TGF-β and the TGF-β Family: Context-Dependent Roles in Cell and Tissue Physiology

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The transforming growth factor-β (TGF-β) is the prototype of the TGF-β family of growth and differentiation factors, which is encoded by 33 genes in mammals and comprises homo- and heterodimers. This review introduces the reader to the TGF-β family with its complexity of names and biological activities. It also introduces TGF-β as the best-studied factor among the TGF-β family proteins, with its diversity of roles in the control of cell proliferation and differentiation, wound healing and immune system, and its key roles in pathology, for example, skeletal diseases, fibrosis, and cancer.

Although initially thought to stimulate cell proliferation, just like many growth factors, it became rapidly accepted that transforming growth factor β (TGF-β) is a bifunctional regulator that either inhibits or stimulates cell proliferation. TGF-β was originally isolated as a cytokine that, together with epidermal growth factor (EGF), induces cellular transformation and anchorage-independent growth of selected fibroblast cell lines (Roberts et al. 1981), yet did not require the presence of EGF to induce phenotypic transformation of other fibroblast cell lines (Shipley et al. 1984). In contrast, TGF-β was also identified as a growth inhibitor secreted from confluent BSC-1 cells, epithelial cells of African green monkey kidney (Tucker et al. 1984). The growth inhibitory activity of TGF-β has been well documented in most cell types, and has been best characterized in epithelial cells. The bifunctional and context-dependent nature of TGF-β activities was further confirmed in a large variety of cell systems and biological responses. For example, TGF-β can inhibit EGF-dependent proliferation of cells in monolayer culture, whereas TGF-β and EGF synergistically enhance anchorage-independent growth of the same cells in soft agar medium (Roberts et al. 1985). Now, it is widely accepted that TGF-β regulates a variety of key events in normal development and physiology, and perturbation of TGF-β signaling has been implicated in the pathogenesis of diseases such as connective tissue disorders, fibrosis, and cancer.

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The identification of TGF-β family members and their signaling components has enabled the characterization of the complex biology of the TGF-β family members. Molecular cloning of TGF-β family members and their signaling mediators started in 1985 with the reported characterization of complementary DNA (cDNA) coding for human TGF-β1 (Derynck et al. 1985). Subsequently, various approaches, based on biochemical purification, developmental genetics, and/or targeted cDNA cloning, led to the identification of polypeptides structurally similar to TGF-β1, which together comprise the members of the TGF-β family. Now that the human and mouse genome sequence projects are completed, it is apparent that mammalian genomes encode 33 TGF-β-related polypeptides. Table 1 shows the 33 known human TGF-β family polypeptides, which include three TGF-β isoforms, activins, nodal, bone morphogenetic proteins (BMPs), and growth and differentiation factors (GDFs). Although mostly studied as homodimers, various heterodimeric combinations of these have also been identified and characterized as biologically active proteins.

In contrast to the large number of TGF-β ligands, fewer receptors and downstream intracellular effectors, termed Smad proteins, mediate transduction of intracellular signaling. In 1991, a cDNA clone for an activin receptor, currently known as ActRII, was isolated (Mathews and Vale 1991). The kinase domain of this receptor closely resembled the *Caenorhabditis elegans daf-1*, a transmembrane serine/threonine-specific protein kinase, which at that time was seen as an orphan receptor, and provided the first indication that TGF-β family members signal through transmembrane serine/threonine kinases. In the following years, seven type I receptors and five type II receptors were identified in mammals and were shown to form heteromeric type I/type II receptor complexes. Genetic analysis in *Drosophila* and *C. elegans* led to a breakthrough in how signals are transduced from the receptors to the nucleus. In *Drosophila*, *mothers against dpp* (*Mad*) was identified as a gene encoding a component that acts epistatically downstream from Decapentaplegic (Dpp; *Drosophila* BMP-2/-4 ligand) (Raftery et al. 1995; Sekelsky et al. 1995). In *C. elegans*, *daf-1* and *daf-4* turned out to also encode serine/threonine transmembrane kinase receptors for TGF-β family members. Screening for mutants with similar phenotypes with *daf-4* revealed three genes, *sma-2*, *sma-3*, and *sma-4*, that were structurally similar to *Mad* of *Drosophila* (Savage et al. 1996). In frog, mouse, and human, genes structurally similar to *Mad* and *sma* were subsequently identified, and the designation "Smad" (Sma and Mad) was adopted. Ligand binding to specific tetrameric type II/type I receptor complexes stabilizes and activates their signaling capacities, and the receptors then transduce the signals by phosphorylating carboxy-terminal serine residues of receptor-regulated (R-) Smads. In most cell types, TGF-βs and activins induce phosphorylation of Smad2 and Smad3 (activin/TGF-β-specific R-Smads), and BMPs induce phosphorylation of Smad1, Smad5, and Smad8 (BMP-specific R-Smads). The activated R-Smads form hetero-oligomeric complexes with a common-partner (co-) Smad, that is, Smad4 in vertebrate cells (Lagna et al. 1996; Zhang et al. 1996; Kawabata et al. 1998). The complexes translocate into the nucleus where they regulate the expression of target genes, such as those encoding inhibitory (I-) Smads, namely, Smad6 and Smad7 in vertebrates, which can inhibit R-Smad activation by the receptors. Finally, TGF-β family proteins were also shown to induce PI3K-Akt signaling and to activate the common mitogen-associated protein (MAP) kinase pathways that are activated by receptor tyrosine kinases, albeit, generally, to a lower extent. Now that essential players in the signaling pathways have been identified, one of the major questions to be addressed in this field is to reveal the precise molecular mechanisms that define the context-dependent dual roles of TGF-β family members.

In this review, we will introduce the TGF-β family members, which in mammals are encoded by 33 genes. We will cluster them into several subgroups based on the structural or sequence similarities of the encoded polypeptides. We further focus on the three TGF-β isoforms, TGF-β1, -β2, and -β3, as the best-studied factors among the TGF-β family proteins. In ad-

Table 1. Names and genes for the TGF-β family proteins

Protein name	Official gene symbol (human)	Protein name synonyms
TGF-β1	*TGFB1*	CIF-A (cartilage-inducing factor-A), differentiation-inhibiting factor
TGF-β2	*TGFB2*	G-TsF (glioblastoma-derived, T-cell suppressor factor), BSC-1 GI (BSC-1 cell-growth inhibitor), polyergin, CIF-B (cartilage-inducing factor-B)
TGF-β3	*TGFB3*	
Inhibin α	*INHA*	Inhibin A and B[a]
Inhibin β_A	*INHBA*	Inhibin A and activin A or AB,[a,b] FRP (follicle-stimulating hormone-releasing protein), EDF (erythroid differentiation factor), XTC-MIF (*Xenopus* XTC cell mesoderm-inducing factor)
Inhibin β_B	*INHBB*	Inhibin B and activin B or AB,[a,b] XTC-MIF
Inhibin β_C	*INHBC*	Activin C[c]
Inhibin β_E	*INHBE*	Activin E[c]
Nodal	*NODAL*	BMP-16 (bone morphogenetic protein-16)
Myostatin	*MSTN*	GDF-8 (growth and differentiation factor-8)
BMP-2	*BMP2*	
BMP-3	*BMP3*	Osteogenin
BMP-4	*BMP4*	BMP-2B
BMP-5	*BMP5*	
BMP-6	*BMP6*	Vgr1 (Vg1-related protein)
BMP-7	*BMP7*	OP-1 (osteogenic protein-1)
BMP-8A	*BMP8A*	OP-2
BMP-8B	*BMP8B*	OP-3
BMP-9	*GDF2*	GDF-2
BMP-10	*BMP10*	
GDF-1	*GDF1*	
GDF-3	*GDF3*	Vgr2
GDF-5	*GDF5*	CDMP-1 (cartilage-derived morphogenetic protein-1), BMP-14
GDF-6	*GDF6*	CDMP-2, BMP-13
GDF-7	*GDF7*	CDMP-3, BMP-12
GDF-9	*GDF9*	
GDF-9B	*BMP15*	BMP-15
GDF-10	*GDF10*	BMP-3b
GDF-11	*GDF11*	BMP-11
GDF-15	*GDF15*	Placental TGF-β, placental BMP (PLAB), PDF (prostate-derived factor), NAG-1 (nonsteroidal anti-inflammatory drug-activated gene-1), MIC-1 (macrophage inhibitory cytokine-1)
MIS (Müllerian-inhibiting substance)	*AMH*	AMH (anti-Müllerian hormone)
Lefty A	*LEFTY2*	EBAF (endometrial bleeding-associated factor), TGF-β4, Stra3
Lefty B	*LEFTY1*	

[a]Inhibin A or B is a heterodimer of inhibin α and inhibin β_A or β_B, respectively.
[b]Activin A or B is a homodimer of inhibin β_A or β_B, respectively. Activin AB is a heterodimer of inhibin β_A and β_B.
[c]Activin C or D is a homodimer of inhibin β_C or β_D, respectively.

dition to the original "transforming" potential, we introduce their diverse roles in the control of cell proliferation, differentiation, wound healing, and the immune system, and TGF-β's key roles in the context of pathological processes in vivo, for example, connective tissue disorders, fibrosis, and cancer.

TGF-β FAMILY LIGANDS IN MAMMALS

The name TGF-β derives from the transforming activity of the cytokine, which induces anchorage-independent growth when administered to cells together with EGF (Roberts et al. 1981) or without the need to add EGF (Shipley et al. 1984), depending on the cell system. At first, two distinct transforming growth factors, that is, TGF-α and -β, were identified and isolated. TGF-α is related to EGF and binds to the EGF receptor, whereas TGF-β is structurally distinct from TGF-α. The most striking characteristic, which distinguished the two cytokines at that time, was that TGF-β is a 25-kd disulfide-linked dimer that is reduced to a 12.5-kd band on gels following treatment with reducing agents, for example, β-mercaptoethanol (Roberts et al. 1983), whereas TGF-α was a monomeric protein of smaller size (Roberts et al. 1980).

All TGF-β family members are encoded by much larger precursor polypeptides whose sequences have been deduced through cDNA cloning. The precursor polypeptides are composed of three segments: an amino-terminal signal peptide that is removed during translocation of the protein into the lumen of the rough endoplasmic reticulum, a large precursor segment or prosegment, and the carboxy-terminal TGF-β family monomer polypeptide for the active and fully mature TGF-β family protein (112 amino acid residues in the case of TGF-β). The prosegments vary in length from 150 to 450 residues and are remarkably not conserved in sequence among TGF-β family members, even among the three TGF-β isoforms (Derynck et al. 1988). Indeed, the sequence similarities among TGF-β-related proteins pertain exclusively to the sequences of the mature polypeptides that follow the prosegments. In the precursor, the prosegment is immediately followed by a cleavage site for a furin protein convertase that precedes the carboxy-terminal mature TGF-β family polypeptide. In the case of the three TGF-β isoforms, the prosegments are also known as latency-associated peptides (LAPs). Following secretion, the LAPs remain noncovalently associated with the mature TGF-β dimer and keep the TGF-βs latent, that is, unable to bind and activate the receptors, in complexes that are called "small latent complexes" (SLCs). Whereas these prosegments are likely to play important roles in folding and transport of the TGF-β family proteins, they were shown to remain associated following secretion with a number of mature TGF-β family proteins, such as with BMP-4, BMP-7, BMP-9 (also known as GDF-2), BMP-10, GDF-5, GDF-11, myostatin (also known as GDF-8), and Müllerian-inhibiting substance (MIS, also known as anti-Müllerian hormone, AMH) (Wilson et al. 1993; Wolfman et al. 2003; Brown et al. 2005; Ge et al. 2005; Sengle et al. 2008). However, only some of the TGF-β family members are produced as latent forms that are unable to bind the receptors. With the exception of the TGF-β1 complex, the roles of the prosegments for other TGF-β family proteins have been poorly characterized, even for the closely related TGF-β2 and -β3.

The latency and activation mechanisms that result in the release of active TGF-β protein have been primarily studied in the case of TGF-β1, with additional knowledge gained on the activation of myostatin (Wolfman et al. 2003). The prosegment of TGF-β1 contains a proline-rich loop, or latency lasso, which surrounds a TGF-β1 monomer like a straitjacket (Shi et al. 2011). Comparison between TGF-β1 LAP and the BMP-9 prosegment, which does not confer latency, suggests conformational differences (Mi et al. 2015). Further analysis of the prosegments of other TGF-β family members, especially nonlatent ones, will reveal functional conservation among them.

For TGF-β to bind to its cell-surface receptors, the LAP needs to be released from the mature peptide. Both the TGF-β1 LAP and -β3 LAP contain an integrin recognition motif Arg-Gly-Asp, or RGD, and bind to several different integ-

rins, for example, $\alpha_v\beta_6$ and $\alpha_v\beta_8$. Biochemical and structural studies revealed that contractile force is necessary for the release of mature TGF-β1 after complex formation between integrin $\alpha_v\beta_6$ and TGF-β1 LAP (Annes et al. 2004; Shi et al. 2011). In agreement with these findings, mice with RGD-to-RGE mutation in TGF-β1 LAP (Yang et al. 2007) and integrin β_6-deficient mice (*Itgb6*$^{-/-}$) (Munger et al. 1999) recapitulate aspects of the phenotype of *Tgfb1*$^{-/-}$ mice, indicating the importance of the interaction between LAP and integrins in latent TGF-β1 activation. The SLC covalently attaches the large latent TGF-β-binding protein (LTBP) through LAP, thus, forming the large latent complex (LLC). The association of LTBP with the SLC is also important for proper TGF-β1 function. Mice expressing a mutant TGF-β1 precursor (C33S) with a prosegment that is unable to bind LTBP, instead of the wild-type TGF-β1 precursor, are hypomorphic for TGF-β1; *Tgfb1*$^{C33S/C33S}$ mice show decreased levels of active TGF-β1, decreased TGF-β signaling, and formation of gastrointestinal tumors (Yoshinaga et al. 2008). LTBPs are efficiently incorporated in the extracellular matrix (ECM) (Taipale et al. 1994; Dallas et al. 1995), where latent TGF-β, as a part of LLC, is stored for future mobilization and activation. Thus, the regulation of latency of TGF-β has substantial biological significance, although it has received little attention. The control of TGF-β latency has been reviewed elsewhere (Horiguchi et al. 2012).

The mammalian genome encodes three different TGF-β isoforms, TGF-β1, -β2, and -β3. The mature proteins are highly conserved in sequence and are marked by the presence of nine cysteine residues. The three-dimensional structure of TGF-β2 reveals that four cysteine pairs are formed intramolecularly, but the sixth cysteine at position 77 forms the single intermolecular disulfide bridge that results in dimer formation (Fig. 1) (Daopin et al. 1992; Schlunegger and Grütter 1992).

Another distinct subfamily is the inhibin β (or activin β) family. Inhibins are heterodimeric

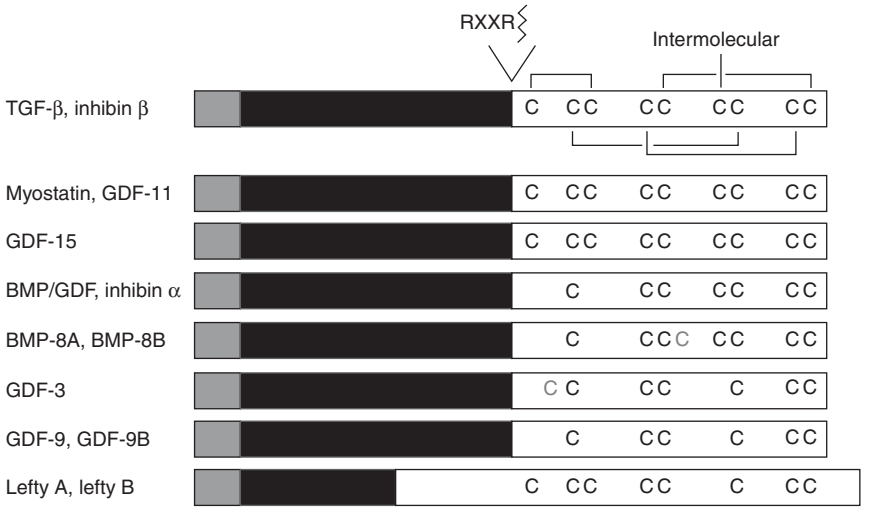

Figure 1. Comparison of the polypeptide organization of TGF-β family proteins. The precursor protein is cleaved by a furin protein convertase, which mainly recognizes the Arg-X-X-Arg (RXXR) motif, but also the Arg-X-Lys/Arg-Arg (RXK/RR) motif. The mature polypeptides (white) contain seven or nine cysteines conserved in most TGF-β family members. These cysteines engage in inter- and intramolecular disulfide bonds. Human BMP-8A, BMP-8B, and GDF-3 have an additional cysteine residue, which is shown in gray, whereas five members lack the cysteine that mediates the disulfide bond in ligand dimerization. Signal peptide, gray; prosegment, black; mature polypeptide, white; TGF-β, transforming growth factor β; BMP, bone morphogenetic protein; GDF, growth and differentiation factor.

proteins consisting of an inhibin α chain and an inhibin β chain, whereas activins are homodimers of inhibin/activin β chains. The four known β chains are closely related to each other and have the corresponding set of nine cysteines, which is apparent in the TGF-βs. Inhibin α, in contrast, is more divergent and has seven cysteines, corresponding to the seven carboxy-terminal cysteines seen in all TGF-β proteins.

All other ligands in the TGF-β family, with five exceptions discussed further below, have a characteristic seven-cysteine pattern that corresponds to the cysteine pattern observed in TGF-β and inhibin β, but without its amino-terminal two cysteines (Fig. 1). In these cases, the disulfide-linked dimerization is mediated by the fourth cysteine in each monomer. Many of these proteins have been named BMP or GDF followed by a number, based on their sequence relationship to other BMPs or GDFs, which were often identified in the same laboratories.

Within this large group of ligands, there is a "core" BMP/GDF subfamily of proteins with similar or related activities. These are structurally closely related to each other and are shown as the top 12 ligands in Figure 2. These BMPs and GDFs signal through heteromeric complexes that comprise the BMP type I receptors, including ALK-1 (activin receptor-like kinase 1), ALK-2, ALK-3/BMPRIA, or ALK-6/BMPRIB. In contrast, some ligands that have the seven-cysteine pattern, like the BMPs and GDFs, do not signal through the same BMP/GDF receptor complexes and intracellular mediators as BMPs and GDFs. Most notably, nodal signals through ALK-4, the major type I receptor for activins, which is also known as ActRIB, whereas myostatin signals through ALK-4/ActRIB or ALK-5, the TGF-β type I receptor also known as TβRI.

As with the heterodimeric inhibins, BMP and/or GDF heterodimers have been identified and shown to be fully active. In mesodermal differentiation, BMP-4/-7 and -2/-7 heterodimers potently induce mesoderm in frog (Suzuki et al. 1997; Eimon and Harland 1999) and zebrafish (Little and Mullins 2009). In cultured cells, BMP-2/-7, -4/-7, and -2/-6 heterodimers are more active than the corresponding homodimers in ectopic bone-formation assays (Aono et al. 1995; Israel et al. 1996). BMP-2/GDF-6 heterodimer is also able to regulate ectodermal cell fate determination (Chang and Hemmati-Brivanlou 1999), and BMP-7/GDF-7 heterodimer is more potent than the corresponding homodimers in neuronal differentiation (Butler and Dodd 2003).

Finally, some TGF-β family proteins, lefty A and lefty B, GDF-3, and GDF-9 and GDF-9B (also known as BMP-15), lack the fourth cysteine in the seven-cysteine pattern and thus have eight or six cysteines (Fig. 1). As this missing cysteine mediates in other ligands the disulfide bond dimerization, one should assume that these TGF-β family proteins do not form disulfide-bonded dimers and have alternative means of interacting with other TGF-β family proteins. Lefty is found to associate with nodal and to inhibit nodal signaling through a dual mechanism involving its interaction with nodal and the EGF-CFC coreceptors, Cripto-1, FRL-1, or Cryptic, which are required for nodal signaling (Chen and Shen 2004; Tabibzadeh and Hemmati-Brivanlou 2006). GDF-3, which also lacks the cysteine required for intermolecular disulfide formation, may also function as an inhibitor of TGF-β/BMP signaling, similarly to lefty. Indeed, GDF-3 was reported to function as a BMP antagonist (Levine and Brivanlou 2006), whereas it was also reported to act as a nodal-like ligand (Chen et al. 2006; Levine et al. 2009) and to signal possibly through ALK-7, a type I receptor for activins (Andersson et al. 2008). GDF-9B has been reported to bind to BMP type I receptor ALK-6/BMPRIB and phosphorylate Smad1, Smad5, and Smad8, whereas GDF-9 activates Smad2 through ALK-5/TβRI (Moore et al. 2003; Mazerbourg et al. 2004; Peng et al. 2013). In addition, a GDF-9/-9B heterodimer is more bioactive for ovarian functions (Peng et al. 2013). Further characterization of the functions of this subfamily of TGF-β family proteins is expected.

COMPLEXITY OF NOMENCLATURE

The name "TGF-β family" derives from the identification of TGF-β1 as a founder member and its activity in the initial phenotypic trans-

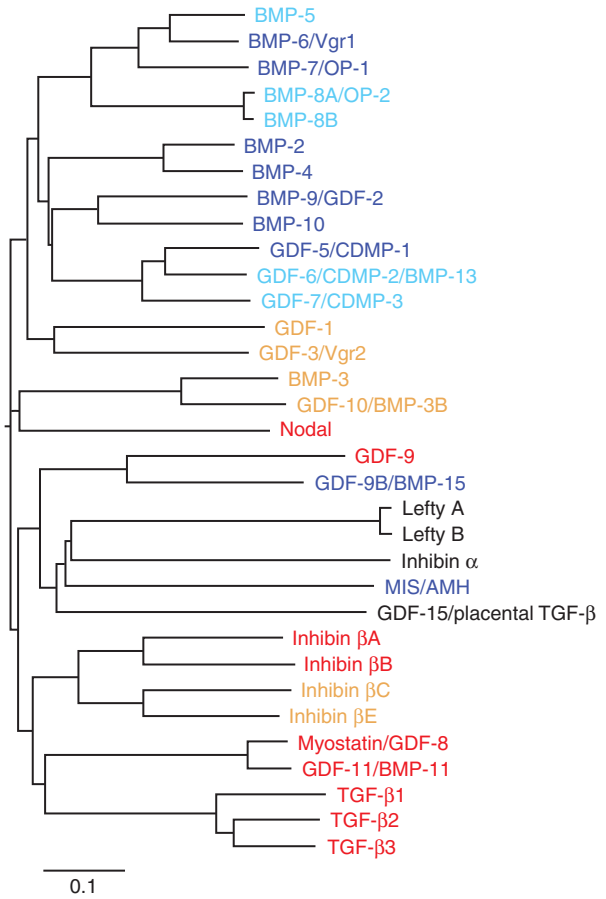

Figure 2. Phylogenetic tree of the 33 TGF-β family polypeptides in human. The amino acid sequences of 33 TGF-β family polypeptides, encoded by their corresponding human genes, were obtained from NCBI's protein database (www.ncbi.nlm.nih.gov), and the carboxy-terminal mature polypeptides were assigned based on validated or predicted furin cleavage sites. The sequences were aligned by Clustal Omega (www.clustal.org/omega) (Sievers et al. 2011) and the phylogenetic tree was illustrated using FigTree v1.4.2 (tree.bio.ed.ac.uk/software/figtree). Ligands that signal through activin- or TGF-β-activated R-Smads or BMP-activated R-Smads are shown in red or blue, respectively. Ligands that may activate these two types of R-Smads, but whose receptors and Smad-signaling pathways have not been fully determined, are shown in orange or light blue, respectively. BMP, Bone morphogenetic protein; OP, osteogenic protein; GDF, growth and differentiation factor; CDMP, cartilage-derived morphogenetic protein; MIS/AMH, Müllerian-inhibiting substance/anti-Müllerian hormone; TGF-β, transforming growth factor β.

formation assay. However, this name may be somewhat misleading. The three TGF-β isoforms regulate a wide variety of cellular processes, both in development and in the adult organisms, and their effects are context-dependent. The three TGF-β isoforms have been established as some of the most potent growth inhibitors, and inhibit proliferation of most types of cells.

The identification of the BMP/GDF subfamily was based on the isolation and characterization of BMP-2 and BMP-4 with their ability to elicit ectopic bone formation and affect skeletal morphogenesis. However, most BMP/GDF proteins were assigned their names because of their sequence similarities to the other BMPs/GDFs, whereas their activities remain largely uncharacterized. It requires substantial

caution to assume their biological functions merely because they share a name, especially for BMPs or GDFs. Recently, several BMP/GDF ligands were rediscovered as critical regulators of distinct physiological and pathological processes. For example, BMP-9 and BMP-10, which were identified as ligands of the ALK-1 receptor (David et al. 2007), play essential roles in the maintenance of homeostasis of vascular system and heart development (David et al. 2009; Pardali et al. 2010). Additionally, BMP-6 was identified as an endogenous ligand that regulates hepcidin expression and iron metabolism in vivo (Andriopoulos et al. 2009; Meynard et al. 2009). GDF-9 and the closely related GDF-9B are secreted by oocytes and regulate female fertility in several mammals.

Myostatin/GDF-8 (encoded by the *Mstn* gene) is now established as a key regulator of skeletal muscle mass; it inhibits muscle differentiation and regeneration, and induces muscle atrophy through receptor complexes of the ActRII or ActRIIB type II receptors with ALK-4 or -5 type I receptors that activate the Smad2/3 pathway (McPherron et al. 1997; Lee and McPherron 2001). As shown in *Mstn*-null mice and in loss-of-function mutations in cattle with double-muscling phenotype, mutations in the *Mstn* gene result in increased muscle mass with a combination of muscle cell hyperplasia and hypertrophy (Grobet et al. 1997; Kambadur et al. 1997; McPherron and Lee 1997; McPherron et al. 1997). GDF-11, which is highly related to myostatin, also signals through ActRII and/or ActRIIB (Oh et al. 2002). Similar to myostatin, GDF-11 has been reported to inhibit myoblast differentiation and regulate the same target genes (Egerman et al. 2015). It is worth noting that soluble ActRIIB-Fc, a ligand trap for myostatin, activin, and GDF-11, prevents muscle wasting and reverses loss of skeletal muscle and cardiac atrophy in tumor-bearing mice with cachexia (Zhou et al. 2010). Recently, GDF-11 has been identified as a circulating factor in young mice, which reverses age-related dysfunction in mouse cardiac and skeletal muscle (Loffredo et al. 2013; Sinha et al. 2014). Whereas these reports claimed that serum levels of GDF-11 decrease with age and that treating older mice with GDF-11 improved muscle regeneration, a contradictory report claims that serum levels of GDF-11 increase with age (Egerman et al. 2015). Further work on the biological activity of GDF-11 is required to confirm the significance of GDF-11 as a potential youth-promoting factor. In contrast, GDF-5 expression was shown to be associated with muscle hypertrophy through activation of BMP signaling (Sartori et al. 2013). Another TGF-β family protein, MIS/AMH, is secreted by Sertoli cells in the testis, and plays important roles in early gonadal development and sex differentiation through inhibition of the Müllerian duct development. MIS signals through its type II receptor, MISRII, and BMPRIA/ALK-3 in vivo, which subsequently activates the Smad1 pathway (Jamin et al. 2002). Mutations in the gene encoding MIS (Behringer et al. 1994) or MISRII (Imbeaud et al. 1995) result in persistent Müllerian duct syndrome. Intriguingly, the sex-determining roles of MIS/MISRII signaling are also conserved in fish, non-mammalian vertebrates that lack a Müllerian duct (Morinaga et al. 2007).

Figure 2 and Table 1 show the 33 known human TGF-β family polypeptides with their evolutionary relationship calculated based on sequence similarities. Because multiple and often parallel approaches have led to the identification of TGF-β family proteins, it is not surprising that several ligands became known with multiple names (Table 1). Now, most investigators have settled on a common name for each individual TGF-β family protein; this nomenclature is used in Figure 2 and Table 1.

With genome-wide and transcriptome analyses gaining in popularity, much research has involved the control of TGF-β family members. However, some discrepancies between the "official gene symbols" and the commonly used names for TGF-β family proteins have caused confusion for researchers and students who enter the field. For example, BMP-9 is the accepted and commonly used name for the protein, but its official gene symbol is GDF2. Lefty A and lefty B are widely used names for the proteins, but the corresponding official gene symbols are Lefty2 and Lefty1, respectively.

TGF-β SIGNALING IN THE CONTROL OF GENE EXPRESSION

More than 30 years of research on these multifunctional ligands has revealed different and sometimes opposite responses to TGF-β, which strongly depend on the cellular context. Most information on the activities of TGF-β ligands derives from studies using cells cultured in media that are supplemented with cytokine-containing serum, thus allowing for signaling cross talk. Although Smads directly target and bind regulatory gene sequences, they either activate or repress gene expression in cooperation with high affinity DNA-binding transcription factors and transcription coregulators that are controlled by and/or dependent on other signaling pathways, therefore providing scenarios for highly context-dependent transcription responses controlled by signaling cross talk. Furthermore, Smad activation and Smad activities are regulated by other kinase and ubiquitylation pathways. Additionally, Smad activation in response to TGF-β ligand-induced receptor activation is complemented by TGF-β/BMP-induced activation of non-Smad-signaling pathways that may be separately controlled. The combination of this signaling cross talk at multiple levels, together with differences in the physiology of different cell types, provides the basis for the high context dependence of the responses to TGF-β.

Several genes, such as the *SMAD7* gene encoding the inhibitory Smad7, *SERPINE1* encoding plasminogen activator inhibitor 1, or *CDKN1A* encoding the cyclin-dependent kinase inhibitor $p21^{Cip1/Waf1}$, have been established as direct Smad target genes that are generally and rapidly induced after TGF-β treatment in many cell types (Lund et al. 1987; Datto et al. 1995b; Nakao et al. 1997; Koinuma et al. 2009b). The development of DNA microarrays, which allows simultaneous monitoring of thousands of transcripts, has revealed "synexpression groups" of genes that are coregulated (Niehrs and Pollet 1999). FoxO and C/EBPβ proteins were identified as some of the first examples of transcription factors that control the expression of TGF-β/Smad-regulated synexpression groups (Gomis et al. 2006a,b). Furthermore, chromatin immunoprecipitation (ChIP) with promoter array analysis (ChIP-chip) and ChIP followed by sequencing (ChIP-seq) revealed chromatin-binding landscapes of Smad proteins (Chen et al. 2008; Koinuma et al. 2009a,b; Qin et al. 2009). Some of these are defined through Smad binding with lineage-specific transcription factors at corresponding enhancer regions (Chen et al. 2008; Lee et al. 2011; Morikawa et al. 2011; Mullen et al. 2011; Trompouki et al. 2011). Forced expression of different lineage-specific transcription factors then redirects Smads to novel binding regions of different lineages (Mullen et al. 2011; Trompouki et al. 2011). These findings suggest that the binding of Smad complexes to target DNA sequences is defined through cooperation with other DNA-binding transcription cofactors in two different ways: (1) cell-type- or lineage-specific transcription factors, or pioneer factors, open up local chromatin structure to make it accessible for Smads, and (2) DNA-binding cofactors that are induced and activated in a context-dependent manner strengthen the interaction between Smads and DNA. The control of gene expression by Smads has been reviewed elsewhere (Morikawa et al. 2013; Gaarenstroom and Hill 2014).

In addition to genes that encode proteins, TGF-β also regulates expression of noncoding RNAs, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). miRNAs are ~19–25 nucleotides long (with an average of 22 nucleotides), and are transcribed as long primary transcripts with hairpin structures (pri-miRNAs). Most pri-miRNAs derive from introns of coding genes or are independently transcribed as noncoding transcripts. In the nucleus, a microprocessor complex containing the RNase III enzyme Drosha and the double-stranded RNA (dsRNA)-binding protein DGCR8 (DiGeorge syndrome critical region 8) specifically recognizes and cleaves pri-miRNAs to generate precursor miRNAs (pre-miRNAs), which are exported to the cytoplasm, where another RNase III Dicer generates a duplex of 19–22 nucleotides long. One strand of the duplex is incorporated into the RNA-induced

silencing complex, which posttranscriptionally regulates hundreds of target mRNAs (Blahna and Hata 2013).

TGF-β regulates directly or indirectly the expression of pri-miRNA transcript and the generation of mature miRNAs. Among them, miRNA-200 family (miR-200a, -200b, -200c, -141, and -429) and miR-205 are markedly down-regulated in cells that have undergone epithelial-mesenchymal transition (EMT) in response to TGF-β. TGF-β indirectly represses miR-200 family members through induction of ZEB1 (also known as δEF1) and ZEB2 (also known as Smad-interacting protein 1, or SIP1) transcription factors, and miR-200 family members target and inhibit the expression of ZEB proteins, which stabilize regulatory networks for TGF-β-induced EMT (Gregory et al. 2008; Park et al. 2008). In addition, TGF-β activates processing of pri-miRNAs. R-Smads interact with RNA helicase p68 (also known as DDX5), a component of the Drosha microprocessor complex, to promote pri-miRNA processing by Drosha (Davis et al. 2008). The role of TGF-β signaling in the control of miRNA generation is more extensively discussed elsewhere (Butz et al. 2012; Hata and Lieberman 2015).

Similarly to miRNAs, the emerging physiological and pathological roles of lncRNAs have started to attract the interest of investigators. LncRNAs are noncoding RNAs usually longer than 200 nucleotides. Several lncRNAs have been reported to play essential roles in TGF-β-regulated biological processes. Future work will confirm their biological significance.

CONTROL OF CELL PROLIFERATION BY TGF-β

TGF-β ligands convey strong growth inhibitory activity in most of the cell types, and this inhibition is reversible after ligand removal (Tucker et al. 1984; Roberts et al. 1985; Ohta et al. 1987; Kimchi et al. 1988). Although the antiproliferative response is observed in many cell types, including epithelial, endothelial, hematopoietic, and glial cells, most knowledge about the molecular mechanisms comes from studies in epithelial cells and keratinocytes (Siegel and Massagué 2003, 2008). The extent of the growth inhibitory response to TGF-β varies depending on the cell type and the particular cell system studied, and reaches a growth arrest in some cell types (Tucker et al. 1984). Confirming observations in cell culture, evidence for an antiproliferative effect of TGF-β1 in vivo was first obtained with polymer beads impregnated with TGF-β1 and implanted near the epithelial end buds of immature mammary glands (Silberstein and Daniel 1987). Also, intravenous injection of TGF-β1 or -β2 inhibits proliferation of the regenerating rat liver (Russell et al. 1988). The cytostatic effects by TGF-β are mediated mainly through two sets of mechanisms: induction of cyclin-dependent kinase inhibitors and elimination of proliferative drivers. TGF-β induces expression of $p15^{Ink4b}$, $p21^{Cip1/Waf1}$, and $p27^{Kip1}$ (Hannon and Beach 1994; Polyak et al. 1994; Datto et al. 1995a), and inhibits the expression of mediators that contribute to cell proliferation, such as c-Myc (Pietenpol et al. 1990), Cdc25A (Iavarone and Massagué 1997), and Id proteins (Kang et al. 2003; Anido et al. 2010). In addition to direct effects of TGF-β signaling on cell-cycle control, TGF-β opposes the action of specific mitogens, such as EGF in keratinocytes (Coffey et al. 1988), or platelet-derived growth factor (PDGF) in rat fibroblast and embryonic cells (Roberts et al. 1985; Anzano et al. 1986). How TGF-β signaling interferes with the growth stimulation resulting from growth factor-induced receptor tyrosine kinase signaling has remained largely unexplored.

In contrast to the growth inhibition in many cell types, TGF-β can also promote cell proliferation in other cell types under certain conditions, as initially reported (Roberts et al. 1981). TGF-β has been shown to stimulate proliferation of several cell types, including chondrocytes, osteoblasts under some conditions, mesenchymal stem cells (MSCs), some fibroblasts, and endothelial cells under some conditions (Roberts et al. 1981; Goumans et al. 2002; Jian et al. 2006). As mentioned, the effects are often context-dependent because TGF-β can induce both growth promotion and growth inhibition in the same cells depending on the cell culture

conditions (Roberts et al. 1985). Thus, not only may different cell types respond differently to TGF-β, but also the same cell type may show opposite responses under different experimental conditions. The molecular mechanisms responsible for inducing proliferation are less defined than those that lead to inhibition of proliferation. In some contexts, the growth-promoting effect seems to be secondary to induction of other cytokines such as PDGF (Leof et al. 1986; Matsuyama et al. 2003; Bruna et al. 2007). The control of cell proliferation has been reviewed elsewhere (Siegel and Massagué 2003, 2008).

CONTROL OF CELL DIFFERENTIATION AND STEMNESS BY TGF-β PROTEINS

TGF-β family members control the development, differentiation, and function of diverse cell types. For example, activin A, which uses similar repertoires of receptors and intracellular signaling pathways as TGF-β, was identified as a critical mesoderm-inducing factor in vivo (Asashima et al. 1990) as well as an erythroid differentiation factor (EDF) (Eto et al. 1987; Yu et al. 1987) and a nerve cell survival factor (Schubert et al. 1990). Additionally, the Spemann's organizer in early embryonic development secretes extracellular antagonists, such as follistatin, which prevents activin signaling, and the BMP inhibitor noggin (Smith and Harland 1992; Hemmati-Brivanlou et al. 1994), which are critical for the establishment of morphogen gradients of activins and BMPs.

Similar to the developmental roles of activin, TGF-β ligands are implicated in differentiation toward a wide variety of lineages, including immune cells (Li and Flavell 2008), blood cells (Blank and Karlsson 2015), and neural/neuronal cells (Krieglstein et al. 2011). Consistently, TGF-β2-deficient mice show defects in multiple organs, including heart, lung, craniofacial, limb, spinal cord, eye, inner ear, and urogenital tracts (Sanford et al. 1997), and TGF-β3-deficient mice display defects during pulmonary and palate development, suggesting its critical roles in EMT (Kaartinen et al. 1995), whereas TGF-β1-deficient mice show a phenotype in the immune system (Shull et al. 1992). In addition, mice deficient in TGF-β2 and -β3 expression reveal defects in the central nervous system (Vogel et al. 2010).

Among a wide variety of effects on many lineages, TGF-β ligands play essential roles in determining the direction and extent of mesenchymal differentiation, as first shown in ectoderm explants from frog embryos (Kimelman and Kirschner 1987; Rosa et al. 1988). In cultured cells, TGF-β1 inhibits differentiation toward adipocytes (Ignotz and Massagué 1985) and skeletal myocytes (Florini et al. 1986; Massagué et al. 1986). In contrast, TGF-β1 and -β2 enhance differentiation toward chondrocytes, consistent with their independent identification and purification as cartilage-inducing factors A and B (CIF-A and CIF-B), respectively (Seyedin et al. 1985). Additionally, TGF-β promotes or represses the expression of differentiation markers by bone matrix-depositing osteoblasts, depending on their extent of differentiation. Inhibition of endogenous TGF-β and activin signaling in human MSCs by pharmacological inhibition of the ALK-4, -5, and -7 type I receptor kinases, induces osteoblast maturation (Maeda et al. 2004), and transcriptome analysis further supports the notion that inhibition of ALK-5/TβRI-mediated TGF-β signaling enhances adipogenic and osteogenic differentiation, and prevents chondrogenic differentiation (Ng et al. 2008).

Complementing the control of MSC differentiation toward specific lineages, TGF-β helps maintain MSCs in an undifferentiated state (Jian et al. 2006). In serum-free media with basic fibroblast growth factor (FGF-2), PDGF-BB, and insulin, TGF-β1 enables multiple passages of MSCs, and inhibition of any of these signaling pathways decreases proliferation of MSCs, suggesting a requirement of these tyrosine kinase-activated pathways for MSC proliferation (Ng et al. 2008). Thus, the cooperation of TGF-β with other cytokines plays important roles in the maintenance and expansion of MSC populations before differentiation commitment, while also restricting their differentiation potential, inhibiting differentiation along the osteoblast, myoblast, and adipocyte lineages.

TGF-β signaling also controls the maintenance of the undifferentiated state and pluripotency of embryonic stem cells (ESCs). In mouse ESCs, leukemia inhibitory factor (LIF), an interleukin 6 (IL-6) class cytokine, and BMP-4 play essential roles in the maintenance of pluripotency. However, in this model of "naïve pluripotency," the role of TGF-β and activin signaling remains to be better defined. Whereas kinase inhibitors for TGF-β/activin type I receptors were reported to help maintain the naïve pluripotent state (Hassani et al. 2012), nodal/activin signaling was also shown to enhance self-renewal of mouse ESCs (Ogawa et al. 2007).

Human ESCs, which share characteristics with mouse epiblast stem cells derived from postimplantation embryos, represent a more mature stage in pluripotency, and are "primed" for differentiation. These cells are usually maintained with FGF-2 and activin A (Beattie et al. 2005; James et al. 2005; Vallier et al. 2005), which stabilize the primed pluripotent state. They no longer require LIF, which has essential roles in the naïve state of mouse ESCs. Defined culture conditions were reported to achieve a naïve pluripotent state for human ESCs and, in this context, two groups used TGF-β or activin ligands (Gafni et al. 2013; Theunissen et al. 2014) rather than their inhibitor(s). The roles of TGF-β family proteins in stem cells are reviewed elsewhere (Oshimori and Fuchs 2012; Sakaki-Yumoto et al. 2013).

Accumulating evidence also indicates essential roles of TGF-β/activin signaling in the maintenance of stem-cell-like properties of some cancer-initiating cell populations, such as glioma-initiating cells (Ikushima et al. 2009; Penuelas et al. 2009), mammary cancer stem cells (Mani et al. 2008), pancreatic cancer–initiating cells (Lonardo et al. 2011), and leukemia-initiating cells in chronic myeloid leukemia (Naka et al. 2010). Consistent with these observations, pharmacological inhibition of TGF-β/activin type I receptor kinases reduces cancer progression in some animal models (Ikushima et al. 2009; Penuelas et al. 2009; Naka et al. 2010; Lonardo et al. 2011). On the other hand, TGF-β was also shown to reduce the cancer-initiating cell populations in certain cancers, including breast cancer, pancreatic cancer, and diffuse-type gastric cancer (Tang et al. 2007; Ehata et al. 2011; Hoshino et al. 2015). These observations suggest that targeting the TGF-β/activin-signaling pathways could be an attractive therapeutic basis for some advanced cancers, although inhibition of these pathways in normal tissues may increase the risk for development of other tumors. The roles of TGF-β in cancer stem cells and cancer progression are reviewed elsewhere (Caja et al. 2012; Katsuno et al. 2013).

TGF-β CONTROLS WOUND HEALING

Normal wound healing is a complex process that involves (1) cell migration and inflammation, (2) proliferation of fibroblasts with formation of granulation tissue and ECM deposition, and (3) remodeling of scar tissue for an extended time period. TGF-βs have been shown to regulate these different steps through effects on multiple cell types, and to promote the wound healing process in vivo. Indeed, early studies, initiated shortly after the isolation and purification of TGF-β1, revealed that TGF-β1 strongly accelerates wound healing in vivo (Sporn et al. 1983).

TGF-β1 expression and activation are rapidly induced in response to injury, progressing outward from the site of injury (Kane et al. 1991). Platelets, which immediately after wounding facilitate the formation of the hemostatic plug, store large amounts of TGF-β1 (Assoian et al. 1983) that is then released at the site of injury. Because TGF-β1 acts as a potent chemoattractant for monocytes (Wahl et al. 1987) and fibroblasts (Postlethwaite et al. 1987), the platelet-derived TGF-β1 is able to attract both cell populations to sites of inflammation and repair. Accordingly, administration of TGF-β1 into wound chambers, or to incisional wounds, stimulates the accumulation of granulation tissue and the cellularization of the wound bed, and accelerates the wound healing response in experimental models (Sporn et al. 1983; Roberts et al. 1986; Mustoe et al. 1987). TGF-β1 is also a potent inducer of the expres-

sion of major ECM proteins, such as fibronectin and collagens (Ignotz and Massagué 1986; Roberts et al. 1986), thus promoting ECM deposition at the site of wound healing. Furthermore, TGF-β also induces inhibition of ECM degradation as a result of repressed expression of metalloproteinases and induction of tissue inhibitor of metalloproteinase synthesis (Edwards et al. 1987).

The repression of the epithelial phenotype with its apical-basal polarity and epithelial junction architecture in response to TGF-β, and concomitant induction of mesenchymal traits with increased cell motility also contribute to epithelial wound healing. At the site of injury, epithelial cells undergo partial or complete EMT as a result of increased TGF-β signaling, therefore promoting epithelial resurfacing, and then reacquire their epithelial phenotype (Kalluri and Weinberg 2009). TGF-β has also been shown to induce the expression of α-smooth muscle actin (Desmouliere et al. 1993), a marker of myofibroblasts. In addition, TGF-β treatment enhances acquisition of the myofibroblastic phenotype, and costimulation with FGF-2 is shown to inhibit the process (Shirakihara et al. 2011). This and other observations raise the possibility that increased TGF-β stimulation during wound healing may result in or contribute to the generation of myofibroblasts.

Although TGF-β1, -β2, and -β3 act through the same receptors and Smad2/3 pathway, they show differences in the control of wound healing that remain to be fully explained. Administration of TGF-β1 or -β2 promotes ECM deposition in the early stages of wound healing, but wounds treated with TGF-β1 or -β2 do not differ from control wounds in the final quality of scarring (Shah et al. 1995). However, inhibition of TGF-β/Smad3 signaling, for example, in the absence of Smad3 expression or in the presence of an antibody to TGF-β1 and -β2, enhances the quality of wound healing and reduces scarring (Ashcroft et al. 1999; Amendt et al. 2002). Additionally, administration of TGF-β3, but not TGF-β1 nor -β2, also reduces cutaneous scarring (Shah et al. 1995). Thus, recombinant TGF-β3 could be considered to promote scarless wound healing, which is supported by results from prophylactic use of recombinant TGF-β3 ligand in phase I/II trial (Ferguson et al. 2009).

TGF-β CONTROLS THE IMMUNE SYSTEM

TGF-β acts as a potent immunosuppressive cytokine through effects on both cell differentiation and cell proliferation. For example, TGF-β inhibits proliferation of T-lymphocytes (Kehrl et al. 1986) and thymocytes (Ristow 1986), and TGF-β2 was isolated as glioblastoma-derived T-cell suppressor factor, based on the observation that glioblastoma is frequently accompanied by immunosuppression (de Martin et al. 1987). The key roles of TGF-β1 in suppressing immune responses also explain the rapid neonatal development of overt inflammation in TGF-β1-deficient mice (Shull et al. 1992). In contrast, TGF-β2- and -β3-deficient mice display developmental defects (Kaartinen et al. 1995; Sanford et al. 1997), emphasizing their roles in development rather than in immune regulation. Consistent with the phenotype of *Tgfb1*$^{-/-}$ mice, T-cell-specific expression of a dominant-negative TGF-β type II receptor (TβRII) mutant, and T-cell-specific ablation of TβRII or TβRI/ALK-5 show that inhibition of TGF-β signaling in T cells causes neonatal lethal inflammatory disease.

Conversely, TGF-β1 promotes T-cell differentiation. Similarities in the autoimmune phenotype of mice lacking expression of TGF-β1 (*Tgfb1*$^{-/-}$) or Foxp3, the transcription factor required for $CD4^+CD25^+$ regulatory T cells (Treg) (Brunkow et al. 2001), led to the discovery that TGF-β, in combination with IL-2, induces Treg cell differentiation through induction of Foxp3 expression in $CD4^+$ T cells (Chen et al. 2003; Liu et al. 2008). TGF-β is also essential for the generation of IL-17-expressing proinflammatory T helper cells (T_H17), in combination with IL-6 or -21 (Veldhoen et al. 2006; Korn et al. 2007). Thus, TGF-β can induce both regulatory/inhibitory and proinflammatory T cells, depending on whether proinflammatory cytokines such as IL-6 are present. The roles of TGF-β in the immune system are reviewed elsewhere (Li and Flavell 2008; Flavell et al. 2010).

ANOMALOUS TGF-β ACTIVATION IN CONNECTIVE TISSUE AND SKELETAL DISEASES

Gene mutations that result in dysregulation of TGF-β latency and confer increased release of activated TGF-β have been implicated in the pathogenesis of connective tissue diseases and skeletal diseases. Camurati–Engelmann disease (CED) is characterized by a progressive diaphyseal dysplasia with hyperostosis and sclerosis of the diaphyses of long bones. Most individuals in CED families have a mutation in the region of the *TGFB1* gene locus that encodes LAP (Janssens et al. 2000; Kinoshita et al. 2000), which affects LAP dimerization and results in hyperactivation of TGF-β signaling (Saito et al. 2001; Tang et al. 2009), whereas no mutations are found in the domain encoding the mature TGF-β1 peptide. As an underlying mechanism, increased TGF-β may promote migration of bone marrow stromal cells to sites of bone resorption (Tang et al. 2009). Accordingly, a type I receptor kinase inhibitor partially rescues the uncoupled bone remodeling in a mouse model for CED with the mutant TGF-β1 precursor gene (Tang et al. 2009).

Mutations in the gene encoding fibrillin-1 have been causally linked to Marfan syndrome (Neptune et al. 2003), and their etiology highlights important roles of TGF-β in Marfan syndrome or Marfan-like connective tissue disorders. The fibrillins, which form extracellular microfibrils, associate with LTBP-1, which interacts with the LAP in the LLC, consequently linking this complex to elastic microfibrils (Isogai et al. 2003). The absence of fibrillin-1 in *Fbn1*-deficient mice results in excessive TGF-β activity in vivo, which could be at the basis of the pathogenesis of emphysema and other manifestations of Marfan syndrome (Neptune et al. 2003). Additionally, mutant fibrillin-1 disturbs the microfibril structure (Judge et al. 2004; Charbonneau et al. 2010) and activates TGF-β signaling, which may then over time generate the pathological features of Marfan syndrome.

Clinically overlapping conditions, which result in aortic aneurysms, for example, in Loeys–Dietz syndrome, familial thoracic aortic aneurysms and dissections, and aneurysms-osteoarthritis syndrome, are also caused by enhanced TGF-β signaling. Mutations that affect TGF-β-signaling pathway components have been reported; these include mutations in loci encoding *TGFB2* (Boileau et al. 2012; Lindsay et al. 2012) or *TGFB3* (Bertoli-Avella et al. 2015), as well as other signaling components or regulators, that is, *TGFBR1* (Loeys et al. 2005, 2006), *TGFBR2* (Mizuguchi et al. 2004; Loeys et al. 2005, 2006), *SMAD3* (van de Laar et al. 2011), and *SKI* (Doyle et al. 2012). These syndromes share some clinical features in the aorta, including dilation of the aortic root or ascending aorta and degeneration of the medial layer of the aorta. Another common feature is paradoxical activation of TGF-β signaling in aortic lesions, although they harbor loss-of-function mutations in TGF-β-signaling components. The molecular mechanisms that explain the paradoxical activation have not been elucidated. The dysregulation of TGF-β signaling in connective tissue and skeletal diseases has been discussed elswhere by Gallo (Lindsay and Dietz 2011; Akhurst 2012).

INCREASED TGF-β SIGNALING DRIVES FIBROSIS

A pivotal role of TGF-β in fibrogenesis was first revealed when subcutaneous injection of purified TGF-β1 was seen to induce fibrotic lesions at the injection site (Roberts et al. 1986). This potent effect was further confirmed by the fibrotic response in lungs of rodents following intratracheal administration of adenovirus that expresses active TGF-β1 (Sime et al. 1997), whereas neutralization of TGF-β with antiserum ameliorated experimental fibrosis in the kidney, heart, and liver (Border et al. 1990; Denis 1994; Kuwahara et al. 2002). In addition, inhibitors or antagonists of the TGF-β/Smad pathway, such as soluble TβRII (George et al. 1999) or adenovirally expressed Smad7 (Nakao et al. 1999), were shown to prevent liver and lung fibrosis in mice, respectively. Most fibrogenic effects resulting from increased TGF-β signaling are thought to be mediated by the Smad pathway because *Smad3*-null mice are resistant to bleomycin-induced lung fibrosis

(Zhao et al. 2002), radiation-induced skin fibrosis (Flanders et al. 2002), and tubulointerstitial fibrosis following unilateral ureteral obstruction (Sato et al. 2003). In addition, phenotypes of integrin $\alpha_v\beta_6$-deficient mice support a role of integrin-mediated TGF-β activation in progressive fibrosis because bleomycin-induced inflammation can occur without progression to fibrosis in these mice (Munger et al. 1999). These and other observations suggest that dysregulation and sustained activation of TGF-β/Smad3 signaling play essential roles in the initiation and maintenance of the fibrotic tissue phenotype.

The profibrotic effects of TGF-β involve a combination of mechanisms and cell types, including enhanced infiltration and/or proliferation of preexisting fibroblasts, generation of myofibroblasts, increased ECM synthesis, and inhibition of collagenolysis, all of which are also apparent in wound healing. In addition, lineage tracing strongly suggests important contributions of mesenchymal cells arising through TGF-β-induced EMT. For example, in a model of tubulointerstitial renal fibrosis, the use of the promoter region of the gene encoding γ-glutamyltranspeptidase, a proximal tubule marker, for cell lineage tracing suggests that 36% of renal fibroblasts derive through EMT from renal tubular epithelium (Iwano et al. 2002). Additionally, using the promoter of surfactant protein C, a type II alveolar epithelial cell marker, for lineage tracing highlights a substantial contribution of EMT of alveolar epithelial cells in TGF-β1-induced pulmonary fibrosis (Kim et al. 2006). Endothelial cells have also been identified as a source of fibroblasts in fibrosis. In heart fibrosis, for example, 27%–35% of all fibroblasts are estimated to originate from endothelial cells through endothelial-mesenchymal transition (Zeisberg et al. 2007).

Although TGF-β has been well established as a key profibrotic cytokine, its activity is attenuated or aggravated by several other cytokines. For example, IL-1 and tumor necrosis factor-α, which cross talk with TGF-β signaling at multiple levels, are well-characterized profibrotic cytokines. Conversely, some members of the nuclear receptor superfamily, such as the vitamin D receptor, PPAR-γ (peroxisome proliferator-activated receptor-γ), and NR4A1, directly repress fibroblast activation induced by TGF-β (Wu et al. 2009; Ding et al. 2013; Palumbo-Zerr et al. 2015).

TGF-β SIGNALING IN CANCER PROGRESSION

TGF-β induces an antiproliferative response in many cell types, including both normal epithelial cells and transformed cells. Initial reports showed that antisense inhibition of TGF-β enhances tumorigenicity in vivo (Wu et al. 1992), and that certain tumor cells can become unresponsive to TGF-β, which was primarily assessed by its effects on proliferation (Shipley et al. 1986; Kimchi et al. 1988). It was, therefore, assumed that disruption of TGF-β signaling is implicated in the pathogenesis of tumor development. In line with this hypothesis, inactivating mutations and deletions in the *TGFBR2* and *SMAD4* gene loci were found in cancers (Markowitz et al. 1995; Hahn et al. 1996) and thought to drive tumorigenesis (reviewed in Garraway and Lander 2013; Vogelstein et al. 2013).

As advocated by Vogelstein and colleagues, the multistep tumorigenesis model (Fearon and Vogelstein 1990; Vogelstein et al. 2013) involves the requirement of sequential genetic alterations over time, which enables the development of tumors and promotes cancer progression. In colon cancers, for example, gatekeeping mutations most often occur in the adenomatous polyposis coli (*APC*) gene, and second mutations in another gene, such as *KRAS*, then stimulate clonal growth and expansion. Mutations in genes such as *PIK3CA*, *SMAD4*, and *TP53* follow this mutation process, resulting in more invasive and metastatic tumors. Conceptually similarly, TGF-β's function as a suppressor of epithelial cell tumorigenesis may need to be alleviated at an early stage in tumor development, which does not preclude roles of TGF-β signaling at later stages.

On the other hand, tumor cells show increased expression of TGF-β, most commonly TGF-β1, when compared with normal sur-

rounding tissue, and secrete TGF-β ligands. Additionally, elevated TGF-β expression correlates with tumor progression and poorer prognosis, indicating pro-oncogenic roles for TGF-β at late stages. Substantial evidence now supports the notion that the increased TGF-β expression by tumor cells promotes tumor progression by enhancing migration, invasion, and survival of tumor cells during later stages of tumorigenesis, through stimulating ECM deposition and tissue fibrosis, perturbing immune and inflammatory function, stimulating angiogenesis, promoting EMT that enables increased migration and invasion, and maintaining cancer stem cells. Platelets attached to tumor cells in the circulation provide an additional source of TGF-β that promotes an invasive mesenchymal-like phenotype and enhances metastasis in vivo through stimulating the TGF-β/Smad and NF-κB pathways in the tumor cells (Labelle et al. 2011).

With the sequential acquisition of genomic mutations, tumor cells were shown to display a range of mutations in different signaling pathways, which lead to changes in TGF-β responsiveness. This is illustrated by a model system consisting of a set of human breast epithelial MCF10A–derived cell lines, in which TGF-β functions as a tumor suppressor early in the carcinogenic process, but switches to a pro-oncogenic agent in the later stage. Intriguingly, in the most aggressive cell line, M-IV, which resembles a high-grade breast carcinoma, loss of TGF-β response does not affect primary tumorigenesis, but suppresses metastasis (Tang et al. 2003).

As illustrated with these observations, TGF-β signaling controls both the initial development of tumors as well as the cancer progression of a large variety of tumor types. Its roles in tumor suppression and its extensive roles in cancer progression through direct effects on the cancer cells themselves, as well as the microenvironment, are the subject of many reviews (Bierie and Moses 2006; Ikushima and Miyazono 2010; Pickup et al. 2013).

CONCLUSIONS

The TGF-β family comprises a large number of secreted and structurally related proteins with multiple roles in developmental patterning, tissue differentiation, and maintenance of homeostasis. As discussed, the biological effects of TGF-β family proteins are contextual, and even the same cell type may show different or opposite responses to the ligand under different biological contexts. Some TGF-β family members are made available to cells with concentration gradients in vivo, resulting in graded effects of cell differentiation and function by the responsive cells. The activation of distinctive sets of genes by the ligand in a dosage-dependent manner plays essential roles in the specification of cell fates during normal development. Cells also respond to TGF-β family proteins in combination with other external stimuli. Costimulation by other cytokines modifies the responsiveness to the ligand, as observed in the immune system. In addition, the complexity of the TGF-β family may reflect some functional redundancy. It is, therefore, possible that some family members could play redundant roles in the regulation of some biological processes. Therefore, detailed profiling of expression and activation of all TGF-β family ligands in each context could lead to a better understanding of the complex nature of TGF-β family members.

Recent advances in single-cell assays have revealed intertumor heterogeneity between tumors of the same histopathological subtype, and intratumor genetic and epigenetic heterogeneity within individual cancer masses. In addition, characterization of the transcriptome and histone modification markers in normal cells, such as mouse ESCs, similarly suggests substantial cell-to-cell variability even in cells with identical genetic backgrounds. It is, therefore, possible that the heterogeneity could contribute to different responsiveness to TGF-β family members. Thus, spatial and temporal profiling of signaling at the single-cell level will help us to understand the complex mechanisms of TGF-β family members.

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