TGF-β/TGF-β receptor system and its role in physiological and pathological conditions

Juan F. SANTIBAÑEZ*, Miguel QUINTANILLA† and Carmelo BERNABEU‡

*Institute for Medical Research, University of Belgrade, 11129 Belgrade, Serbia, †Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Autónoma de Madrid, Madrid, Spain, and ‡Centro de Investigaciones Biológicas, CSIC and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28040 Madrid, Spain

ABSTRACT

The TGF-β (transforming growth factor-β) system signals via protein kinase receptors and Smad mediators to regulate a plethora of biological processes, including morphogenesis, embryonic development, adult stem cell differentiation, immune regulation, wound healing and inflammation. In addition, alterations of specific components of the TGF-β signalling pathway may contribute to a broad range of pathologies such as cancer, cardiovascular pathology, fibrosis and 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.

INTRODUCTION

Since the discovery of the first member of the TGF-β (transforming growth factor-β) superfamily in the early 1980s, 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 signalling

Key words: cancer, cardiovascular disease, fibrosis, reproductive disorder, Smad protein, transforming growth factor-β (TGF-β), transforming growth factor-β receptor.

Abbreviations: ACE, angiotensin-converting enzyme; ACVR/ActR, activin receptor; ALK, activin-like kinase; AMH, anti-Mullerian hormone; AngII, angiotensin II; AS-ODN, antisense oligonucleotide; AV, arteriovenous malformation; BDA, brachydactyly type A2; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; BPD, bronchopulmonary dysplasia; caALK2, constitutively active ALK2; CED, Camurati–Engelmann disease; Co-Smad, co-operating Smad; COPD, chronic obstructive pulmonary disease; CTGF, connective tissue growth factor; CV, cardiovascular; DMD, Duchenne muscular dystrophy; DN, diabetic nephropathy; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; FOP, fibrodysplasia ossificans progressiva; FPAH, familial PAH; FSH, follicle-stimulating hormone; GDF, growth/differentiation factor; GM-CSF, granulocyte/macrophage colony-stimulating factor; GS, glycine/serine; HDAC, histone deacetylase; I-Smad, inhibitory Smad; IPAH, idiopathic PAH; JPS, juvenile polyposis syndrome; HGF, hepatocyte growth factor; HHT, hereditary haemorrhagic telangiectasia; HMPA, hereditary mixed polyposis syndrome; HNPCC, hereditary non-polyposis colorectal cancer; IFP, idiopathic pulmonary fibrosis; JP, juvenile polyposis; LAP, latency-associated peptide; LSD, Loeys–Dietz syndrome; MGP, matrix Gla (γ-carboxylated glutamate) protein; MH, Mad homology; MIF, Müllerian inhibitory factor; MIS, Müllerian inhibitor substance; MSI, microsatellite instability; PAH, pulmonary arterial hypertension; PMDS, persistent Müllerian duct syndrome; POF, premature ovarian failure; R-Smad, receptor-associated Smad; RAAS, renin–angiotensin–aldosterone system; SMC, smooth muscle cell; SNP, single nucleotide polymorphism; TAAD, thoracic aortic aneurysm syndrome; TGF-β, transforming growth factor-β; TBRI etc., type I TGF-β receptor etc.

Correspondence: Professor Carmelo Bernabeu (email bernabeu.c@cib.csic.es).
pathway are involved in human diseases including CV (cardiovascular), fibrosis, reproductive, cancer or wound-healing disorders. Some of these diseases are hereditary conditions such as HHT (hereditary haemorrhagic telangiectasia), familial primary pulmonary hypertension and JP (juvenile polyposis).

In the present review, first we will consider 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. Secondly, we will describe the role of TGF-β signalling in the normal physiology and how its malfunction results in diseases affecting different organs, including, among others, CV system, pulmonary, bone, muscle and reproductive disorders and cancer. Finally, we will summarize the current knowledge about the therapeutic approaches that target different components of the TGF-β signalling pathway. The use of antibodies, AS-ODNs (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.

THE TGF-β SIGNALLING PATHWAY

The TGF-β system includes several components involved at different levels of a standard outside-inside signalling pathway, namely, soluble factors, specific membrane receptors and intracellular mediators.

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 the TGF-β family are implicated, among others, in morphogenesis, embryonic development, adult stem cell 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 fibroblasts [1]. So far, more than 40 members of this family are known, which have, in common, their dimeric structure and the presence of a cysteine knot structural motif [2]. These proteins cluster in several subfamilies, such as TGF-βs, BMPs (bone morphogenetic proteins), GDFs (growth and differentiation factors), MIF (Müllerian inhibitory factor), activins or inhibins (Figure 1).

Among the TGF-βs, six distinct isoforms with a variable degree of homology have been discovered, although only the TGF-β1, TGF-β2 and TGF-β3 isoforms are expressed in mammals, and their human genes are located on chromosomes 19q13, 1q41 and 14q24, respectively [3–5]. BMPs were originally identified as a family of proteins that induced 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 [6,7]. 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 the BMP2/BMP4 group, the BMP5–BMP8 group, the OP-1 (osteogenic protein-1) group and the BMP9/BMP10 group [6,7]. 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 the left–right axial structures in early embryonic development [8]. GDFs are classified within the BMPs family and include, at least, 11 components: GDF1–GDF3, GDF5–GDF11 and GDF15 [9,10]. MIF, also known as AMH (anti-Müllerian hormone) or MIS (Müllerian inhibitory substance) has been mainly studied for its regulatory role in male sex differentiation. MIF is implicated in the regression of the Müllerian ducts in male fetuses and in the development and function of the gonads of both sexes [11,12]. Activins are structurally related proteins involved...
in the control of cell proliferation, differentiation, apoptosis, metabolism, homeostasis, differentiation, immune response and endocrine function [13]. Activins are produced in the gonads, pituitary gland, placenta and other organs. Activins enhance FSH (follicle-stimulating hormone) biosynthesis and secretion, and participate in the regulation of the menstrual cycle. Activins are secreted as homodimers or heterodimers of inhibin subunits. Although four inhibin 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 [14] (Supplementary Figure S1 at http://www.clinsci.org/cs/121/cs1210233add.htm). 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 [14]. Inhibins are closely related to activins. Inhibins are disulphide-linked heterodimers consisting of α-subunit and either a βA or βB subunit to form inhibin A and inhibin B respectively (Supplementary Figure S1).

**TGF-β receptors**

All TGF-β family members bind cell-surface serine/threonine kinase receptors types I and II (TBRI and TBRII respectively), which form heteromeric complexes in the presence of dimerized ligands. Seven TBRIIs, also named ALKs (activin-like receptor kinases), as well as five different TBRIIs have been described (Figure 2 and Table 1). In addition, TGF-β ligands may interact with the co-receptors endoglin and betaglycan [known as TBRIIs (type III TGF-β receptors)] [15–17]. Soluble ligands bind first to the constitutively active TBRII, followed by the interaction and phosphorylation of a GS (glycine/serine)-rich domain of the TBRI to produce an activated ligand–receptor complex [18]. Then, the activated TBRI phosphorylates the downstream effector Smads. Both type I and II kinase receptors are themselves phosphorylated at tyrosine and serine/threonine residues, probably implicated in a cross-talk activity regulation of a variety of signal transduction pathways.

The activity of TGF-β kinase receptors can be regulated by the auxiliary TBRIII endoglin or betaglycan [16,17]. Endoglin and betaglycan are type I integral membrane proteins with large extracellular domains and short cytoplasmic domains lacking kinase signalling motifs. The ubiquitous betaglycan binds with high-affinity to several members of the TGF-β family, including TGF-β1, TGF-β2, TGF-β3, activin-A, BMP2, BMP4, BMP7 and GDF5 [16]. Betaglycan increases ligand binding to the respective cognate TBRIIs and TBRIIs to modulate their signalling [16,18,19]. 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, activin-A, BMP2 and BMP7, requiring the presence of the corresponding signalling receptors; by contrast, endoglin can bind directly to TGF-β3 and BMP9 independently of the kinase receptors [17]. Endoglin enhances TGF-β1-, BMP7- and BMP9-dependent Smad1/Smad5 responses, while it inhibits the TGF-β3/Smad3 pathway [16,17,20,21]. Endoglin and betaglycan belong to the ZP (zona pellucida) family of proteins, which are characterized by the proteolytic cleavage of their extracellular domain [22,23]. Of note, the released soluble protein may act by antagonizing the effects of the corresponding membrane-associated TBRIII [16,17] (Supplementary Figure S2 at http://www.clinsci.org/cs/121/cs1210233add.htm).

**Smad family-dependent signal transduction**

Intracellular TGF-β signalling is mediated by the Smad family of proteins. Members of the Smad family are well conserved and can be classified into three groups: (i) R-Smads (receptor-associated Smads); (ii) Co-Smads (co-operating Smads) and (iii) I-Smads (inhibitory Smads) [24,25]. The activated receptor complexes transduce intracellular signalling by the type I receptor phosphorylation of the R-Smads at their C-terminal domains [15]. The unphosphorylated R-Smads are transcriptionally inactive and sequestered in the cytoplasm by specific retention proteins such as SARA (Smad anchor for receptor activation) [15] or endofin [6]. In humans, five different R-Smads have been described that are substrates for activated TGF-β receptors (Smad1, Smad2, Smad3, Smad5 and Smad8). Smad2 and Smad3 are substrates for receptors activated by TGF-βs and activins, whereas Smad1, Smad5 and Smad8 mediate pathways activated by BMPs, GDFs and MIFs (Figure 2). Each R-Smad contains two highly conserved domains at the N-terminus and the C-terminus, named MH1 and MH2 respectively (where MH is Mad homology), and a linker domain. MH1 can interact with DNA and with other proteins and possesses a NLS (nuclear localization signal), whereas MH2 mediates homo- or hetero-oligomerization of the Smads and the transactivation of Smad nuclear complexes. The highly variable linker region, located between the MH1 and MH2 domains, is enriched in proline residues and has potential serine/threonine substrates for phosphorylation [25]. Upon ligand activation of the TGF-β receptor complex, the TBRI phosphorylates R-Smad at a serine-rich C-terminal motif, and then the phospho-R-Smad associates with Smad4 (mammalian Co-Smad). This Smad complex is shuttled into the nucleus where, in collaboration with other transcription factors, it binds and regulates promoters of different target genes [15].
The TGF-β signalling pathway

The upper panel represents the different ligands, signalling receptors (TBRI and TBRII), auxiliary receptors (TBRIII) and Smad proteins (R-Smad, Co-Smad and I-Smad). The bioavailability of the ligand and the core signalling receptor formed by the heterodimeric association between TBRI and TBRII determines the specificity of the signalling. The type I receptor acts downstream of TBRII by phosphorylating specific R-Smads. Thus activation of ALK1, ALK2, ALK3 and ALK6 leads to phosphorylation of Smad1, Smad5 and Smad8, whereas activation of ALK4, ALK5 and ALK7 phosphorylates Smad2 and Smad3. Phosphorylated (-P) R-Smads associate with Smad4 in heterogeneous complexes that are translated 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. ACVR, activin receptor; GTM, general transcription machinery.

Two of these genes are I-Smads, Smad6 and Smad7. The induced expression of these inhibitory Smads produces a negative-feedback regulation of TGF-β signalling [26]. I-Smads contain a characteristic C-terminal MH2 domain, but they lack the conserved MH1 domain. Smad6 preferentially inhibits BMP signalling by disrupting the Smad1–Co-Smad interaction and forming an inactive Smad1–Smad6 complex. In addition, Smad7 inhibits R-Smad phosphorylation by binding to the TGF-β, activin and BMP type I receptors [27]. Once in the nucleus, the phospho-R-Smad–Co-Smad complex can bind, through the MH1 domain, to SBEs (Smad-binding elements) in the DNA. Smad3 recognizes a 5-bp -GTCTG-, whereas Smad4, as well as Smad1, Smad5 and Smad8, 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, although of low affinity, 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 [15,26,27]. Regarding the R-Smad mediators involved, TGF-β superfamily members are classified into the TGF-β/Nodal/activin group (Smad2 and Smad3) and the BMP/GDF group (Smad1, Smad5 and Smad8) (Figure 2).

### Regulation of the TGF-β pathway

All components of the TGF-β pathway are subjected to fine tuning, at different levels, in the modulation of TGF-β family signal transduction. TGF-βs are secreted in an inactive form [28]. Thus the TGF-β1, TGF-β2 and TGF-β3 proteins are synthesized as propeptide precursors containing a prodomain [also named LAP (latency-associated peptide)] and the mature domain. Then, a cleavage by the convertase family of endoproteases occurs between LAP and the mature homodimer protein, but LAP remains associated with the mature domain forming a SLC (small latent complex). TGF-βs are maintained in this inactive form in a multiprotein complex that involves interaction with ECM (extracellular matrix) proteins and integrins. The bioavailability of active TGF-βs depends on proteolytic processing that releases the cytokines. Although all members of the 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 [28]. 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. On the basis of the size of their cysteine knot, a common arrangement of six half cysteine residues that form three intrachain disulfide bonds, the BMP antagonists can be classified into four subfamilies: (i) Dan; (ii) Chordin; (iii) twisted gastrulation; and (iv) Noggin. These BMP antagonists have differential affinities for the different BMPs [6,7].

Ligand access to, and signalling by, the kinase receptors can be modulated by several auxiliary receptors, including the TBRIII members endoglin and betaglycan, the repulsive guidance family of glycosylphosphatidylinositol-anchored proteins DRAGON, RGMa (repulsive guidance molecule a) 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 to post-translational modifications, including phosphorylation, sumoylation and ubiquitylation, which are enzymatically reversible and regulate their stability and availability [18]. Another level of regulation is the internalization and recycling of the ligand–receptor complexes via either lipid rafts/caveolae or clathrin-coated vesicles, which can modulate signalling as well as protein degradation in the proteasome [18,32,33]. On the other hand, a negative feedback loop has been well documented in which TGF-βs signalling 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 signalling 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 signalling showing high preference for the type I BMP receptors ALK1, ALK3, ALK5 and ALK6 [10].

In addition to the canonical Smad pathway, the TGF-β signal is determined by a cross-talk with non-Smad pathways such as MAPK (mitogen-activated protein kinase), NF-κB (nuclear factor κB), Rho-like GTPase, PI3K (phosphoinositide 3-kinase)/Akt or hypoxia/HIF-1 (hypoxia-inducible factor-1) [10,34,35].

**TGF-β SYSTEM IN HEALTH AND DISEASE**

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

**Cardiovascular system**

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

HHT, or Rendu–Osler–Weber syndrome, is inherited as an autosomal dominant trait involving mainly mutations in the ENG (endoglin) 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), whereas 1–2% carries a mutation in SMAD4. Interestingly, the same SMAD4 mutations also lead to JP (juvenile polyposis), resulting in a combined syndrome of JP–HT (JP and HHT) [38]. Additional HHT loci have been mapped to chromosomes 5q (HHT3) and 7p (HHT4) [39,40], 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 AVMs (arteriovenous malformations) in lung, liver or brain. The pathogenic mechanism underlying the generation of telangiectasia and AVMs appears to be a haploinsufficiency of the HHT genes in endothelia, and recent findings from transgenic animal models of HHT support the existence of a second hit that triggers the generation of these vascular lesions [40–42].

LSD (Loeys–Dietz syndrome) is an autosomal-dominant genetic syndrome caused by mutations in the genes encoding ALK5 (LSD type I) or TGFBR2 (encoding TBRII; LSD type II) [43]. LDS is characterized by vascular effects (cerebral, thoracic, and abdominal arterial aneurysms and/or dissections) and skeletal manifestations (pectus excavatum, pectus carinatum, scoliosis, arachnodactyly, joint laxity and talipes equinovarus). Approximately 75% of affected individuals have LDS type I with craniofacial manifestations (ocular hypertelorism, craniosynostosis and bifid uvula/cleft palate). LDS type II patients (25%) show cutaneous manifestations (velvety and translucent skin; easy bruising; widened and atrophic scars). The majority of identified mutations are either adjacent to or within the serine/threonine kinase domains of ALK5 and TBRII. In three quarters of LSD cases, the disorder is the result of a de novo gene mutation.

Familial TAAD (thoracic aortic aneurysm syndrome) 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].

PAH (pulmonary arterial hypertension) is a disease characterized by elevated pulmonary artery pressure leading to right heart failure. PAH demonstrates abnormal remodelling of small peripheral resistance vessels in the lung, involving proliferation and migration of vascular SMCs (smooth muscle cells), endothelial cells and fibroblasts. Two types of PAH have been described, sporadic or IPAH (idiopathic PAH) and hereditary or FPAH (familial PAH). Both IPAH and FPAH are genetically related with a heterozygous germline mutation in BMPR2 (encoding the type II BMP receptor). Mutations in BMPR2 have been associated with 80% of FPAH and 15–40% of IPAH. 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 PAH [45,46].

Atherosclerosis is the primary cause of CV disease and stroke. Atherosclerosis development and progression is a complex process involving endothelial dysfunction, vascular inflammation and accumulation of lipids and...
Table 2  Human disease-causing mutations in TGF-β family ligands, receptors and signalling proteins

<table>
<thead>
<tr>
<th>Protein/gene (OMIM ID #)</th>
<th>Disease (OMIM ID#; Genecard; reference)</th>
<th>Line of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ligand</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β1 (#190180)</td>
<td>Camurati–Engelmann disease (#131300)</td>
<td>Germline</td>
</tr>
<tr>
<td>TGF-β2 (#190220)</td>
<td>Peter’s anomaly (#604229)</td>
<td></td>
</tr>
<tr>
<td>TGF-β3 (#190230)</td>
<td>Arthrogrypotic right ventricular dysplasia-1 (#107970)</td>
<td></td>
</tr>
<tr>
<td>BMP4 (#112262)</td>
<td>Anophthalmia–microphthalmia and/or retinal dystrophy (#607932); cleft lip and cleft palate (#600625)</td>
<td>Germline</td>
</tr>
<tr>
<td>BMP7 (#112267)</td>
<td>Ocular, brain, ear, palate and skeletal anomalies [125]</td>
<td>Germline</td>
</tr>
<tr>
<td>BMP13/GDF6 (#601147)</td>
<td>Klippel–Feil syndrome (#118100); spondylocostal dysostosis-4-isolated (#122600); microphthalmia (#613094)</td>
<td>Germline</td>
</tr>
<tr>
<td>BMP14/GDF5 (#601146)</td>
<td>Hunter–Thompson type chondrodysplasia (#201250); Grebe-type chondrodysplasia (#200700); brachydactyly type C (#113100); multiple synostosis syndrome (#186500); Du Pan syndrome (#228900)</td>
<td>Germline</td>
</tr>
<tr>
<td>BMP15 (#300247)</td>
<td>Ovarian dysgenesis (#300510); POIF (#300510)</td>
<td>Germline</td>
</tr>
<tr>
<td>GDF1 (#602880)</td>
<td>Tetralogy of Fallot (human congenital heart defects) (#187500)</td>
<td>Germline</td>
</tr>
<tr>
<td>GDF3 (#606522)</td>
<td>Microphthalmia, anophthalmia and colobomata [126]</td>
<td>Germline</td>
</tr>
<tr>
<td>GDF5 (#601146)</td>
<td>Du Pan type chondrodysplasia (#328090); brachydactyly type C (#113100); proximal symphalangism (#185800)</td>
<td>Germline</td>
</tr>
<tr>
<td>GDF6 (#601147)</td>
<td>Klippel–Feil syndrome (#118100); spondylocostal dysostosis 4 (#122600)</td>
<td>Germline</td>
</tr>
<tr>
<td>GDF8/Myostatin (#601788)</td>
<td>Muscle hypertrophy [127]; DMD (#310200)</td>
<td>Germline</td>
</tr>
<tr>
<td>GDF9 (#601918)</td>
<td>POIF (#300510)</td>
<td>Germline</td>
</tr>
<tr>
<td>Inhibin α (#147380)</td>
<td>POIF [128]</td>
<td>Somatic</td>
</tr>
<tr>
<td>AMH (#600957)</td>
<td>Persistent Mullerian duct syndrome (#261550)</td>
<td>Germline</td>
</tr>
<tr>
<td><strong>Type I receptor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALK1/ACVR1L1 (#601284)</td>
<td>HHT (HHT2) (#600376); gonadotrophi tumour; A482V mutation of unknown significance [129]</td>
<td>Germline</td>
</tr>
<tr>
<td>ALK2/ACVR1 (#6012576)</td>
<td>FOP (#1035100)</td>
<td>Germline</td>
</tr>
<tr>
<td>ALK3/BMPRIA (#601299)</td>
<td>JP (#174900); hereditary mixed polyposis (#610069); JP of infancy (#612242)</td>
<td>Germline</td>
</tr>
<tr>
<td>ALK4/ACVR1B (#601300)</td>
<td>Pancreatic carcinoma (#266550)</td>
<td>Somatic</td>
</tr>
<tr>
<td>ALKS/TGFBR1 (#190181)</td>
<td>Loey–Dietz syndrome (#609192); type 2 Marfan syndrome</td>
<td>Germline</td>
</tr>
<tr>
<td>ALK6/BMPRIA (#603248)</td>
<td>Frachydactyly (#112600); chondrodysplasia, acromesomelic, with genital anomalies [130]</td>
<td>Germline</td>
</tr>
<tr>
<td><strong>Type II receptor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACVR2/ActRIIA (#102581)</td>
<td>Human pituitary tumours (45 %) [129]</td>
<td>Polymorphism</td>
</tr>
<tr>
<td>ACVR2B/ActRIIB (#602730)</td>
<td>Left-right axis malformations, visceral heterotaxy (autosomal type 4) (HT4X) (#602730)</td>
<td>Germline</td>
</tr>
<tr>
<td>BMPR2/BMPRII (#600799)</td>
<td>Familial primary pulmonary hypertension (PAP) (#178600)</td>
<td>Germline</td>
</tr>
<tr>
<td>TGFBR2/TBR2 (#190182)</td>
<td>Multiple cancers (colorectal, gastric, endometrial, prostate, breast, lung, hepatocellular, lymphoma, pancreatic and cervical cancer, and glioma) (#190182); Loey–Dietz syndrome (#610380); atherosclerosis [131]; Marfan syndrome (#154700)</td>
<td>Somatic/germline</td>
</tr>
<tr>
<td>AMHR2/MSRRII (#600956)</td>
<td>Persistent Mullerian duct syndrome (#261550)</td>
<td>Germline</td>
</tr>
<tr>
<td><strong>Type III receptor</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endoglin/CD105/ENG (#131195)</td>
<td>HHT (#187300)</td>
<td>Germline</td>
</tr>
<tr>
<td>Betaglycan/TGFBRIII (#600742)</td>
<td>Possible association between gene expression levels of TGFBR2 and bone mineral density (#612728)</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Smad</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smad2 (#601366)</td>
<td>Colorectal cancer [132]; colon cancers [133]</td>
<td>Somatic</td>
</tr>
<tr>
<td>Smad3 (#603109)</td>
<td>Osteoarthritis [134]</td>
<td>Germline</td>
</tr>
<tr>
<td>Smad4 (#600933)</td>
<td>JPS (#174900); pancreatic and colorectal cancer [135]</td>
<td>Germline</td>
</tr>
<tr>
<td>Smad8 and Smad9 (#603295)</td>
<td>Primary pulmonary hypertension (#178600)</td>
<td>Germline</td>
</tr>
</tbody>
</table>

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 proatherogenic changes in the blood vessel wall. Conversely, viral delivery of TGF-β1 in an atherosclerotic mouse model [Ldlr (low-density lipoprotein receptor)−/−] suppressed formation of

© The Authors Journal compilation © 2011 Biochemical Society
atherosclerotic lesions. In humans, common genetic variants in the TGFβ1 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. Similarly to TGF-β1, activin 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 (γ-carboxylated glutamate) protein; an 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 remodelling often leading to limb loss or death [50]. A critical role for TGF-β in restenosis has been supported by several experimental approaches [51]. Thus overexpression of TGF-β1 in arteries induces neoimal hyperplasia and fibrosis, and animal models with targeted disruption of TGF-β prevent neointima formation and the constrictive remodelling associated with angioplasty [51,52]. In addition, Smad7 overexpression attenuates collagen deposition, remodelling and contribution of adventitial fibroblasts to neointima formation after balloon angioplasty [53]. On the contrary, BMP4 may contribute to graft neointimal atrophy by inhibiting 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 TGFβ1, 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 RAAS (renin–angiotensin–aldosterone system) contributes to arterial hypertension. Interestingly, TGF-β signalling is up-regulated by the RAAS axis, and ACE (angiotensin-converting enzyme) 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 AngII (angiotensin II)-induced aldosterone in human adrenocortical cells, suggesting that BMP6 may be involved in aldosterone breakdown induced by chronic treatment with AngII receptor antagonists [57].

Pre-eclampsia 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 up-regulated and play a major pathogenic role, through an anti-angiogenic effect [41].

Heart disease involves cardiac remodelling, 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 remodelling 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 signalling pathways. In humans, heterozygous loss-of-function mutations in the GDF1 contribute to cardiac defects in TOF (tetralogy of Fallot) [59], whereas mutations in TGFβ3 have been related with the ARVD (arrhythmogenic right ventricular dysplasia-1) [60]. Moreover, TGF-β1 may act as a downstream target of angiotensin signalling, mediating AngII-induced hypertrophy. AngII promotes cardiomyocyte and fibroblast proliferation concomitant with an increased expression of ECM proteins [37,58], and clinical trials have documented the beneficial effects of AngII 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 TGFβ1 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 NSTE-ACS (non-ST-elevation acute coronary syndrome) have significantly elevated circulating levels of GDF15. In addition, patients who had died from a myocardial infarction show markedly enhanced GDF15 levels in the ischaemic myocardium, suggesting that GDF15 may be a biomarker for heart diseases [64].

**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 DN (diabetic nephropathy), congestive heart failure, rheumatoid arthritis, Marfan syndrome, hypertrophic scars, SSc (systemic sclerosis), myocarditis and Crohn’s disease [37,65,66]. Multiple lines of evidence show the involvement of TGF-β as a critical regulator of both physiological fibrogenesis and pathological fibrosis. The fibrotic reaction is characterized by an increased production of ECM components, such as fibronectin, collagen, laminin and
vimentin 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 CTGF (connective tissue growth factor). TGF-β induces CTGF expression in fibroblasts, which promotes collagen synthesis and microfibril 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 fibroblast differentiation. Indeed, TGF-β signalling through Smad3 directly promotes expression of type I collagen, a major component of the ECM, during fibrosis [68]; Smad3 is required for AngII-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 (epithelial–mesenchymal transition) in alveolar epithelial cells and transdifferentiation of quiescent hepatic stellate cells into myofibroblasts, respectively [70]. In addition, EMT may contribute to TGF-β-induced cardiac fibrosis [71,72]. Progression of 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 4-fold in the urine of diabetic compared with non-diabetic patients, suggesting that overproduction of TGF-β1 in the kidneys may contribute to DN [65,74].

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 the 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 BPD (bronchopulmonary dysplasia), emphysema, COPD (chronic obstructive pulmonary disease) 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 signalling 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 airway disease [76]. Genetic studies have identified TGF-β as a promising candidate gene related to COPD based on association analyses between SNPs (single nucleotide polymorphisms) in the TGFBI 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 remodelling and fibrosis may be provoked by cigarette smoke [75].

Asthma is a chronic inflammatory disorder of the airways whose distinctive trait is the presence of structural changes (remodelling) of the airway wall. Airway remodelling is characterized by subepithelial fibrosis with thickening of basement membrane in areas of proximal airways. TGF-β1 signalling 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-β1 signalling [75].

IPF (idiopathic pulmonary fibrosis) is one form of DPLD (diffuse parenchymal lung diseases) 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 of patients [79].

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 haematopoiesis. Bone tissue is continuously remodelled 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. Tgfb1-knockout mice survivors 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 the Acvr2 (type IIA activin A receptor) or ActivinA2b (type IIB activin A receptor) gene show multiple bone defects. Some Acrv2-null mice exhibit hypoplasia of the mandible and other skeletal abnormalities [8]. Bmpr1b (type IB BMP receptor)-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 signalling in several hereditary diseases affecting bone and muscle [37].

CED (Camurati–Engelmann disease) 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 transcriptions [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 SNPs associated with osteoporosis have been identified in TGFB1 (encoding TBR1), TGFB2, SMAD2, SMAD3, SMAD4 and SMAD7 [84,85].

BDA (brachydactyly type A2) 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 BMP1R/BMPR1B gene, acting in dominant-negative manner, are responsible for bone malformation in BDA [86]. Mutations in GDF5 cause BDC (brachydactyly type C), an autosomal dominant disorder characterized by an abnormal shortness of the fingers and toe defects [87] as well as AMDH (acromesomelic chondrodysplasia Hunter–Thompson) type, an autosomal-recessive form of dwarfism, characterized by normal axial skeletons and missing or fused skeletal elements within the hands and feet. In addition, a mutation in GDF5 (R438L) is responsible for proximal sympalangism, showing fusion of carpal and tarsal bones and ankylosis of the proximal interphalangeal joints. Du Pan syndrome originates from 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 FOP (fibrodysplasia ossificans progressiva), 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 caALK2 (constitutively active 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. SCDO4 (spondylocostal dystostosis 4) 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]. DMD (Duchenne muscular dystrophy) 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 DMD (dystrophin) gene, but additional polymorphisms in GDF8/MSTN (encoding myostatin) were identified in DMD patients [94]. GDF8/myostatin is a muscle-specific ligand that negatively regulates muscle growth. Accordingly, GDF8/MSTN mutations are associated with gross muscle hypertrophy [95], and disruption of endogenous Gdf8/Mstn 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.

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 signalling results in several reproductive disorders.
POF (premature ovarian failure) 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 and GDF9 are crucial in folliculogenesis and follicle differentiation in mammals [99]. A heterozygous mutation in BMP15 has been identified in two sisters with primary amenorrhea, and the mutant BMP15 protein (Y235C) antagonizes the activity of normal BMP15 leading to a decreased proliferation of granulosa cells [100]. Additional BMP15 and GDF9 variants have been identified in POF patients [99, 101]. Most of these mutations involve the propeptide region and lead to a defective production of bioactive protein, 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 INHA (inhibin α) gene has been identified in patients with POF, resulting in an inactive mutant protein (A257T).

The PMDS (persistent Müllerian duct syndrome) is a rare form of inherited male pseudohermaphroditism characterized by the presence of a uterus and sometimes other Müllerian duct derivatives in otherwise normally masculinized XY males [11]. The phenotype can be produced by a mutation in the gene encoding 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 [12]. 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 [12].

**TGF-β in cancer**

TGF-β has a tumour-suppressive role at early stages of tumour 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 types 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, tumour cells evade the TGF-β suppressive action by different mechanisms, and, paradoxically, TGF-β becomes a pro-oncogenic factor that stimulates tumour cell growth and invasiveness at later stages of tumorigenesis. The pro-oncogenic activities of TGF-β are exerted at both compartments, the tumour and the stroma. Thus TGF-β stimulates tumour cell proliferation by inducing the production of autocrine mitogenic growth factors, such as PDGF-B (platelet-derived growth factor B). It also induces EMTs 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 tumour stroma, TGF-β stimulates the generation of myofibroblasts from mesenchymal precursors, the so-called cancer-associated fibroblasts that facilitate tumour cell proliferation, invasion and promote neoangiogenesis. In addition, 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 here.

Several components of the TGF-β signalling pathway are inactivated in subsets of pancreatic, colorectal, gastric, ovarian and head and neck tumours, which disable the tumour suppressive action of TGF-β [108]. In individuals with familial syndromes, germline mutations in some of these tumour suppressor genes have also been found. In the present paper, 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.

HNPPC (hereditary non-polyposis colorectal cancer) 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, MSI (microsatellite instability). HNPPC 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-bp polyadenine repeat in TBRII (BART-II). BART-II mutations that lead to a truncated receptor lacking the transmembrane and the intracellular kinase domains have been identified at a high frequency in HNPPC [110] and other MSI-associated cancers [108].

JPS (juvenile polyposis syndrome) 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 (now known as SMAD4) 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 approximately 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].

HMPS (hereditary mixed polyposis syndrome) is another autosomal dominantly inherited syndrome characterized by mixed ‘adenomatous/hyperplastic/atypical juvenile’ polyps. Germline BMPRIA mutations have also been found in some HMPS cases. 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 tumourigenesis [109].

THERAPEUTIC INTERVENTIONS TARGETING THE TGF-β SIGNALLING PATHWAY

As explained above, the TGF-β signalling pathway is an attractive target for therapy in a number of diseases, including fibrotic and CV 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 signalling may be beneficial for therapeutic purposes as in the case of recombinant human BMPs that activate the bone regenerative properties of the BMP pathway [6,114]. A summary of the current clinical trials is shown (Table 3 and Figure 3). Further information can be obtained from the NIH webpage (http://clinicaltrials.gov).

Ligand traps

Neutralizing antibodies

The pan-TGF-β antibody GC-1008 (Genzyme) was tested in a Phase I clinical study of patients with renal cell carcinoma or malignant melanoma (NCT00356460). Treatment was well tolerated with mainly grades 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.

Antisense

Another approach, which has entered clinical trials, inhibits TGF-β function by means of AS-ODNs. The anti-tumorigenic effect of AS-ODNs was supported by Phase I/II trials with the TGFβ2 antisense compound AP12009 (Trabedersen; Antisense Pharma). In comparison with standard chemotherapy, treatment with AP12009 resulted in prolonged survival of patients with anaplastic astrocytoma, and a Phase III trial 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 compared with 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 [117].

A Phase II trial with Lucanix (NovaRx), a TGF-β2 antisense gene-modified allogeneic tumour vaccine, has been 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 GM-CSF (granulocyte/macrophage colony-stimulating factor) and antisense TGFβ2 autologous tumour cell vaccine for the treatment of advanced metastatic carcinoma. Is it expected that GM-CSF overexpression stimulates expression of tumour antigens in tumour cells and dendritic cell migration, whereas TGF-β2 blockade by TGFβ2 antisense may allow for a better immune response at the vaccine site.

Soluble receptors

TGF-β1 is one of the main mediators in the fibrotic process, associated with both scarring and a long list of pathologies related to chronic inflammation affecting several organs and tissues. ISDIN (Barcelona, Spain) has developed a 14-mer peptide (P144) from human betaglycan designed to block the interaction between TGF-β1 and the signalling 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 muscle growth [118]. A Phase II trial is ongoing in patients with DMD (NCT01099761), and a Phase I trial is ongoing in healthy postmenopausal women with unexplained weight loss (NCT00952887).

Receptor inhibitors
Most of the current strategies to inhibit TGF-β signalling at the receptor kinase level include the use of small molecule inhibitors, which bind to the ATP-binding domain of the receptors [119]. 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/Smad3 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 EBV (Epstein–Barr virus)-positive lymphoma (NCT00368082). Autologous or allogeneic LMP (latent membrane protein)-specific cytotoxic T-cells have been retrovirally genetically modified to express a dominant negative TBRII (DNRII) to render them resistant to the immunosuppressive effects of TGF-β.
Table 3  Selected clinical studies using agents targeting the TGF-β pathway

<table>
<thead>
<tr>
<th>Type of compound</th>
<th>Drug name</th>
<th>Target</th>
<th>Clinical trial/study (ClinicalTrials.gov Identifier)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human anti-TGF-β mAb (pan-neutralizing IgG4)</td>
<td>GC1008</td>
<td>TGF-β1</td>
<td>Phase I on malignant carcinoma, and renal cell carcinoma (NCT00356460) &lt;br&gt; Phase II on relapsed malignant pleural (NCT01112293) &lt;br&gt; Phase I in treatment of resistant idiopathic focal segmental glomerulosclerosis (NCT00464321)</td>
</tr>
<tr>
<td>Human anti-TGF-β1 mAb CAT-192</td>
<td>AP12009 (Trabedersen)</td>
<td>TGF-β2</td>
<td>Phase I on pancreatic and colorectal neoplasms, and melanoma (NCT00844064) &lt;br&gt; Phase II on glioblastoma and anaplastic astrocytoma (NCT00431561) &lt;br&gt; Phase III on anaplastic astrocytoma (NCT00761280)</td>
</tr>
<tr>
<td>TGF-β2 AS-ODN</td>
<td>Lucanix</td>
<td>TGF-β2</td>
<td>Phase III on lung neoplasm carcinoma, non-small cell lung Stage IIIA (T3, N2 only) carcinoma, non-small cell lung Stage IIIB carcinoma, and non-small cell lung Stage IV (NCT00676507)</td>
</tr>
<tr>
<td>TBRI and TBRII inhibitor</td>
<td>TGFβR2 and ALK5</td>
<td>Phase II for the treatment of skin fibrosis in systemic sclerosis (NCT00574613 and NCT00781053)</td>
<td></td>
</tr>
<tr>
<td>Soluble activin type IIB receptor</td>
<td>AC-031</td>
<td>GDF8/myostatin</td>
<td>Phase II DMD (NCT01099761) &lt;br&gt; Phase I in healthy postmenopausal women with weight loss (NCT00952887)</td>
</tr>
<tr>
<td>Human anti-ALK1 mAb</td>
<td>PF03446962</td>
<td>ALK1</td>
<td>Phase I on advanced solid tumours (NCT00557856)</td>
</tr>
<tr>
<td>Human/murine chimaeric anti-endoglin mAb</td>
<td>TRC105</td>
<td>Endoglin/CD105</td>
<td>Phase I on cancer, neoplasm metastasis (NCT00582985) &lt;br&gt; Phase I of degenerative lumbar disc disease, spondylolisthesis and spinal stenosis (NCT00405600) &lt;br&gt; Phase II to degenerative disc disease (NCT00707265) &lt;br&gt; Phase I of osteoarthritis (NCT00243295) &lt;br&gt; Phase II for osteoporosis (NCT00752557)</td>
</tr>
<tr>
<td>Human recombinant BMP2</td>
<td>rhBMP2</td>
<td>BMP receptors</td>
<td>Phase II for the treatment of osteoarthritis of the knee (NCT0111104)</td>
</tr>
<tr>
<td>Human recombinant BMP7</td>
<td>rhBMP7</td>
<td>BMP receptors</td>
<td>Phase II for the treatment of degenerative disc disease (NCT00813813; NCT01124006) &lt;br&gt; Phase I/II for the treatment of early lumbar disc degeneration (NCT01158924)</td>
</tr>
</tbody>
</table>

Tumours 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 [17,36]. 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 tumours 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 grades 1–2 toxicity, including fatigue, anaemia, proteinuria and diarrhoea. One patient with hormone refractory prostate cancer obtained a complete PSA (prostate-specific antigen) response, and three patients had prolonged stable disease [120].

When increased BMP signalling contributes to disease pathogenesis, inhibitors may offer therapeutic benefit, as it is the case of 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 signalling from ALK1, ALK2, BMPR-IA/ALK3 and BMPR-IB/ALK6 and blocks BMP-induced Smad1/Smad5/Smad8 phosphorylation. In addition, Dorsomorphin-optimized derivatives LDN-193189 or DM-3189 with higher activity and specificity for type I BMP 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 caALK2, leading to a reduction in ectopic ossification and functional impairment in mice [6].

© The Authors Journal compilation © 2011 Biochemical Society
**Recombinant ligands**

On the basis of animal studies demonstrating dramatic increases in fusion mass and quality of bone regeneration using BMPs [6], clinical trials in humans have been initiated. Although animal models have shown osteoinduction 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 (recombinant human osteogenic protein-1)]. BMP2 is being used in the following trials: Phase I of degenerative lumbar disc disease, spondylolysisis and spinal stenosis (NCT00405620); in Phase II treatment 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 (NCT01111104). In addition, two clinical studies in Phase II are in progress by using GDF5/BMP14 for the treatment of degenerative disc disease (NCT00813813 and NCT01124006) and one in Phase I/II for the treatment of early lumbar disc degeneration (NCT01158924).

**Other preclinical approaches to regulate TGF-β signalling**

Disruption of the intracellular Smad signalling may become a relevant strategy to control TGF-β superfamily signalling. 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 [119]. HGF (hepatocyte growth factor) exerts anti-fibrotic effects by opposing TGF-β1/Smad signalling. Induction of the inhibitory Smad7 by HGF, treatment with the HGF synthetic analogue BB3 (Angion Biomedica) and gene transfer of Smad7 showed antifibrotic properties in animal models [121,122]. Also, Paclitaxel/Taxol, an anticancer drug that stabilizes microtubules, attenuated hepatic fibrosis by inhibiting TGF-β signalling [123].

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-Abl (c-Abelson) 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 [119].

A growing interest exists in using commonly used drugs for anti-TGF-β therapy (Table 3). For example, the antihypertensive drug losartan, an AngII receptor blocker, has been reported to antagonize TGF-β signalling through inhibition of the renin–angiotensin axis. Accordingly, losartan reduced renal wall thickness by suppression of local TGF-β signalling 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 DN and that, by lowering TGF-β1 production, the ACE inhibitor therapy may protect the kidney. Moreover, Tranilast, which is currently used for the treatment of asthma, allergic rhinitis and atopic dermatitis by inhibiting mast cell degranulation, has shown a potent antifibrotic effect in sclerotic fibroblasts and in animal models of fibrosis. In addition, Tranilast showed beneficial effects preventing stricture 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 HDAC (histone deacetylase) inhibitors. In cancer, the loss of TGF-β signalling occurs early in carcinogenesis and contributes to tumour 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 therapy, aims to activate the tumour-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 (trichostatin A) and sodium butyrate successfully restores the expression of TGFBR2 [124].

**CONCLUSIONS**

The core molecular components of the TGF-β/receptors/Smad signalling 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 signalling in vitro and in vivo, including the ligand bioavailability and sensing by receptors as well as the characterization of possible cross-talks with other signalling pathways. These studies may provide novel molecular targets for treatment of diseases caused by malfunctioning of the TGF-β signalling pathway such as CV, fibrosis, reproductive, cancer or...
wound-healing disorders. In addition, identification of small molecules (inhibitors and potentially activators) of TGF-β signalling using high-content screenings raises new hopes for future therapeutic interventions.

ACKNOWLEDGEMENTS

We acknowledge the contributions of many researchers that, although relevant to the issues dealt with in the present review, could not be included due to space limitations. The CIBER de Enfermedades Raras is an initiative of the Instituto de Salud Carlos III (ISICIII) of Spain.

FUNDING

Our work was supported by the Spanish Ministry of Science and Innovation [grant numbers SAF2010-19222 (to C.B.) and SAF2010-19152 (to M.Q.)], Genoma España [MEICA (to C.B.)], Centro de Investigación Biomédica en Red (CIBER) de Enfermedades Raras (to C.B.), and the Ministry of Science and Technological Development, Republic of Serbia [grant number 175062 (to J.F.S.)].

REFERENCES


© The Authors Journal compilation © 2011 Biochemical Society
SUPPLEMENTARY ONLINE DATA

TGF-β/TGF-β receptor system and its role in physiological and pathological conditions

Juan F. SANTIBÁÑEZ*, Miguel QUINTANILLA† and Carmelo BERNABEU‡

∗Institute for Medical Research, University of Belgrade, 11129 Belgrade, Serbia, †Instituto de Investigaciones Biomédicas Alberto Sols, Consejo Superior de Investigaciones Científicas (CSIC)-Universidad Autónoma de Madrid, Madrid, Spain, and ‡Centro de Investigaciones Biológicas, CSIC and Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), 28040 Madrid, Spain

Figure S1 Schematic diagram of the dimeric structure of inhibins and activins
Monomers are linked by disulfide bonds (black line).

Figure S2 Generation of soluble type III receptors by proteolytic processing of membrane-bound endoglin or betaglycan
The MT1-MMP (membrane-type metalloprotease-1) has been reported to cleave the juxtamembrane region (green arrowhead), leading to the secretion of the large ectodomain of endoglin or betaglycan. Several functions reported for these soluble type III receptors are indicated. The EC (extracellular) TM (transmembrane) and CYT (cytoplasmic) domains as well as the juxtamembrane ZP motif are indicated. Adapted from Lopez-Novoa and Bernabeu, 2010 [41].

Received 22 February 2011/9 March 2011; accepted 11 March 2011
Published on the Internet 27 May 2011, doi:10.1042/CS20110086

Correspondence: Professor Carmelo Bernabeu (email bernabeu.c@cib.csic.es).