TGF-β/TGF-β RECEPTOR SYSTEM AND ITS ROLE IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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Abbreviations: ACE, angiotensin converting enzyme; ALK, activin like kinase; AMH, anti-mullerian hormone; AMDH, acromesomelic chondrodysplasia Hunter-Thompson type; AMH, anti-mullerian hormone; ARVD, arrhythmogenic right ventricular displasia; AS-ODNs, antisense oligonucleotides; AVM, arterionevous malformation; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; COPD, chronic obstructive pulmonary disease; CV, cardiovascular; EMT, epithelial-mesenchymal transition; FSH, follicle stimulating hormone; GDF, growth/differentiation factor; HDAC, histone deacetylase; JPS, juvenile polyposis syndrome; HHT, hereditary haemorrhagic telangiectasia; JP, juvenile polyposis; LAP, latency associated peptide; LLC, large latent complex; LSD, Loeys-Dietz syndrome; MAPK, mitogen-activated protein kinase; MIF, mullerian inhibitory factor; MIM, mendelian inheritance in man; MSC, mesenchymal stem cells; OP, osteogenic protein; PAH, pulmonary arterial hypertension; PP2A, protein protein phosphatase 2A; Co-Smad, cooperating Smad; I-Smad, inhibitory Smad; R-Smad, receptor associated Smad; SMC, smooth muscle cells; shRNA, short hairpin RNA; SpCC, spindle cell carcinoma; TAAD, thoracic aortic aneurysm syndrome; TGF-β, transforming growth factor-β

ABSTRACT

The transforming growth factor-β (TGF-β) system signals via protein kinase receptors and Smad mediators to regulate a plethora of biological processes, including morphogenesis, embryonic development, adult stem cells differentiation, immune regulation, wound healing or inflammation. In addition, alterations of specific component of the TGF-β signaling pathway may contribute to a broad range of pathologies such as cancer, cardiovascular pathology, fibrosis or congenital diseases. The knowledge about the mechanisms involved in TGF-β signal transduction has allowed a better understanding of the disease pathogenicity as well as the identification of several molecular targets with great potential in therapeutic interventions.

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INTRODUCTION

Since the discovery of the first member of the TGF-β superfamily in the early 1980´s, a steadily growing number of related members have been identified and functionally characterized in vertebrates and invertebrates. In mammals, the TGF-β family regulates many cellular functions including cell growth, differentiation, adhesion, migration, and apoptosis. TGF-β signalling is also essential for embryonic development, including germ-layer specification and patterning. Alterations of the TGF-β signaling pathway are involved in human diseases such as cardiovascular, fibrosis, reproductive, cancer or wound-healing disorders. Some of these diseases are hereditary conditions such as hereditary haemorrhagic telangiectasia, familial primary pulmonary hypertension or juvenile polyposis.

Here, first we will review the process of intracellular signal transduction from the soluble factors to the membrane-bound receptors and downstream into the nucleus. This pathway is relatively simple and well conserved in evolution. We will briefly describe the molecular components that make up the core pathway: ligands, receptors and SMADs. Second, we will describe the role of TGF-β signaling in the normal physiology and how its malfunction results in diseases affecting different organs, including, among others, cardiovascular system, pulmonary, bone, muscle and reproductive disorders or cancer. Finally, we will summarize the current knowledge about the therapeutic approaches that target different components of the TGF-β signaling pathway. The use of antibodies, antisense oligonucleotides, soluble receptors, recombinant ligands or chemical kinase inhibitors in preclinical and clinical studies will be discussed. To conclude, we will take a look at future challenges in the field.

1. The TGF-β signaling pathway. The transforming growth factor beta (TGF-β) system includes several components involved at different levels of a standard outside-inside signaling pathway, namely, soluble factors, specific membrane receptors and intracellular mediators.

1.1. Soluble factors of the TGF-β family proteins. The TGF-β family includes a large number of factors structurally and functionally related which act as multifunctional regulators of a wide range of biological processes. The members of TGF-β family are implicated, among others, in morphogenesis, embryonic development, adult stem cells differentiation, immune regulation, wound healing, inflammation and cancer. The first member of the family, TGF-β, was discovered in 1983 because of its ability to induce (‘transform’) the growth of cultured rat fibroblasts [1,2]. So far, more than 40 members of this family are known which cluster in several subfamilies, such as TGF-βs, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), activins, inhibins, Mullerian inhibitory factor (MIF) or Nodal (Figure 1).

TGF-βs. Six distinct isoforms of TGF-β with a variable degree of homology have been discovered, although only TGF-β1, TGF-β2, and TGF-β3 isoforms are expressed in mammals [3,4] and their human genes are located on chromosomes 19q13, 1q41, and 14q24, respectively [5]. The three TGF-β1, TGF-β2, TGF-β3 proteins are synthesized as pro-peptide precursors with a signal domain, followed by the prodomain (also named latency associated peptide -LAP-) and the mature domain. The signal domain is removed in the endoplasmic reticulum, and then a cleavage by the convertase family of endoproteases occurs between LAP and the mature homodimer protein [6]. Then, LAP remains associated with the mature domain forming a small latent complex (SLC) until bioactive TGF-β is released. After secretion, SLC is covalently associated, through LAP, with latent TGF-β binding proteins (LTBPs), forming the large latent

complex (LLC), which also binds to the extracellular matrix (ECM) proteins, such as fibronectin and fibrillin. Although all members of TGF-β family are synthesized as precursor proteins containing LAP, the capacity of LAP to maintain the ligands in a latent form is not conserved among all family proteins [7].

BMPs. BMPs were originally identified as a family of proteins that induces the formation of bone and cartilage when implanted at ectopic sites in rats. Members of the BMP family have been found in vertebrates as well as in invertebrates and are known to exhibit a wide range of biological effects on various cell types [8]. BMPs regulate the transcription of several genes involved in osteogenesis, neurogenesis, and ventral mesoderm specification. Members of the BMP family can be classified into several subgroups, including BMP2/4 group, BMP5/6/7/8 group, osteogenic protein-1 (OP-1) group, and BMP9/10 group [8]. Nodal (also known as BMP16) plays a critical role in early stages of development for cell fate determinations as well as in cell differentiation. Studies of the mouse Nodal gene suggest that it may be essential for mesoderm formation and subsequent organization of left-right axial structures in early embryonic development [9]. **GDFs** were discovered when searching for additional members of the TGF-β superfamily [10], and are classified within the BMPs family. So far, at least eleven components of GFDs have been described: GDF1-3, GDF5-11 and GDF15 [11].

MIF. MIF, also known as anti-Müllerian hormone (AMH) has been mainly studied for its regulatory role in male sex differentiation. MIF is implicated in the regression of Müllerian ducts in male fetuses and in the development and function of gonads of both sexes [12,13].

Activins. Activins are structurally related proteins involved in the control of cell proliferation, differentiation, apoptosis, metabolism, homeostasis, differentiation, immune response, and endocrine function [14]. Activins are produced in the gonads, pituitary gland, placenta, and other organs. Activins enhance follicle-stimulating hormone (FSH) biosynthesis and secretion, and participates in the regulation of the menstrual cycle. Activins possess a cysteine knot scaffold and are secreted as homodimers or heterodimers of inhibin β-subunits. Although four β-subunit genes (βA, βB, βC, and βE) have been described in humans, only dimers composed of βA/βA (activin A), βB/βB (activin B), and βA/βB (activin AB) subunits have been shown to be biologically active [15] (Supplementary Figure 1).

Inhibins. Inhibins were originally characterized as proteins produced by the gonads that act in an endocrine manner to negatively regulate FSH synthesis and secretion from the anterior pituitary. As such, inhibins are essential for normal reproductive and endocrine function [15]. Inhibins are closely related to activins. Inhibins are disulfide-linked heterodimers comprising an α-subunit and either a βA or βB subunit to form inhibin A and inhibin B, respectively (Supplementary Figure 1).

1.2. The TGF-β Receptors. All TGF-β family members bind cell surface serine/threonine kinase receptors types I and II, which form heteromeric complexes in the presence of dimerized ligands. Seven type I TGF-β receptors, also named activin like-receptor kinase (ALKs), as well as five different type II TGF-β receptors have been described (Figure 2 and Table 1). In addition, TGF-β ligands may also interact with the co-receptors endoglin and betaglycan (known as type III TGF-β receptors), which modulate the receptor kinase transduction [16-18]. Soluble ligands bind first to the constitutively active type II receptor, followed by the interaction and

phosphorylation of a glycine/serine (GS) rich domain of the type I receptor to produce an activated ligand-receptor complex [19]. This phosphorylation of the type I receptor disrupts the interaction between the kinase domain and a TGF-β signaling inhibitor, FKBP12. In addition, the phosphorylation of the GS domain increases its acidic nature, allowing the interaction with the basic domain of the downstream effectors Smads, which are then, phosphorylated by type I receptors. Both types I and II kinase receptors are themselves phosphorylated in tyrosine residues as well as in serine/threonine residues, probably implicated in a cross-talk activity regulation of a plethora of signal transduction pathways.

The activity of TGF-β kinase receptors can be also regulated by the type III receptors (TβRIII) endoglin or betaglycan [17,18]. Endoglin and betaglycan are type I integral membrane proteins with large extracellular domains, regular hydrophobic transmembrane domains, short cytoplasmic domains and lacking kinase signaling motifs. The ubiquitously betaglycan is a major TGF-β binding molecule at the cell surface that binds with high affinity several members in the TGF-β family, including TGF-β1, TGF-β2, TGF-β3, Activin-A, BMP2, BMP4, BMP7 and GDF5 [17]. Betaglycan interacts with, and presents ligand to, the type II TGF-β receptor. This presenting ability of betaglycan increases ligand binding to their respective cognate type I and type II TGF-β superfamily receptors to enhance their signaling. In addition, betaglycan stimulates BMP signaling by regulating the traffic and cell localization of interacting receptors [19]. However, betaglycan can inhibit both BMP and activin signaling, promoting the binding of inhibin to the corresponding type II signaling receptors [20]. At variance with betaglycan, endoglin is predominantly expressed in vascular endothelial cells, a cell type that has little or no betaglycan expression. Endoglin interacts with TGF-β1, ActivinA, BMP2 and BMP7, requiring the presence of the corresponding signaling receptors; by contrast, endoglin can bind directly TGF-β3 and BMP9 independently of the kinase receptors [18]. Endoglin enhances TGF-β1/Smad2 and TGF-β1-, BMP7- and BMP9-dependent Smad1/5 responses, while inhibits TGF-β/Smad3 pathways [17,18,21,22]. Endoglin and betaglycan belong to the zona pellucida (ZP) family of proteins characterized by the proteolytic cleavage of their extracellular domain [23,24]. Of note, the released soluble protein may act antagonizing the effects of the corresponding membrane-associated TβRIII [17,18] (Supplementary Figure 2).

1.3. Smad family dependent signal transduction. Intracellular TGF-β signaling is mediated by the Smad family of proteins. Members of the Smad family are well conserved and can be classified in three groups: i) Receptor associated Smads (R-Smad); ii) Cooperating Smads (Co-Smad); and iii) Inhibitory Smads (I-Smads) [25]. The activated receptor complexes transduce intracellular signaling by the type I receptor phosphorylation of the R-Smads in their carboxy-terminal domains [16]. The unphosphorylated R-Smads are transcriptionally inactive and sequestered in the cytoplasm by specific retention proteins such as SARA (Smad anchor for receptor activation) [16] or endofin [8]. In humans, five different R-Smads have been described that are substrate for activated TGF-β receptors (Smad1, 2, 3, 5, and 8). Smad2 and Smad3 are substrate for TGF-β and activins, whereas Smad1, 5 and 8 are substrate for BMP, GDF and MIFs routes (Figure 2). Each R-Smad contains three distinct domains, two highly conserved domains at the N-terminus and the C-terminus, named MH1 and MH2, respectively and a linker domain. MH1 can interact with DNA and with other proteins and possesses a nuclear localization signal (NLS), while MH2 mediates homo- or hetero-oligomerization of the Smads and the transactivation of Smad nuclear complexes. The highly variable linker region is located between

MH1 and MH2 domains, is enriched in prolines and has potential serine/theronine substrates for phosphorylation [26]. Upon ligand activation of the TGF-β receptor complex, the type I receptor phosphorylates R-Smad at a serine rich C-terminal motif, and then the phosphoR-Smad associates with Smad4 (mammalian Co-Smad). Interestingly, Co-Smad lacks the C-terminal motif for type I receptor phosphorylation [15]. The activated Smad complex consists of a trimer of single Co-Smads and homo or hetero-dimers of R-Smads. This Smad complex is shuttled into the nucleus where, in collaboration with other transcription factors, binds and regulates promoters of different target genes [16]. Two of these genes are I-Smads, Smad6 and Smad7. The induced expression of these inhibitory Smads produces a negative-feedback regulation of the TGF-β signaling [27]. I-Smads contain a characteristic C-terminal MH2 domain, but they lack the conserved MH1 domain . Smad6 preferentially inhibits BMP signaling by disrupting the Smad1/Co-Smad interaction and forming an inactive Smad1/Smad6 complex. In addition, Smad7 inhibits R-Smad phosphorylation by binding the TGF-β, activin and BMP type I receptors [28]. Once in the nucleus, the phosphoR-Smad/Co-Smad complex can bind, through the MH1 domain, with weak affinity to Smad-binding elements (SBEs) in the DNA. Smad3 recognizes a 5 base-pair -GTCTG-, whereas Smad4 and Smad1,-5 and 8 recognize non-consensus GC-rich motifs. In Smad2, a 30 amino acid insertion in the MH1 domain disables its individual binding to DNA. The binding of Smad complexes to DNA is crucial for the transcriptional activation of Smad target genes, but requires additional interactions with other transcription factors to form a large transcriptional complex with high-affinity for chromatin [16,27,28].

Regarding the Smad mediators involved, TGF-β superfamily members are classified into TFG-β/Nodal/Activin group and BMP/GDF group. TFG-β/Nodal/Activin signals are transduced through type I and type II receptors through R-Smad proteins Smad2 and Smad3, while BMP/GDF signals are transduced via type I and type II receptors through R-Smad proteins Smad1, Smad5 and Smad8 [16,27,28,25] (Figure 2).

1.4. Regulation of the TGF-β pathway. All components of the TFG-β pathway are subjected to a fine tuning, at different levels, in the modulation of TGF-β superfamily signal transduction. TGF-βs are secreted and maintained as an inactive form in the LLC that binds to ECM proteins [7], and whose bioavailability depends on the activation of a proteolytic cleavage or integrin interaction. Unlike TGF-β, BMPs are secreted in their active form and their activity is regulated by BMP antagonists, proteins that bind directly to BMPs and prevent them from interacting with their respective type I and type II receptors. Based on the size of their cysteine knot, a common arrangement of six half cysteine residues that form three intra-chain disulphide bonds, the BMP antagonists can be classified in four subfamilies: i) Dan; ii) Chordin; iii) Twisted gastrulation; and iv) Noggin. These BMP antagonists have differential affinities for the different BMPs.

Ligand access to, and signaling by, the kinase receptors can be modulated by several auxiliary receptors, including the TβRIII members endoglin and betaglycan, the repulsive guidance family of glycosylphosphatidylinositol-anchored proteins DRAGON, RGMa, and hemojuvelin, neuropilin, and the member of the α2-macroglobulin/C3, C4, C5 family of thioester containing proteins, CD109 [29-31].

TGF-β receptors and Smads are subject of post-translational modifications, including phosphorylation, sumoylation and ubiquitylation, which are enzymatically reversible and

regulate these proteins stability and availability, resulting as key determinants to the TGF-β cellular responses [19]. Another point of modulation is the regulation of the internalization and recycling of the ligand/receptor complexes via lipid rafts/caveolae, which can lead to protein degradation in the proteasome [32,33]. On the other hand, a negative feedback loop has been well documented, where TGF-βs signaling induces the expression of inhibitory proteins, including I-Smads or Smurf ubiquitinases (E3-ubiquitin ligases that selectively target the receptors and Smad proteins for degradation). Thus, BMP signaling induces Smad6 and Smurf1, whereas TGF-β1 induces Smad7 and either Smurf1 or Smurf2. In turn, Smad7 inhibits both BMP and TGF-β pathways, whereas Smad6 is more selective for BMP signaling showing high preference for BMP type I receptors ALK1, ALK3, ALK3 and ALK6 [11].

In addition to the canonical Smad pathway, the TGF-β signal is determined by a crosstalk with non-Smad pathways such as MAP kinase (MAPK), NF-kB, Rho-like GTPase, phosphatidylinositol-3-kinase (PI3K)/AKT or hypoxia/HIF-1 [11,34,35].

2. TGF-β system in health and disease. The TGF-β system regulates a plethora of biological processes, including morphogenesis, embryonic development, wound healing or inflammation. Alterations of specific component of the TGF-β signaling pathway may contribute to a broad range of inherited and non-inherited pathologies such as cardiovascular pathology, fibrosis, cancer or congenital diseases. A summary of diseases affecting components of the TGF-β system is listed in Table 2.

2.1. Cardiovascular System. In the cardiovascular system (CV), components of the TGF-β pathway have been implicated in several processes influencing vascular cell proliferation and migration, arteriogenesis, angiogenesis, cardiac development and a variety of cardiovascular pathologies [36,37]. Genetic studies in mouse have provided much evidence for the TGF-β involvement in vascular morphogenesis and dysfunction [36]. Studies in humans have shown the TGF-β involvement in hereditary and non hereditary CV diseases:

Hereditary Hemorrhagic Telangiectasia (HHT), or **Rendu-Osler-Weber syndrome,** is inherited as an autosomal dominant trait involving mainly mutations in *ENDOGLIN* (*ENG*) or *ALK1* genes, whose protein products influence TGF-β family signalling in vascular endothelial cells. More than 90% of HHT patients carry a pathogenic mutation in *ENG* (HHT1) or *ALK1* (HHT2), whilst 1-2% carries a mutation in *SMAD4.* Interestingly, the same *SMAD4* mutations also lead to Juvenile Polyposis (JP) resulting in a combined syndrome of JP and HHT (JP-HT) [38]. Additional HHT loci have been mapped to chromosome 5q (HHT3) and to chromosome 7p (HHT4) [39], whose genes are predicted to encode new components of the TGF-β/BMP signalling pathways. HHT is associated with frequent epistaxis, telangiectases in skin and mucosa, and arteriovenous malformations (AVMs) in lung, liver, or brain. The pathogenic mechanism underlying the generation of telangiectasia and AVMs appears to be haploinsufficiency of the HHT genes in endothelia and recent data of transgenic animal models of HHT support the existence of a second hit that triggers the generation of these vascular lesions [40-42].

Loeys-Dietz syndrome (LSD) is an autosomal dominant genetic syndrome caused by mutations in the genes encoding *ALK5* (LSD type I) or *TGFBR2* (LSD type II) [43]. LDS is characterized by vascular findings (cerebral, thoracic, and abdominal arterial aneurysms and/or dissections)

and skeletal manifestations (pectus excavatum, pectus carinatum, scoliosis, arachnodactyly, joint laxity, talipes equinovarus). Approximately 75% of affected individuals have LDS type I with craniofacial manifestations (ocular hypertelorism, craniosynostosis, bifid uvula/cleft palate). LDS type II patients (25%) show cutaneous manifestations (velvety and translucent skin; easy bruising; widened, atrophic scars). The majority of identified mutations are either adjacent to or within the serine/threonine kinase domains of ALK5 and TGFBRII. In three quarters of LSD cases, the disorder is the result of a *de novo* gene mutation.

Familial thoracic aortic aneurysm syndrome (TADD) is an autosomal dominant disorder of large arteries. CV manifestations include dilatation of the aorta at the level of either the ascending aorta or the sinuses of Valsalva and aneurysms and dissections of the thoracic aorta involving either the ascending or descending aorta. Familial TAAD demonstrates genetic heterogeneity and the majority of individuals diagnosed have an affected parent. Two recurrent missense mutations affecting the kinase domain of TBRII that lead to familial TAAD have been described. *TGFBR2* mutations leading to aneurysms and dissections occur predominantly in the functionally important kinase domain and are predicted to cause loss of function [44].

Pulmonary Arterial Hypertension (PHA) is a disease characterized by elevated pulmonary artery pressure leading to right heart failure. PHA demonstrates abnormal remodeling of small peripheral resistance vessels in the lung, involving proliferation and migration of vascular smooth muscle cells, endothelial cells and fibroblasts. Two types of PHA have been described, sporadic or idiopathic PAH (IPAH) and hereditary or familial PAH (FPAH). Both IPAH and FPAH are genetically related with a heterozygous germ-line mutation in *BMPR2*. Mutations in the *BMPR2* have been associated with 80% of familial PAH and 15-40% of idiopathic PAH. Some specific mutations have been identified that affect the ligand binding, transmembrane or cytoplasmic domains of BMPR2. Genetic heterogeneity may occur in some cases of severe unexplained PAH. In this regard, mutations in *ALK1* have also been reported in a few HHT families with clinical and histological features of severe PAH, suggesting that mutations in *ALK1* may contribute to PHA [45,46].

Atherosclerosis is the primary cause of cardiovascular disease and stroke. Atherosclerosis development and progression is a complex process involving endothelial dysfunction, vascular inflammation, and accumulation of lipids and cellular debris within the intima of medium and large sized arteries, resulting in plaque formation and acute and chronic luminal obstruction. Several lines of research support a protective role of TGF-β in atherosclerosis. In animal models, deletion of a single *Tgfb1* allele gene or reduced availability of TGF-β1 leads to pro-atherogenic changes in the blood vessel wall. Conversely, viral delivery of TGF-β1 in an atherosclerotic mouse model (*Ldlr*$^{-/-}$) suppressed formation of atherosclerotic lesions. In humans, common genetic variants in the *TGFB1* promoter (such as A-800G and T-509C) have been associated with altered plasma levels of TGF-β1, which may contribute to atherosclerosis disease status). Activin, similarly to TGF-β1, appears to have a protective role in atherosclerosis development. By contrast, BMP2, BMP4 and BMP6 levels are increased in advanced atherosclerotic lesions and these elevations are associated with increased levels of calcification, suggesting a role for BMPs in promoting advanced atherosclerotic lesions [47,48]. Indeed, MGP (matrix GLA protein; inhibitor of BMP) transgenic or MGP-deficient mice have shown that inhibition of BMP protects animals from developing atherosclerosis and vascular calcifications [49].

Arterial occlusive diseases are treated by various open and endovascular approaches including endarterectomy, atherectomy, bypass graft, balloon angioplasty or stent angioplasty. Upon intervention, in a significant number of patients, restenosis displays an exuberant fibrotic reaction, intimal proliferation, and vascular remodeling often leading to limb loss or death [50]. A critical role for TGF-β in restenosis has been supported by several experimental approaches. Thus, overexpression of TGF-β1 in arteries induces neo-intimal hyperplasia and fibrosis [51] and animal models with targeted disruption of TGF-β prevent neointima formation and the constrictive remodeling associated with angioplasty [52]. Also, Smad7 overexpression attenuates collagen deposition, remodeling and contribution of adventitial fibroblasts to neointima formation after balloon angioplasty [53]. On the contrary, BMP4 may contribute to graft neointimal atrophy by inhibiting smooth muscle cells (SMC) proliferation and increasing SMC death in restenosis [54].

Hypertension is a predominant risk factor for stroke, coronary heart disease, arterial aneurysm and chronic kidney disease. In humans, polymorphisms in *TGFB1,* resulting in increased TGF-β1 expression, correlate with an elevation in arterial pressure Moreover, TGF-β neutralizing antibodies are sufficient to decrease blood pressure and subsequent renal failure in a rat hypertension model [37,55]. Activation of the renin-angiotensin-aldosterone system (RAAS) contributes to arterial hypertension. Interestingly, TGF-β signaling is upregulated by the RAAS axis and angiotensin converting enzyme (ACE) inhibitors and angiotensin I receptor antagonists reduce renal TGF-β1 production. Administration of TGF-β neutralizing antibodies and an ACE inhibitor, showed a synergistic effect inhibiting renal injury and proteinuria in rats [37,56]. BMP6 can enhance angiotensin II-induced aldosterone in human adrenocortical cells, suggesting that BMP6 may be involved in aldosterone breakdown induced by chronic treatment with angiotensin II receptors antagonists [57].

Preeclampsia is a systemic syndrome of pregnancy, clinically characterized by new onset of hypertension and proteinuria associated with significant morbidity and mortality to both mothers and fetuses. In these patients, plasma levels of soluble endoglin are upregulated and play a major pathogenic role, through an anti-angiogenic effect [41].

Heart disease involves cardiac remodeling, associated with cardiac hypertrophy and interstitial fibrosis, which alters the structural characteristics of the myocardium, leading to the loss of normal cardiac function. TGF-β1 is a pivotal modulator of cardiac remodeling by mediating cardiomyocyte growth, myofibroblast activation and ECM production, which underlie the development of myocardial fibrosis [37,58]. Multiple cardiac disorders have been linked to alterations in TGF-β/BMP signaling pathways. In humans, heterozygous loss-of-function mutations in the *GDF1* contribute to cardiac defects in **tetralogy of Fallot** (TOF) [59], while mutations in *TGFB3* have been related with the **arrhythmogenic right ventricular dysplasia-1** (ARVD) [60]. Moreover, TGF-β1 may act as a downstream target of angiotensin signaling, mediating Angiotensin II-induced hypertrophy Angiotensin II promotes cardiomyocyte and fibroblast proliferation concomitant with an increased expression of ECM proteins [37, 58] and clinical trials have documented the beneficial effects of angiotensin II inhibition in patients with myocardial infarction and heart failure. Of note, increased circulating levels of TGF-β1 are found in patients with dilated, hypertrophic and restrictive cardiomyopathy. Compatible with this finding, polymorphisms in *TGFB1* that result in increased TGF-β1 expression have been linked to dilated cardiomyopathy [61]. Similarly, a polymorphism in *BMP10*, which leads to increased

BMP10 levels, was identified in patients with dilated cardiomyopathy [62]. GDF15 has been involved in heart diseases and in vitro experiments suggest that GDF15 is a cardioprotective cytokine [63]. Patients with **non-ST-elevation acute coronary syndrome** (NSTE-ACS) have significantly elevated circulating levels of GDF15. In addition, patients who had died from a myocardial infarction show markedly enhanced GDF15 levels in the ischemic myocardium, suggesting that GFD15 may be a biomarker for heart diseases [64].

2.2. Fibrotic and inflammatory diseases. Abnormal TGF-β regulation and function are implicated in a growing number of fibrotic and inflammatory pathologies, including pulmonary fibrosis, liver cirrhosis, glomerulonephritis and diabetic nephropathy, congestive heart failure, rheumatoid arthritis, Marfan syndrome, hypertrophic scars, systemic sclerosis (SSc), myocarditis and Crohn's disease [37,65,66]. Multiple lines of evidence involve TGF-β as a critical regulator of both physiologic fibrogenesis and pathological fibrosis. The fibrotic reaction is characterized by an increased production of ECM components, such as fibronectin, collagen, laminin and vitronectin, as well as proliferation, migration and accumulation of mesenchymal cells. These processes result in the activation of local fibroblasts to differentiate into myofibroblasts, which are a specialized type of ECM producing cells [65]. An important effector of TGF-β-induced fibrosis is connective tissue growth factor (CTGF). TGF-β induces CTGF expression in fibroblasts, which promotes collagen synthesis and myofibroblast differentiation [65]. Moreover, CTGF binds directly to TGF-β, and enhances its activity resulting in increased binding to TBRI and TBRII [67]. Smad3 has been identified as an intracellular mediator of TGF-β-induced fibrosis. Indeed, TGF-β signaling through Smad3 directly promotes expression of type I collagen, a major component of the ECM, during fibrosis [68]; Smad3 is required for angiotensin II-induced vascular fibrosis; and *Smad3*$^{-/-}$ knockout mice are resistant to bleomycin- and TGF-β-mediated pulmonary fibrosis and to skin injury from ionizing radiation [69].

TGF-β plays a key role in pulmonary and hepatic fibrosis, not only through its ability to attract fibroblasts and stimulate their proliferation, but also through induction of EMT in alveolar epithelial cells and transdifferentiation of quiescent hepatic stellate cells into myofibroblasts, respectively [70]. In addition, the endothelial to mesenchymal transition may contribute to TGF-β-induced cardiac fibrosis [71,72]. Progression of diabetic nephropathy (DN) to end-stage kidney disease is manifested by the scarring of the renal glomerulus, followed by a fibrotic process in the tubulointerstitial region [73]. Elevated levels of the three isoforms of TGF-β are observed in glomerular and tubulointerstitial compartments of patients with established DN, suggesting that increased renal TGF-β levels closely correlate with the degree of mesangial matrix expansion, interstitial fibrosis, and renal insufficiency. Moreover, TGF-β1 was increased four-fold in the urine of diabetic versus non-diabetic patients, suggesting that overproduction of TGF-β1 in the kidneys, may contribute to DN [65,74].

2.3. Pulmonary diseases. The lung is the main organ of respiration in air-breathing animals. In mammals, the exchange of gases with the blood takes place in alveoli, which are hollow spherical outcroppings of the respiratory bronchioles. Disturbances to the alveolar architecture have serious consequences, as exemplified by human diseases such as **bronchopulmonary dysplasia** (BPD), **emphysema**, **chronic obstructive pulmonary disease** (COPD) and **asthma** [75,76].

BPD is a chronic lung disease in prematurely born infants, which is an important cause of morbidity and mortality. Patients who survive with BPD often show obstructive airway disease, pulmonary hypertension, and delay of growth and development [77]. Although TGF-β is an important mediator during development of the normal early lung patterning, excessive TGF-β signaling may negatively affect alveologenesis during pulmonary development. Thus, in premature babies with lung injury, the level of TGF-β in the bronchoalveolar lavage is increased and correlates with the severity of BPD. Furthermore, increased TGF-β levels have been observed in the peripheral areas of lungs from babies with BPD [76]. Also, overexpression of TGF-β1 in animal models induces a pathology that closely resembles BPD in human neonates [78]. These results suggest that TGF-β is a therapeutic target for the treatment of BPD.

COPD is the fourth leading cause of death in the developed world; it is characterized by irreversible airflow obstruction due to chronic bronchitis, emphysema, and/or small airways disease [76]. Genetic studies have identified TGF-β as a promising candidate gene related to COPD based on association analyses between SNPs in the TGFB1 gene and COPD phenotypes and a case-control study In addition, increased expression of TGF-β1 and decreased expression of the inhibitory Smad6 and Smad7 in the airway epithelium of patients with chronic bronchitis or COPD has been reported. Interestingly, increased TGF-β1 expression in airway epithelial cells from patients with COPD and smokers correlated with the burden of cigarette smoking, suggesting that TGF-β effects in airway remodeling and fibrosis may be provoked by cigarette smoke [75].

Asthma is a chronic inflammatory disorder of the airways characterized by structural changes (remodeling) of the airway wall. Airway remodelling is characterized by subepithelial fibrosis with thickening of basement membrane in areas of proximal airways. TGF-β1 signaling is increased in the lungs of asthmatics, which is in agreement with the increased activity of this cytokine in asthma pathogenesis. Conversely, experiments with animal models suggest that airway remodelling in asthma may be prevented or reversed using agents which block TGF-β signaling [75].

Idiopathic pulmonary fibrosis (IPF) is one form of **diffuse parenchymal lung diseases** (DPLD) characterized by progressive dyspnea and whose pathogenesis and mortality correlate with TGF-β1 levels. Although TGF‑β1 polymorphism does not predispose to the development of IPF, increased TGF-β1 levels play an important role in the progression of fibrosis and might cause shorter survival [79].

2.4. Bone and muscle diseases. Bones constitute a mineralized organ that plays key roles in human physiology providing mechanical support to the movement, regulating blood calcium levels, protecting various organ systems and sheltering hematopoiesis. Bone tissue is continuously remodeled by the mineralization activity of osteoblasts and the bone resorbing activity of osteoclasts [80]. TGF-β superfamily members are abundantly expressed in the bone environment where they regulate important processes. TGF-β1 has been implicated in the regulation of osteoblast proliferation, differentiation and apoptosis. Survivors of TGF-β1 knock-out mice have reduced bone growth and mineralization, as well as decreased serum levels of bone alkaline phosphatase, a bone turnover marker [37]. Severe anomalies in bone development were observed in BMP2 and BMP4 knockout mice [81] and mice lacking *Acvr2* or *Acvr2b* gene show multiple bone defects. Some *Acvr2*-null mice exhibit hypoplasia of the mandible and other

skeletal abnormalities [8]. *Bmpr1b*-deficient *Gdf5*-null mice are viable, but exhibit short limbs and abnormal digit cartilage [82]. In humans, genetic studies have shown the involvement of TGF-β superfamily signaling in several hereditary diseases affecting bone and muscle [37].

Camurati-Engelmann disease (CED) is an autosomal dominant disorder characterized by hyperostosis of the long bones and the skull, proximal muscle weakness, severe limb pain, a wide-based waddling gait, and joint contractures. More than 90% of CED patients have mutations in *TGFB1*. The majority of these mutations lead to single amino-acid substitutions in the carboxy terminus of TGF-β1 LAP, which may disrupt LAP dimerization and binding to active TGF-β1, leading to increased active TGF-β1 release from the cell and TGF-β-mediated transcription [78,83].

Osteoporosis is the most common age-related skeletal chronic disorder, characterized by reduced bone mass and increased risk of low-trauma fractures. Fragility fractures in osteoporosis represent a major cause of morbidity and mortality. *TGFB1* is a candidate target gene in osteoporosis with relevant polymorphisms located in the promoter region (-1348C/T and -509C/T) and in exon 1 (29T/C, L10P and 74G/C, R25P). Additional single-nucleotide polymorphisms associated with osteoporosis have been identified in *TGFBR1*, *TGFBR2*, *SMAD2*, *SMAD3*, *SMAD4* and *SMAD7* [84,85].

Brachydactyly type A2 (BDA) is an autosomal dominant malformation characterized by shortening and deviation of the index fingers and the first and second toes. Mutations in the GS or kinase domains of the *BMPR1B* gene, acting in dominant-negative manner, are responsible for the bone malformation in BDA [86]. Mutations in *GDF5* cause **Brachydactyly type C** (BDC), an autosomal dominant disorder characterized by an abnormal shortness of the fingers and toes defects [87] as well as **acromesomelic chondrodysplasia Hunter-Thompson** type (AMDH), an autosomal recessive form of dwarfism, characterized by normal axial skeletons and missing or fused skeletal elements within the hands and feet. Also, a mutation in GDF5 (R438L) is responsible for **proximal symphalangism**, showing fusion of carpal and tarsal bones and ankylosis of the proximal interphalangeal joints. **Du Pan Syndrome** is originated by defects in GDF5. This syndrome, also known as fibular hypoplasia and complex brachydactyly is a rare autosomal recessive condition characterized by absence of the fibulae and severe acromesomelic limb shortening with small, non-functional toes [88].

Mutations in ALK2 cause **fibrodysplasia ossificans progressiva** (FOP), an autosomal dominant disorder with skeletal malformations and progressive ossification in muscular tissues [89]. A heterozygous mutation in ALK2 (R206H) is frequently found in individuals with FOP and results in hyperactivation of the ALK2 kinase. Interestingly, a transgenic mouse model, expressing constitutively active (ca) ALK2 in muscle, mimics the phenotype of human FOP and intramuscular expression of caALK2 results in ectopic bone formation, joint fusion and functional impairment [90].

A heterozygous mutation in *GDF6* is responsible for the autosomal dominant **Klippel-Feil syndrome** [91], characterized by fusion of vertebral bodies C2 and C3. **Spondylocostal dystostosis** 4 (SCDO4) presents hemivertebrae and rib malformations, as well as heterozygosity for a mutant GDF6 allele, predicted to result in K424R substitution at a highly conserved residue in the propeptide domain [92].

Skeletal muscle is a form of striated muscle tissue existing under control of the somatic nervous system. GDF8/Myostatin is highly expressed in skeletal muscle and has been implicated in human muscle diseases characterized by fibrosis such as muscular dystrophy [93]. **Duchenne muscular dystrophy** (DMD) is the most common inherited lethal myopathy. DMD patients lose their ability to walk by the age of 12 years and die during their twenties due to either cardiac or respiratory failure. The disorder is caused by pathogenic mutations in the dystrophin gene, but additional polymorphisms in *GDF8/Myostatin* were identified in DMD patients [94]. GDF8/Myostatin is a muscle-specific ligand that negatively regulates muscle growth. Accordingly, *GDF8/Myostatin* mutations are associated with gross muscle hypertrophy [95] and disruption of endogenous *GDF8/Myostatin* by gene targeting in mice results in increased muscle mass and stronger muscle [96]. These observations suggest that myostatin is a potential therapeutic target for treating the symptoms in muscular dystrophy patients.

2.5. Reproductive disorders. TGF-β superfamily members play critical roles in the female reproductive system, regulating ovarian follicle development, primordial follicle recruitment, gonadotropin receptor expression, granulose and theca cell proliferation, oocyte maturation, ovulation, luteinization, and corpus luteum formation [97]. Not surprisingly, deregulation of TGF-β superfamily signaling results in several reproductive disorders.

Premature ovarian failure (POF) is characterized by cessation of menstruation for at least 4 months and symptoms of hypoestrogenism and elevated gonadotropins before the age of 40 years. POF occurs in up to 1% of women and is a common cause of infertility [98]. BMP15 is crucial in folliculogenesis and follicle differentiation in mammals [99]. A heterozygous mutation in *BMP15* has been identified in two sisters with primary amenorrhoea and the mutant BMP15 protein (Y235C) antagonizes the activity of normal BMP15 leading to a decreased proliferation of granulosa cells [100]. Additional *BMP15* variants have been identified in POF patients [101]. Most of these mutations involve the propeptide region and lead to a defective production of bioactive BMP15, providing further support for their implication in the development of POF. Moreover, inhibin-A is one of the most important regulators of the female reproductive cycle [102] and one mutation in the inhibin alpha gene (*INHA*) has been identified in patients with FOP, resulting in an inactive mutant protein (A257T).

The **persistent Mullerian duct syndrome** (PMDS) is a rare form of inherited male pseudohermaphroditism characterized by the presence a uterus and sometimes other Müllerian duct derivatives in otherwise normally masculinized XY males [12]. The phenotype can be produced by a mutation in the gene encoding anti-mullerian hormone (AMH, PMDS-I) or by a mutation in the AMH receptor (PMDS-II). Mutations in either *AMH* or *AMHR2* produce indistinguishable clinical symptoms. AMH is expressed almost exclusively by the somatic cells of the gonads from both sexes. In males, AMH is highly expressed by Sertoli cells from their differentiation to the onset of puberty, whereas in females, AMH is synthesized by granulose cells of growing follicles from birth to menopause [13]. In PMDS-I, markedly low levels of circulating AMH are observed, due to homozygous or heterozygous mutations. In PMDS-II, mutations in *AMHR2* may lead to a soluble unstable receptor or to the disruption of the substrate-binding site in the kinase domain [13].

2.6. TGF-β in Cancer. TGF-β has a tumor suppressive role at early stages of tumor development by virtue of its potent growth inhibitory effect on epithelial and lymphoid tissues, from which most human cancers arise. In addition to the control of cell cycle, TGF-β exerts other

type of effects in individual cells and tissues in order to protect them from tumorigenesis. For example, TGF-β induces either apoptosis or replicative senescence depending on the cell type, and inhibits the production of paracrine mitogenic factors by stromal cells. It is also involved in the maintenance of genomic stability and, even, in the preservation of the normal tissue architecture; i.e., in the colon [103]. However, tumor cells evade the TGF-β suppressive action by different mechanisms and, paradoxically, TGF-β becomes a pro-oncogenic factor that stimulates tumor cell growth and invasiveness at later stages of tumorigenesis. The pro-oncogenic activities of TGF-β are exerted at both compartments, the tumor and the stroma. Thus, TGF-β stimulates tumor cell proliferation by inducing the production of autocrine mitogenic growth factors, such as platelet-derived growth factor B (PDGF-B). It also induces epithelial-mesenchymal transitions associated with the acquisition of motility and invasive properties, and promotes the formation of distal metastasis by a variety of mechanisms [104]. In addition, in the tumor stroma, TGF-β stimulates the generation of myofibroblasts from mesenchymal precursors, the so called cancer-associated fibroblasts that facilitate tumor cell proliferation, invasion and promote neoangiogenesis. Also, by its immunosuppressive actions TGF-β helps cancer cells to evade the immune surveillance. There are recent and excellent reviews addressing the wide range of TGF-β roles in cancer as well as the molecular basis involved [105-107], and, therefore, these topics will not be discussed herein.

Several components of the TGF-β signalling pathway are inactivated in subsets of pancreatic, colorectal, gastric, ovarian, and head and neck tumors, which disable the tumor suppressive action of TGF-β [108]. In individuals with familial syndromes, germline mutations in some of these tumor suppressor genes have also been found. In this article, we only will review germline inactivation of components of the TGF-β pathway that predispose to cancer. Interestingly, all the familial syndromes bearing mutations in genes of the TGF-β/BMP system predispose to colorectal or gastric cancer.

Hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome is an autosomal dominant disorder caused by mutations in the DNA mismatch repair system, mainly the MLH1 and MSH2 genes, that leads to replication errors and hence microsatellite instability (MSI). HNPCC accounts for 1-6% of the total colorectal cancer incidence worldwide [109]. MSI predominantly affects mono, di- and tri-nucleotide tracts and short sequence repeats by accumulating mutations at these repeats. The TGFBR2 gene contains such “microsatellite-like” sequences in exon 3 that encodes a 10-base pair polyadenine repeat in TBRII (BAT-RII). BAT-RII mutations that lead to a truncated receptor lacking the transmembrane and the intracellular kinase domains have been identified at a high frequency in HNPCC [110] and other MSI-associated cancers [108].

Juvenile polyposis syndrome (JPS) is also an autosomal dominant syndrome characterized by multiple hamartomatous polyps occurring throughout the gastrointestinal tract. Unlike adenomatous polyps, hamartomatous polyps (dilated cystic glands with retention of mucus) are considered to be non-neoplastic. However, JPS is associated with increased risk of developing gastric, colorectal and pancreatic malignancies [111]. Two genes of the TGF-β/BMP signalling system, MADH4 and BMPRIA, encoding Smad4 and a transmembrane type I receptor for BMPs (ALK3), respectively, are responsible for JPS. Germline BMPRIA and MADH4 mutations account each for about 20% of JPS cases. MADH4 mutations are clustered in the region encoding the protein MH2 domain involved in Smad oligomerization. In BMPRIA, most

alterations are microdeletions or non-sense mutations that lead to the synthesis of a truncated protein [108]. Germline mutations in the ENG gene encoding the TGF-β co-receptor endoglin have also been found at low frequency in JPS patients who do not have BMPRIA and MADH4 mutations. Nevertheless, there is no consensus to consider ENG as a JPS susceptibility gene [112, 113].

Hereditary mixed polyposis syndrome (HMPS) is another autosomal dominantly inherited syndrome characterized by mixed "adenomatous/hyperplastic/atypical juvenile" polyps. Germline BMPRIA mutations have also been found in some HMPS families. The fact that BMPRIA mutations are also involved in JPS, and that JPS and HMPS are two well defined clinical entities, suggests that inactivation of the type I BMP receptor is the initiating event for both disorders that predispose to colorectal tumorigenesis [109].

3. Therapeutic interventions targeting the TGF-β signaling pathway. As explained above, the TGF-β signaling pathway is an attractive target for therapy in a number of diseases, including fibrotic and cardiovascular diseases or cancer. Depending on the specific disease, the therapeutic approach may involve the inhibition of the pathway or its enhancement. Inhibition treatments include ligand traps, such as ligand specific neutralizing antibodies, soluble ligand receptors, antisense-dependent silencing of ligands and chemical inhibitors that block kinase activity of TGF-β family members receptors. Conversely, increased ligand-dependent signaling may be beneficial for therapeutic purposes as it is the case of recombinant human BMPs that activate the bone regenerative properties of the BMP pathway [114]. A summary of the current clinical trials is shown in Table 3 and Figure 3. Further information can be obtained from the NIH webpage (http://clinicaltrials.gov).

3.1. Ligands traps.

3.1.1. Neutralizing antibodies. The pan-TGF-β antibody GC-1008 (Genzyme Inc.) was tested in a phase I clinical study of patients with renal cell carcinoma or malignant melanoma (NCT00356460). Treatment was well tolerated with mainly grade 1-2 toxicity including skin rash, fatigue, headache and gastrointestinal symptoms. Some patients achieved stable disease or improved [115]. A phase II trial is ongoing for the treatment with GC-1008 of Pleural Malignant Mesothelioma (NCT01112293) and a phase I clinical study has been completed for the treatment of Focal Segmental Glomerulosclerosis (NCT00464321), which is associated with nephrotic syndrome in children and adolescents and it is an important cause of kidney failure in adults. Moreover, GC-1008 is ready to enter in phase I trials for idiopathic pulmonary fibrosis.

3.1.2. Antisense. Another approach which has entered clinical trials inhibits TGF-β function by means of antisense oligonucleotides (AS-ODNs). The anti-tumorigenic effect of AS-ODNs was supported by phase I/II trials with the *TGFB2* antisense compound AP12009 (Trabedersen; Antisense Pharma). In comparison to standard chemotherapy, treatment with AP12009 resulted in prolonged survival of patients with anaplastic astrocytoma, and a phase III is ongoing (NCT00761280) [116]. Accordingly, patients with high-grade glioma showed a higher survival rate at 24 months and showed significantly more responders after 14 months when treated with AP12009 as compared to standard chemotherapy protocols; a phase II trial is completed (NCT00431561). Another phase I study (NCT00844064) to evaluate the treatment with AP12009 of pancreatic neoplasms, melanoma and colorectal neoplasms is ongoing.

A phase II trial with Lucanix (NovaRx Corporation), a TGF-β2 antisense gene-modified allogeneic tumor vaccine, is completed in patients with advanced non-small cell lung cancer (NCT01058785) and a phase III is ongoing (NCT00676507). Interestingly, a novel approach is being tested in a phase I trial (NCT00684294), using a combination of GMCSF and antisense *TGFB2* autologous tumor cell vaccine for the treatment of Advanced Metastatic Carcinoma. Is it expected that GMCSF overexpression stimulates expression of tumor antigens in tumor cells and dendritic cell migration, whereas TGF-β2 blockade by *TGFB2* antisense may allow for a better immune response at the vaccine site.

3.1.3. Soluble receptors. TGF-β1 is one of the main mediators in the fibrotic process, associated to both scarring and a long list of pathologies related to chronic inflammation affecting several organs and tissues. ISDIN has developed a 14mer peptide (P144) from human betaglycan designed to block the interaction between TGF-β1 and the signaling receptors, thus modulating TGF-β1 biological effects. Two phase II clinical studies are ongoing for the treatment of skin fibrosis in Systemic Sclerosis (NCT00574613 and NCT00781053).

Aceleron-Pharma has generated a recombinant fusion protein by joining a portion of the human ActRIIB receptor to a portion of the human immunoglobulin (ACE-031). This creates a decoy version of ActRIIB which interferes with ligands, such as GDF8/Myostatin, thus allowing the muscle growth [117]. A phase II trial is ongoing in patients with Duchenne Muscular Dystrophy (NCT01099761), and a phase I trial is ongoing in healthy postmenopausal women with unexplained weight loss (NCT00952887).

3.1.4. Receptors inhibitors. Most of the current strategies to inhibit TGF-β signaling at the receptor kinase level include the use of small molecule inhibitors, which bind to the ATP binding domain of the receptors [118]. A number of companies have developed ATP-mimetic drugs that target the kinase catalytic site of TBRI/ALK5. Although these small inhibitors are not completely specific, they are very effective at inhibiting Smad2/3 phosphorylation. Preclinical studies in vitro and in vivo have shown the efficacy of these compounds in prevention and cure of several experimental diseases. The TBRI/ALK5 and TBRII dual inhibitor LY2157299 is now in phase I trial in patients with metastatic malignancies to determine the safety and pharmacokinetics of the compound.

An interesting approach is ongoing in a phase I trial to relapse Epstein-Barr virus (EBV)-positive lymphoma (NCT00368082). Autologous or allogeneic latent membrane protein (LMP)-specific cytotoxic T-cells have been retroviral genetic modified to express a dominant negative TBRII (DNRII) to render them resistant to the immunosuppressive effects of TGF-β.

Tumors require new blood vessels to support their ability to grow and metastasize. New treatments aimed at preventing these blood vessels have the ability to improve the clinical management of cancer. Since its expression is mostly restricted to endothelial cells, ALK1 and endoglin represent promising targets for anti-angiogenic therapies in cancer [18,36]. Although no in vitro data about specific ALK1 inhibitors have been published so far, a clinical phase I study testing a human anti-ALK1 antibody PF-03446962 (Pfizer) in patients with advanced solid tumors is ongoing (NCT00557856). A phase I trial using a human/murine chimeric anti-endoglin monoclonal antibody TRC105 (Tracon Pharmaceuticals Inc.) in patients with solid cancer is ongoing (NCT00582985). Treatment was well tolerated with mainly grade 1-2 toxicity including fatigue, anemia, proteinuria and diarrhea. One patient with hormone refractory prostate cancer

obtained a complete prostate-specific antigen (PSA) response and 3 patients had prolonged stable disease [119].

When increased BMP signaling contributes to disease pathogenesis, inhibitors may offer therapeutic benefit, as it is the case of fibrodysplasia ossificans progressiva (FOP). Inhibition of Smad phosphorylation by BMPR-I intracellular kinase domains with small molecules may provide more efficient signal transduction pathway inhibition. Preclinical studies have shown the efficacy of Dorsomorphin, which selectively inhibits BMP signaling from ALK1, ALK2, BMPR-IA/ALK3 and BMPR-IB/ALK6, and blocks BMP-induced Smad1/5/8 phosphorylation Also, Dorsomorphin optimized derivatives LDN-193189 or DM-3189 with higher activity and specificity for BMP type I receptors have been developed LDN-193189 has shown promising results in a mouse model of FOP; it inhibits activation of Smad1/5/8 induced by the caALK2, leading to a reduction in ectopic ossification and functional impairment in mice [8].

3.2. Recombinant ligands. Based on animal studies demonstrating dramatic increases in fusion mass and quality of bone regeneration using BMPs [8], clinical trials in humans have been initiated. Although animal models have shown osteo-induction with various BMPs, including recombinant human BMP2, BMP7 and BMP9, as well as whole BMP extract from human bone, clinical trials have primarily been limited to BMP2 and BMP7 (rhOP-1). BMP2 is being used in the treatment phase I of degenerative Lumbar Disc Disease, Spondylolisthesis and Spinal Stenosis (NCT00405600); in phase II of Degenerative Disc Disease (NCT00707265); in phase I Osteoarthritis (NCT00243295); and in Osteoporosis (NCT00752557). A phase II trial is ongoing for the treatment of Osteoarthritis of the Knee with BMP7 (NCT0111104). In addition, two clinical studies in phase II are in progress by using GDF5/BMP14 for the treatment of Degenerative Disc Disease (NCT00813813; NCT01124006), and one in phase I/II for the treatment of early Lumbar Disc Degeneration (NCT01158924).

3.3. Other preclinical approaches to regulate TGF-β signaling. Disruption of the intracellular Smad signaling may become a relevant strategy to control TGF-β superfamily signaling. Several preclinical studies have assessed the efficacy of endogenous/synthetic Smad inhibitors, Smad sequestration or targeting degradation in several diseases in vitro and in vivo [118]. Hepatocyte growth factor (HGF) exerts anti-fibrotic effects by opposing TGF-β1/Smad signaling. Induction of the inhibitory Smad7 by HGF, treatment with the HGF synthetic analog BB3 (Angion Biomedica Corp) and gene transfer of Smad7 showed antifibrotic properties in animal models [120,121]. Also, Paclitaxel/Taxol, an anticancer drug that stabilizes the microtubules, attenuated hepatic fibrosis by inhibiting TGF-β signaling [122].

In addition to Smads, other signalling pathways downstream of TGF-β provide novel opportunities for TGF-β-targeting therapies. This is the case of the protein tyrosine kinase c-Abelson (c-Abl) that is activated by TGF-β in fibroblasts and mediates some of the Smad-independent profibrotic effects. In Systemic Sclerosis c-Abl was found to be constitutively phosphorylated in the skin lesions of patients. Interestingly, Imatinib has been shown to block the induction of c-Abl activity and fibrotic gene responses elicited by TGF-β in explanted Systemic Sclerosis fibroblasts. Moreover, the anti-TGF-β effects of Imatinib are also associated with the inhibition of Smad1 activation [118].

A growing interest exists in using commonly used drugs for anti-TGF-β therapy (Table 3). For example, the anti-hypertensive drug Losartan, an angiotensin II receptor blocker, has been

reported to antagonize TGF-β signaling through inhibition of the renin-angiotensin axis. Accordingly, Losartan reduced aortic wall thickness by suppression of local TGF-β signaling in a mouse model of Marfan syndrome. A pathogenic role for excess TGF-β1 levels in diabetic nephropathy has been postulated. In order to counteract the excess of TGF-β in this disease, a clinical study using the ACE inhibitor captopril was carried out. Serum TGF-β1 levels decreased significantly in the captopril-treated group. Furthermore, the captopril-treated patients showed a better preserved renal function. These results suggest that TGF-β1 plays a pivotal role in the progression of diabetic nephropathy and that, by lowering TGF-β1 production, the ACE inhibitor therapy may protect the kidney. Morover, Tranilast, which is currently used for the treatment of asthma, allergic rhinitis and atopic dermatitis, inhibiting mast cell degranulation, has shown a potent anti-fibrotic effect in sclerotic fibroblasts and in animal models of fibrosis. In addition, Tranilast showed beneficial effects preventing strictures progression in the treatment of Crohn's disease [66]. These effects are likely mediated by the Tranilast inhibition of TGF-β1 secretion and Smad activation.

Another line of investigation is based on histone deacetylase (HDAC) inhibitors. In cancer, the loss of TGF-β signaling occurs early in carcinogenesis and contributes to tumor progression. The loss of TGF-β responsiveness frequently involves the *TGFBR2,* whose expression is silenced through epigenetic mechanisms. Thus, re-expression of *TGFBR2,* by using HDAC inhibitors as epigenetic therapies, aims to activate the tumor suppressive role of the TGF-β signal pathway. Indeed, the treatment of cancer cell lines resistant to TGF-β-induced growth inhibition, with HDAC inhibitors 5 aza-2′ deoxycytidine, MS275, TSA and sodium butyrate successfully restores the expression of *TGFBR2* [123].

Conclusions

The core molecular components of the TGF-β/Receptors/SMAD signaling pathway and the phylogenetically conserved mechanisms of intracellular signal transduction have been well characterized. Nonetheless, one of the current challenges is to understand the differential functional responses of cell context-dependent inputs into the core pathway. A better comprehension as to how modulatory stimuli shape a functional cell response will require a further detailed analysis of the endogenous signaling *in vivo* and *in vitro*, including the ligand bioavailability and sensing by receptors as well as the characterization of possible cross-talks with other signaling pathways. These studies may provide novel molecular targets for treatment of diseases caused by malfunctioning of the TGF-β signaling pathway such as cardiovascular, fibrosis, reproductive, cancer or wound-healing disorders. In addition, identification of small molecules (inhibitors and potentially activators) of TGF-β signaling using high-content screenings raises new hopes for future therapeutic interventions.

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REFERENCES

1 Anzano, M. A., Roberts, A. B., Smith, J. M., Sporn, M. B. and De Larco, J. E. (1983) Sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type alpha and type beta transforming growth factors. Proc. Natl. Acad. Sci. USA **80**, 6264–6268

2 Sporn, M. B. (2006) The early history of TGF-β, and a brief glimpse of its future. Cytokine & Growth Factor Reviews **17**, 3-7

3 Govinden, R. and Bhoola, K.D. (2003) Genealogy, expression, and cellular function of transforming growth factor-beta. Pharmacol. Ther. **98**, 257-65

4 Funkenstein, B., Olekh, E. and Jakowlew, S. B. (2010) Identification of a novel transforming growth factor-beta (TGF-beta6) gene in fish: regulation in skeletal muscle by nutritional state. BMC Mol. Biol. **11**, 37-53

5 Roberts, A. B. (1998) Molecular and cell biology of TGF-β. Miner. Electrolyte Metab. **24**, 111-119

6 Dubois, C. M., Laprise, M. H., Blanchette, F., Gentry, L. E. and Leduc, R. (1995) Processing of transforming growth factor β1 precursor by human furin convertase. J. Biol. Chem. **270**, 10618-10624

7 Rifkin, D. B. (2005) Latent transforming growth factor-β (TGF-β) binding proteins: orchestrators of TGF-β availability. J. Biol. Chem. **280**, 7409-7412

8 Miyazono, K., Kamiya, Y. and Morikawa, M. (2010) Bone morphogenetic protein receptors and signal transduction. J. Biochem. **147**, 35-51

9 Schier, A. F. (2009) Nodal morphogens. Cold Spring Harb. Perspect. Biol. **1**, a003459

10 Lee, S. J. (1990) Identification of a novel member (GDF-1) of the transforming growth factor-β superfamily. Mol. Endocrinol. **4**, 1034–1040

11 Moustakas, A. and Heldin, C. H. (2009) The regulation of TGFbeta signal transduction. Development. **136**, 3699-3714

12 Josso, N., Belville, C., di Clemente, N. and Picard, J.Y. (2005) AMH and AMH receptor defects in persistent Müllerian duct syndrome. Hum. Reprod. Update **11**, 351-356

13 di Clemente, N. and Belville, C. (2006) Anti-Mullerian hormone receptor defect. Best Pract. Res. Clin. Endocrinol. Metab. **20**, 599-610

14 Xia, Y. and Schneyer, A. L. (2009) The biology of activin: recent advances in structure, regulation and function. J. Endocrinol. **202**, 1-12

15 Stenvers, K. L. and Findlay, J. K. (2010) Inhibins: from reproductive hormones to tumor suppressors. Trends Endocrinol. Metab. **21**, 174-180

16 Shi, Y. and Massagué, J. (2003) Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell **113**, 685-700

17 Gatza, C. E., Oh, S. Y. and Blobe, G. C. (2010) Roles for the type III TGF-beta receptor in human cancer. Cell Signal **22**, 1163-1174

18 Bernabeu, C., Lopez-Novoa, J. M. and Quintanilla, M. (2009) The emerging role of TGF-beta superfamily coreceptors in cancer. Biochim Biophys Acta **1792**, 954-973

19 Kang, J. S., Liu, C. and Derynck, R. (2009) New regulatory mechanisms of TGF-beta receptor function. Trends Cell Biol. **19**, 385-394

20 Lewis, K. A., Gray, P. C., Blount, A. L., MacConell, L. A., Wiater, E., Bilezikjian, L. M. and Vale, W. (2000) Betaglycan binds inhibin and can mediate functional antagonism of activin signalling. Nature **404**, 411-414

21 Santibanez, J. F., Letamendia, A., Perez-Barriocanal, F., Silvestri, C., Saura, M., Vary, CP., Lopez-Novoa, J. M., Attisano, L. and Bernabeu, C. (2007) Endoglin increases eNOS expression by modulating Smad2 protein levels and Smad2-dependent TGF-beta signaling. J. Cell Physiol. **210**, 456-468

22 Blanco, F.J., Santibanez, J. F., Guerrero-Esteo, M., Langa, C., Vary, C. P, and Bernabeu, C. (2005) Interaction and functional interplay between endoglin and ALK-1, two components of the endothelial transforming growth factor-beta receptor complex. J. Cell Physiol. **204**, 574-584

23 Jovine, L., Qi, H., Williams, Z., Litscher, E. and Wassarman, P. M. (2002) The ZP domain is a conserved module for polymerization of extracellular proteins. Nat. Cell Biol. **4**, 457-461

24 Llorca, O., Trujillo, A., Blanco, F. J. and Bernabeu, C. (2007) Structural model of human endoglin, a transmembrane receptor responsible for hereditary hemorrhagic telangiectasia. J. Mol. Biol. **365**, 694-705

25 Huminiecki, L., Goldovsky, L., Freilich, S., Moustakas, A., Ouzounis, C. and Heldin, C. H. (2009) Emergence, development and diversification of the TGF-b signalling pathway within the animal kingdom. BMC Evol. Biol. **9**, 28

26 Ross, S. and Hill, C. S. (2008) How the Smads regulate transcription. Int. J. Biochem. Cell Biol. **40**, 383-408.

27 Itoh, S. and ten Dijke, P. (2007) Negative regulation of TGF-beta receptor/Smad signal transduction. Curr. Opin. Cell Biol. **19**, 176-184

28 Derynck, R. and Akhurst, R. J. (2007) Differentiation plasticity regulated by TGF-beta family proteins in development and disease. Nat. Cell Biol. **9**, 1000-1004

29 Corradini, E., Babitt, J. L. and Lin, H. Y. (2009) The RGM/DRAGON family of BMP co-receptors. Cytokine Growth Factor Rev. **20**, 389-398

30 Glinka, Y., Stoilova, S., Mohammed, N. and Prud'homme, G. J. (2010) Neuropilin-1 exerts coreceptor function for TGF-beta-1 on the membrane of cancer cells and enhances responses to both latent and active TGF-beta. Carcinogenesis 2010 Dec 24. [Epub ahead of print].

31 Bizet, A. A., Liu, K., Tran-Khanh, N., Saksena, A., Vorstenbosch, J., Finnson, K. W., Buschmann, M. D. and Philip, A. (2011) The TGF-β co-receptor, CD109, promotes internalization and degradation of TGF-β receptors. Biochim. Biophys. Acta. 2011 Feb 2. [Epub ahead of print].

32 Di Guglielmo, G. M., Le Roy, C., Goodfellow, A. F. and Wrana, J. L. (2003) Distinct endocytic pathways regulate TGF-β receptor signalling and turnover. Nat. Cell Biol. **5**, 410-421

33 Santibanez, J. F., Blanco, F. J., Garrido-Martin, E. M., Sanz-Rodriguez, F., del Pozo, M. A. and Bernabeu, C. (2008) Caveolin-1 interacts and cooperates with the transforming growth factor-beta type I receptor ALK1 in endothelial caveolae. Cardiovasc. Res. **77**, 791-799

34 Zhang, Y. E. (2009) Non-Smad pathways in TGF-β signaling. Cell Res. **19**, 128-139

35 Sánchez-Elsner, T., Botella, L. M., Velasco, B., Corbí, A., Attisano, L. and Bernabéu, C. (2001) Synergistic cooperation between hypoxia and transforming growth factor-beta pathways on human vascular endothelial growth factor gene expression. J. Biol. Chem. **276**, 38527-38535

36 Pardali, E., Goumans, M. J. and ten Dijke, P. (2010) Signaling by members of the TGF-beta family in vascular morphogenesis and disease. Trends Cell Biol. **20**, 556-567

37 Gordon, K. J. and Blobe, G. C. (2008) Role of transforming growth factor-beta superfamily signaling pathways in human disease. Biochim. Biophys. Acta **1782**, 197-228

38 Gallione C, Aylsworth, A. S., Beis, J., Berk, T., Bernhardt, B., Clark, R. D., Clericuzio, C., Danesino, C., Drautz, J., Fahl, J. et al. (2010) Overlapping spectra of SMAD4 mutations in juvenile polyposis (JP) and JP-HHT syndrome. Am. J. Med. Genet. A. **152A**, 333-339

39 Bayrak-Toydemir, P., McDonald, J., Akarsu, N., Toydemir, R. M., Calderon, F., Tuncali, T., Tang, W., Miller, F. and Mao, R. (2006) A fourth locus for hereditary hemorrhagic telangiectasia maps to chromosome 7. Am. J. Med. Genet. A. **140**, 2155-2162

40 Shovlin, C.L. (2010) Hereditary haemorrhagic telangiectasia: pathophysiology, diagnosis and treatment. Blood Rev. **24**, 203-219.

41 López-Novoa, J. M. and Bernabeu, C. (2010) The physiological role of endoglin in the cardiovascular system. Am. J. Physiol. Heart Circ. Physiol. **299**, H959-H974

42 Abdalla, S. A. and Letarte, M. (2006) Hereditary haemorrhagic telangiectasia: current views on genetics and mechanisms of disease. J. Med. Genet. **43**, 97-110

43 Loeys, B. L., Chen, J., Neptune, E. R., Judge, D. P., Podowski, M., Holm, T., Meyers, J., Leitch, C. C., Katsanis, N., Sharifi, N. et al. (2005) A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2, Nat. Genet. **37**, 275–281

44 Pannu, H., Fadulu, V. T., Chang, J., Lafont, A., Hasham, S. N., Sparks, E., Giampietro, P. F., Zaleski, C., Estrera, A.L., Safi, H.J. et al. (2005) Mutations in transforming growth factor-beta receptor type II cause familial thoracic aortic aneurysms and dissections. Circulation **112**, 513–520

45 Eickelberg, O. and Morty, R. E. (2007) Transforming Growth Factor β/Bone Morphogenic Protein Signaling in Pulmonary Arterial Hypertension: Remodeling Revisited. Trends Cardiovasc. Med. **17**, 263–269

46 Machado, R. D., Aldred, M. A., James, V., Harrison, R. E., Patel, B., Schwalbe, E. C, Gruenig, E., Janssen, B., Koehler, R., Seeger, W. et al. (2006) Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension, Hum. Mutat. **27**, 121–132

47 Grainger, D. J. (2004) Transforming growth factor beta and atherosclerosis: so far, so good for the protective cytokine hypothesis. Arterioscler. Thromb. Vasc. Biol. **24**, 399-404

48 Grainger, D. J. (2007) TGF-beta and atherosclerosis in man. Cardiovasc. Res. **74**, 213-222

49 Yao, Y., Bennett, B. J.,Wang, X., Rosenfeld, M. E., Giachelli, C., Lusis A. J. and Boström K. J. (2010) Inhibition of Bone Morphogenetic Proteins Protects Against Atherosclerosis and Vascular Calcification. Circ. Res. **107**, 485-494

50 Bauters, C. and Isner, J. M. (1997) The biology of restenosis, Prog. Cardiovasc. Dis. **40**, 107–116

51 Nabel, G., Shum, L., Pompili, V. J., Yang, Z. Y., San, H., Shu, H. B., Liptay, S., Gold, L., Gordon, D., Derynck, R. and Nabel G. J. (1993) Direct transfer of transforming growth factor beta 1 gene into arteries stimulates fibrocellular hyperplasia. Proc. Natl. Acad. Sci. USA **90**, 10759–10763

52 Ryan, S. T., Koteliansky, V. E., Gotwals, P. J. and Lindner, V. (2003) Transforming growth factor-beta-dependent events in vascular remodeling following arterial injury. J. Vasc. Res. **40**, 37-46

53 Mallawaarachchi, C. M, Weissberg, P. L. and Siow, R. C. (2005) Smad7 gene transfer attenuates adventitial cell migration and vascular remodeling after balloon injury. Arterioscler. Thromb. Vasc. Biol. **25**, 1383–1387

54 Hsieh, P. C. H., Kenagy, R. D., Mulvihill, E. R., Jeanette, J. P., Wang, X., Chang C. M., Yao, Z., Ruzzo, W. L., Justice, S., Hudkins, K. L. et al. (2006) Bone morphogenetic protein 4: Potential regulator of shear stress-induced graft neointimal atrophy. J. Vasc. Surg. **43**, 150-158

55 Lavoie, P., Robitaille, G., Agharazii, M., Ledbetter, S., Lebel, M. and Larivière R. (2005) Neutralization of transforming growth factor-beta attenuates hypertension and prevents renal injury in uremic rats. J. Hypertens. **23**, 1895–1903

56 Benigni, A., Zoja, C., Corna, D., Zatelli, C., Conti, S., Campana, M., Gagliardini, E., Rottoli, D., Zanchi, C., Abbate, M. et al. (2003) Add-on anti-TGF-β antibody to ACE inhibitor arrests progressive diabetic nephropathy in the rat. J. Am. Soc. Nephrol. **14**, 1816-1824

57 Otani, H., Otsuka, F., Inagaki, K., Suzuki, J. and Makino, H. (2010) Roles of bone morphogenetic protein-6 in aldosterone regulation by adrenocortical cells. Acta Med. Okayama **64**, 213-218

58 Goumans, M. J., Liu, Z. and ten Dijke, P. (2009) TGF-beta signaling in vascular biology and dysfunction. Cell Res. **19**, 116-127

59 Karkera, J. D., Lee, J. S., Roessler, E., Banerjee-Basu, S., Ouspenskaia, M. V., Mez, J., Goldmuntz, E., Bowers, P., Towbin, J., Belmont, J. W. et al. (2007) Loss-of-function mutations in growth differentiation factor-1 (GDF1) are associated with congenital heart defects in humans. Am. J. Hum. Genet. **81**, 987-994

60 Beffagna, G., Occhi, G., Nava, A., Vitiello, L., Ditadi, A., Basso, C., Bauce, B., Carraro, G., Thiene, G., Towbin, J. A. et al. (2005) Regulatory mutations in transforming growth factor-beta-3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. Cardiovasc. Res. **65**, 366-373

61 Holweg, C. T., Baan, C. C., Niesters, H. G., Vantrimpont, P. J., Mulder, P. G, Maat, A. P., Weimar, W. and Balk, A. H. (2001) TGF-beta1 gene polymorphisms in patients with end-stage heart failure. J. Heart Lung Transplant. **20**, 979–984

62 Nakano, N., Hori, H., Abe, M., Shibata, H., Arimura, T., Sasaoka, T., Sawabe, M., Chida, K., Arai, T., Nakahara, K.I. et al. (2007) Interaction of BMP10 with Tcap may modulate the course of hypertensive cardiac hypertrophy. Am. J. Physiol. Heart Circ. Physiol. **293**, H3396–H33403

63 Wollert, K. C. (2007) Growth-differentiation factor-15 in cardiovascular disease. From bench to bedside, and back. Basic Res. Cardiol. **102**, 412–415

64 Kempf, T., von Haehling, S., Peter, T., Allhoff, T., Cicoira, M., Doehner, W., Ponikowski, P., Filippatos, G. S., Rozentryt, P., Drexler, H. et al. (2007) Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. J. Am. Coll. Cardiol. **50**, 1054-1060

65 Pohlers, D., Brenmoehl, J., Löffler, I., Müller, C.K., Leipner. C., Schultze-Mosgau, S., Stallmach, A., Kinne, R.W. and Wolf, G. (2009) TGF-beta and fibrosis in different organs - molecular pathway imprints. Biochim. Biophys. Acta **1792**, 746-756

66 Varga J. and Pasche, B. (2008) Antitransforming growth factor-β therapy in fibrosis: recent progress and implications for systemic sclerosis. Curr. Opin. Rheumatol. **20**, 720–728

67 Abreu, J. G., Ketpura, N. I., Reversade, B. and De Robertis, E. M. (2002) Connective-tissue growth factor (CTGF) modulates cell signaling by BMP and TGF-beta. Nat. Cell Biol. **4**, 599-604

68 Verrecchia, F. and Mauviel, A. (2007) Transforming growth factor-beta and fibrosis. World J. Gastroenterol. **13**, 3056-3062.

69 Flanders, K. C., Sullivan, C. D., Fujii, M., Sowers, A., Anzano, M. A., Arabshahi, A., Major, C., Deng, C., Russo, A., Mitchell, J. B. and Roberts, A. B. (2002) Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. Am. J. Pathol. **160**, 1057–1068

70 Willis, B. C. and Borok, Z. (2007) TGF-β-induced EMT: mechanisms and implications for fibrotic lung disease. Am. J. Physiol. Lung Cell Mol. Physiol. **293**, L525-L534

71 Goumans, M. J., van Zonneveld, A. J. and ten Dijke, P. (2008) Transforming growth factor beta-induced endothelial-to-mesenchymal transition: a switch to cardiac fibrosis? Trends Cardiovasc. Med. **18**, 293-298

72 Leask, A. (2010) Potential therapeutic targets for cardiac fibrosis: TGFbeta, angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation. Circ. Res. **106**, 1675-80.

73 Brosius, F. C. 3rd. (2008) New insights into the mechanisms of fibrosis and sclerosis in diabetic nephropathy. Rev. Endocr. Metab. Disord. **9**, 245–254

74 Alsaad, K. O. and Herzenberg, A. M. (2007) Distinguishing diabetic nephropathy from other causes of glomerulosclerosis: an update. J. Clin. Pathol. **60**, 18–26.

75 Makinde, T., Murphy, R. F. and Agrawal, D. K. (2007) The regulatory role of TGF-β in airway remodeling in asthma. Immunol. Cell Biol. **85**, 348–356

76 Morty, R. E., Konigshoff, M. and Eickelberg, O. (2009) Transforming Growth Factor-β Signaling across Ages. From Distorted Lung Development to Chronic Obstructive Pulmonary Disease. Proc. Am. Thorac. Soc. **6**, 607–613

77 Jobe, A. H. and Bancalari, E. (2001) Bronchopulmonary dysplasia. Am. J. Respir. Crit. Care Med. **163**, 1723–1729

78 Gauldie, J., Galt, T., Bonniaud, P., Robbins, C., Kelly, M. and Warburton, D. (2003) Transfer of the active form of transforming growth factor-beta 1 gene to newborn rat lung induces changes consistent with bronchopulmonary dysplasia. Am. J. Pathol. **163**, 2575-2584

79 Xaubet, A., Marin‐Arguedas, A., Lario, S., Ancochea, J., Morell, F., Ruiz-Manzano, J., Rodriguez-Becerra, E., Rodriguez-Arias, J. M., Inigo, P., Sanz, S. et al. (2003) Transforming growth factor‐beta1 gene polymorphisms are associated with disease progression in idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. **168**, 431-435

80 Cohen Jr, M. M. (2006) The new bone biology: pathologic, molecular, and clinical correlates, Am. J. Med. Genet. A. **140**, 2646–2706

81 Bandyopadhyay, A., Tsuji, K., Cox, K., Harfe, B. D., Rosen, V. and Tabin, C. J. (2006) Genetic analysis of the roles of BMP2, BMP4, and BMP7 in limb patterning and skeletogenesis. PLoS Genet. **2**, e216

82 Yi, S. E., Daluiski, A., Pederson, R., Rosen, V. and Lyons, K. M. (2000) The type I BMP receptor BMPRIB is required for chondrogenesis in the mouse limb. Development **127**, 621-630

83 Wallace, S. E. and Wilcox, W. R. (update 2010) Camurati-Engelmann Disease. In: Pagon, R. A., Bird, T. C., Dolan, C. R., Stephens, K. editors. GeneReviews [Internet]. University of Washington, Seattle (WA); 1993-2004

84 Marini, F. and Brandi, M. L. (2010) Genetic Determinants of Osteoporosis: Common Bases to Cardiovascular Diseases? Int. J. Hypertens. **2010**, 394579

85 Watanabe, Y., Kinoshita, A., Yamada, T., Ohta, T., Kishino, T., Matsumoto, N., Ishikawa, M., Niikawa, N. and Yoshiura, K. (2002) A catalog of 106 single-nucleotide polymorphisms (SNPs) and 11 other types of variations in genes for transforming growth factor-beta1 (TGF-beta1) and its signaling pathway. J. Hum. Genet. **47**, 478–483

86 Lehmann, K., Seemann, P., Boergermann, J., Morin, G., Reif, S., Knaus, P. and Mundlos, S. A. (2006) A novel R486Q mutation in BMPR1B resulting in either a brachydactyly type C/symphalangism-like phenotype or brachydactyly type A2. Eur. J. Hum. Genet. **14**, 1248-1254

87 Yang, W., Cao, L., Liu, W., Jiang, L., Sun, M., Zhang, D., Wang, S., Lo, W. H. Y., Luo, Y. and Zhang, X. (2008) Novel point mutations in GDF5 associated with two distinct limb malformations in Chinese: brachydactyly type C and proximal symphalangism. J. Hum. Genet. **53**, 368-374

88 Douzgou, S., Lehmann, K., Mingarelli, R., Mundlos, S. and Dallapiccola, B. (2008) Compound heterozygosity for GDF5 in Du Pan type chondrodysplasia. Am. J. Med. Genet. A. **146A**, 2116-2121

89 Bocciardi, R., Bordo, D., Di Duca, M., Di Rocco, M. and Ravazzolo, R. (2009) Mutational analysis of the ACVR1 gene in Italian patients affected with fibrodysplasia ossificans progressiva: confirmations and advancements. Eur. J. Hum. Genet. **17**, 311-318

90 Yu, P. B., Deng, D. Y., Lai, C. S., Hong, C. C., Cuny, G. D., Bouxsein, M. L., Hong, D. W., McManus, P. M., Katagiri, T., Sachidanandan, C. et al. (2008) BMP type I receptor inhibition reduces heterotopic ossification. Nat. Med. **14**, 1363-1369

91 Tassabehji, M., Fang, Z. M., Hilton, E. N., McGaughran, J., Zhao, Z., de Bock, C. E., Howard, E., Malass, M., Donnai, D., Diwan, A. et al. (2008) Mutations in GDF6 are associated with vertebral segmentation defects in Klippel-Feil syndrome. Hum. Mutat. **29**, 1017-1027

92 Asai-Coakwell, M., French, C. R., Ye, M., Garcha, K., Bigot, K., Perera, A. G., Staehling-Hampton, K., Mema, S. C., Chanda, B., Mushegian, A. et al. (2009) Incomplete penetrance and phenotypic variability characterize Gdf6-attributable oculo-skeletal phenotypes. Hum. Mol. Genet. **18**, 1110-1121

93 Elkasrawy, M. N. and Hamrick, M. W. (2010) Myostatin (GDF-8) as a key factor linking muscle mass and bone structure. J. Musculoskelet. Neuronal Interact. **10**, 56-63

94 Nishiyama, A., Takeshima, Y., Saiki, K., Narukage, A., Oyazato, Y., Yagi, M. and Matsuo, M. (2007) Two novel missense mutations in the myostatin gene identified in Japanese patients with Duchenne muscular dystrophy, BMC Med. Genet. **8**, 19

95 Schuelke, M., Wagner, K. R., Stolz, L. E., Huber, C., Riebel, T., Komen, W., Braun, T., Tobin, J. F. and Lee, S.J. (2004) Myostatin mutation associated with gross muscle hypertrophy in a child. New Engl. J. Med. **350**, 2682-2688

96 McPherron, A. C., Lawler, A. M. and Lee, S. J. (1997) Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature **387**, 83-90

97 Richards, J. S. and Pangas, S. A. (2010) The ovary: basic biology and clinical implications. J. Clin. Invest. **120**, 963-972.

98 Sinha, P. and Kuruba, N. (2007) Premature ovarian failure. J. Obstet. Gynaecol. **27**, 16–9

99 Shimasaki, S., Moore, R. K., Otsuka, F. and Erickson, G. F. (2004) The bone morphogenetic protein system in mammalian reproduction. Endocr. Rev. **25**, 72–101

100 Di Pasquale, E., Beck-Peccoz, P. and Persani, L. (2004) Hypergonadotropic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. Am. J. Hum. Genet. **75**, 106-111

101 Tiotiu, D., Alvaro-Mercadal, B., Imbert, R., Verbist, J., Demeestere, I., De Leener, A., Englert, Y., Vassart, G., Costagliola, S. and Delbaere, A. (2010) Variants of the BMP15 gene in a cohort of patients with premature ovarian failure. Hum. Reprod. **6**, 1581–1587

102 Chand, A. L., Ooi, G. T., Harrison, C. A., Shelling, A. N. and Robertson, D. M. (2007) Functional analysis of the human inhibin alpha subunit variant A257T and its potential role in premature ovarian failure. Hum. Reprod. **22**, 3241–3248

103 Roberts, A. B. and Wakefield, L. M. (2003) The two faces of transforming growth factor beta in carcinogenesis. Proc. Natl. Acad. Sci. USA. **100**, 8621-8623

104 Nguyen, D. X., Bos, P. D. and Massagué, J. (2009) Metastasis: from dissemination to organ-specific colonization. Nat. Rev. Cancer. **9**, 274-284

105 Bierie, B. and Moses, H. L. (2010) Transforming growth factor beta (TGF-beta) and inflammation in cancer. Cytokine Growth Factor Rev. **21**, 49-59.

106 Massagué, J. (2008) TGFbeta in Cancer. Cell **134**, 215-230

107 Ikushima, H. and Miyazono, K. (2010) TGFbeta signalling: a complex web in cancer progression. Nat. Rev. Cancer **10**, 415-424

108 Levy, L. and Hill, C. S. (2006) Alterations in components of the TGF-beta superfamily signaling pathways in human cancer. Cytokine Growth Factor Rev. **17**, 41-58

109 Cheah, P. Y. (2009) Recent advances in colorectal cancer genetics and diagnostics. Crit. Rev. Oncol. Hematol. **69**, 45-55

110 Parsons, R., Myeroff, L. L., Liu, B., Willson, J. K., Markowitz, S. D., Kinzler, K. W. and Vogelstein, B. (1995) Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. Cancer Res. **55**, 5548-5550

111 Zbuk, K. M. and Eng, C. (2007) Hamartomatous polyposis syndromes. Nat. Clin. Prac. Gastroenterol. Hepatol. **4**, 492-501

112 Howe, J. R., Haidle, J. L., Lal, G., Bair, J., Song, C., Pechman, B., Chinnathambi, S. and Lynch, H. T. (2007) ENG mutations in MADH4/BMPR1A mutation negative patients with juvenile polyposis. Clin. Genet. **71**, 91-92

113 Pérez-Gómez, E., del Castillo, G., Santibáñez, J., López-Novoa, J. M., Bernabéu, C. and Quintanilla, M. (2010) The role of the TGF-beta coreceptor endoglin in cancer. ScientificWorldJournal **10**, 2367-2384

114 Otten, J., Bokemeyer, C. and Fiedler, W. (2010) Tgf-Beta superfamily receptors-targets for antiangiogenic therapy? J. Oncol. **2010**, 317068

115 Morris, J. C., Shapiro, G. I., Tan, A. R., Lawrence, D. P., Olencki, T. E., Dezube, B. J. Hsu, F. J., Reiss, M. and Berzofsky, J. A. (2008) Phase I/II study of GC1008: a human anti-transforming growth factor-β (TGFβ) monoclonal antibody (MAb) in patients with advanced malignant melanoma (MM) or renal cell carcinoma (RCC). J. Clin. Oncol. **26**, 9028

116 Bogdahn, U., Hau, P., Stockhammer, G., Venkataramana, N. K., Mahapatra, A. K., Suri, A., Balasubramaniam, A., Nair, S., Oliushine, V., Parfenov, V. et al. (2011). Targeted therapy for high-grade glioma with the TGF-β2 inhibitor trabedersen: results of a randomized and controlled phase IIb study. Neuro. Oncol. **13**, 132-142.

117 Cadena, S. M., Tomkinson, K. N, Monnell. T. E., Spaits, M. S., Kumar, R., Underwood, K. W., Pearsall, R. S, and Lachey, J. L. (2010) Administration of a Soluble Activin Type IIB Receptor Promotes Skeletal Muscle Growth Independent of Fiber Type. J. Appl. Physiol. **109**, 635-642

118 Pennison, M. and Pasche, B. (2007) Targeting transforming growth factor-beta signaling. Curr. Opin. Oncol. **19**, 579-585

119 Seon, B. K., Haba, A., Matsuno, F., Takahashi, N., Tsujie, M., She, X., Harada, N., Uneda, S., Tsujie, T., Toi, H. et al. (2011) Endoglin-targeted cancer therapy. Curr. Drug Deliv. 8, 135-143.

120 Nie, J., Dou, X., Hao, W., Wang, X., Peng, W., Jia, Z., Chen, W., Li, X., Luo, N., Lan, H. Y. and Yu, X. Q. (2007) Smad7 gene transfer inhibits peritoneal fibrosis. Kidney Int. **72**, 1336-1344

121 Shukla, M. N., Rose, J. L., Ray, R., Lathrop, K. L., Ray, A. and Ray, P. (2009) Hepatocyte growth factor inhibits epithelial to myofibroblast transition in lung cells via Smad7. Am. J. Respir. Cell Mol. Biol. **40**, 643-653

122 Zhou, J., Zhong, D. W., Wang, Q. W., Miao, X. Y. and Xu, X. D. (2010) Paclitaxel ameliorates fibrosis in hepatic stellate cells via inhibition of TGF-beta/Smad activity. World J. Gastroenterol. **16**, 3330-3334

123 Chowdhury, S., Ammanamanchi, S. and Howell, G. M. (2009) Epigenetic Targeting of Transforming Growth Factor beta Receptor II and Implications for Cancer Therapy. Mol. Cell Pharmacol. **1**, 57-70

FIGURE LEGENDS

Figure 1. Schematic representation of the mammalian TGF-β family. The figure represents the four major groups of TGF-β family members, including TGF-βs, Activins/Inhibins, BMPs and AMH/MIS.

Figure 2. The TGF-β signaling pathway. The upper panel represents the different ligands, signaling receptors (type I and II), auxiliary receptors (type III) and Smad proteins (R-Smad, Co-Smad and I-Smad). The bioavailability of the ligand and the core signaling receptor formed by the heterodimeric association between type I and type II receptors determines the specificity of the signaling. The type I receptor acts downstream of the type II by phosphorylating specific R-Smads. Thus, activation of ALK1, ALK2, ALK3 and ALK6 leads to phosphorylation of Smad1, Smad5 and Smad8, while activation of ALK4, ALK5 and ALK7 phosphorylates Smad2 and Smad3. Phosphorylated (-P) R-Smads associate with Smad4 in heteromeric complexes that are translocated to the nucleus where they regulate specific gene expression responses by binding to gene promoters together with other DNA binding transcription factors. The red star indicates the Ser/Thr kinase activity in the receptors. GTM, general transcription machinery.

Figure 3. Therapeutic targets in the TGF-β signaling pathway. The figure represents several therapeutic approaches that target different components of the TGF-β signaling pathway, including antibodies, antisense oligonucleotides, soluble receptors, recombinant ligands or chemical kinase inhibitors. For further details of the specific drugs, see Table 3.

Table 1. TGF-β Signaling and Auxiliary Receptors, their Ligands and R-Smads		
Type I receptors	**Ligands**	**R-Smads**
ALK1/ ACVRL1	TGF-β, BMP9, BMP10	Smad1,5,8
ALK2/ ACVR1	BMPs, GDFs	Smad1,5,8
ALK3/ BMPR1A	BMPs	Smad1,5,8
ALK4/ ACVR1B	Activins, Myostatin/GDF8, GDF11	Smad2,3
ALK5/ TGFBR1	TGF-βs, Myostatin/GDF8, GDF11	Smad2,3
ALK6/ BMPR1B	BMPs	Smad1,5,8
ALK7/ ACVR1C	BMP16/Nodal	Smad2,3
Type II receptors		
TGFBR2/TBRII	TGF-βs	
BMPR2/ BMPRII	BMPs, GDFs	
ACVR2/ ActRIIA	Activins, BMP2, BMP4, BMP7, GDFs	
ACVR2B/ ActRIIB	Activins, BMP2, BMP4, BMP7, GDFs, BMP16/Nodal	
AMHR2/ AMHR II	AMH	
Type III receptors		
Betaglycan	TGF-β1, TGF-β2, TGF-β3, Activin-A, BMP2, BMP4, BMP7, GDF5	
Endoglin	TGF-β1, TGF-β3, Activin-A, BMP2, BMP7, BMP9	

Table 2. Human disease-causing mutations in TGF-β family ligands, receptors, and signaling proteins

Protein/Gene (OMIM ID#)	Disease (OMIM ID#; Genecard; References)	Line of mutation
Ligands		
TGF-β1 **(#190180)**	Camurati-Engelmann disease **(#131300)**	Germ line
TGF-β2 **(#190220)**	Peters anomaly **(#604229)**	Chromosomal aberration
TGF-β3 **(#190230)**	Arrhythmogenic right ventricular dysplasia-1 **(#107970)**	Germ line
BMP4 **(#112262)**	Anophthalmia-microphthalmia and/or retinal dystrophy **(#607932)** Cleft lip and cleft palate **(#600625)**	Germ line
BMP7 **(#112267)**	Ocular, brain, ear, palate, and skeletal anomalies (Wyatt et al., 2010; *Hum. Mutat.* **31**, 781-787)	Germ line
BMP13/GDF6 **(#601147)**	Klippel-Feil syndrome **(#118100)** Spondylocostal dysostosis-4 - isolated **(#122600)** Microphthalmia **(#613094)**	Germ line
BMP14/ GDF5 **(#601146)**	Hunter-Thompson type chondrodysplasia **(#201250)** Grebe type chondrodysplasia **(#200700)** Brachydactyly type C **(#113100)** Multiple-synostosis syndrome **(#186500)** Du Pan syndrome **(#228900)**	Germline
BMP15 **(#300247)**	Ovarian dysgenesis **(#300510)** Premature ovarian failure 4 **(#300510)**	Germ line
GDF1 **(#602880)**	Tetralogy of Fallot (human congenital heart defects) **(#187500)**	Germ line
GDF3 **(#606522)**	Microphthalmia, anophthalmia and colobomata (Ye et al., 2010; *Hum. Mol. Genet.* **19**, 287-298)	Germ line
GDF5 **(#601146)**	Du Pan type chondrodysplasia **(#228900)** Brachydactyly type C **(#113100)** Proximal symphalangism **(#185800)**	Germ line
GDF6 **(#601147)**	Klippel-Feil syndrome **(#118100)** Spondylocostal dystostosis 4 **(#122600)**	Germ line
GDF8/Myostatin **(#601788)**	Muscle hypertrophy (Schuelke et al., 2004; *N. Engl. J. Med.* **350**, 2682-2688) Duchenne muscular dystrophy **(#310200)**	Germ line
GDF9 **(#601918)**	Premature ovarian failure (**#300510**)	Germ line
Inhibin alpha **(#147380)**	Premature ovarian failure (Chand et al., 2010; *Hum. Reprod. Update*. **16**, 39-50)	Somatic
Anti-Mullerian Hormone **(#600957)**	Persistent mullerian duct syndrome **(#261550)**	Germ line

Type I receptors		
ALK1/ ACVRL1 **(#601284)**	Hereditary hemorrhagic telangiectasia (HHT2) (**#600376**) Gonadotroph tumor; A482V mutation of unknown significance (D'Abronzo et al., 1999; *J. Clin. Endocrinol. Metab.* **84**, 1716-1721)	Germline
ALK2/ ACVR1 **(#102576)**	Fibrodysplasia ossificans progressiva (FOP) (**#135100**)	Germ line
ALK3/ BMPR1A **(#601299)**	Juvenile polyposis **(#174900)** Hereditary mixed polyposis **(#610069)** Juvenile polyposis of infancy **(#612242)**	Germ line
ALK4/ ACVR1B **(#601300)**	Pancreatic carcinoma **(#260350)**	Somatic
ALK5/TGFBR1 **(#190181)**	Loeys-Dietz syndrome **(#609192)** Type 2 Marfan syndrome	Germ line
ALK6/BMPR1B **(#603248)**	Frachydactyly **(#112600)** Fhondrodysplasia, acromesomelic, with genital anomalies (Demirhan et al., 2005; *J. Med. Genet.* 42, 314-317)	Germ line
TypeII receptors		
ACVR2/ ActRIIA **(#102581)**	Human pituitary tumors (45%) (D'Abronzo et al., 1999; *J. Clin. Endocrinol. Metab.* **84**, 1716-1721)	Polymorphism
ACVR2B/ ActRIIB **(#602730)**	Left-right axis malformations, visceral heterotaxy (autosomal type 4) (**HTX4**) **(#602730)**	Germ line
BMPR2/BMPRII **(#600799)**	Familial primary pulmonary hypertension (PAH) **(#178600)**	Germ line
TGFBR2/TBR2 **(#190182)**	Multiple cancers (colorectal, gastric, endometrial, prostate, breast, lung, hepatocellular, lymphoma, pancreatic,cervical, glioma) **(#190182**) Loeys-dietz syndrome **(#610380)** Atherosclerosis (McCaffrey, 2009; *Front. Biosci. (Schol Ed)*. **1**, 236-245) Marfan syndrome **(#154700)**	Somatic / Germ line
AMHR2/MISRII **(#600956)**	Persistent Müllerian duct syndrome **(#261550)**	Germ line
Type III receptors		
Endoglin/CD105/ENG **(#131195)**	Hereditary hemorrhagic telangiectasia **(#187300)**	Germ line
Betaglycan/ TGFBRIII **(#600742)**	Possible association between gene expression levels of TGFBR3 and bone mineral density **(#612728)**	Unknown
Smads		
Smad2 **(#601366)**	Colorectal cancer (Takagi et al., 1998; *Br. J. Cancer* **78**, 1152-1155) Colon cancers (Takenoshita et al., 1998; *Carcinogenesis* 19, 803-807)	Somatic
Smad3 **(#603109)**	Osteoarthritis (Yao et al., 2003; *Eur. J. Hum. Genet.* **11**, 714-717)	Germline
Smad4 **(#600993)**	Juvenile polyposis syndrome (**#174900**) Pancreatic, colorectal cancer (Schutte et al., 1999; *Ann. Oncol.* **10** Suppl 4, 56-59.)	Germline
Smad8 and Smad9 **(#603295)**	Primary pulmonary hypertension **(#178600)**	Germline

Table 3. Selected clinical studies using agents targeting the TGF-β pathway

Type of compounds	**Drug**	**Target**	**Clinical Trial/Study** (ClinicalTrials.gov Identifier)
Human anti-TGF-β mAb (pan-neutralizing IgG4)	GC1008	TGF-βs	Phase I on malignant carcinoma, and renal cell carcinoma (NCT00356460) Phase II on Relapsed Malignant Pleural (NCT01112293) Phase I in treatment of Resistant Idiopathic Focal Segmental Glomerulosclerosis (NCT00464321)
Human Anti-TGF-β1 mAb	CAT-192	TGF-β1	Phase I/II in Patients With Early Stage Diffuse Systemic Sclerosis (NCT00043706)
TGF-β2 antisense ODN	AP12009 (Trabersden)	TGF-β2	Phase I on pancreatic and Colorectal Neoplasms, and melanoma (NCT00844064) Phase II on Glioblastoma and Anaplastic Astrocytoma (NCT00431561) Phase III on Anaplastic Astrocytoma (NCT00761280)
TGF-β2 antisense gene-modified allogeneic tumor cell vaccine	Lucanix	TGF-β2	Phase III on Lung Neoplasm Carcinoma, Non-Small-Cell Lung: Stage IIIA (T3, N2 Only) Carcinoma, Non-Small-Cell Lung: Stage IIIB Carcinoma, Non-Small-Cell Lung: Stage IV (NCT00676507)
Type I and II TGF-β receptors inhibitor	LY21109761	TGFBR2 and ALK5	Phase II on metastatic melanoma (NCT00383292)
Peptide Inhibitor of TGF-β1	P144	TGF-β1	Phase II for the treatment of Skin Fibrosis in Systemic Sclerosis (NCT00574613 and NCT00781053)
Soluble Activin Type IIB Receptor	AC-031	GD8/Myostatin	Phase II Duchenne Muscular Dystrophy (NCT01099761) Phase I in healthy postmenopausal women with weight loss (NCT00952887)
Human Anti-ALK1 mAb	PF03446962	ALK1	Phase I on Advanced Solid Tumors (NCT00557856)
Human/murine chimeric anti-endoglin mAb	TRC105	Endoglin/CD105	Phase I on Cancer, Neoplasm Metastasis (NCT00582985)
Human recombinant BMP2	RhBMP2	BMP receptors	Phase I of degenerative Lumbar Disc Disease, Spondylolisthesis and Spinal Stenosis (NCT00405600) Phase II to Degenerative Disc Disease (NCT00707265) Phase I of Osteoarthritis (NCT00243295) Phase II for Osteoporosis (NCT00752557)
Human recombinant BMP7	rhBMP7	BMP receptors	Phase II for the treatment of Osteoarthritis of the Knee (NCT0111104)
Human recombinant GDF5/BMP14	rhGDF5/BMP14	BMP receptors	Phase II the treatment of Degenerative Disc Disease (NCT00813813; NCT01124006) Phase I/II for the treatment of early Lumbar Disc Degeneration (NCT01158924)

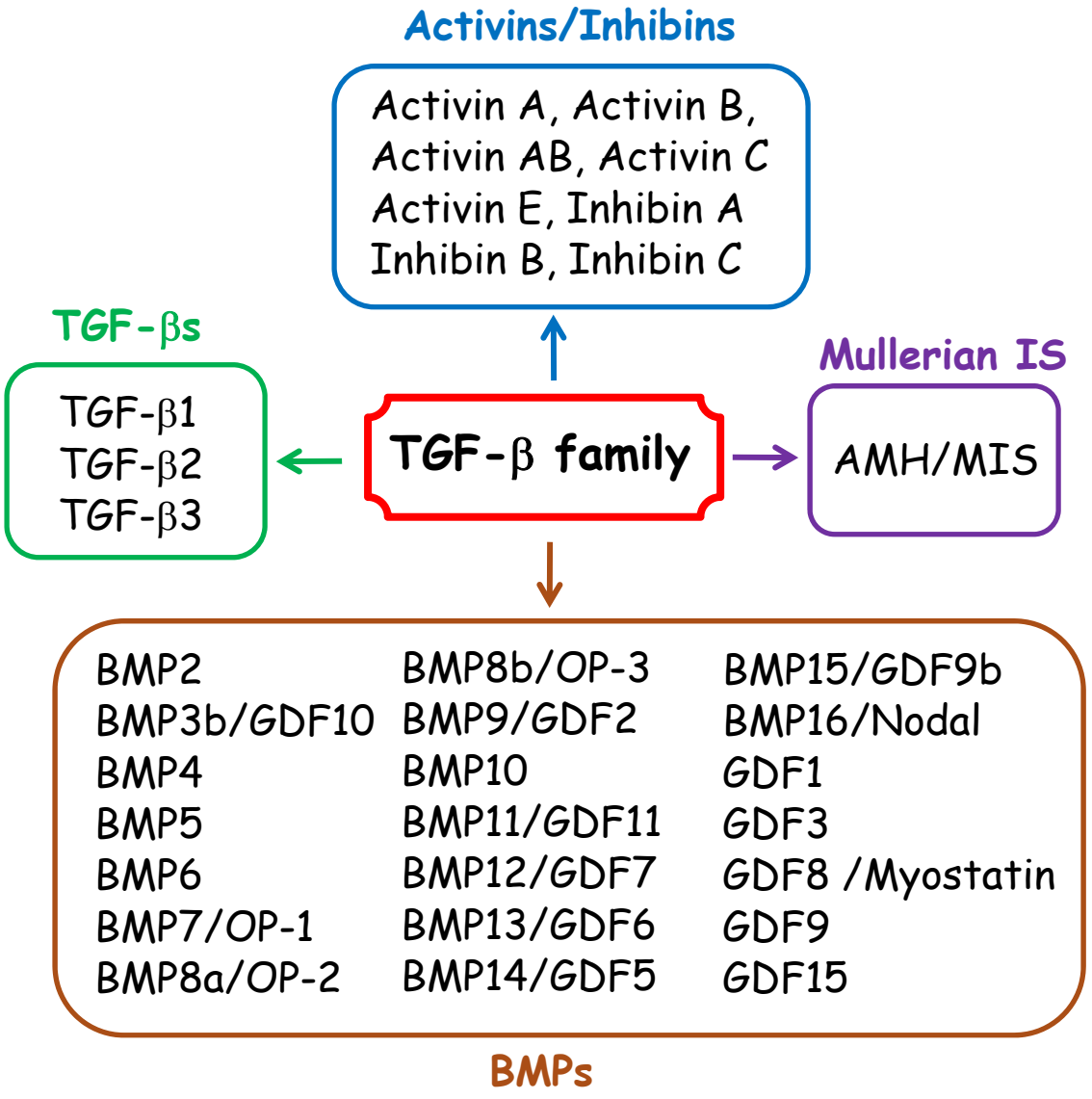
Activins/Inhibins
Activin A, Activin B,
Activin AB, Activin C
Activin E, Inhibin A
Inhibin B, Inhibin C
TGF-βs
TGF-β1
TGF-β2
TGF-β3
TGF-β family
Mullerian IS
AMH/MIS
BMP2
BMP3b/GDF10
BMP4
BMP5
BMP6
BMP7/OP-1
BMP8a/OP-2
BMP8b/OP-3
BMP9/GDF2
BMP10
BMP11/GDF11
BMP12/GDF7
BMP13/GDF6
BMP14/GDF5
BMP15/GDF9b
BMP16/Nodal
GDF1
GDF3
GDF8 /Myostatin
GDF9
GDF15
BMPs

Figure 1

TGF-β system	TGF-β/Activin/Nodal	BMPs/GDFs
Ligands	TGF-Bs, Activins, Myostatin, Nodal	BMPs, GDFs, AMH
Co-Receptors/ Type III Receptors	Betaglycan	Endoglin
Type I Receptors	ALK4/5/7	ALK1/2/3/6
Type II Receptors	TGFBR2/ACVR2/ ACVR2B	BMPR2/ACVR2/ ACVR2B/AMHR2
R-Smads	Smad2/3	Smad1/5/8
I-Smads	Smad7	Smad6
Co-Smad	Smad4	

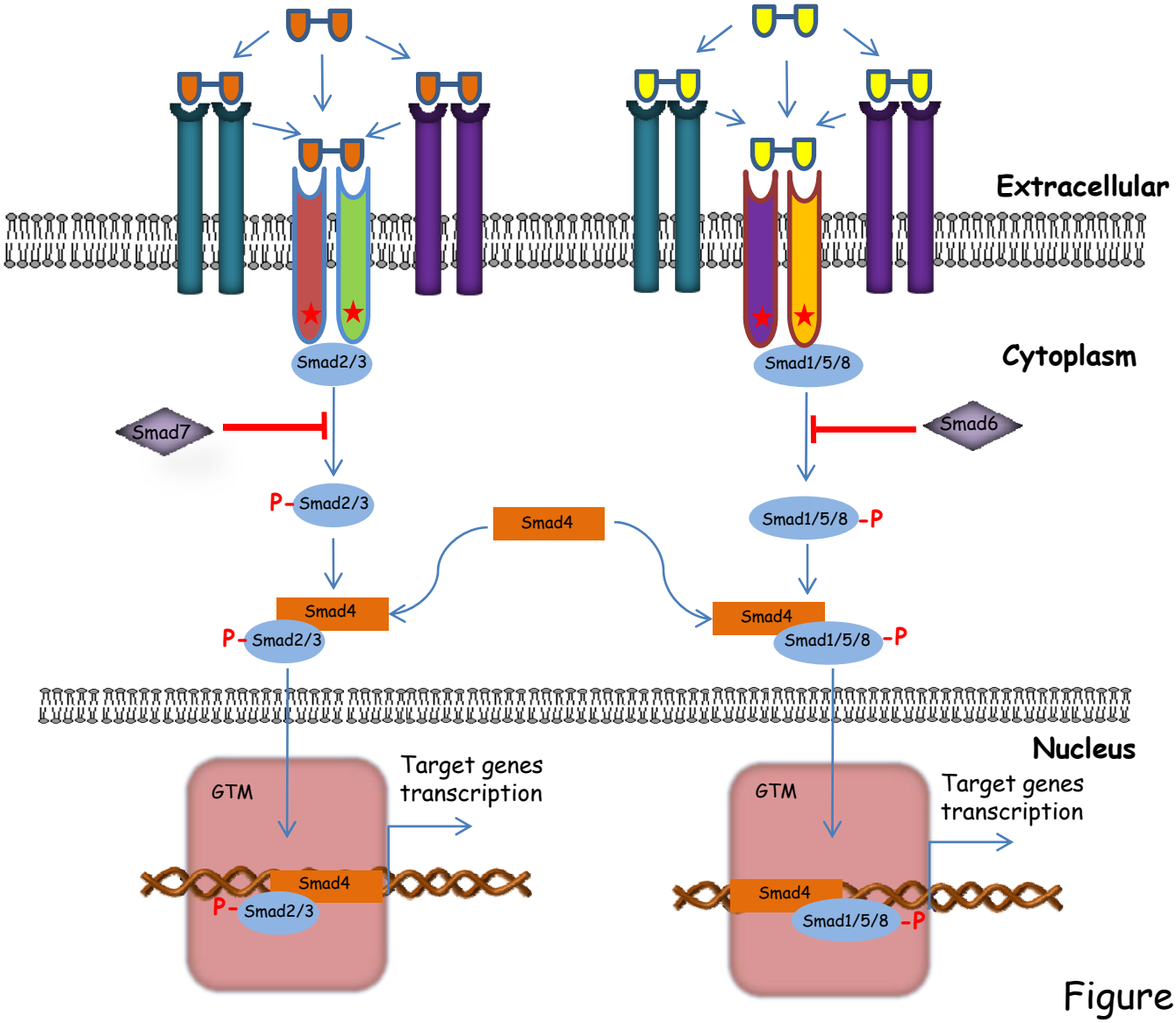

Figure 2

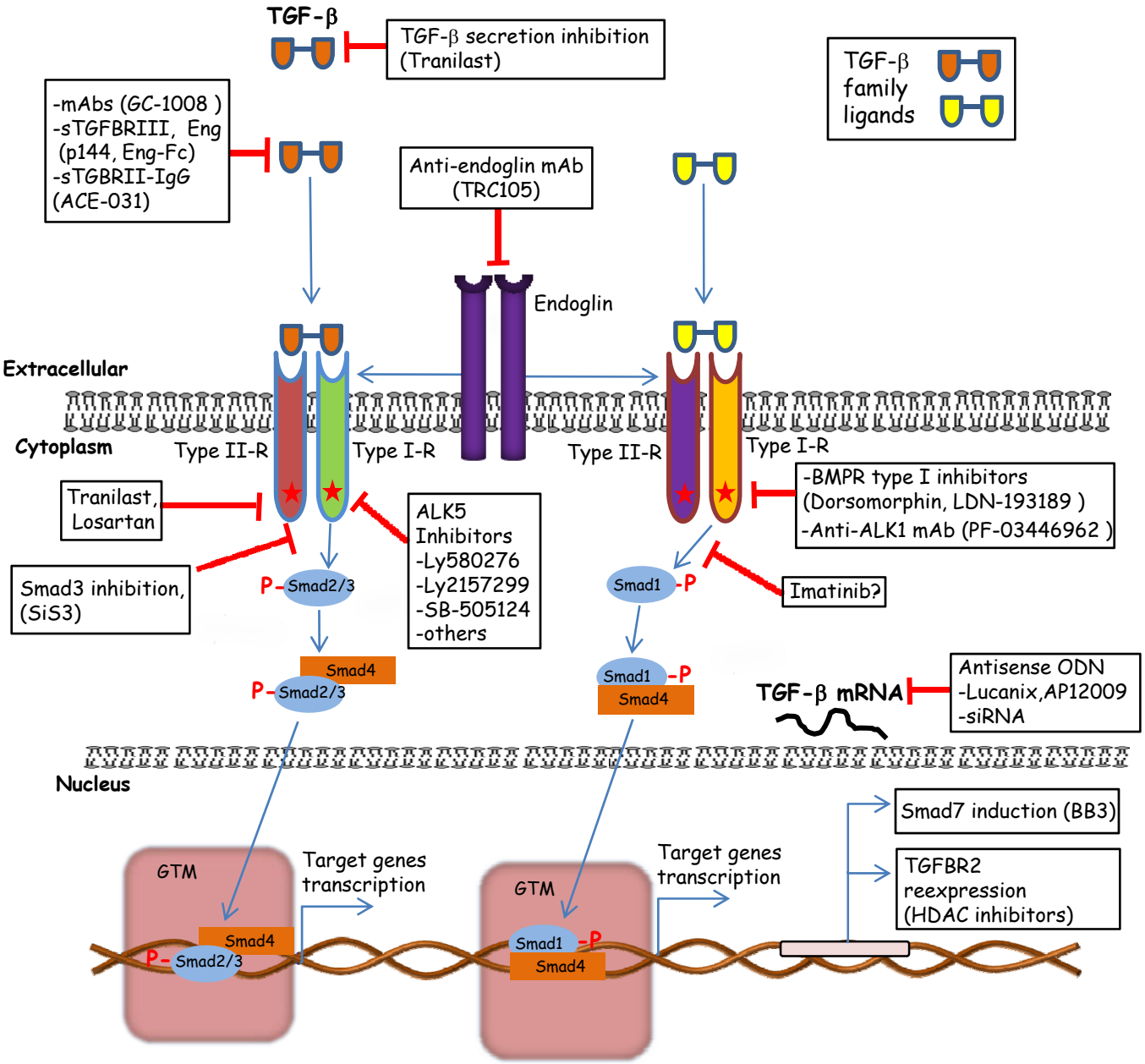
TGF-β
TGF-β secretion inhibition (Tranilast)
TGF-β family ligands
-mAbs (GC-1008)
-sTGFBRIII, Eng (p144, Eng-Fc)
-sTGBRII-IgG (ACE-031)
Anti-endoglin mAb (TRC105)
Endoglin
Extracellular
Cytoplasm
Type II-R
Type I-R
Type II-R
Type I-R
Tranilast, Losartan
Smad3 inhibition, (SiS3)
ALK5 Inhibitors
-Ly580276
-Ly2157299
-SB-505124
-others
-BMPR type I inhibitors (Dorsomorphin, LDN-193189)
-Anti-ALK1 mAb (PF-03446962)
Imatinib?
P-Smad2/3
Smad4
P-Smad2/3
Smad1-P
Smad1-P
Smad4
TGF-β mRNA
Antisense ODN
-Lucanix,AP12009
-siRNA
Nucleus
GTM
Target genes transcription
Smad4
P-Smad2/3
GTM
Target genes transcription
Smad1-P
Smad4
Smad7 induction (BB3)
TGFBR2 reexpression (HDAC inhibitors)

Figure 3