EBP to this sequence of the promoter. This element co-localizes with the previously reported TGF--responsive element

identified in the rat *COL1A1* promoter [73]. The role of p38 in the down-regulation of the rat COL1A1 promoter activity by TNF-α has been recently suggested [74].

3.3. Transcriptional regulation of COL1A2 by TNF-α

Initial observations demonstrated that a 135-bp region of the COL1A2 promoter within 330 bp of the transcription start site could confer responsiveness to TNF-α [56]. Although no specific transcription factor could be identified by Inagaki et al. [55, 56], they postulated that unknown factors interacting with both box B and the upstream Sp1 binding sites present in box A were necessary for TNF-α responses [55, 56]. More recently, Kouba et al., identified the specific TNF-α response element (TαRE) that allows *COL1A2* transcriptional inhibitory response to TNF-α in human dermal fibroblasts [75]. Specifically, the TαRE was found to reside between nucleotides -271 and -235 relative to the transcription initiation site. Electrophoretic mobility supershift assays (EMSA) identified NF-kB1 and RelA NF- kB family members as transcription factors binding the TαRE and mediating TNF-α repression of *COL1A2* promoter activity. Precise nucleotide requirement for NF-αB binding to the TαRE was determined in nucleotide substitution experiments and clearly indicated that TNF-α and TGF-β responses involve closely located, yet distinct, elements within the -271/-235 segment of *COL1A2* promoter [75]. The crucial role of NF-αB signaling pathway in the downregulation of *COL1A2* promoter mediated by TNF-α was further confirmed by the same group by using a gene knockout approach. In NF-αB essential modulator$^{-/-}$ (NEMO$^{-/-}$) fibroblasts, lack of NF-αB activation in response to TNF-α prevented repression of basal *COL1A2* gene expression. Similar regulatory mechanisms take place in dermal fibroblasts, as evidenced using transfected dominant negative forms of IKK-β, critical kinases upstream NF-αB pathway [67].

4. Antagonistic effect of TNF-α on TGF-β-induced type I collagen gene expression (Figure 3)

Antagonistic activities of pro-inflammatory cytokines against TGF-β are playing an essential role in maintaining tissue homeostasis and extracellular matrix deposition [2, 76]. Several studies have addressed the molecular mechanisms underlying the antagonistic activities exerted by pro-inflammatory cytokines and TGF-β. For example, differential induction of c-Jun and JunB, transcription factors of the AP-1 family that exhibit antagonistic transcriptional activities, underlies the differential regulation of matrix metalloproteinase-1 gene expression by TGF-β and pro-inflammatory cytokines, including TNF-α and IL-1 [76, 77, 78, 79]. More recently, it was shown that several signaling pathways, such as those for NF-κB and JAK (Janus kinase)/STAT (signal transducer and activator of transcription), activated in response to various stimuli, such as cytokines, shear stress, and UV light, lead to an increased expression of inhibitory SMAD7, which, in turn, prevents signaling from the TGF-β receptors [80, 81]. NF-κB has been suggested to be part of the signals responsible for SMAD7 gene activation by TNF-α [82], although it is clearly a cell-type specific mechanism [78, 83]. Alternatively, induction of c-Jun by cytokines has been shown to directly interfere with the SMAD pathway either by preventing SMAD3 binding to cognate DNA sequences or by sequestering the transcriptional coactivator p300 [23, 78, 84, 85]. In this respect, activation of JunB expression in response to SMAD signaling downstream of the TGF-β receptors was recently shown to be part of a negative autoregulatory loop attenuating SMAD-specific transcriptional responses [24]. Using a gene knockout approach, to elucidate the specific roles played by the Jun-N-terminal kinase (JNK) and NF-kB pathways downstream of TNF-α in the context of α(2) type I collagen gene (COL1A2) expression, Verrecchia et al.,. clearly establish a link between previous reports about the interference of c-Jun and JNK with Smad signaling and the inhibition of TGF-β-induced type I collagen gene expression by TNF-α.

Specifically, one of the proposed mechanisms by which Jun proteins interfere with the Smad pathway involves direct Smad-Jun interaction, not compatible with Smad/DNA complex formation [23]. Also, overexpression of constitutively active forms of either MEKK1 or MKK4, kinases involved in JNK activation, enhance Smad-Jun associations and repress Smad-dependent transcription [84]. Using independent approaches to interfere with the JNK and NF-κB pathways, such as expression of dominant-negative mutant forms of MKK4 and IKK-β in normal human dermal fibroblasts, or genetically modified, immortalized, mouse fibroblast lines established from *JNK*$^{-/-}$ and *NEMO*$^{-/-}$ embryos, these authors provide compelling evidence for a central role of JNK in allowing the antagonistic activity of TNF-α and c-Jun against TGF-β-induded type I collagen gene expression, *COL1A2* promoter transactivation, and Smad signaling. On the other hand, NF-κB activity, although critical for the inhibitory effect of TNF-α on basal *COL1A2* promoter activity, plays no role in mediating the former phenomenon [67].

5. Conclusion

Excessive accumulation of collagen, especially type I collagen, is a major pathological feature in diseases characterized by tissue fibrosis. Several pharmacological agents have been tested for the effects on collagen metabolism [86]. Some of these agents, which modulated collagen gene expression either at transcriptional or translational level, are already in clinical use or are currently being tested. Unfortunately, to date, no treatment modality specifically reduces collagen deposition in tissues. Understanding of the mechanisms of TGF-β-mediated up-regulation of type I collagen gene expression provides novel and promising opportunities for treatment of fibrotic diseases.

One approach could be to reduce TGF-β gene expression, either by suppressing the initiation of gene transcription or by altering mRNA stability. TGF-β mRNA expression is reduce by anti-sense oligonucleotide [87, 88], INF-γ [89], and anti-oxidants such as δ-

tochoferol [90]. Another approach could directly target circulating TGF-β. Thus, administration of anti-TGF-β antiserum, or the use of a chimeric protein composed of the extracellular domain of the TGF-β type II receptor linked to the Fc portion of IgG that binds and inactivates TGF-β, reduce matrix accumulation in rats with glomerulonephritis [91, 92, 93]. Other options might include inhibiting the local action of TGF-β on target cell, by interfering with post-receptor signaling. Two molecules are potent inhibitors of TGF-β/Smad-mediated type I collagen expression. Firstly, Smad7, induced by INF-γ, blocks TGF-β/Smad signaling pathway by preventing phosphorylation and/or nuclear translocation of receptor-associated Smads. Secondly, JNK, activated by TNF-α, blocks TGF-β/Smad signaling pathway by induction of c-Jun phosphorylation which in turn, directly interfere with the Smad pathway either by preventing Smad3 binding to cognate DNA sequences or by sequestering the transcriptional coactivator p300 [23, 78, 84, 85]. In this context, 5-FU, which has been tried with some success in the treatment of keloids and hypertrophic scars, has been recently identified as a potent inhibitor of TGF-β/Smad signaling, capable of blocking TGF-β-induced, Smad-driven, upregulation of *COL1A2* gene expression in a JNK-dependent manner [94].

Figures

Figure 1: The TGF-β signal transduction machinery

Figure 2: The TNF-α signal transduction machinery

Figure 3: Antagonistic effect of TNF-α on TGF-β-induced type I collagen gene expression

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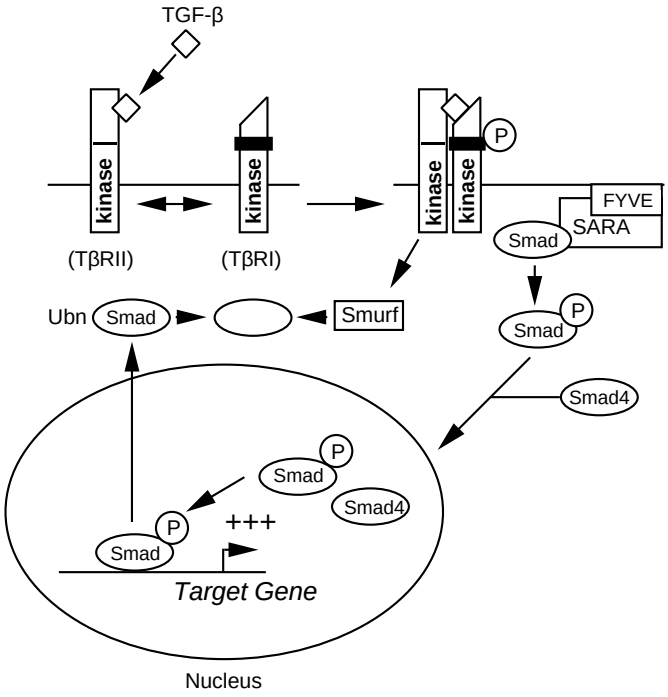

Figure 1

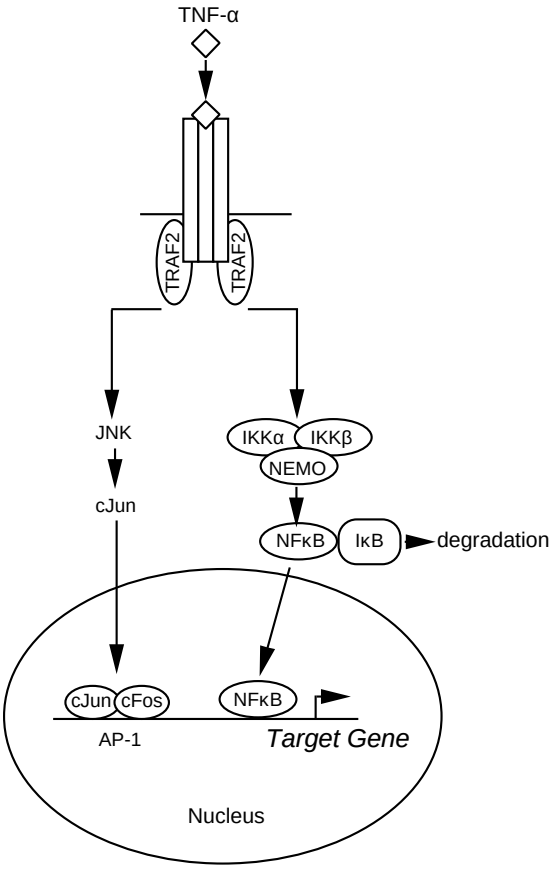

Figure 2

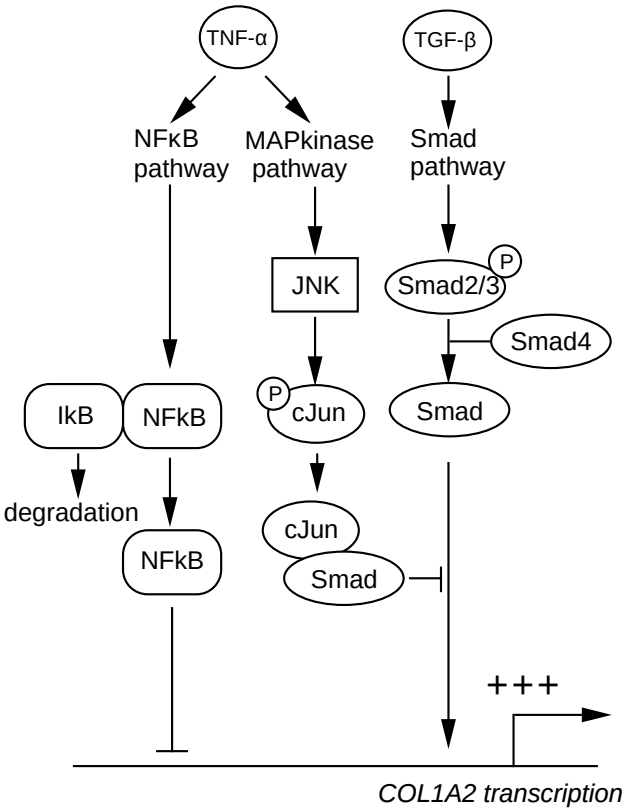

Figure 3