Feature

Endless healing: TGF-β, SMADs, and fibrosis

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Received 23 August 2001; accepted 29 August 2001

First published online 12 September 2001

1. Introduction

The human body consists of two major compartments, a cellular and an extracellular compartment. Most types of injury confer damage to both of these compartments, and the following healing process therefore involves regeneration of both the cellular and extracellular mass, the extracellular matrix (ECM). Irrespective of the affected tissue, this wound healing process follows a conserved sequence of events, including initiation and execution of (i) the coagulation cascade, (ii) an inflammatory response (associated with angiogenesis, formation of granulation tissue, and reepithelialization of denuded areas), and (iii) a fibroproliferative response (including proliferation of mesenchymal cells and increased synthesis of ECM) [1]. In normal wound healing, a network of negative feedback mechanisms activated after successful healing is responsible for the proper termination of the proliferative and fibrotic processes, thus restoring tissue integrity. If these feedback mechanisms fail to operate, however, continuous ECM secretion and deposition will lead to perturbation of normal tissue architecture and 'endless healing', with the eventual development of tissue fibrosis. Alternatively, such 'endless healing' may also be due to repeated injuries leading to continuous activation of the fibroproliferative response. Although the exact molecular mechanisms leading to such unregulated and continuous deposition of ECM molecules remain enigmatic, accumulating evidence from multiple in vivo and in vitro observations suggests that transforming growth factor (TGF)-β is a key soluble mediator in the development of fibrosis (for a review, see [2]).

2. The TGF-β system

TGF-β represents the prototypic member of a large family of polypeptide growth factors, which, due to ubiquitous expression of its cell surface receptors, exerts multiple biological effects on most cell types. TGF-β plays an important and nonredundant role in embryonic development and cellular differentiation, regulates cellular proliferation, induces synthesis of ECM proteins, and modulates the immune response, thus identifying it as a key regulator of both cellular and extracellular homeostasis. Interestingly, TGF-β can lead to completely opposite biological effects, depending on the cell type investigated, e.g. stimulation of proliferation in fibroblasts vs. inhibition of proliferation in epithelial cells. The cloning and characterization of three membrane-bound TGF-β receptors, designated TβRI, TβRII, and TβRIII (betaglycan) represented a milestone in the field of signal transduction; TβRI and TβRII were the first membrane-bound receptors exhibiting intrinsic serine–threonine kinase activity [3,4]. This discovery thus introduced a completely novel intracellular signaling mechanism activated by extracellular ligands, apart from the then well-known tyrosine kinase receptor system.

Many of the biological effects induced by TGF-β are associated with activation of diverse transcription factor systems, including activating protein-1 (AP-1) and mitogen-activated protein kinase (MAPK) family members. Beginning in 1995, the cloning of Smad molecules led to the discovery and designation of a novel and previously unknown class of transcription factors, which directly interacted with the signal transducing receptor isotype TβRI. To date, sequence and functional analysis of eight Smad isoforms has led to their classification into three subgroups; (i) receptor-regulated Smads, which directly function as substrate molecules for phosphorylated type I receptors (Smad1, 5, 8 for BMP ligands and Smad2 and 3 for TGF-β ligands), (ii) the common Smad4, which is thought to be the binding partner for all receptor-regulated Smads, facilitating their translocation into the nucleus, and (iii) inhibitory Smads (Smad6 and 7), which inhibit the association of receptor-regulated Smads with the type I receptors and thereby antagonize TGF-β-induced effects [5,6].

In the current model of TGF-β signal transduction, biological effects of TGF-β are induced after binding of active TGF-β to the ligand binding receptor isotype, TβRII. This leads to formation and stabilization of a heterotetrameric complex of TβRI and TβRII, followed by transphosphorylation of TβRI by the constitutively active TβRII kinase in its intracellular juxtamembrane region rich in glycine and serine residues, termed the GS domain. Subsequent phosphorylation of the receptor-associated cytoplasmic effector molecules Smad2 or Smad3 by TβRI then leads to heterooligomerization of phosphorylated Smad2/3 with the common Smad4, and modulation of gene transcription in the nucleus (Fig. 1) [6,7]. TβRIII (betaglycan) is a widely expressed heparan- and chondroitin sulfate proteoglycan that binds TGF-β with high affinity through its protein core. Betaglycan facilitates ligand binding to TβRII, but recent reports have indicated that it may play a far more complex role, exhibiting positive and/or negative effects on TGF-β signaling, depending on the cell type ([8,9], and O. Eickelberg and R.G. Wells, submitted).

3. TGF-β and fibrosis: mechanisms and hypotheses

The pathophysiology of tissue fibrosis is characterized by two related events, (i) transdifferentiation of fibroblasts into

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PII: S0014-5793(01)02875-7

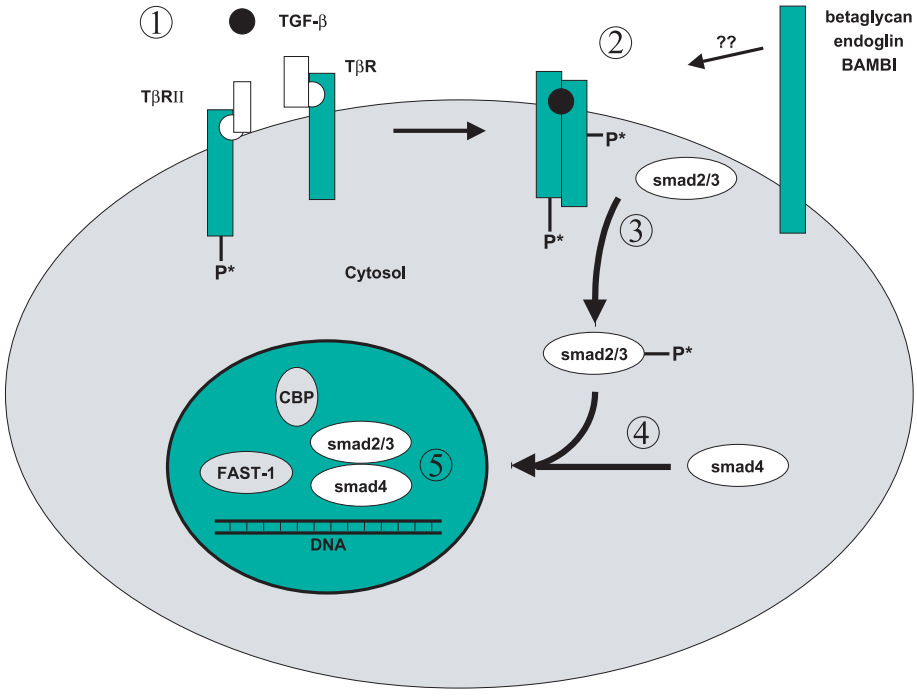

Fig. 1. TGF-β signal transduction. TGF-β signal transduction is initiated by binding of TGF-β ligand to TβRII. TβRI is then recruited into the receptor complex, followed by stabilization of TβRI/TβRII heterotetrameric complexes, and phosphorylation of the kinase domain of TβRI by the constitutively autophosphorylated TβRII kinase. Phosphorylated TβRI then recruits and phosphorylates Smad2/3 molecules, which, upon dissociation from TβRI, form dimers with Smad4. Smad2/3-Smad4 heterodimers translocate to the nucleus where they recruit cofactors in order to activate or repress gene transcription. Regulation of TGF-β activity can occur at multiple levels, including: (1) availability of active ligand through TGF-β binding proteins or soluble receptors, (2) formation of active receptor complexes through interaction with betaglycan, endoglin, or the pseudoreceptor BAMBI, (3) Smad2/3 phosphorylation through competition for TβRI by the inhibitory Smads 7 and 8, (4) formation of Smad2/3-Smad4 dimers through mutations in Smad4, and (5) activity and recruitment of transcriptional activators or repressors.

'activated' myofibroblasts, and (ii) enhanced synthesis and secretion of ECM molecules by these cells. The pathological hallmark of fibrosis is the accumulation of excess amounts of ECM in the affected tissue, due to both quantitative and qualitative changes in ECM composition [1]. TGF-β has been shown to play a pivotal role in the initiation and degree of fibrosis in a variety of organ systems, including the lung, liver, and kidney, and attempts to inhibit or antagonize TGF-β activity have led to promising results in downregulating or reversing tissue fibrosis [2].

In this issue of FEBS letters, Ricupero et al. describe antifibrotic effects of a dietary flavonoid, apigenin, in a cell culture model using human embryonic lung fibroblasts [10]. Flavonoids represent a widespread class of natural compounds with potent antioxidant, antiproliferative, and antifibrotic effects. To date, more than 4000 different flavonoid substances have been isolated and identified from a multitude of plant species. In the plant kingdom, flavonoids protect against UV light and pathogenic microorganisms, and some of the conspicuous colors seen in plants are due to the chemical nature of flavonoids [11]. For centuries, flavonoids in herbal extracts have been used in Chinese and Japanese medicine, particularly in patients with chronic hepatitis or cirrhosis. In this issue's study, Ricupero et al. demonstrate that apigenin decreases proliferation and collagen synthesis of lung fibroblasts in a time- and dose-dependent manner. Apigenin acts as a TGF-β inhibitor, attenuating TGF-β-induced collagen and α-smooth muscle actin (α-SMA) mRNA synthesis. This inhibition seemed pathway specific, since TGF-β-induced connective tissue growth factor (CTGF) expression was unaffected by apigenin [10]. The study therefore suggests that TGF-β simultaneously activates at least two different and independent signal transduction mechanisms in lung fibroblasts, one leading to increased expression of collagen and α-SMA, the other leading to increased CTGF expression. Since TGF-β-induced CTGF expression is reported to be Smad3-and Smad4-dependent [12], these results suggest that apigenin may specifically interfere with Smad2, but not Smad3 activity. As such, apigenin may represent a useful compound to further dissect TGF-β-induced signaling pathways, as well as a modulator of the fibrotic response, especially in light of its nontoxic and non-mutagenic nature.

As aforementioned, Smad molecules are directly phosphorylated by TβRI, and represent the immediate substrate molecules of activated TGF-β receptors. However, TGF-β is also known to activate other signal transduction pathways, including the MAPK or AP-1 pathways, and this may occur independent of Smad phosphorylation and activation. Theoretically, the preferential activation of one TGF-β signaling pathway vs. another (e.g. Smad2 vs. Smad3, or AP-1 vs. Smads) through biochemical or pharmaceutical modulation can therefore potentially modify the cellular response to TGF-β.

A recent article by Reisdorf et al., suggested such mechanisms during the development of skin fibrosis [13]. In their study, the authors used a pig model of γ-irradiation-induced skin fibrosis, which is characterized by excess proliferation of fibroblasts, transdifferentiation of fibroblasts into activated

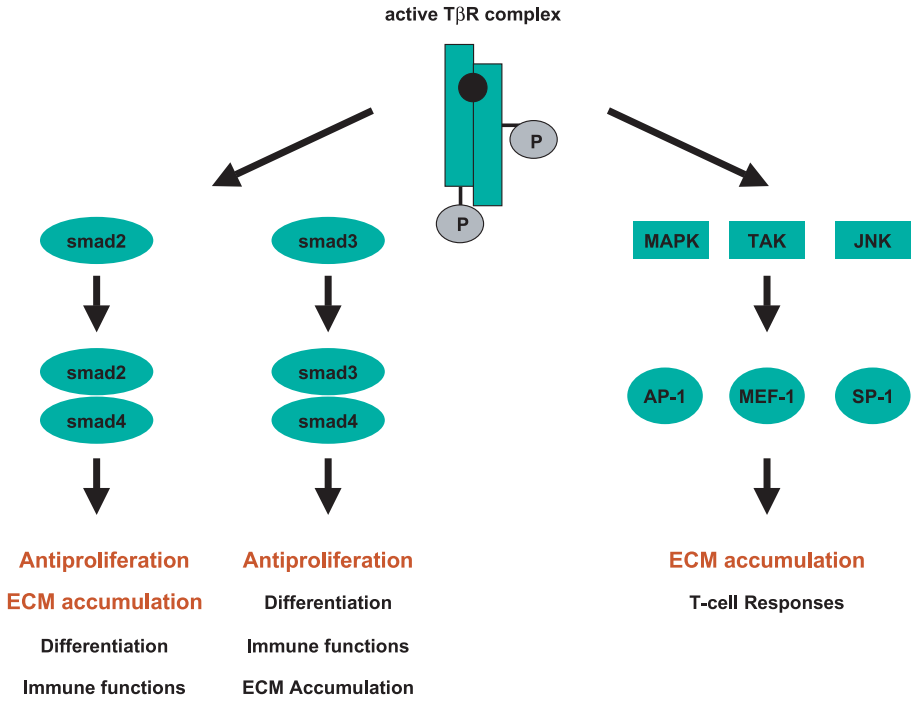

Fig. 2. A proposed model for the contribution of different TGF-β signaling pathways to the cellular response. In most cells, several signal transduction pathways, including the MAPK and AP-1 families are expressed and active along with the Smad pathway. The cellular response to TGF-β therefore depends on the fine-tuned activation of coexisting pathways and crosstalk between pathways, implying that modulation of one pathway vs. another will affect the outcome of the cellular response to TGF-β.

myofibroblasts, and TGF-β-induced increased ECM deposition in irradiated skin areas. Reisdorf et al. isolated primary skin fibroblasts and myofibroblasts from non-irradiated, control areas and fibrotic areas, respectively, and assessed their responses to TGF-β, hypothesizing that molecular events triggered during the irradiation process altered TGF-β responsiveness of these cells. Both cell populations consistently revealed similar expression levels of TβRI, TβRII, Smad2, 3, and 4. Myofibroblasts, however, exhibited a significant decrease in TGF-β-dependent growth inhibition, reporter gene expression, and Smad3/4 DNA binding activity. This decrease in TGF-β-induced responses was independent of differential expression of the inhibitory Smad7, but coincided with reduced nuclear translocation of Smad3. Thus, the authors argue that a relative loss of Smad3 activity leads to a decrease in proliferative and transcriptional responses to TGF-β, whereas fibrotic mechanisms remain unaffected by Smad3 dysfunction [13].

Such an uncoupling of the profibrotic and antiproliferative responses of TGF-β due to altered activity of Smad isoforms has been proposed earlier by Anita Roberts's group. Mice lacking Smad3 exhibit accelerated cutaneous wound healing, and keratinocytes derived from Smad3 null mice demonstrate a reduced sensitivity to TGF-β-mediated growth inhibition. Matrix deposition in incisional wounds, however, occurred at similar levels in wild-type and Smad3 null mice, providing further evidence that Smad3 activity is required for proliferative, but not matrix effects in response to TGF-β [14]. In contrast, analysis of CCl_4-induced liver fibrosis in Smad3 null mice suggested that Smad3 seems to be required for maximal collagen type I induction during fibrosis [15], although this may include effects other than merely TGF-β-dependent mechanisms. Analyses of mouse embryonic fibroblasts derived from Smad2 and Smad3 null animals has suggested that c-fos, Smad7, and TGF-β1 induction by TGF-β is Smad3-dependent, whereas MMP-2 expression is Smad2-dependent (Table 1). Fibronectin induction was independent of either Smad, whereas growth inhibition in a variety of cell types was Smad2/3-dependent [16].

Taken together, these studies have conclusively suggested that it is possible to alter a particular response to TGF-β without affecting others by selective inactivation or overexpression of a particular signaling molecule within the TGF-β pathway. In addition to the differential contribution of Smad2 and Smad3, the specific outcome of a TGF-β-initiated cellular response seems also dependent on the simultaneous activation of Smad-independent signaling pathways, such as the MAPK or AP-1 pathways (Fig. 2, Table 1). The specific

Table 1
Biological effects induced by TGF-β, which are dependent on specific signaling compounds within the TGF-β system

Signaling intermediate	Required for expression of, mediates
Smad2	PAI-1 [16], MMP-2 [16], Smad4 [19,20], TGF-β [19,20], Mix.2 [21,22], antiproliferative effects of TGF-β [16]
Smad3	CTGF [12], TGF-β [14,16], Collagen type I [15], c-fos [16], Smad7 [16], PAI-1 [16,25], p15 [23], Mix.2 [24], antiproliferative effects of TGF-β [13–16,23,25,26]
AP-1/JunD/MAPK	Collagen deposition [18], Fibronectin [27], IL-6 [28], PAI-1 [29], TGF-β1 [30,31]

contribution of each of these pathways, as well as crosstalk between pathways, ultimately determines the outcome of a given cell's response to TGF-β. This entails that the specific availability and activation of such pathways determines whether TGF-β treatment will result in an antiproliferative response or a fibrotic response, as seen in the case of lung fibrosis.

Whereas Smads have been implicated in the pathophysiology of lung fibrosis [17], stimulation of collagen and ECM deposition by TGF-β is mediated by JunD, a member of the AP-1 family of transcription factors [18]. It remains to be analyzed whether, in patients with lung fibrosis, the preferential activation of one signal transduction pathway, such as the AP-1 pathway, has facilitated the development of fibrosis. Hypothetically, the preferential activation of one pathway (such as AP-1) vs. the inactivation of another (such as Smads) may shift the biological response evoked by TGF-β to a fibrotic phenotype, whereas this response in an environment of relatively abundant Smads could lead to a predominantly antiproliferative response, and apigenin may present as a useful tool to investigate such mechanisms [10]. As such, further clarification of the differential contributions of TGF-β-induced signaling systems to the cellular response over the next years will more than likely enrich our understanding of the tissue specific effects of TGF-β, and may lead to the discovery of novel therapeutic options for the treatment of fibrotic diseases.

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