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Abstract

Since its discovery in the early 80s, Transforming Growth Factor-β (TGF-β) has emerged as a family of growth factors involved in essential physiological processes, including embryonic development, differentiation, tissue repair and cell growth control. Knockout experiments for the three mammalian isoforms of TGF-βs in mice have demonstrated their importance in regulating inflammation and tissue repair. Also, TGF-β has been implicated in the pathogenesis of human diseases, including tissue fibrosis and carcinogenesis where, in the latter case, it may exert both tumor suppressor and pro-oncogenic activities depending on the stage of the tumor. Cellular signaling by TGF-β family members is initiated by the assembly of specific cell surface serine/threonine kinase type receptors that activate transcription factors of the Smad family.

1. Introduction

The Transforming Growth Factor-β (TGF-β) family comprises over 30 members throughout the animal reign that include TGF-βs *stricto sensu*, Activins and Bone Morphogenic Proteins (BMPs). They are critical in governing cell fate determination and patterning in the developing embryo, and regulate a broad spectrum of biological responses in the adult [1]. Their pleiotropic activities include context-specific inhibition or stimulation of cell proliferation, control of extracellular matrix (ECM) synthesis and degradation, control of mesenchymal-epithelial interactions during embryogenesis, mediation of cell and tissue

responses to injury, and modulation of immune functions. Deregulation of TGF-β signaling has been implicated in developmental disorders and in several human diseases, including cancer, tissue fibrosis, and autoimmune disorders.

2. Structure and Signaling

The three mammalian TGF-βisoforms, TGF-β1, TGF-β2 and TGF-β3, encoded by three distinct genes, are structurally nearly identical (nine conserved cysteine residues, 76 to 80% amino acid homology), and synthesized by a wide variety of cell types including platelets, macrophages, fibroblasts and tumor cells. They are secreted as latent precursor molecules (LTGF-β) requiring activation into a mature form for receptor binding and subsequent activation of signal transduction pathways (Figure 1, reviewed in [2]). The LTGF-β molecules consist of 390 to 414 amino acids. They contain an amino-terminal hydrophobic signal peptide region, the latent-associated peptide (LAP) region, of 249 residues, and the C-terminal, potentially bioactive region that contains 112 amino acids per monomer. They are characterized by a knot composed of six cysteines that form three intrachain disulfide bonds stabilizing several β-sheet bands. A seventh cysteine residue participates in an interchain disulfide bond with an identical monomeric TGF-β chain to generate a mature TGF-β dimer. LTGF-β is usually secreted as a large latent complex (LLC) covalently bound via the LAP region to LTGF-β-binding protein (LTBP) or as a small latent complex (SLC) without LTBP. The LAP confers latency to the complex whereas LTBP serves to bind TGF-β to the extracellular matrix and to enable its proteolytic activation. Conformational changes of the latent TGF-β complex induced by either cleavage of the LAP by various proteases such as plasmin, thrombin, plasma transglutaminases or endoglycosylases, or by physical interactions of the LAP with other proteins, such as thrombospondin-1, leads to the release of bioactive, mature TGF-β.

Upon activation, TGF-β transduces its signal across the plasma membrane by binding to specific serine/threonine kinase receptors [3, 4]. The TGF-β receptor family consists of two structurally similar sub-families, type I and type II receptors (TβRI and TβRII), with small cysteine-rich extracellular regions and intracellular portions consisting mainly of their kinase domains. TGF-β binds to TβRII, forming a heterodimeric complex which can recrute TβRI and activate it by phosphorylating serine and threonine residues within a region rich in

glycine and serine residues (GS domain) preceding the receptor kinase domain. Additionally, betaglycan, a transmembrane proteoglycan also known as TβRIII, allows high-affinity binding of TGF-β to TβRII but, thus far, is not known to transduce signal. In unstimulated cells, TβRI is stabilized in an inactive conformation by its association with FKBP12 (FK506 binding protein 12).

Unless TGF-β is trapped, for example by association with the extracellular matrix proteoglycan decorin, signal transduction from the TGF-β receptors to the nucleus is predominantly mediated by phosphorylation of evolutionarily conserved cytoplasmic mediators of the Smad family [3] (Figure 2). The latter is divided into three functional groups: receptor-associated Smads (R-Smads), which directly interact with activated type I receptors in a ligand-specific manner, co-Smads, such as Smad4, a common mediator of all TGF-β family members, and inhibitory Smads (I-Smads), including Smad7 [5].

R-Smads are characterized by two highly conserved Mad-homology (MH) domains, MH1 in their N-terminal end and MH2 in their C-terminus, bound by a variable proline-rich linker region. In response to TGF-β, TβRI phosphorylates Smad2 and Smad3 on two serine residues within a conserved –SSXS motif at the extreme C-terminus of the MH2 domain. Microtubules play an important role in guiding R-Smads to the plasma membrane, where a FYVE-domain protein, Smad-Anchored for Receptor Activation (SARA), presents the Smad2 and Smad3 to the receptor. Upon phosphorylation by TβRI, R-Smads partner with the co-Smad, Smad4, and translocate into the nucleus where they activate downstream transcriptional responses. Active nuclear import processes regulated by Ran and importins allow for rapid, efficient, and controlled, nuclear import of Smad complexes. Smad4 only translocates to the nucleus when complexed with R-Smads, whereas ligand-activated Smad2 and Smad3 may translocate into the nucleus in a Smad4-independent fashion. In the absence of Smad4, however, neither Smad2 nor Smad3 are capable of transcriptional activity, suggesting that the principal function of Smad4 is to regulate transcription rather than to transmit signals from the cytoplasm to the nucleus.

R-Smad/Smad4 complexes may then function as transcription factors, binding DNA either directly or in association with other DNA binding proteins [3]. Maximal affinity of recombinant Smad3 and Smad4 is observed with the CAGAC nucleotidic sequence, via their MH1 domain. A 30-aa insertion within the Smad2 MH1 domain prevents direct DNA binding of Smad2. The latter requires a nuclear DNA-binding protein of the Fast family to bind DNA in association with Smad4, to activate transcription in response to TGF-β. By associating with

various DNA-binding partners, the Smads can achieve high-affinity, selective interactions with cognate DNA. The transactivating role of the Smad proteins has been ascribed to their MH2 domain. In order to fully activate transcription of their target promoters, Smad complexes must recruit additional factors, such as transcription factors like AP-1, TFE3, or CBFA/AML, DNA-binding adaptors like FAST1, and co-activators such as CREB-binding protein (CBP) and p300 [3]. Smads also bind transcriptional repressors such as TG-3 interacting factor (TGIF), Ski and SnoN [6], which modulate the interactions of Smads with the transcriptional activators p300/CBP and recruit histone deacetylases that inhibit gene transcription.

Smad7 binds activated T□RI, thereby preventing phosphorylation of Smad2/3 [7]. It also recruits two ubiquitin-ligases E3 Smurf1 and Smurf2, to the activated T□RI, leading its proteasomal degradation. Smad7 may function as a negative feedback loop, as its expression is induced by TGF-□ in a Smad-dependent manner. Finally, several proteins, such as STRAP or YAP-65, stabilize the Smad7-T□RI association, thereby preventing R-Smad phosphorylation by T□RI and subsequent intracellular signaling [8, 9].

Aside from the Smads that are highly specific substrates for the TGF-□ receptor kinases, other signaling pathways may also be activated by TGF-□ in a context- and cell type-specific manner. These include the Mitogen Activated Protein Kinase (MAPK) cascades (p38, ERK and JNK), phosphatidyl-inositol-3-kinase, and PP2A/p70s6K, though the molecular details of such couplings are still obscure. The relative importance and interplay of these various pathways in the changing responses of cells to TGF-□ are just beginning to be probed [10, 11].

3. Biological functions

Active on all cell types, TGF-□ may stimulate or inhibit cell proliferation, differentiation, motility, adhesion or death, depending on the type and developmental state of a cell. These functions participate in the control of both normal tissue homeostasis and in the development of various pathological situations [1].

Inhibition of cell proliferation is central to the TGF-□ response of epithelial, endothelial, hematopoietic, neural and certain types of mesenchymal cells. TGF-□ mediated growth arrest involves distinct mechanisms, such as gene responses that inhibit cyclin-dependent kinases

(cdks), such as induction of $p15^{INK4}$ and/or $p21^{WAF1}$, or down regulation of c-Myc or that of Cdc25A [12].

Another key function for TGF-β is to regulate the expression of proteins of the extracellular matrix (ECM), including fibrillar collagens and fibronectin. TGF-β also represses ECM degradation, by inhibiting the expression of metalloproteinases and serine proteases, and by enhancing the expression of proteases inhibitors such as the tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitors (PAIs). TGF-β is therefore considered a potent anabolic factor that enhances connective tissue deposition and repair [13]. Of note, TGF-β3, which is mostly expressed during embryonic life, exhibits unique anti-scarring properties, as exemplified in knockout animals in which embryonic wound healing is not scarless, as opposed to embryos expressing TGF-β3 [14].

TGF-β is also critical for maintenance of proper immune functions and knockout mice lacking TGF-β1 die a few weeks after birth from aberrant regulation of the immune response, which culminates in lethal cardiopulmonary inflammation. In this context, TGF-β has been shown to play pivotal role in multiple stages of T cell apoptosis, selection, activation and clearance [15].

4. Medical applications

Fibrosis:

TGF-β drives pro-fibrotic responses *in vitro* and *in vivo*, by enhancing ECM gene expression and repressing that of catabolic enzymes, enhancing fibroblast proliferation and inducing the myofibroblast phenotype. Overexpression of TGF-β and deregulated Smad signaling, involving increased Smad2/3 phosphorylation and/or nuclear accumulation, or decreased expression of Smad7, have been identified in fibrosis [16].

Cancer:

Escape of epithelial cells from TGF-β growth control is a hallmark of many cancers. Indeed, the role of TGF-β signaling as a tumor suppressor pathway in early carcinogenesis is best illustrated by the presence of inactivating mutations in genes encoding TGF-β receptors and Smads in human carcinomas, and by studies of tumor development in mouse models [10]. In contrast, TGF-β may become pro-oncogenic at later stages of carcinogenesis, as it exacerbates the malignant phenotype of transformed and tumor-derived cells. High levels of TGF-β expression are correlated with the advanced clinical stage of a tumor. Tumor derived TGF-β could contribute to tumor growth indirectly by suppressing immunosurveillance, or by

stimulating the production of pro-angiogenic factors. Its ability to induce an epithelial to mesenchymal transition in various tumor cells may also contribute to increased invasiveness and metastatic activity.

Therapeutic potential:

Since TGF-β plays opposite roles on malignant progression depending on the stage of carcinogenesis, therapeutic approaches targeting the TGF-β pathway would have to be context-specific. Chemoprevention would aim at enhancing TGF-β signaling in order to promote its tumor supressor activities, whereas treatment of advanced cancer would require strategies aimed at inactivating TGF-β or its downstream signals.

Among the therapeutic approaches tested thus far to antagonize the deleterious effects of TGF-β in fibrosis or advanced stage cancer, one may cite the use of TβRII-IgG Fc chimera, TGF-β neutralizing antibodies or natural antagonists such as decorin, as well as targeted overexpression of either Smad7 or dominant-negative receptor mutants [14, 16-18]. Interestingly, it has been shown that the anti-tumor effect of chemopreventive drugs such as retinoids or tamoxifen correlates with their ability to induce TGF-β production and activation by epithelial cells. Molecules capable of enhancing Smad signaling or inhibiting Smad7 would bypass defects in upstream receptors, and thus may serve as chemopreventive agents. Caution must however be exercised as such approach may accelerate advanced stages of carcinogenesis [10, 17].

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Figure legends

Figure 1: TGF-β1 maturation

TGF-β1 is synthesized as pre-pro-TGF-β, that is cleaved by endopeptidases in the Golgi apparatus to form a small latent complex (SLC) containing a mature 24 kDa TGF-β1 homodimer non covalently associated with two 80 kDa latency-associated peptides (LAP). It is usually secreted as a large latent complex (LLC), covalently bound with latent TGF-β binding protein (LTBP). Final activation involves the release of mature TGF-β1 from the LLC.

Figure 2: The Smad pathway

TβRI is stabilized in an inactive conformation by its association with FKBP12. Upon TGF-β ligation to TβRII, the latter phosphorylates TβRI, which in turn phosphorylates Smad2/3. R-Smads are presented to the TβRI by a membrane-bound protein, SARA. Activated R-Smads bind Smad4 and translocate to the nucleus to act as transcription factors, controlled by a balance between transcriptional co-activators (co-A) or co-repressors (co-R). Inhibitory Smad7 binds activated TβRI, thereby preventing phosphorylation of R-Smads, or recruits the ubiquitine ligases Smurf1 and Smurf2 to induce proteasomal degradation of the receptor complexes.

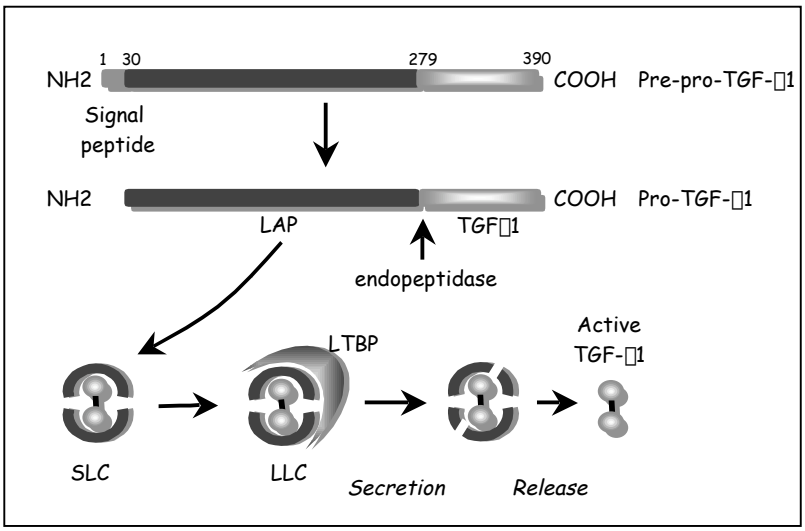

Figure 1

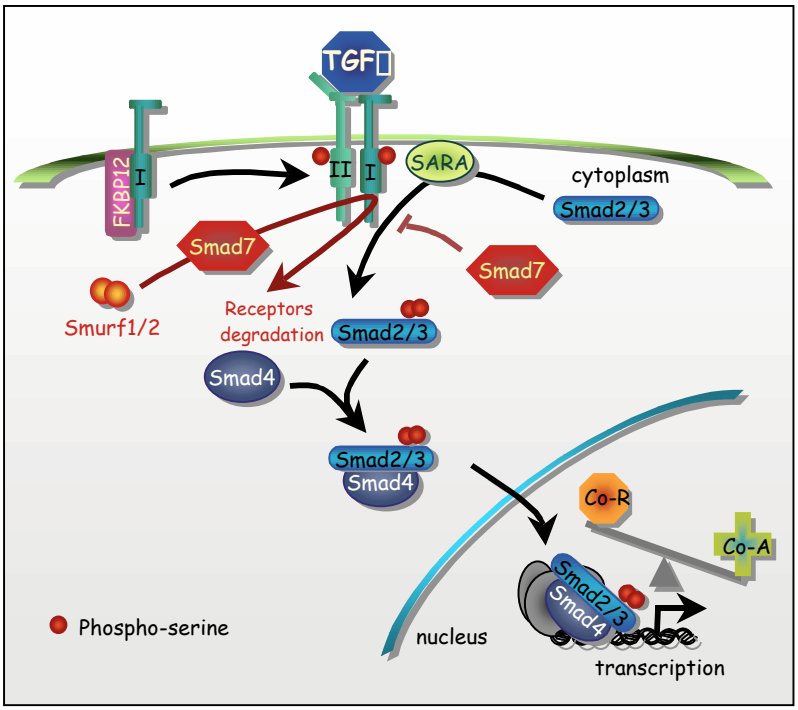

Figure 2