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Role of SMAD/TGF-β signaling pathway in connective tissue diseases

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Abstract

Several members of the TGF-β super family play important roles in connective tissue growth. The bone morphogenetic proteins (BMPs) induce early cartilage formation and MMP13 is a major enzyme targeting cartilage for the degradation of types II, IV, and IX collagen, proteoglycan, osteonectin and perlecan. In this review, we discuss the crosstalk between SMAD/TGF-β signaling pathway and therapeutic applications in connective tissue diseases.

Keywords: SATF3; Endotoxemia; Proinflammatory cytokine; HMGB1

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Introduction

Transforming growth factor β (TGFβ) is the prototype member of a highly evolutionary conserved secreted family of cytokines. The TGFβ family consists of TGFβ, activins, inhibins, nodals, anti-mullerian hormone (AMH) and bone morphogenetic proteins (BMPs). TGFβ family members affect various biological processes including cell proliferation, differentiation, migration, adhesion, apoptosis and extracellular matrix (ECM) production [1]. Consequently, TGFβ members play crucial role in embryonic development, adult tissue homeostasis and in the pathogenesis of a variety of diseases such as cancer, autoimmune diseases, fibrosis and cardiovascular diseases. They all exert their cellular effects via heteromeric complexes of type I and type II serine/threonine kinase receptors located at the plasmamembrane, and the Smad transcription factors, which have a pivotal role in intracellular signaling [2].

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TGFβ family members act in a highly contextual manner and can either stimulate or inhibit angiogenesis. Genetic studies in mice and human have robustly confirmed the notion that TGFβ signaling is essential for regulation of vasculogenesis and angiogenesis. Inactivation of different components of TGFβ signaling in mice results in embryonic lethality mainly due to defects in the vascular system [3].

All members of the TGF-β family of cytokines share conserved structural motifs and signal through similar mechanisms, although the exact signaling pathways activated by any given ligand differ. Signaling is initiated by binding of the ligand to tetrameric complexes formed by two type I and two type II receptors, all possessing serine, threonine, and tyrosine kinase activity [4].

Ligand binding induces autophosphorylation of type II receptors, which in turn phosphorylate and activate type I receptors. Activated type I receptors phosphorylate, and thus activate receptor-regulated Smad proteins (R-Smads). Phosphorylated R-Smads bind the common Smad (co-Smad, known as Smad4 or DPC4), translocate into the nucleus and modulate gene expression by interacting with other transcription factors to regulate target gene expression [5].

Extracellular matrix–dependent regulation of TGF-β bioavailability

Connective tissue disorders are a family of diseases characterized by alterations in the composition, structure, or turnover of the extracellular matrix (ECM), a complex mixture of carbohydrates and proteins that includes collagens, proteoglycans, and glycoproteins [6] that is secreted by cells in multicellular organisms. The specific properties of each type of connective tissue (i.e., cartilage, bone, ligaments, skin, muscle, and vessel walls) are dependent on the tissue-specific molecular composition of the ECM. In addition to providing physical support to cells, tissues, and organs, the ECM participates in regulating complex cellular behaviors including proliferation, migration, apoptosis, and differentiation [3].

Dynamic remodeling of the ECM through regulated deposition, assembly, and degradation is critical for all ECM functions. ECM exerts its influence on surrounding cells through its interaction with matrix-sensing receptors on the cell surface, such as integrins [4], and by regulation of the bioavailability of signaling molecules through regulated sequestration, release, and activation [7]. The importance of the ECM to

normal development and homeostasis is highlighted by the number of disorders that are either caused or exacerbated by abnormal ECM function [8].

TGF-β ligands (TGF-β1, TGF-β2, and TGF-β3) are secreted as biologically inactive latent complexes that are converted into an active form through a tightly regulated process that depends on interactions with components of the ECM [9]. TGF-β latent complexes (referred to as the small latent complex, or SLC) are comprised of TGF-β dimers and latency-associated peptide (LAP) dimers. LAPs are translated as prodomains from the same genes and mRNAs encoding the corresponding TGF-βs and are referred to as β1-LAP for TGF-β1, β2-LAP for TGF-β2, and β3-LAP for TGF-β3. LAP prodomains are cleaved during posttranslational processing and remain associated with the corresponding TGF-β molecule through noncovalent interactions [10].

Disruption of noncovalent interactions between TGF-β and LAP molecules, through LAP degradation [11], chemical modification, such as that caused by reactive oxygen species or low pH [12], and/or through binding-induced conformational change [13], results in conversion of latent TGF-β into active TGF-β. TGF-β-LAP dimers (SLC) covalently bind to one of the latent TGF-β binding proteins (LTBP-1, -3, and -4) through disulfide bonds formed between LAP and LTBPs, resulting in the large latent complex, or LLC. Most cell-types do not secrete TGF-β as a SLC but rather already in complex with LTBP proteins. Interactions between LTBPs and ECM components, in particular with fibrillin microfibrils, are thought to be important for proper localization, concentration, and activation of latent TGF-β [7].

Roles of TGF-B and BMP signaling skeletal development

Bone is described as a hard connective tissue because the terminally differentiated osteoblasts that form much of the bone tissue produce an ECM that mineralizes, and provides bone with its rigid structure. Bone tissue forms through two distinct mechanisms that either depend exclusively on osteoblasts or additionally on chondrocytes [5]. Most flat bones, such as craniofacial bones and part of the clavicle, are intramembranous, developing by direct differentiation of mesenchymal progenitors into osteoblasts. The entire appendicular and most of the axial skeleton have an endochondral origin, forming through a cartilage template that is subsequently replaced by osteoblasts and bone [6]. TGF-β and BMP signaling are crucial for the development of both bone types [11].

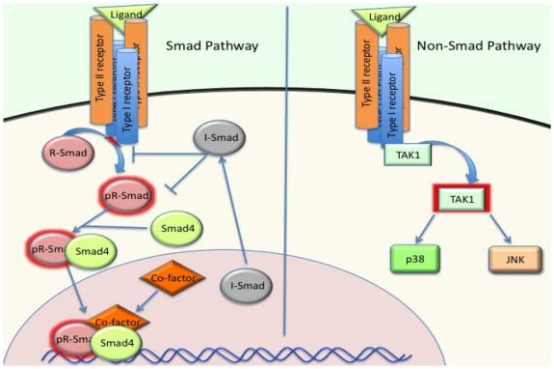
Although BMP signaling promotes differentiation and maturation of osteoblasts, TGF-β exerts a dual role during osteogenesis by inducing commitment to the osteoblast lineage while at the same time preventing terminal differentiation [14].

Digit malformations caused by disruption of TGF-β signaling

Depending on the specific perturbation, disruption in the BMP signaling pathway can cause various types of BD, all characterized by reduction or absence of phalanges in one or more digits and resulting in shortened toes and/or fingers [11]. Although BD can occur as an isolated malformation, more commonly it is one of several features of syndromes caused by mutations that impair BMP ligand or receptor function, for example, in AMDs.

SMAD proteins

The first description of SMAD proteins was the finding of Mothers Against Dpp (MAD) in Drosophila, which modified the phenotype of decapentaplegic (dpp; a BMP ligand) mutants [3]. Later studies identified Sma proteins in C. elegans as closely related to MAD, and both mediated signaling downstream of serine/threonine kinase receptors of TGFβ superfamily proteins [7]. Therefore, homologs of Mad and Sma have been named Smad. So far, 8 mammalian Smad proteins have been isolated, designated Smad1 through Smad8. The SMAD proteins are divided into 3 groups according to their functions. The first group is the receptor-regulated Smads (RSmads), which include Smad1, 2, 3, 5 and 8. These Smad proteins bind to membrane bound serine/threonine receptors, and are activated by the kinase activity of the receptors. The second group includes only one member, Smad4. Smad4 acts as a co-factor that binds to the activated R-Smads to form a complex that translocates into the nucleus. Therefore, Smad4 has been named Co-Smad. The third group comprises the inhibitory Smads (I-Smads), which includes Smad6 and Smad7. These two Smads exert an inhibitory effect on the signaling cascade by various mechanisms (Figure below).



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Smad proteins also share similar structures. A typical Smad structure includes N-terminal MH1 domain, linker region and a C-terminal MH2 domain. The MH1 domain is highly conserved in all R-Smads and Smad4, but not in I-Smads. The major function of the MH1 domain is to mediate DNA binding of Smad proteins. The linker region is highly variable in different Smads. It is the target of regulation by other intracellular proteins through phosphorylation, ubiquitination, or sumoylation. The MH2 domain is present in all Smads. Activation of R-Smads is through the phosphorylation of a Ser-X-Ser motif in the MH2 domain by activated receptors. The MH2 domain is also responsible for Smad protein interactions with other intracellular proteins and transcriptional activation of target genes [11]. Different receptors in the TGFβ superfamily have different preferences for binding to R-Smad proteins. For example, Smad1, 5 and 8 mediate BMP signaling by interacting with the BMP receptors ALKs 1, 2, 3, and 6, whereas Smad2 and 3 mediate TGFβ and Activin signaling through the TGFβ/activin receptors ALKs 4 and 5. Smad6 is more specific for the inhibition of BMP signaling, whereas Smad7 has inhibitory effects on both BMP and TGFβ signaling. The consensus understanding so far is that R-Smads require Smad4 binding before they can translocate into the nucleus, Recent evidence has challenged this dogma, as Smad4 conditional deletion in mice did not cause significant skeletal defects [7]; while, conditional deletion of Smad1/5/8 led to lethality at birth due to severe chondrodysplasia [6].

TGFβ signaling in mural cells

TGFβ signaling besides operating as a rheostat that controls EC differentiation, viability and function, it plays also important role in smooth muscle cell (SMC) differentiation and function. Several studies provided evidence that SMCs are a major cell type through which TGFβ affects vascular development and disease. TGFβ upregulates differentiation markers and induces contractile function in SMC precursors [14]. TGFβ potentiates vascular SMC (VSMC) differentiation by increasing the expression of α-smooth muscle actin (SMA) and smooth muscle myosin [15]. At low concentrations it promotes proliferation of SMC, whereas, at high concentrations it inhibits SMC proliferation and migration [16].

It was shown that TGFβ-induced expression of SMC marker genes involves activation of protein kinase N (PKN) and p38 MAPK pathways resulting in differentiation of SMCs [3]. TGFβ1 regulates phenotype transition in SMC by regulation of specific contractile

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proteins such as αSMA, SM22 α and calponin 1 in different cells including 10T1/2 cells, neural crest cells and fibroblasts [2]. In the neural crest stem cell line Monc-1, RhoA modulates TGFβ1-induced SMC differentiation. In 10T1/2 cells, ERK1/2 activity and PI3K/AKT are involved in TGFβ1-regulated differentiation [8].

Genetic studies in mice revealed that inactivation of critical TGFβ signaling molecules in all cells results in failure of vascular development which correlates with a lack of SMC investment of nascent vessels [1]. In adult arteries, SMCs express TGFβ receptors and appear to respond to TGFβ by synthesizing ECM that contributes to the growth of atherosclerotic and restenotic lesions [17]. Lineage-specific ablation of TGFβ signaling in VSMCs resulted in alterations in vascular morphogenesis in mice, including impaired elastogenesis and vessel widening [18]. TGFβ actions on SMC are both direct and indirect, mediated through downstream effects of TGFβ signaling in other cell types. For example, TGFβ acts directly on ECs, with consequent paracrine effects on SMCs. TGFβ could act directly on cells of the immune system, with effects on SMC mediated through alterations in plasma cytokines [19].

BMPs play also important role in SMC function. BMPs were reported to regulate migration, proliferation, and apoptosis of VSMCs and to modulate the expression of SMC phenotypic markers in vitro. Interestingly, the effects of BMPs on VSMCs vary, depending on the anatomic origin of the cells, their state of differentiation or dedifferentiation, and culture conditions. BMP2, BMP4 and to a lesser extent BMP7 are reported to inhibit VSMC proliferation [20]. It was shown that BMP2 and BMP7-induced apoptosis of pulmonary SMCs (PASMCs) [1].

It was also shown that BMP2 inhibits SMC proliferation without stimulating ECM synthesis and that adenovirus-mediated transfer of the BMP2 gene inhibited injury-induced intimal hyperplasia suggesting the possibility of therapeutic application of BMP2 for the prevention of vascular proliferative disorders [2]. BMP7 inhibits TGFβ and platelet-derived growth factor (PDGF)-BB-induced SMC growth and stimulates the expression of SMC-specific markers by inducing p21 and I-Smad expression. In addition, BMP7 exhibited anti-inflammatory activity by downregulating intercellular adhesion molecule-1 (ICAM-1) expression [1].

These results suggest that BMP7 may be used in clinic in order to prevent vascular proliferative disorders and to maintain vascular integrity. In another study, BMP2 was shown to be expressed by human aortic SMCs (HASMC) and to induce migration of

HASMC in a concentration- and time-dependent manner. In addition, BMP2 synergistically induced PDGF-induced chemotaxis. In contrast, BMP2 had no significant effect on HASMC proliferation [5]. Both BMP7 and BMP4 promote apoptotic cell death in human primary PASMCs by activating caspase-8 and caspase-9. PASMCs expressing mutant forms of BMPRII, identified in idiopathic pulmonary arterial hypertension (IPAH) patients were resistant to BMP-mediated proapoptotic effects [3]. Interestingly it was shown that pulmonary microvascular ECs (PMVEC) secrete BMP4 in response to hypoxia and promote proliferation and migration of VSMCs in a BMP4-dependent fashion [18]. It was shown that BMP4 inhibited proliferation of PASMCs isolated from proximal pulmonary arteries, but stimulated proliferation of PASMCs from peripheral arteries, and conferred protection from apoptosis. Although these differences were not caused by differential activation of BMP signaling pathways, the pro-proliferative effect of BMP4 on peripheral PASMCs was found to be p38MAPK/ERK-dependent [19].

Dysregulation of TGF-β or BMP Signaling in Catabolic Bone Diseases

Signaling through TGF-β and BMP pathways has been implicated in catabolic bone diseases, such as osteoarthritis and osteoporosis, that are common in the population but with limited therapeutic options. These conditions, unlike the genetic disorders already described in this review, appear to have a complex genetic basis with contributions from multiple genes and interacting pathways [20].

Arthritis is a chronic, destructive disease affecting articular cartilage and subchondral bone leading to loss of joint function and pain. Osteoarthritis (OA) is the most common form with increased prevalence in the aged population and characterized by progressive degeneration of articular cartilage, aberrant chondrocyte maturation, and osteophyte formation in subchondral bone, resulting in remodeling and functional loss of the joint [16]. Proinflammatory cytokines (TNF-α, IL-1) and matrix metalloproteinases (MMPs) play central roles in OA [21].

Elevated BMP-2 expression was found in articular chondrocytes of OA mouse models [19]. In cartilage, BMP-2 has both anabolic and catabolic effects by stimulating expression of ECM proteins, such as collagen type II, and matrix degrading enzymes, such as MMPs [17]. Single-nucleotide polymorphisms (SNPs) in BMP2 were found in OA patients but their functional relevance is unknown (Valdes et al. 2004, 2006). The

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The role of TGF-β signaling in human OA pathology is not well understood, but increasing evidence supports its function in maintaining articular cartilage. Age-related loss of TβRI expression, which may shift signal transduction from TβRI and Smad2 and Smad3 to ALK-1 and Smad1, Smad5 and Smad8, was found in two independent OA mouse models [11]. This resulted in the formation of transcriptionally active Runx2–Smad1 or Runx2–Smad5 complexes, which in turn activated terminal chondrocyte maturation and MMP13 expression. MMP13 is the main collagenase that cleaves type II collagen in articular cartilage leading to OA.

Expression of a truncated form of TβRII or silencing Smad3 expression [22] also results in an OA phenotype in mice, further showing the importance of TGF-β signaling in the maintenance of joint homeostasis. OA is observed in all characterized etiologies of LDS (mutations in TGFBR1, TGFBR2, SMAD3, and TGFB2), but appears to be particularly frequent in patients with SMAD3 mutations.

Vascular muscle and TGFβ signaling

EC-Mural cell interactions are important not only for the growth and maintenance of intact vessels but also for physiological vessel function. Vessel tone regulation is controlled by the contractile state of SMC. EC regulate the SMCs contractile function by releasing a number of vasoactive soluble factors [4]. These factors stimulate either contraction or relaxation of mural cells and thus modulate capillary flow. Nitric oxide (NO) and prostacyclin (PGI2) exert vasodilatory effects on SMCs [8]. NO has also antiproliferative effects on SMC. EC dysfunction, including decreased endothelial nitric-oxide synthase (eNOS) activity and loss of bioactive NO, plays a prominent role in the development of pulmonary arterial hypertension (PAH) [11]. On the other hand endothelin (ET)-1 is a potent vasoconstrictor which stimulates contraction of mural cells via ET-A and ET-B receptors and release of NO from ECs via endothelial ET-B receptors [7].

Several studies have suggested that TGFβ plays an important role in the regulation of vascular tone and reactivity, since TGFβ signaling is involved in regulating the expression of both vasodilators and vasoconstrictors. It was demonstrated that TGFβ/ALK5/Smad3 pathway induces EC expression of ET-1, resulting in decreased EC migration and proliferation. In addition, TGFβ1 induces PGI2 production by ECs [110], and increases EC levels of cycloxygenase-1 [4] and prostacyclin-stimulating factor, which induce synthesis of the vasodilatory molecule prostacyclin. In addition, iloprost, a prostacyclin analog induces increased TGFβ1 expression by SMCs, suggesting a potential feedback mechanism. TGFβ regulates also eNOS activity, the enzyme responsible for baseline NO signaling in ECs.

In contrast, addition of a synthetic NO donor diethylenetriamine nitric oxide adduct (DETA)/NO to cultured human coronary SMCs was shown to induce TGFβ mRNA and protein expression in human coronary smooth muscle cells [7]. Another study has demonstrated that aortas from NO synthase deficient mice displayed enhanced basal TGFβ1 activity; ECs from these animals showed increased Smad phosphorylation and transcriptional activity. In the same study it was shown that NO reduced the half-life of ectopically expressed Smad2 by enhancing its ubiquitination. Thus, NO pathway in ECs interferes with TGFβ/Smad2 signaling by directing the proteasomal degradation of activated Smads and may act as a feedback mechanism to limit activation of NO by TGFβ [4].

These results suggest that, since TGFβ signaling influences both vasodilators and vasoconstrictors, misregulation of TGFβ signaling may result in perturbation of the vessel tone. Consistent with this notion it was shown that intravascular infusion of TGFβ in mice results in decreased vascular resistance and vasolidation. In addition, it was suggested that chronically increased levels of TGFβ1 in the brain of Alzheimer leads to impaired constriction of cerebral vessels due to impaired ET-1 mediated contractions [12]. The role of TGFβ signaling in hypertension will be further discussed in later sections.

Conclusions

Many diseases and syndromes are caused by mutations in the SMAD/TGF-β signaling pathway, reflecting the fundamental functions of these signaling molecules and their pathways in development, patterning, function, and homeostasis in connective tissue. Both pathways have synergistic and overlapping effects but also possess discrete functions that are revealed in pathway-specific human genetic diseases of connective tissues. This pathway needs careful investigation to neutraliz its effects.

Competing interests

The authors declare that they have no competing interests.

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