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BMP-7 as antagonist of organ fibrosis

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TABLE OF CONTENTS

1. ABSTRACT

Fibrosis is a scarring process that is a common feature of chronic organ injury. It is characterized by elevated activity of transforming growth factor-beta resulting in increased and altered deposition of extracellular matrix and other fibrosis-associated proteins. Recent work has demonstrated that bone morphogenetic protein-7 blocks transforming growth factor-beta signaling. Moreover, member of the CCN family, Endoglin, Sclerostin, Sclerostin domain-containing proteins, Gremlin, Noggin, Chordin, and Kielin/Chordin-like protein influence the biological activity of both cytokines. As a consequence, they modulate cellular proliferation, migration, adhesion and extracellular matrix production. This tight protein network consisting of transforming growth factor-betas, bone morphogenetic proteins and various binding partners includes potential novel molecular targets and biomarkers useful for prognostication, disease monitoring and therapy. We here summarize recent advances in understanding bone morphogenetic protein-7 function and signaling and the current attempts to use this critical modulator as a pharmacological device to reverse transforming growth factor-beta-induced fibrogenesis.

2. INTRODUCTION

Since the first identification of human bone morphogenetic protein-7 (BMP-7, formerly known as osteogenic protein-1 or OP-1) as a factor involved in bone formation in 1990 (1), this member of the transforming growth factor-beta (TGF-beta) superfamily has addressed the curiosity of many scientists. BMP-7 physiologically acts as a major and essential morphogen and survival factor in the development of kidney, bone and eye. This is substantiated by the finding that respective homozygous null mice exhibit arrested kidney development and dysplastic kidneys, and die soon after birth (2, 3). Although, the precise physiological function in kidney and other organs has not been completely assigned, it is obvious that BMP-7 is an endogenous regulator of organ homeostasis and regeneration (4, 5). Moreover, the finding that recombinant BMP-7 (rBMP-7) reduces the severity of injury after acute and chronic organ failure (6) by counteracting TGF-$beta_1$-mediated profibrotic effects, this member of the TGF-beta superfamily, including its signaling pathways and biological modifiers have become attractive targets for modulation of profibrotic TGF-$beta_1$ activity in experimental and clinical settings of various

Table 1. BMP and GDF family members

BMP member[1]	Alternative names	Chromosomal localization	Functions and activities
BMP-1	Tolloid, PCP[2]	8p21	laminin-5 processing, chordin antagonist, metalloprotease that proteolytically removes the C-propeptides of procollagens I-III, determination of dorsal-ventral pattering in embryogenesis, activator of other members of the TGF-beta superfamily
BMP-2	BMP2A	20p12	bone and cartilage formation, binds to members of the CCN family (i. e. NOV), stimulate the entire process of stem cell differentiation *in vitro*
BMP-3	osteogenin	4q21	bone formation (controversially discussed), antagonistic activity against BMP-2
BMP-3B	GDF10	10q11.1	inducer of endochondral bone formation, antagonistic activity against BMP-2
BMP-4	BMP2B, BMP2B1	14q22-q23	formation of teeth, limbs and bone, tooth development, limb formation, bone induction, and fracture repair, stimulate the entire process of stem cell differentiation *in vitro*, potentiates growth factor-induced proliferation of mammary epithelial cells, cofactor in angiogenesis, binds to chordin-like 1
BMP-5	MGC34244	6p12.1	cartilage development, chondrocyte differentiation (*in vitr*o and *in vivo*), osteoclast generation, formation of multiple skeletal features
BMP-6	Vgr-1, GDF3	6p24-p23	osteoblast differentiation, osteoclast generation, promotes E-cadherin expression, inhibits growth of mature human B cells
BMP-7	OP-1	20q13	osteoblast differentiation, osteogenic transformation, cartilage repair, counteract epithelial-to-mesenchymal transition, counteracts TGF-beta-mediated fibrosis, stimulate the entire process of stem cell differentiation *in vitro*, inhibits smooth muscle cell proliferation, binds members of the CCN family (i. e. CTGF)
BMP-8	OP-2, BMP-8B	1p35-p32	bone and cartilage development, induce ectopic bone growth
BMP-9	GDF2	10q11.22	induce the expression of choline acetyltransferase and vesicular acetylcholine transporter, regulator of acetylcholine synthesis, differentiating factor for cholinergic central nervous system neurons; anti-angiogenic factor, interferes with IGF-I signaling
BMP-10		2p13.2-2p14	essential in normal embryonic heart development
BMP-11	GDF11	12q13.13	regionalizes the anterior-posterior axis, involved in retina development
BMP-12	GDF7, CDMP3	2p24-p23	inhibits terminal differentiation of myoblasts
BMP-13	CDMP2, GDF6	8q22.1	inhibits terminal differentiation of myoblasts; regulate growth and maintenance of articular cartilage
BMP-14	GDF5, CDMP1, LAP4, LPS-associated protein 4	20q11.2	long bone fracture healing
BMP-15	GDF9B	Xp11.2	oocyte and follicular development
BMP-16	Nodal	10q22.1	inducing and patterning mesoderm and endoderm, regulating neurogenesis and left-right axis asymmetry; mesoderm formation
GDF8	Myostatin, MSTN	2q32.2	not known
GDF15	Mic-1, PLAB, PDF	19p13.2-p13.1	associated with pregnancy, involvement in iron metabolism

[1]Although BMPs are consecutively numbered, the nomenclature partially overlaps with those of the GDF subfamily. In regard to fibrogenesis, it is most interestingly that BMP-7 counteracts TGF-beta activity and inhibits EMT. [2]Abbreviations used are: CDMP: cartilage-derived morphogenetic protein; GDF: growth/differentiation factor; LAP: lipopolysaccharide-associated protein; MIC: macrophage-inhibiting cytokine; OP: osteogenic protein; PCP: procollagen C proteinase; PDF: prostate-derived factor; PLAB: bone morphogenetic protein from placenta; Vgr-1: VG1-related sequence.

acute and chronic diseases. It is now generally assumed that in normal tissue a balance of biological active TGF-beta$_1$ and BMP-7 exists that shifts toward TGF-beta$_1$ during inflammation and fibrogenesis. This balance is further modulated by several extracellular proteinogenic modifiers and the overall regulation of this network is still elusive and topic of many past and ongoing investigations. Key questions addressed were its physiological functionality in embryogenesis and development, its extra- and intracellular-signaling pathways, its competition with TGF-beta pathways, its regulation by secreted modulator proteins, and its potential versatility in different animal models relevant for fibrogenesis and clinical applications.

The concept that BMP-7 counteracts the profibrogenic activity of TGF-beta$_1$ was confirmed in independent studies. Moreover, it was found that BMP-7 is effective in inhibition of epithelial-to-mesenchymal transition (EMT) which triggers the fibrogenic response by generation of fibroblasts. These are the two general mechanisms causally involved in initiation and progression of fibrosis. Both processes, EMT and activation of fibroblasts, induce a tight network of genes. These lead to loss of cell-cell adhesion and E-cadherin expression, elevated and *de novo* expression of specific mesenchymal markers (e. g. beta-catenin, SNAIL, fibroblast specific protein 1 = FSP1), and production of typical profibrotic extracellular matrix (ECM) molecules (e.g. collagen type I and III, fibronectin) and intermediate filament proteins (e. g. alpha-smooth muscle actin = alpha-SMA, desmin).

Undoubtedly, recent advances and emerging insights in TGF-beta-/BMP-signaling and the identification of several independent proteins that bind and modulate the activity of TGF-beta or BMP, regulate their cellular secretion, interfere with receptor binding and alter the biological balance of both cytokines have exposed attractive novel targets in the treatment of fibrotic lesions.

3. INDIVIDUAL SECTIONS

3.1. Structural insights of BMPs

At least 35 structurally related members of the transforming growth factor-beta (TGF-beta) superfamily have been identified that are subdivided in (i) TGF-betas, (ii) activins/inhibins, (iii) bone morphogenetic proteins (BMPs)/growth and differentiation factors (GDFs) and (iv) the more distantly related group of GDNFs (Figure 1). Like

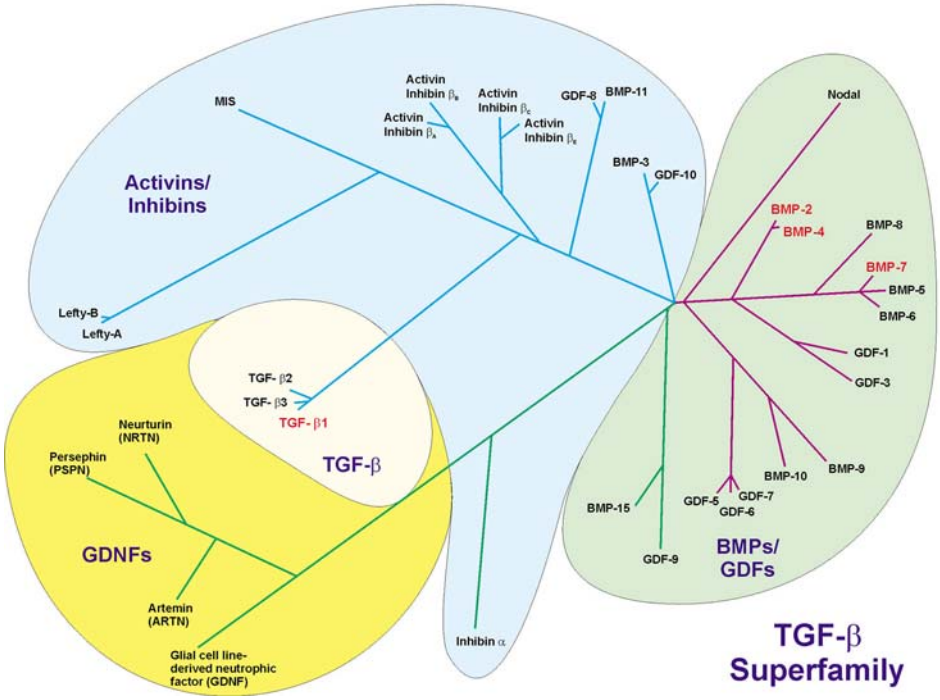

Figure 1. The TGF-beta superfamily. Based on their structural features the 35 mammalian members of the TGF-beta family are subdivided into (i) TGF-betas, (ii) activins/inhibins, (iii) bone morphogenetic proteins (BMPs)/growth and differentiation factors (GDFs), and (iv) the more distantly related group of GDNF ligands. The affiliation to one of these subgroups is ambiguous and handled somewhat irregular. TGF-beta$_1$, BMP-7, BMP-2 and BMP-4 (all marked in red) are those cytokines with outstanding importance in control of fibrogenesis.

the classical TGF-betas, the different BMPs/GDFs (Table 1) bind to two different serine/threonine kinase receptors, and mediate their signals through Smad-dependent and -independent pathways (7). The BMPs were originally identified and characterized two decades ago from bovine bone matrix by their ability to induce cartilage and bone formation (8). Structurally, BMPs and other TGF-beta family members are synthesized as larger monomeric pre-pro-forms consisting of a signal sequence, a long latency-associated peptide (LAP) and the mature cytokine that shows the highest degree of conservation (Figure 2). After synthesis, the precursors dimerize before enzymatic cleavage at characteristic R-X-X-R proteolytic processing sites, which leads to the release of the biologically active (mature) carboxy-terminal domain. The individual monomers of each dimer are linked by an intermolecular disulphide bond, while the monomers are characterized by a tight network of three (BMPs, GDFs) or four (TGF-betas, inhibin-betas) intramolecular disulphide bonds (Figure 3A), resulting in the typical butterfly-like structures that are characteristic for members of the TGF-beta superfamily (Figures 3B and 3C).

The different ligands act as morphogens during embryonic development, organogenesis, bone formation, and are indispensable in other physiological processes. For example, BMP-7 (OP-1) plays a key role in transformation of mesenchymal cells into bone and cartilage and rBMP-7 was effective in the repair of a resistant tibial non-union (9). Therefore, recombinant BMP-7 was introduced as a novel surgically effective therapeutic. Moreover, BMP-7 reduced the severity of injury after ischemic acute renal failure in rats (6). Recently, the concept that BMP-7 treatment abolishes the formation of EMT-derived fibroblasts by directly counteracting TGF-beta-induced Smad signaling has been established in various organs (10).

3.2. BMP signaling: Modes of signal transmission and their regulation

3.2.1. The BMP subgroups

Based on functional and structural aspects, especially with respect to the fibrotic response, the different members of the BMP subfamily can be divided into several subgroups. A first group, i.e. the BMP-2/4 group, includes BMP-2, BMP-4, and their *Drosophila* ortholog decapentaplegic (*dpp*). The second group, i.e. the osteogenic protein-1 (OP1) group, encompasses BMP-5, BMP-6, BMP-7, BMP-8 (OP-2) and the dipteran homolog that is known as the glass bottom boat *(gbb)-60A* gene product. GDF-5, also termed cartilage-derived morphogenetic protein-1 (CDMP-1), GDF-6 (CDMP-2 or BMP-13), and GDF-7 (BMP-12) form the third BMP group (GDF-5 group) (11). Expression of members of the BMP-2/4 and the OP-1 group has been reported in kidney, lung and liver, all representing tissues that are susceptible for fibrogenesis. Therefore, it is commonly suggested and in part experimentally shown that these groups are involved in control processes, which regulate organ injury and fibrogenesis. Comparable to TGF-beta-signaling, BMP-signal transduction is subject to diverse levels of

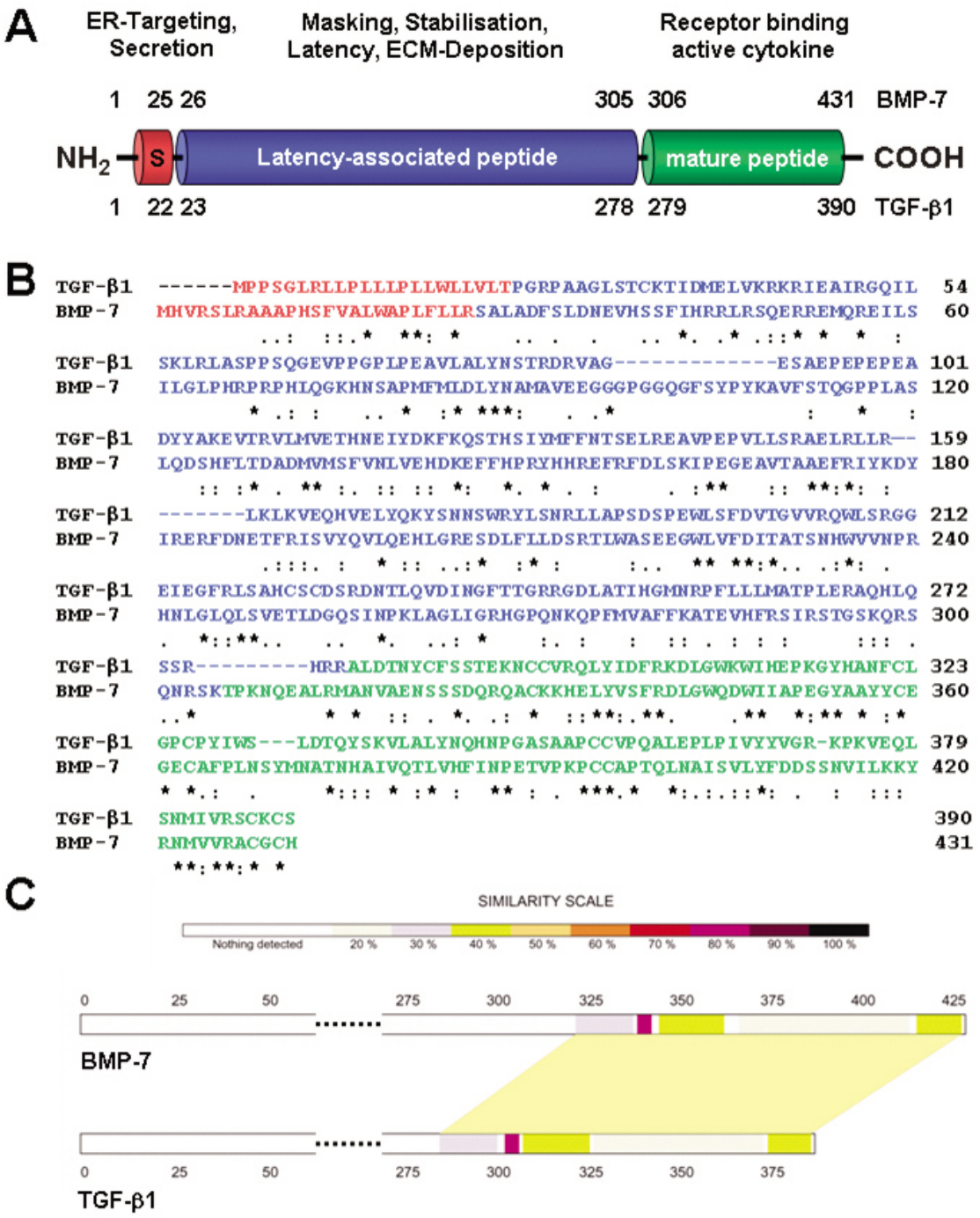

Figure 2. Structural features of BMP-7 and TGF-beta$_1$. (A) Both, BMP-7 and TGF-beta$_1$ share the same modular structure typical for members of the TGF-beta superfamily. The N-terminal leader sequence (*in red*) is necessary for targeting to endoplasmic reticulum (ER) and subsequent cellular secretion. The latency-associated peptide (*in blue*) is required for masking (latency), stabilization and extracellular matrix deposition. The mature (biologically active) peptide (*in green*) is located at the C-terminus of the pre-propeptide. Locations of amino acid positions are given for human BMP-7 (Swiss-Prot P18075) and TGF-beta$_1$ (Swiss-Prot P01137). (B) Sequence alignment of human TGF-beta$_1$ and BMP-7. The regions of the signal region, latency-associated peptide, and mature cytokine are given in red, blue, and green, respectively. (C) Schematic overview about sequence similarities between BMP-7 and TGF-beta$_1$. The highest degree of similarity (~40-70%) of both cytokines is found at the C-terminal regions harboring the mature peptides.

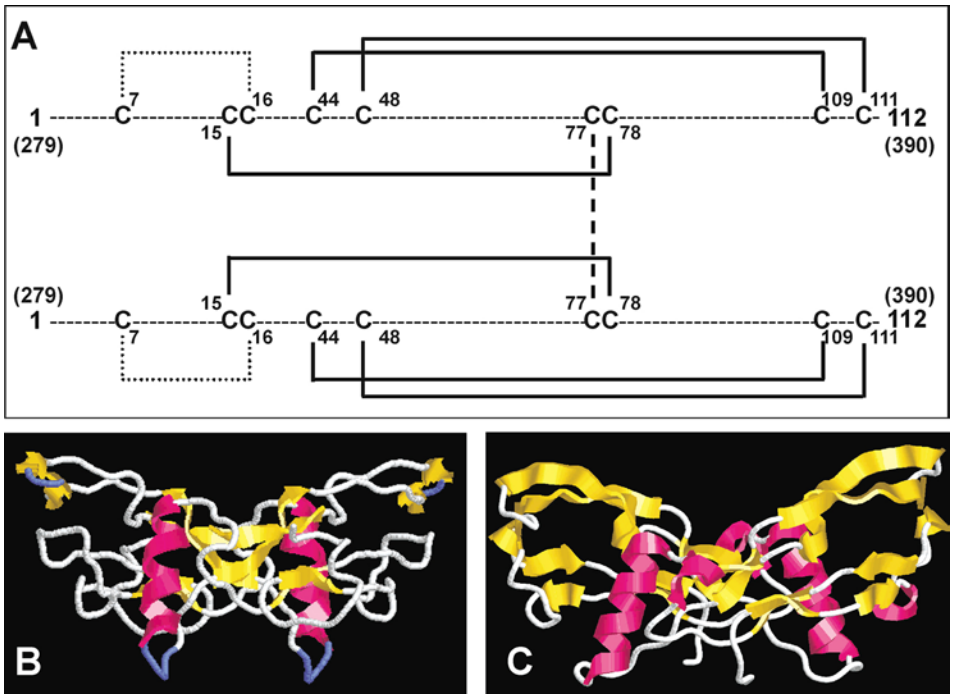

Figure 3. Two- and three-dimensional structure of mature TGF-betas and BMPs. **(A)** Members of the TGF-beta/BMP/GDF family are only active as dimers in which the individual monomers are linked by a single disulphide bond that is in human TGF-beta$_1$ located at position 77 of the mature peptide (position 356 from the Start-ATG). In addition, the TGF-beta monomers contain four intrachain disulphide bonds (*solid lines*) that are conserved in TGF-betas and inhibin-betas, while the other members of this family lack the first bond (*speckled line*). The dimers are bridged by one intermolecular disulphide bond (*dotted line*). The sequence positions of cysteines involved in disulphide bonding correlate to those counted from the beginning of human mature TGF-beta$_1$ (for orientation refer to Figure 2B). (B, C) The tight network of intramolecular disulphide bonds and the single intermolecular linkage of two monomers cause the butterfly-like tertiary fold that is typical for dimers of the TGF-beta family. For this analysis the minimized average nuclear magnetic resonance (NMR) structure of human TGF-beta$_1$ (B) or the crystal structure of human BMP-3 (C) that are deposited in the Brookhaven Protein Databank (PDB) under accession numbers 1KLC and 2QCQ, respectively, were taken for molecular visualization using the RasMol program (Windows version 2.7.4.2). For more structural details, refer to the original literature describing the respective three-dimensional structures of these TGF-beta superfamily members (151, 152).

modulation. These include receptor-binding, receptor activation, modulation of Smad activity and lastly interaction of Smads with other transcription factors. As a consequence, BMP-signaling induces a wide variety of responses with a limited set of molecular components.

3.2.2. BMP receptors

Similar to the prototype ligand TGF-beta, BMPs are bound by a set of membrane inserted type I and type II receptors (12) that are divided into different evolutionarily conserved subgroups (Figure 4). In contrast to TGF-beta, BMPs bind to another subset of type II receptors (Figure 5) that subsequently activate the activin-like receptor-kinase (ALK)-2, ALK-3, and ALK-6 (13, 14). The ligands of the BMP2/4 group preferentially bind to ALK-3 and ALK-6, whereas proteins of the OP-1 group have affinity for ALK-2 and ALK-6 (15). Members of the GDF-5 group bind primarily to ALK-6, although it was recently shown that GDF-9 may signal *via* the classical TGF-beta-receptor ALK-5 (16). Beside these classical BMP receptors, ALK-1 is able to bind BMP-9 and BMP-10 (17, 18). The implication of ALK-1 in BMP-signaling implies another level of complexity into signaling crosstalk (19), since ALK-1 mediates TGF-beta responses during angiogenesis in endothelial cells (20). In this setting, TGF-beta/ALK-1 counteracts TGF-beta/ALK-5 responses (21, 22). The possible activation of ALK-1 by TGF-beta is of great importance for the interplay of TGF-beta *vs.* BMP-type signaling. TGF-beta was shown to mediate activation of intermediates and target genes that were previously categorized as being specific for BMPs *via* ALK-1 (21-25). The corresponding type II receptors for BMP-ligands are BMPRII, ActRII, and ActRIIB, which upon ligand binding activate the type I receptors through phosphorylation (26, 27).

In addition to the signaling receptors, which are essential for ligand responses, BMP-receptor binding and signal transmission is fine tuned by accessory (co-) receptors. These include for example the pseudo receptor BAMBI that is transcriptionally induced by BMPs and binds to the BMP type I receptors ALK-3 and ALK-6 thereby interfering with type I/ type II receptor complex formation (28, 29). In addition, membrane-associated

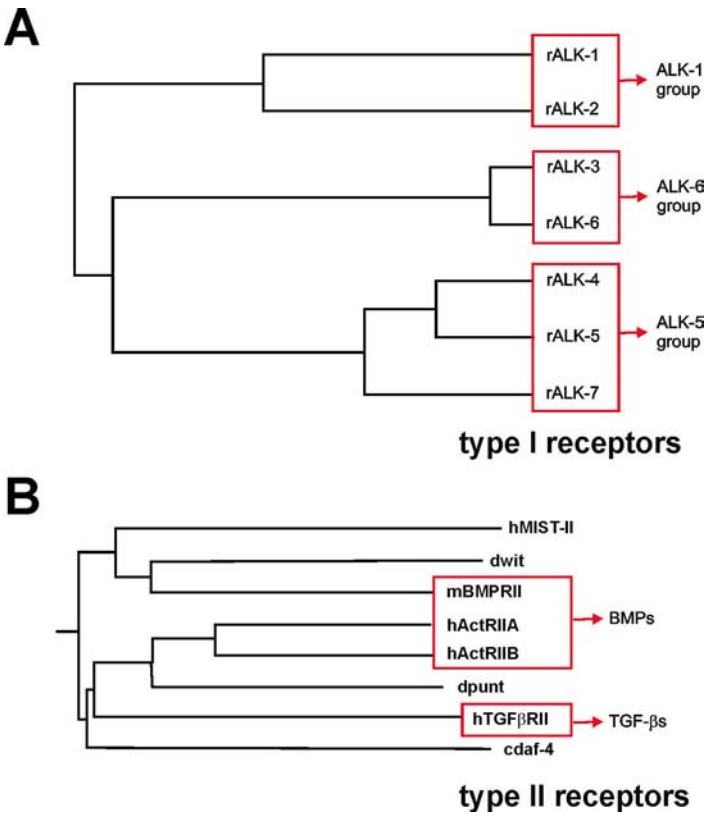

Figure 4. TGF-beta/BMP receptors. (A) The type I receptors are further grouped into three subgroups. The ALK-1 group contains ALK-1 and ALK-2, the ALK-6 group contains ALK-3 and ALK-6, while the ALK-5 group contains ALK-4, ALK-5 and ALK-7. The protein sequences of the different ALKs from rat were taken as input for this dendrogram. (B) The type II receptors BMPRII, ActRIIA and ActRIIB are specific for BMPs, while TGFbetaRII is specific for TGF-betas. In this dendrogram the following proteins were aligned: human ActRIIA (hActRIIA), human ActRIIB (hActRIIB), human TGFbetaRII, murine BMPRII (mBMPRII), human Mast cell immunoreceptor signal transducer (hMIST-II), *Drosphophila melanogaster* receptors Wishful Thinking (dwit) and Punt (dpunt), and *Caenorhabditis elegans* Cell surface receptor of the abnormal dauer formation family member (cdaf-4).

receptors of the repulsive guidance molecule (RGM) family modulate BMP-signaling (30, 31). RGM receptors are critical regulators of iron balance and may cause hemochromatosis upon mutations in RGMc (hemojuvelin). RGMc is essential as co-receptor for BMP-2/4-induced hepcidin expression in hepatocytes (32, 33). Beside afore mentioned receptors, there are two type III TGF-beta receptors, which are not only involved in TGF-beta- but also in BMP-signaling. Betaglycan (also termed TbetaRIII) is more or less ubiquitiously expressed, binds to BMP-2, BMP-4, and BMP-7 and promotes BMP-2-induced EMT (34). Endoglin (CD105) shows a more restricted expression pattern, being highly expressed in endothelial cells, activated macrophages and hepatic stellate cells (35-37). Endoglin binds in the presence of the corresponding type II receptor to BMP-2, BMP-7, and BMP-9 (Figure 6) and increases BMP-7- and BMP-9-mediated responses (38, 24, 17, 18). However, the underlying mechanisms for this activation are currently unknown.

3.2.3. Secreted BMP signaling modulators

The receptor equipment endows a cell with versatile signaling machinery. Moreover, the signal transmission is regulated by ligand affinities and the occurrence of different intracellular pathways. Several secreted proteins of different families encompassing Noggin, Chordin, Gremlin and Dan have been characterized. These proteins antagonize BMP-signaling by binding to the ligands thereby inhibiting association with their cognitive receptors. In a similar fashion, members of the CTGF/CYR61/NOV (CCN) family, that include the connective tissue growth factor (CTGF) and the Nephroblastoma-overexpressed protein (NOV), antagonize BMP responses, whereas the Kielin/Chordin-like protein (KCP) was shown to enhance BMP-7 signal transduction (39). The relevance of these secreted modulators is evident from their regulatory functions under fibrotic conditions (see 3.6).

3.2.4. BMP intracellular signaling pathways

In general, ligand binding to the membrane receptors leads to activation of the type I receptor kinases that in turn phosphorylate intracellular Smad mediators (40). There are two groups of receptor-regulated Smads (RSmads); e.g. the “TGF-beta"-Smads comprising Smad2 and Smad3, and the “BMP”-RSmads (BRSmads)

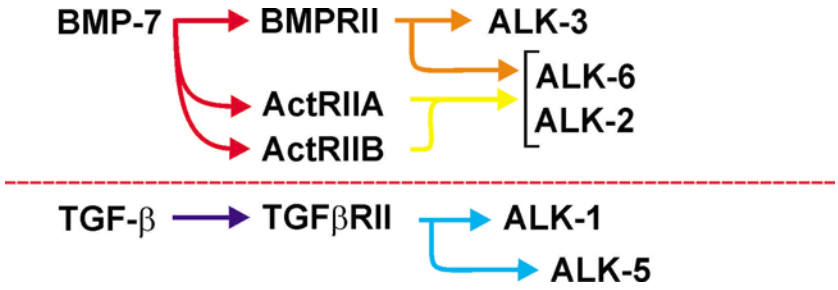

Figure 5. Receptor for BMP-7 and TGF-beta. BMP-7 bind to the type II receptors BMPRII, ActRIIA and ActRIIB that subsequently activate with different specificities the type I receptors ALK-3, ALK-6 or ALK-2. In contrast, TGF-beta binds to TGFbetaRII that subsequently transphosphorylate ALK-1 and ALK-5.

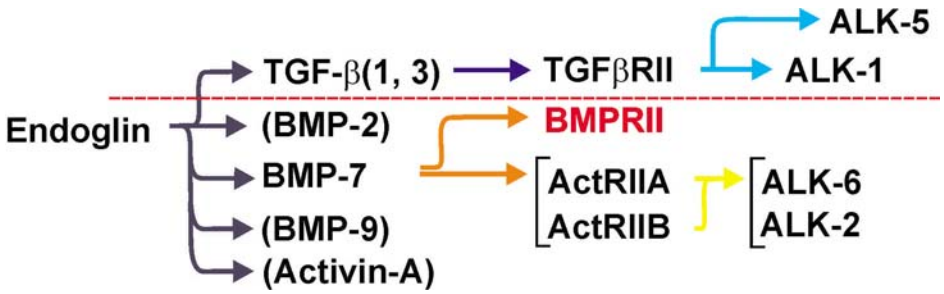

Figure 6. Modulation of BMP/TGF-beta signaling by Endoglin. The accessory type III receptor Endoglin has affinity for individual members of the TGF-beta superfamily that influences the affinity for individual type II receptors. In the presence of Endoglin, BMP-7 signaling is increased.

comprising Smad1, Smad5 and Smad8. The signaling in response to BMPs leads to the activation of the BRSmads through phosphorylation of the most C-terminally located serine residues by the type I receptor kinase (41-43). Structurally, RSmads are composed of three functionally different modules, e.g. the MAD homology domain 1 (MH1), MH2 and the connecting linker region. DNA and co-factor binding is governed by the N-terminal MH1 domain, whereas interaction and phosphorylation by the type I receptor at the SSXS-motif (phosphorylated serines are underlined) occurs within the MH2 domain. The linker region is substrate for mitogen-activated protein (MAP) kinases, which regulate the nuclear translocation of RSmads. Smurf proteins also bind to the linker region and mediate the ubiquitinylation of specific residues within the linker to mark RSmads for degradation (40).

The signal transfer of BMPs to the intracellular side is best characterized for BMP-2 and involves two different modes. In the first mode, BMP-2 binds to preformed type I/type II receptor heteromeric complexes, while in the second mode, BMP-2 binds to type II and type I receptors to form the hetero-oligomeric complexes. The binding of BMP-2 to preformed receptor complexes induces phosphorylation and activation of Smad-dependent pathways, while the sequential recruitment of receptors activates a different, Smad-independent pathway resulting in induction of the p38 MAP kinase (44). Activation of the receptor facilitates the interaction of special receptor binding proteins called X-linked inhibitor of apoptosis (XIAP), TGF-beta-activated kinase 1 (TAK1), TAK interacting protein 1 (TAB1) and consecutive activation of the p38 MAP kinase (45).The RSmads Smad2 and Smad3, which are substrates for the TGF-beta-activated ALK-5 receptor, are differentially activated by TGF-beta and play functionally different roles in several cells (46). In a similar manner Smad1 and Smad5 are differentially activated but this phenomenon has not been investigated in detail. This differentiation may be achieved by the usage of alternative BMP type I receptors or may arise from a differential expression of the Smad protein, itself (47, 48). Although it has been regarded as a paradigm that TGF-beta ligands activate Smad2 and Smad3 and on the other hand BRSmads are specifically activated by the BMP-receptor kinases, it was recently recognized that there are some exceptions to this rule. ALK-1, a receptor of the "BMP-receptor group" transmits signals of TGF-beta *via* phosphorylation of Smad1 and/or Smad5 (43, 49), and GDF-9 has been shown to signal *via* the classical TGF-beta receptor ALK-5 to activate signaling involving Smad2/Smad3 (16). Once activated, Smad proteins interact with the common Smad4 and translocate into the nucleus to regulate transcription of target genes.

Negative and positive crosstalk between the Smad- and the MAPK-pathways is given by the fact, that linker phosphorylation of Smads by MAP kinase modulates the transcriptional activity of Smads (50). Nevertheless, the BMPRII receptor is also able to directly interact with cytoskeletal associated proteins, e.g. LMK1 and Tctex1, similar to the interaction of Endoglin with Tctex2 (51). The binding regulates the overall function of these cytoskeletal-associated proteins (52, 53).

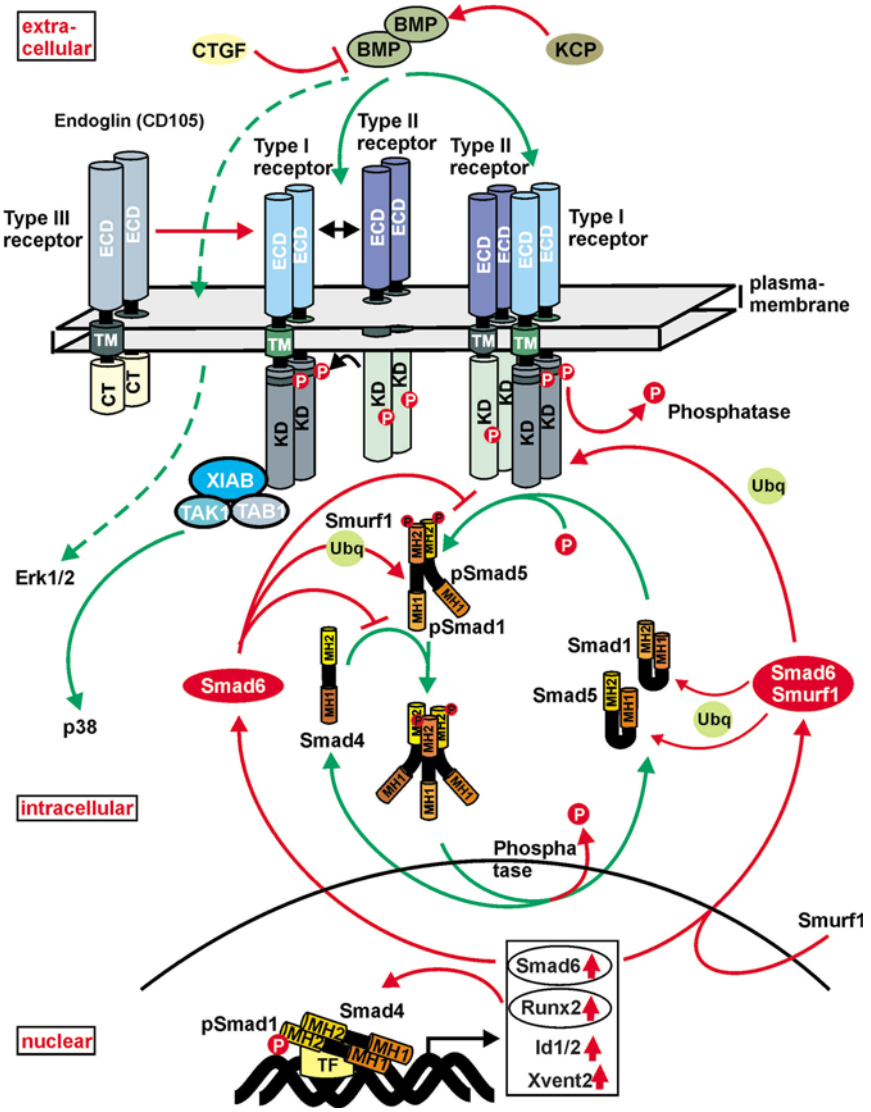

Figure 7. BMP signal transduction. Signaling by members of the BMP-subfamily of ligands is initiated by binding to a heteromeric complex of type I receptors, e.g. ALK-2, ALK-3 and ALK-6, as well as type II receptors, e.g. BMPRII, ActRII and ActRIIB. The access of ligands to the receptors is regulated by secreted proteins like CTGF (negatively) or KCP (positively). In addition, type III receptors like Betaglycan or Endoglin modulate the signal transmission. Intracellular mediators belong to the family of MAP kinases (p38, Erk1/2; *left*) or to the Smad family (*right*), depending on the composition of the formed receptor complex. BRSmads are activated by phosphorylation through the corresponding type I receptor, associate with the co-Smad4 and translocate into the nucleus to regulate transcription of target genes in conjunction with co-repressors and co-activators. The signaling circuit (*green*) is controlled by the inhibitory Smad (i.e. Smad6) at several steps. The activated forms of type I receptors and Smads are deactivated through dephosphorylation by specific phosphatases and these components are marked for degradation with ubiquitin by the ubiquitin ligase Smurf. Abbreviations used are: CTGF: connective tissue growth factor; KCP: Kielin/Chordin-like protein; BMP: bone morphogenetic protein; ECD: extracellular domain; TM: transmembranal domain; KD: kinase domain; P: phosphate; Ubq: ubiquitin; MH1/MH2: MAD homology domain 1/2; TF: transcription factor

3.2.5. BMP-mediated transcriptional control

Once the phosphorylated Smads are translocated into the nucleus, they regulate the transcription of target genes (Figure 7). Since the Smads have only a low intrinsic DNA binding affinity (54), they associate with co-factors to facilitate the integration of different signaling inputs to generate positive and negative gene responses. This interaction is most likely the most important mechanism accounting for the high diversity of gene responses regulated by the few Smad proteins. A growing number of proteins were identified that interact with Smads to regulate transcriptional responses (55). The group of runt domain transcription factors (Runx) are involved in various biological processes, including haematopoiesis and bone formation. The family consists of the three homologous

proteins Runx1, Runx2, and Runx3. Runx2 is transcriptionally induced by BMP-2, involving the transcription factor Dlx5 (56, 57). Upon BMP stimulation, Runx2 and BRSmads physically interact, are subsequently positioned into the nucleus and co-operatively regulate transcription of target genes (58). The CREB binding protein (p300/CBP) that has histone acetylase activity is another transcriptional co-activator. p300/CBP binds to BRSmads and governs their access to transcriptional initiation sites by changing the chromatin structure (59). Tob has been described as a specific negative regulator of BMP-2 responses (60). It is induced by BMP-2, associates with BRSmads and the common Smad4 (co-Smad4) and inhibits transcriptional activity of BRSmads (60). Although c-Ski and the Ski-related protein SnoN are inhibitors of Smad2 and Smad3 (61), they also reduce BMP-signaling mediated by Smad1 and Smad5 (62). The inhibitory effect of c-Ski is mediated by binding to co-Smad4 and recruitment of a histone deacetylase to this complex. This ability is lost upon modification of c-Ski (ARPG mutation), which abolishes binding of c-Ski to co-Smad4 binding (63).

3.2.6. BMP target genes

The best characterized *bona fide* target genes of BMP-signaling are the *Xenopus Vent2* gene and the Id genes, including Id1, Id2, Id3 and Id4 (64-68). The respective promoters contain specific Smad binding elements (SBEs) confering binding of Smad1 and Smad5 that are necessary and sufficient for BMP-responsiveness (69, 70, 66). These elements have been cloned into different reporter systems allowing to monitor BMP activity (71, 72), and to identify further genes that are targets of BMPs (73). Both genes, *Xvent2* and *Id1*, are potently induced by BMPs (74, 75, 76, 24). Another family of target genes of the BMP-signaling cascade are the Runx transcription factors (see also 3.2.5) that are essential for the commitment of the osteogenic program and are in turn pivotal regulators of Smad-signaling itself (see 3.2.7). As a member of the inhibitory Smads (ISmads), Smad6 has been identified to be an essential feed-back regulator of BMP-signaling (77, 78). Smad6 belongs to the inhibitory Smads, e.g. Smad6 and Smad7, and is up regulated as an immediate early gene in response to BMP-stimulation (79). In detail, it was shown that upon BMP-2 administration the Smad6 gene is regulated by Smad1 in co-operation with Runx2 (80). With respect to EMT it is worth to note that BMP4 is able to induce typical marker proteins like SNAIL and SLUG that reduce E-cadherin expression (81), both components of the "EMT proteome" (see 3.5.) and potently up-regulated by TGF-beta (82).

3.2.7. Regulation of intracellular BMP-signaling

As mentioned above BMPs induce their own inhibitor, Smad6. In contrast to Smad7 (83, 84), Smad6 is primarily an inhibitor of BMP-signaling (77, 78) and has multiple capabilities to switch off and modulate the signaling cascade (55). Smad6 directly interacts with the activated type I receptors and inhibits further activation of BRSmads by the receptor (85). Smad6 also binds to BRSmads (Smad1) and abrogates their interaction with co-Smad4 (78). In addition, HECT-type E3 ligases Smurf1 and Smurf2 interact with type I receptors and BRSmads (86). The binding of Smurfs to the activated type I receptor is enhanced by the binding of Smad6 and leads to ubiquitinylation of the receptors, which marks them for degradation (87, 88). A similar mechanism applies to the activated BRSmads that are also bound co-operatively by Smad6/Smurf1 and labelled with ubiquitin for degradation (89, 87). Since phosphorylation is an essential step in the activation of type I receptors and BRSmads, its reversal by specific phosphatases results in deactivation of these components (90). Smads are composed of three structurally and functionally different modules which are not only subject to protein-protein interaction but also substrates for direct post-translational modification, e.g. phosphorylation. Thereby, the Smad proteins function as integrators for signals of different sources, which modulate their activity. Smad phosphorylation by type I receptors occurs at the C-terminal domain of Smads, whereas MAP kinases phosphorylate BRSmads at serine and threonine residues in the linker region (91). Erk1/2 phosphorylation of the Smad1 linker region leads to inhibition of nuclear accumulation of Smad1 (92). Smurf1 binds to this phosphorylated linker region and causes cytoplasmic retention and poly-ubiquitinylation of Smad1 (91) implying that only the phosphorylation at the C-terminal domain of Smads mediates down stream signaling.

3.3. Physiological functions of BMP-7 in normal and fibrotic organs

BMPs have a variety of different functions during embryonic development. In general, they are morphogens acting as graded positional cues to dictate cell fate specification and tissue patterning. They were first purified from bone and thought to play essential functions in chondrogenesis and osteogenesis. In the meantime, several lines of evidence indicate that the BMPs are influencing a wide range of tissues during development and are essential for organ homeostasis. Beside several other BMP knock-out models, an unequivocal demonstration of their multifunctional morphogenic character came from mice that were deficient for BMP-7 (93). These mice clearly revealed that BMP-7 is not only an early inducer of glomeruli formation but is also involved in the formation of other organs and lens formation. The absence of endogenous BMP-7 led to small dysgenic kidneys with less glomeruli combined with hydroureters. Moreover, these mice have defects in eye formation and skeletal patterning, indicating that BMP-7 is also important for eye development and skeletogenesis. Most interestingly, mice lacking BMP-7 showed severe defects in the "*nephrogenic process"* in which metanephric mesenchyme undergoes an epithelial transition to form glomeruli and tubules of the nephron (93). This process is an important mechanism for cellular reorganization during kidney development and it is reasonable that *vice versa* BMP-7 is the driving force involved in controlling the ratio of mesenchymal to epithelial cells in morphogenesis. Following its inductive action in kidney development, BMP-7 in normal kidney continues to be heavily expressed specifically in podocytes, distal tubules and collecting ducts (47). In line, it has been demonstrated that the inhibition of endogenous BMPs in transgenic mice ectopically expressing the BMP antagonist Noggin in the glomerular podocytes resulted in a severe

Table 2. Therapeutic effects of BMP-7 in experimental fibrosis

Organ	Injury model	Treatment	Antifibrotic effects	References
Liver	TAA[1]-treated rats	adenoviral expression of BMP-7	decreased expression of alpha-SMA and type I collagen, decreased hydroxyproline content	(104)
	CCl_4-treated mice	rBMP-7	decreased type III collagen accumulation, reduced number of $FSP1^+$ and $FSP1^+/Alb^+$ fibroblasts, increased serum albumin	(105)
Heart	pressure overload in mice	rBMP-7	reduced accumulation of extracellular matrix and fibroblasts, increased microvascular density, elevated left-ventricular end-diastolic pressure	(106)
	chronic heart rejection in mice	rBMP-7	reduced accumulation of extracellular matrix and fibroblasts, increased microvascular density, decrease in $FSP1^+CD31^+$- and alpha-SMA^+CD31^+-positive cells	(106)
Kidney	STZ-treated C1 mice	rBMP-7	inhibition of glomerular hypertrophy, reduced tubular damage and relative interstitial volume, decreased type III collagen accumulation, reduced serum creatinine	(100)
	STZ-treated mice	transgenic	decreased expression of type I collagen and fibronectin, reduced glomerular and interstitial fibrosis level, elevated activity of renal MMP-2 and MMP-4	(101)
	$MRL/MpJ^{lpr/lpr}$ lupus mice	rBMP-7	reduced glomerular hypertrophy and relative interstitial volume, reduced serum creatinine, decreased interstitial type I collagen	(103)
	$Col4A3^{-/-}$ mice	rBMP-7	reduced tubular atrophy and relative cortical interstitial volume, decreased renal pathology-related mortality rate, reduced serum creatinine, blood urea nitrogen, and urine protein	(103)
	unilateral ureteral ligated rats	rBMP-7	reduced tubular atrophy and relative cortical interstitial volume, decreased interstitial type IV collagen, recovery of glomerular filtration rate	(97)
Lung	asbestos exposure in mice	rBMP-7	reduced hydroxyproline contents	(107)
Eye	capsular injury with hypodermic needle	adenoviral expression of rBMP-7	suppression of injury-induced EMT in lens, increased expression of Id2 and Id3	(108, 153)

[1]Abbreviations used are: Alb^+: albumin positive; $Col4A3^{-/-}$: deficient for the alpha3-chain of type IV collagen; MMP: matrix metalloproteinase; rBMP-7: recombinant BMP-7; STZ: streptozotocin; TAA: thioacetamide.

phenotype that is characterized by mesangial matrix expansion. These results further strengthen the notion that BMPs have an important role in regulating glomerular structural homeostasis (94). The findings that BMP-7 expression is down regulated in diseased kidney and that the balanced administration of recombinant BMP-7 reduces the progression of renal fibrosis in animals with experimental renal diseases further indicate that BMP-7 has therapeutic (antifibrotic) properties (95-98).

Beside the important renal performance of BMP-7, it has been shown that liver regeneration is also affected by this multifunctional cytokine. Systemic application of neutralizing antibodies targeting endogenous BMP-7 after hepatectomy in mice resulted in impaired hepatic regeneration, whereas administration of rBMP-7 led to an enhanced regeneration suggesting that BMP-7 is a physiological regulator of hepatocyte health (7).

In recent studies, it became clear that some of the antifibrotic effects are mediated by the inhibition of profibrogenic TGF-beta (94). This mechanisms of balancing the "Good (i.e. BMP-7) against the Bad (i. e. TGF-beta)" has nowadays attracted many scientists and clinicians and was transferred to other organs, in which the final common pathways by which TGF-beta is establishing fibrosis are more or less the same. Therefore, many studies were recently initiated with the aim to clarify some of the basic aspects of the beneficial molecular and cellular mechanisms of this potential therapeutic.

3.4. Efficacy of BMP-7 as physiological and therapeutic antifibrotic agent

The important role of BMP-7 as a TGF-beta-antagonist in maintenance of organ homeostasis was utilized to use BMP-7 as an antifibrotic agent in different models of organ fibrosis (Table 2). Applications of BMP-7 in several rodent fibrosis models in liver, heart, kidney, lung, and eye revealed a high therapeutic potency without significant side effects or toxicity *in vivo* (99). Thus, renal fibrogenesis associated with ureter obstruction in mice was prevented by systemic application of rBMP-7, and fibrotic symptoms, e.g. interstitial accumulation of type IV collagen or tubular atrophy, were significantly reduced (95, 97). Remarkably, these studies found BMP-7 effectiveness superior to enalapril, a drug used to treat kidney disease related to diabetes. Another mouse model reflecting human diabetic nephropathy confirmed the benefit from BMP-7 in this pathological context (100). CD-1 mice were made diabetic with streptozotocin (STZ) and subsequently developed glomerular hypertrophy in combination with tubulointerstitial fibrosis. Systemic treatment of these mice with rBMP-7 regressed progression of diabetic nephropathy, as indicated by inhibition of glomerular hypertrophy and tubular damage, decrease of interstitial type III collagen, and reduction of serum creatinine, reflecting recovery of renal function. Additionally, transgenic mice expressing human BMP-7 under transcriptional control of a rat phosphoenolpyruvate carboxykinase promoter fragment showed only reduced glomerular fibrosis and expression of extracellular matrix components after STZ treatment (101). Prevention of glomerular sclerosis by BMP-7 treatment, superior to enalapril therapy, was also observed in a diabetic rat model (102) and a general reversion of impaired tubular architecture in a rat model of ischemic acute renal injury or in mice with nephrotoxic serum induced nephritis by BMP-7 is described (6, 99). The antifibrotic efficiency of BMP-7 has also been demonstrated in two genetic models of renal diseases. In both, $MRL/MpJ^{lpr/lpr}$ mice, which develop a

lupus-like disease with progressive renal fibrosis, or mice lacking the type IV collagen-alpha3 gene, which develop progressive renal disease, the systemic administration of rBMP-7 resulted in reversion of glomerular and tubular homeostasis and reduction of serum creatinine (103).

The therapeutic effects of BMP-7 were not only examined in renal fibrosis but also found in hepatic, cardiac and pulmonary fibrosis models. Adenoviral delivery of a construct that constitutively expressed murine BMP-7 in thioacetamide-treated rats, which develop hepatic fibrosis, resulted in a reduced expression of alpha-SMA and type I collagen that was accompanied by a decrease in liver hydroxyproline contents reflecting the antifibrotic potential of BMP-7 in this organ (104). In the same study, these effects were shown to be mediated by antagonism of TGF-beta signaling in hepatic stellate cells that represent the key effector cell during hepatic fibrogenesis. Another chemotoxic animal model of liver fibrosis in which carbon tetrachloride (CCl_4) was utilized as a hepatoxin revealed that rBMP-7 inhibited progression of liver fibrosis in mice by counteracting the TGF-beta-induced EMT of hepatocytes in the injured liver (105). Two different mouse models of cardiac fibrogenesis were used to assess the efficiency of systemic administered rBMP-7 (106). Both models, pressure overload by aortic banding or chronic allograft rejection by heart transplantation of MHC class II-incompatible donors and recipients, are characterized by development of cardiac fibrosis and dysfunction. BMP-7 therapy resulted in reduced accumulation of extracellular matrix and fibroblasts and increased microvascular density. Additionally, the chronic heart rejection model revealed a decreased number of fibroblast specific protein 1 (FSP1) or alpha-SMA positive cells, indicating reversal of TGF-beta-induced EMT. In lung, it was recently demonstrated that rBMP-7 reduces the hydroxyproline content in mice that were exposed to asbestos (107).

In vitro experiments revealed that the underlying mechanisms that regulate the interrelation between TGF-beta and BMP-7 signaling are intracellularly transmitted by Id2, Id3 and Smad6 (104, 108, 109). These studies have already shown that BMP-7 increases the expression of Smad6 and Id proteins in several cellular systems, which directly lead to blockage of collagen expression. Moreover, transient expression of Id proteins had similar effects like overexpression of BMP-7 (104).

In summary, a number of *in vivo* models in different organs demonstrated the high efficiency of BMP-7 as therapeutic agent in fibrotic diseases. Nevertheless, no clinical approach has been made so far to transfer these promising data from animal models into human therapy.

3.5. The complex regulatory network of BMP-7 and TGF-beta in epithelial-to-mesenchymal transition

EMT is the phenomenon whereby fully differentiated epithelial cells transit into a mesenchymal phenotype giving rise to fibroblasts and myofibroblast that play an important role in tissue repair and fibrosis following epithelial injury. TGF-$beta_1$, initially described as an inducer of EMT in normal mammary epithelial cells (110), has since been shown to mediate EMT *in vitro* in different epithelial cells, including renal proximal tubular, lens, alveolar epithelial-, cardiac endothelial- and most recently biliary endothelial cells and hepatocytes (111-116, 106). EMT response to TGF-$beta_1$ in fibrosis is predominantly mediated *via* Smad-dependent pathways, mainly Smad3 (117). In Smad-mediated pathways, TGF-$beta_1$ signals are transduced by transmembrane serine/threonine kinase type II and type I receptors. Upon TGF-$beta_1$ stimulation, the receptors are internalized into early endosomes where Smad anchor for receptor activation (SARA) is localized and modulates the formation of ALK-5 complexes with Smad2 or Smad3. Smad2 and Smad3 are phosphorylated at serine residues by the type I receptor and associated with Smad4 and further translocated to the nucleus where they interact with other transcription factors to regulate the transcription of TGF-beta-responsive genes such as CTGF, alpha-SMA, collagen 1A2 and plasminogen activator inhibitor-1 (PAI-1) (118) (Figure 8). Non-Smad-dependent pathways implicated in TGF-beta-dependent EMT include RhoA, Ras, MAPK, PI3K, Notch, and Wnt. Stimulation of these cooperative pathways usually provides the context for induction and specification of EMT within a particular tissue, with Smads representing the dominant pathway (119). In addition, integrin-linked kinase (ILK), an intracellular serine/threonine kinase that interacts with the cytoplasmic domains of beta-integrins and cytoskeletal proteins, has been identified as a potential downstream mediator of Smad-mediated TGF-beta1 signaling, playing an important role in EMT (120). Modulation of the TGF-beta1-dependent Smad pathway in animal models has provided strong evidence for a role of TGF-beta in fibrotic EMT in vivo. EMT was ameliorated in Smad3 knockout mice (121, 113), and in hepatocytes overexpressing Smad7, an antagonist of TGF-beta signaling (122, 123).

BMP-7 blunts TGF-beta1-induced EMT in adult organ fibrosis by directly counteracting TGF-beta-induced Smad3-dependent EMT, evidenced through the reduction of fibrosis occurring via EMT in vivo (124, 107, 103). In association with Smad2 downregulation, BMP-7 delayed EMT in lens epithelium, whereas overexpression of inhibitory Smad7 blocked EMT and decreased nuclear translocation of Smads2 and -3 (122). The underlying mechanism is thought to involve the induction of Id proteins by BMP-7 (125), which is then inhibited by TGF-beta that promotes EMT. Ids lack a basic DNA binding region, but they possess an HLH dimerization motif that allows them to interact with and inactivate bHLH transcription factors that can potentially inhibit or activate transcription. CTGF, PAI-1 and thrombospondin-1 are among those TGF-beta responsive genes directly down regulated by BMP-7 (126). Blocking of TGF-beta-dependent upregulation of PAI-1 by BMP-7 also results in induced expression of active MMP-2 that promotes degradation of the fibrotic matrix. BMP-7 counteracts TGF-beta1-induced EMT, reversing chronic renal injury through induction of E-cadherin, a key epithelial cell adhesion molecule, through direct antagonism involving Smad signaling pathways as evidenced by co-localization of phospho-Smad 2/3 and Smad 1 in nuclei (103).

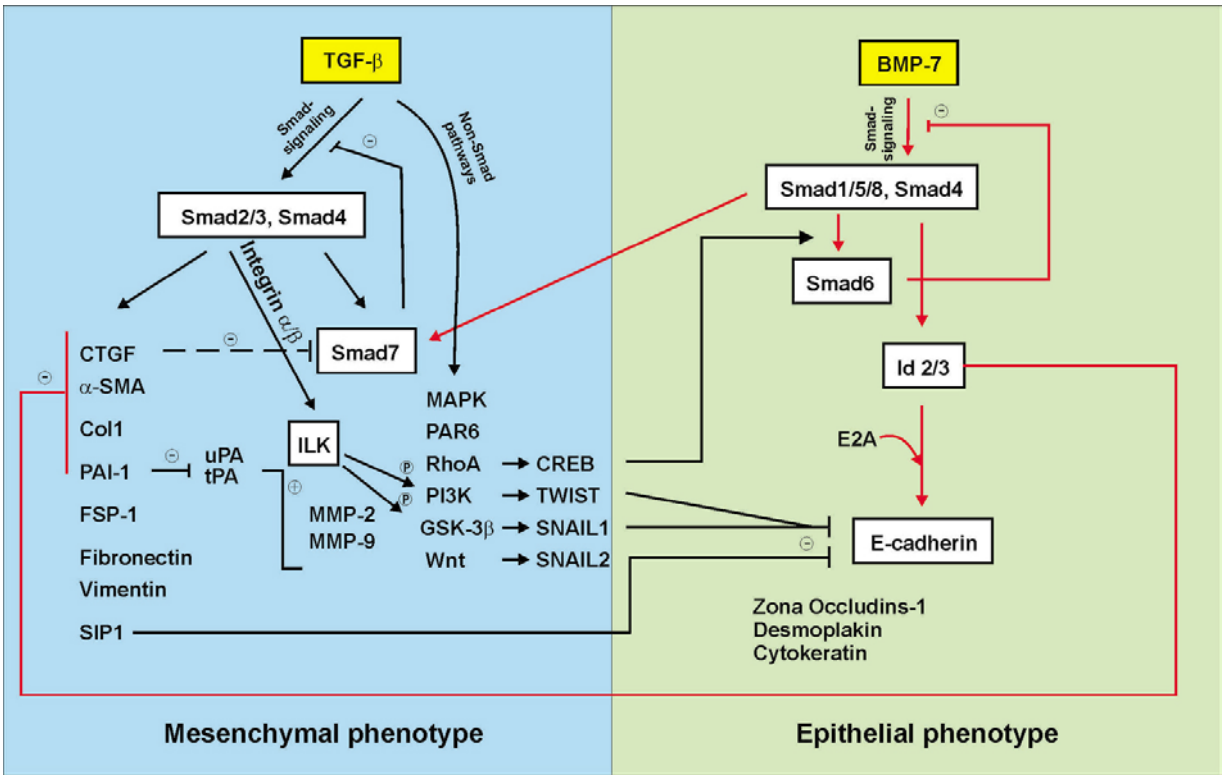

Figure 8. Complex pattern of interaction between TGF-β and BMP-7. BMP-7 supports the epithelial phenotype by inducing the expression of Smad7 through the Smad1/4/GATA complex that inhibits TGF-β signaling, and Id2/3 which inactivate the repressor E2A to permit expression of E-cadherin. Ids also inhibit several TGF-β responsive genes including CTGF and alpha - SMA, Col 1 and PAI-1. On the other hand, TGF-beta supports the mesenchymal phenotype by the rapid induction of CTGF. CTGF binds BMP-7 and inhibits BMP-7 signaling as evidenced by lower levels of pSmad1/5 and Id1 mRNA (140). In addition, CTGF activates several receptor systems that integrate with TGF-β/Smad signaling leading to the induction of transcription repressors that inhibit E-cadherin. CTGF further inhibits the expression level of Smad7, thus enhancing the transcription of TGF-beta-responsive genes. Moreover CREB, activated *via* CTGF, associates itself with the BMP/Smad complex, activating expression of Smad6 that not only inhibits the BMP-7 signaling pathway but also Id2/3 activity that leads to repression of E-cadherin. TGF-beta-induced PAI-1 in turn inhibits the activities of uPA and tPA that can activate the MMPs. Integrin-linked kinase (ILK), an intracellular serine/threonine kinase, associates with beta-integrin and regulates E-cadherin at the transcriptional level *via* the transcriptional repressor SNAIL-1. In addition, ILK phosphorylates Akt (PI3K) and glycogen synthase kinase (GSK), phosphorylation of GSK-3 resulting in nuclear translocation of beta-catenin and activation of the Wnt signaling pathway, which has also been strongly implicated in EMT.

BMP-7 regulates the expression of target genes that are characterized by BMP responsive elements (BRE) in their promoters. One of these BRE binds the Smad1/4/GATA complex, in the presence of GATA transcription factors and thus may enhance Smad7 induction leading to a blockage of TGF-beta signaling and allowing BMP to signal, even at low concentrations (127).

3.6. Functional interplay between BMP-7, connective tissue growth factor (CTGF/CCN2) and other crucial modifiers and regulators in organ fibrosis

We and others have previously reported that hepatocytes substantially synthesize CTGF during culture and in injured liver, and that this cell type is a major source of CTGF in the liver (128-130). CTGF, a designation introduced in 1991 (131) is a 36-38 kD, cysteine-rich, heparin-binding and secreted protein, which was initially identified in the culture supernatant of vascular endothelial cells. It is now classified as the second of six members of the CCN gene family containing CTGF itself, Cysteine-rich protein 61 (CYR61), NOV, and others (132). These proteins share approximately 40 to 60% sequence similarity and are characterized as mosaic proteins that comprise four conserved structural modules (133).

CTGF is suggested as an important downstream modulator protein of the profibrogenic master cytokine TGF-beta, amplifying its pro-fibrogenic action in a variety of tissues (133). Based on this function, CTGF has reached considerable pathophysiological relevance because of its involvement in the pathogenesis of fibrotic diseases, atherosclerosis, skin scarring, and other conditions with excess production of connective tissue (134). The strong expression of CTGF in fibrotic tissue occurs on the level of transcription and is stimulated by specific growth factors such as TGF-beta and endothelin-1, but also by

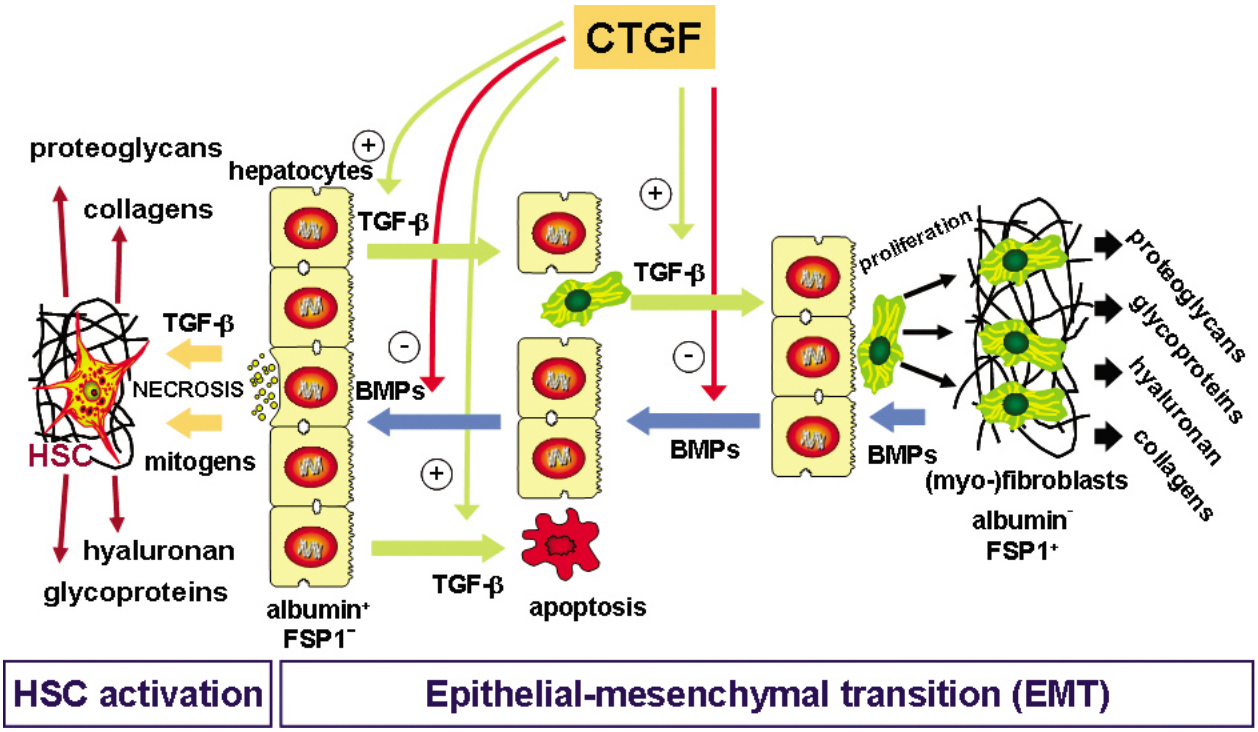

Figure 9. Pathogenetic concept of hepatic fibrogenesis. Upon injury, necrotic hepatocytes release mitogens (i. e. TGF-beta) that activate hepatic stellate cells (HSC). These cells produce large amount of proteoglycans, collagens, glycoproteins, and hyaluronic acid. Hepatocytes are further induced to undergo apoptosis or EMT. The resulting cells (i.e. myofibroblasts) lose their ability to express albumin while they become positive for the fibroblast-specific protein-1 (FSP1). In all these processes, TGF-beta acts profibrogenic while BMPs have opposing effects. The balance of both cytokines is further modulated by CTGF that increases TGF-beta and reduces BMP activities.

environmental influences such as biomechanical stress and hypoxia (133). CTGF gene activation by TGF-beta is mediated by a functional SBE, which resides within the CTGF promoter (135).

The CTGF protein consists of four functionally specialized modules with a proteinase-sensitive hinge region between modules II and III (136). Its molecular mechanism of action is still not known in detail, but its crucial role in fibrogenesis is documented by strong upregulation in fibrotic liver tissue (137, 138, 132), and even more importantly by recent studies, in which knock-down of CTGF by siRNA lead to substantial attenuation of experimental liver fibrosis (139, 140). Recent reports gave evidence that upregulation of CTGF inhibits BMP-7 signal transduction in the diabetic kidney (141). Abreu and coworkers furthermore presented data that describe CTGF as extracellular trapping protein for BMP and TGF-beta thus modulating the activity of these cytokines (142). According to functional studies in *Xenopus laevis*, CTGF directly binds BMP and TGF-beta through their cysteine-rich (CR) domain, thus antagonizing BMP activity by preventing its binding to BMP receptors. Of note, the opposite effect, enhancement of receptor binding, was observed for TGF-beta. These results suggest that CTGF inhibits BMP and activates TGF-beta signals by direct binding in the extracellular space. From this, CTGF would act profibrogenic by shifting the balance toward mesenchymal activity during hepatocellular EMT (143) (Figure 9). However, clarification is still pending.

Comparable to CTGF, there are several other proteinogenic modifiers that interfere with the activity of BMP-7. BMP antagonists identified so far include those of the Dan/Cerberus group (e.g. Gremlin), Noggin, Chordin and Follistatin. Although there is presently only limited information about the affinity and specificity of these modifiers, it is known that Noggin, Chordin, and Follistatin can physically interact with BMP-7. Therefore, it is reasonable that these antagonists interfere with BMP signaling by sequestering BMP-7. The recent finding that Gremlin was up-regulated in asbestos-exposed mouse lungs and combined with a down-regulation of BMP signaling indicated by reduced levels of Smad1/5/8 and enhanced Smad2 phosphorylation suggests that Gremlin is potentially involved in blockade of BMP signaling (109). However, a direct interaction of Gremlin with BMP-7 was not reported. Therefore, the effects of Gremlin on BMP-7 might be attributed as indirect. Another suppressor of BMP-7 activity is Sclerostin (also known as SOST) that was originally identified as the sclerostenosis-causing gene. It contains six conserved cysteine residues and one conserved glycine residue that are essential to form the cystine knot which binds to BMP-7 with high affinity and with unique ligand specificity (144). A similar protein containing such a sclerostin domain that is commonly known as uterine

sensitization-associated gene-1 (USAG-1 or SOSTDC1 for Sclerostin domain-containing protein 1) inhibits BMP-2, BMP-4, BMP-6, and BMP-7 activity in a mouse preosteoblast cell line (145). Interestingly, the ratio of USAG-1 to BMP-7 expression decreased with kidney damage but increased after subsequent kidney regeneration (146).

Additionally, there are several secreted proteins that increase the activity of BMP-7. We have reported that the accessory type III receptor Endoglin enhances BMP-7 signaling and *vice versa* suppresses the activity of TGF-beta$_1$ (24). In this study we further demonstrated that the transient overexpression of Endoglin, previously shown to inhibit TGF-beta$_1$-induced ALK-5/Smad3 signaling, enhanced the BMP-7/Smad1/Smad5 pathway suggesting that Endoglin is another attractive target molecule when a lowered BMP-7 activity should be counteracted. The Kielin/Chordin-like protein (KCP) is a protein that was recently identified as an enhancer of BMP-7 signaling (147). KCP is a high molecular weight protein consisting of a signal peptide, followed by 18 cysteine-rich chordin repeats and a C-terminal von Willebrand factor type D domain. It binds to BMP-7 and enhances binding to the type I receptor. Animals lacking KCP are more susceptible to the development of renal interstitial fibrosis and are molecularly characterized by reduced levels of phosphorylated Smad1 again demonstrating that BMP-7 in conjunction with its modifiers is essential for proper organ development and function (147).

3.8. BMP-7 as a novel diagnostic marker?

Newly recognized pathogenetic mechanisms of fibrosis such as EMT offer several innovative options for therapy of liver fibrogenesis and non-invasive diagnostic strategies. Elevated levels of both BMP-7 (repressor of EMT) and TGF-beta (inducer of EMT) are found in serum and plasma of patients with liver fibrosis, most likely because transcriptional up-regulation in the hepatic cells, release from necrotic hepatocytes and reduced hepatic clearance, which suggests that the determination of BMP-7 alone is not sufficient *per se* to estimate hepatic fibrogenesis (148-150). Therefore, the determination of the TGF-beta/BMP-7 ratio in serum or plasma is potentially promising, since this ratio might reflect the process of EMT and thus at least partially the rate of progression of fibrosis. A decrease of this ratio might indicate those patients with slow progression (*slow fibroser*), an increase a fast progression (*rapid fibroser*).

However, the cytokine ratio in the circulation might be not an accurate reflection of their activity/concentration in the tissue at the immediate environment of epithelial cells and fibroblasts, respectively. Furthermore, it has to be kept in mind that the major fraction of these cytokines determined immunologically with an ELISA is bound to carrier proteins (e.g. alpha$_2$-Macroglobulin) and, thus, in a biologically latent form. Therefore, the protein ratio does not necessarily mimic the diagnostically important activity ratio of these mediators and more well-designed clinical studies are required to identify the diagnostic value of BMP-7.

4. SUMMARY AND PERSPECTIVES

Many independent studies in animals provide supportive evidence for the potential efficacy of recombinant human BMP-7 in the setting of chronic organ damage. In experimental models of kidney injury it counteracts profibrogenic activities of TGF-beta, reduces inflammation, improves blood flow, and inhibits EMT representing the crucial cellular environment in which epithelial cells are metamorphosed into myofibroblasts that lose cell-cell adhesion and express large quantities of alpha-SMA and profibrotic molecules such as collagen types I and III and fibronectin. Similar beneficial effects of BMP-7 for maintenance of tissue homeostasis and regeneration were reported in liver, lung and heart. Therefore, it is reasonable that the rescue of BMP signaling activity is an effective means to treat fibrosis in various tissues and organs. It is noteworthy that the rapidly growing body of literature reporting insights in BMP-7 functionality, its signaling cascade, including receptors and modifiers, intracellular pathways and its signaling crosstalk has shown that BMP-7 is indeed a valuable drug candidate for the treatment of fibrotic lesions. Basic scientists will wait in suspense how these findings will translate into new knowledge to the clinic and help to develop effective novel antifibrotic therapies.

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Abbreviations: ALK: activin-like receptor-kinase: BMP: Bone morphogenetic protein; BRSmads: BMP specific RSmads; CTGF: connective tissue growth factor; CYR61: Cysteine-rich protein 61; ECM: extracellular matrix; EMT: epithelial-to-mesenchymal transition; FSP1: fibroblast specific protein 1; GDF: growth and differentiation factor; Id: inhibitor of differentiation; ILK: Integrin-linked kinase; KCP: Kielin/Chordin-like protein; NOV: Nephroblastoma-overexpressed protein; PAI-1: plasminogen activator inhibitor-1; rhBMP-7: recombinant human BMP-7; RSmad (s): receptor-regulated Smad (s); Runx: runt domain transcription factors; SBE: Smad binding elements; TGF-beta: transforming growth factor-beta

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