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TGF-β Family Signaling in Connective Tissue and Skeletal Diseases

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The transforming growth factor β (TGF-β) family of signaling molecules, which includes TGF-βs, activins, inhibins, and numerous bone morphogenetic proteins (BMPs) and growth and differentiation factors (GDFs), has important functions in all cells and tissues, including soft connective tissues and the skeleton. Specific TGF-β family members play different roles in these tissues, and their activities are often balanced with those of other TGF-β family members and by interactions with other signaling pathways. Perturbations in TGF-β family pathways are associated with numerous human diseases with prominent involvement of the skeletal and cardiovascular systems. This review focuses on the role of this family of signaling molecules in the pathologies of connective tissues that manifest in rare genetic syndromes (e.g., syndromic presentations of thoracic aortic aneurysm), as well as in more common disorders (e.g., osteoarthritis and osteoporosis). Many of these diseases are caused by or result in pathological alterations of the complex relationship between the TGF-β family of signaling mediators and the extracellular matrix in connective tissues.

The transforming growth factor β (TGF-β) family of cytokines comprises the three TGF-β proteins (TGF-β1, TGF-β2, and TGF-β3) and related growth and differentiation factors such as activins, inhibins, bone morphogenic proteins (BMPs), and growth and differentiation factors (GDFs). These molecules play critical roles both in normal development and in several pathological conditions, including inflammation, fibrosis, and cancer (reviewed in Li and Flavell 2006; Gordon and Blobe 2008; Ikushima and Miyazono 2010). This review focuses on the role of these proteins in development and homeostasis of the skeleton and other connective tissues (summarized in Fig. 1) and on the diseases that ensue when these pathways are altered. Aberrant signaling in these pathways has been associated with common connective

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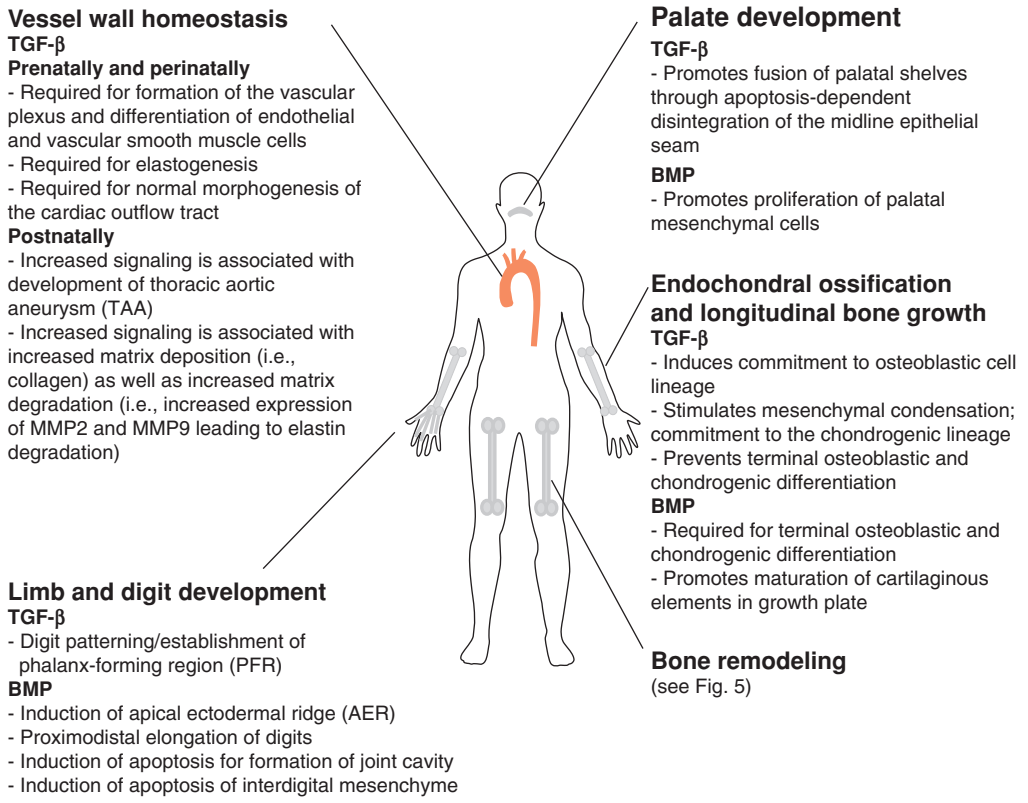

Figure 1. Summary of the roles of transforming growth factor β (TGF-β) and bone morphogenetic protein (BMP) signaling in the development and homeostasis of connective tissue and the skeletal system.

tissue disorders such as osteoarthritis and osteoporosis. In addition, mutations in several members of the TGF-β family, their receptors, or signaling mediators have been shown to cause hereditable connective tissue syndromes that are characterized by defects in the development and homeostasis of soft and hard connective tissues (Table 1).

OVERVIEW OF TGF-β SIGNALING

All members of the TGF-β family of cytokines share conserved structural motifs and signal through similar mechanisms, although the exact signaling pathways activated by any given ligand differ. Signaling is initiated by binding of the ligand to tetrameric complexes formed by two type I and two type II receptors, all possessing serine, threonine, and tyrosine kinase activity. Ligand binding induces autophosphorylation of type II receptors, which in turn phosphorylate and activate type I receptors. Activated type I receptors phosphorylate, and thus activate receptor-regulated Smad proteins (R-Smads). Phosphorylated R-Smads bind the common Smad (co-Smad, known as Smad4 or DPC4), translocate into the nucleus and modulate gene expression by interacting with other transcription factors to regulate target gene expression (reviewed in Schmierer and Hill 2007; Massagué 2012; Hata and Chen 2016). In this review, we have broadly grouped the TGF-β family into two general pathways, TGF-β and its main signaling molecules Smad2 and Smad3 (Fig. 2) and BMP and its main signaling molecules Smad1, Smad5, and Smad8 (Fig. 3).

Seven type I receptors and five type II receptors have been identified in humans (Heldin and Moustakas 2016). Type I receptors are also known as activin receptor-like kinases (ALK-1

Table 1. Connective tissue and skeletal disorders caused by known mutations in the transforming growth factor β (TGF-β) family signaling pathways

Disorder (official symbol) [MIM #]	Gene mutation	Change in signal activity	Clinical manifestation			
			Cardiovascular	Ocular	Skeletal	Other
Acromesomelic dysplasia (AMD)	*GDF5*	↓			All AMD: acromesomelic limbs and brachydactyly	
Hunter–Thompson type (AMDH) [201250]					Joint dislocation in elbow and ankles, also frequently found in hip and knee	
Grebe type (AMDG) [200700]					Ball-shaped reduction of phalanges	
Du Pan syndrome [228900]					Hypo-/aplasia of fibula, short limbs, ball-shaped reduction of phalanges, partial syndactyly	
Brachydactyly (BD)					All BD: shortened digits	
BD type A2 (BDA2) [112600]	*BMPR1B* *GDF5* *BMP2*	↓			Shortening of middle phalanx in second and fifth digit	
BD type B2 (BDB2) [611377]	*NOG*	↑			Shortening of distal phalanges symphalangism and partial syndactyly, fusion of carpal/tarsal bones	
BD type C (BDC) [113100]	*GDF5*	↓			Shortening of middle phalanx in second, third and fifth digit, shortening of first metacarpal, hypersegmentation of second and/or third digit	
Camurati–Engelmann disease (CED) [131300]	*TGFB1*	↑			Sclerosing bone dysplasia, thickening of the diaphyses of the long bones	

Continued

Table 1. *Continued*

Disorder (official symbol) [MIM #]	Gene mutation	Change in signal activity	Clinical manifestation			
			Cardiovascular	Ocular	Skeletal	Other
Fibrodysplasia ossificans progressiva (FOP) [135100]	*ACVR1*	↑			Extra-articular joint ankylosis, tibial osteochondroma, cervical spine and femoral neck malformation, big toe malformation	Postnatal soft tissue ossification
Hereditary hemorrhagic telangiectasia (HHT) HHT1 [187300] HHT2 [600376]	*ENG* ACVRL1	↓	Telangiectases and arteriovenous malformations of skin, mucosa, and viscera			
Loeys–Dietz syndrome (LDS) LDS1 [609192] LDS2 [610168] LDS3 [613795] LDS4 [614816] LDS5	*TGFBR1* *TGFBR2* *SMAD3* *TGFB2* *TGFB3*	↓/↑	Aortic root aneurysm, arterial tortuosity, aneurysm of other vessels	Hyper-telorism	Arachnodactyly, cleft palate/bifid uvula, craniosynostosis, pectus deformity, scoliosis, joint laxity, malar hypoplasia	Increased susceptibility to asthma, allergy, eczema, gastrointestinal inflammation
Marfan syndrome (MFS) [154700]	*FBN1*	↑	Aortic root aneurysm	Ectopia lentis	Arachnodactyly, dolichostenomelia, pectus deformity, scoliosis, joint laxity, malar hypoplasia	
Multiple synostosis syndrome (SYNS) SYNS1 [186500]	*NOG*	↑			Progressive symphalangism, carpal, tarsal, and vertebral fusions	Progressive hearing loss
SYNS2 [610017]	*GDF5*					

Continued

Table 1. *Continued*

Disorder (official symbol) [MIM #]	Gene mutation	Change in signal activity	Clinical manifestation			
			Cardiovascular	Ocular	Skeletal	Other
Orofacial cleft 11 (OFC11) [600625]	*BMP4*	[a]			Nonsyndromic cleft lip with or without cleft palate	
Proximal symphalangism (SYM1)						
SYM1A [185800]	*NOG*	↑			Multiple joint fusions restricted to proximal interphalangeal joints in hand and feet, fusion of carpal/tarsal bones	Common: conductive hearing loss
SYM1B [615298]	*GFD5*					
Sclerosteosis (SOST1) [269500]	*SOST*	-			Sclerosing bone dysplasia, tall stature, syndactyly	
Shprintzen–Goldberg syndrome (SGS) [182212]	*SKI*	↑	Aortic root aneurysm	Hypertelorism	Arachnodactyly, dolichostenomelia, craniosynostosis, pectus deformity, malar hypoplasia, joint laxity	
Stiff skin syndrome (SSS) [184900]	*FBN1*	↑			Syndesmodysplasic, dwarfism, cutaneous nodules in distal interphalangeal joints	Skin fibrosis
Van Buchem disease (VBD) [239100]	*SOST*	-			Sclerosing bone dysplasia, increased inner and outer diameter of metacarpals	

SNPs in components of BMP/TGF-β pathway are further implicated in osteoarthritis and osteoporosis but have not been functionally determined.
[a]Mutant protein activity has not been functionally determined.

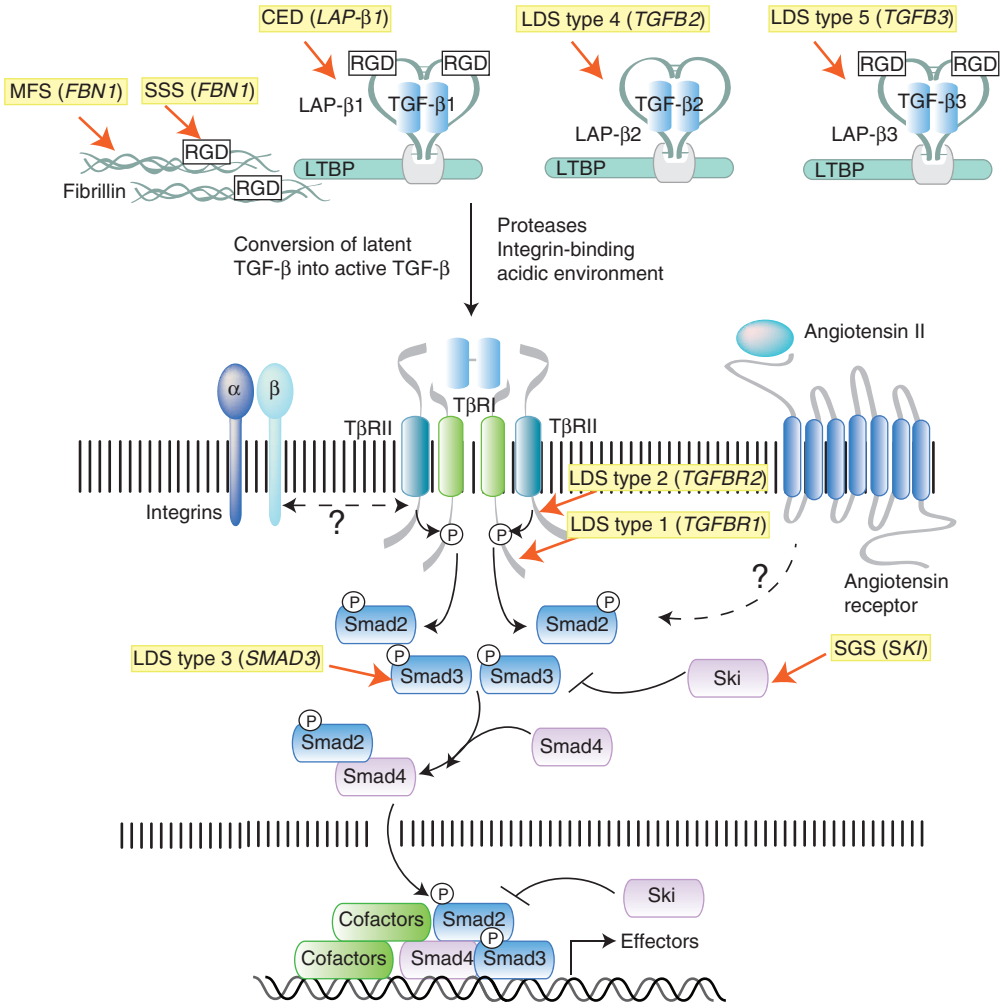

Figure 2. Summary of the transforming growth factor β (TGF-β) signaling pathway and mutations causing connective tissue disorders. TGF-β molecules (TGF-β1, -β2, and -β3) are secreted in latent forms that are unable to interact with the receptors. In the latent form, dimeric TGF-β molecules are noncovalently associated with dimeric latency-associated peptides (LAPs), which are made from the same gene as the corresponding TGF-β molecule. This complex, which is referred to as the small latent complex (SLC), is then covalently linked by disulfide bonds to a member of the latent TGF-β binding protein (LTBP) family, to form the larger complex called large latent complex (LLC). The LLC complex interacts noncovalently with various components of the extrcellular matrix (ECM), including fibrillin microfibrils and integrins, the major adhesion molecule linking cells to the ECM. Both fibrillin and TGF-β (TGF-β1 and -β3, but not TGF-β2) contain an arginine-glycine-aspartic acid (RGD) domain that can bind integrin molecules. Cross talk between LTBP, fibrillin, and integrins is thought to be critical for proper localization, sequestration, and conversion of latent TGF-β to active TGF-β. Once activated, dimeric TGF-β binds to a tetrameric receptor complex formed by two type I (TβRI) and type II (TβRII) receptor subunits, leading to direct phosphorylation of R-Smads (Smad2 or Smad3), complex formation with co-Smad (Smad4), and induction or repression of target gene expression. Heterozygous inactivating mutations in both "positive" and "negative" regulators of this pathway have been identified as the cause of genetic disorders that are characterized by pathological alterations in the connective tissue due to misregulated expression of TGF-β gene targets (i.e., tissue metalloproteinases, collagen, integrins, and several other ECM components). The syndromes associated with these mutations, and the human gene mutated in each condition, are highlighted in yellow.

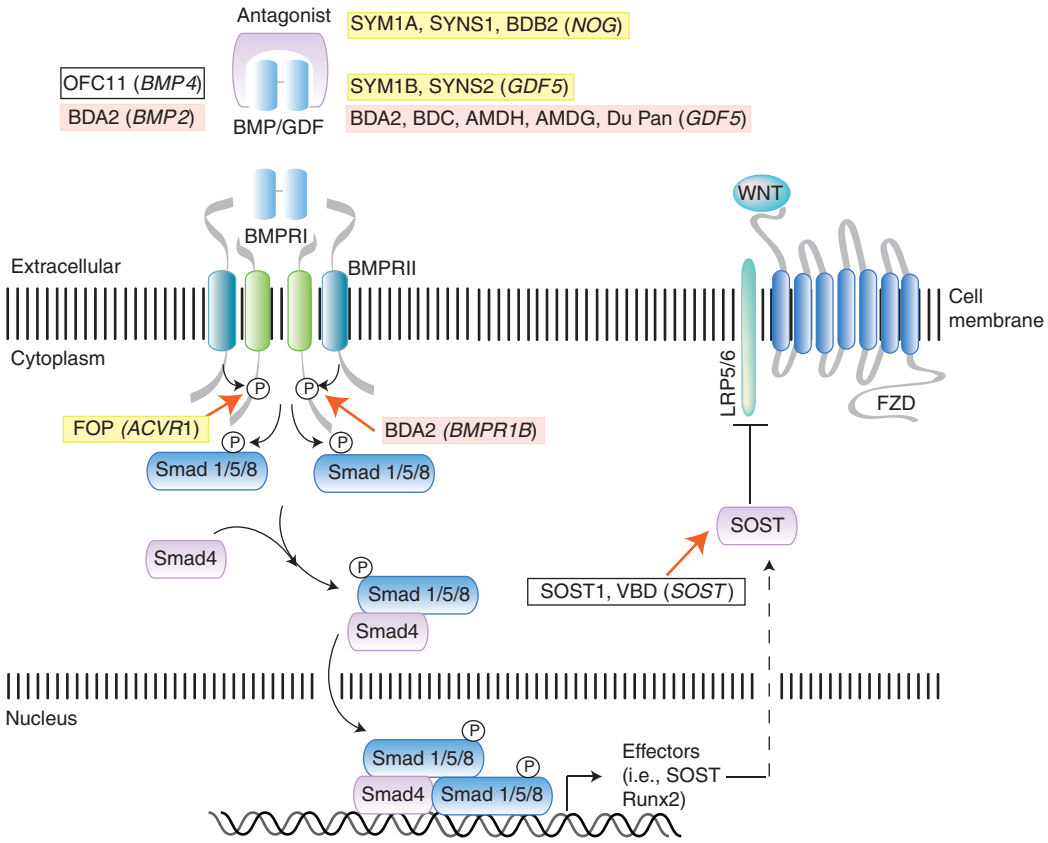

Figure 3. Summary of the bone morphogenetic protein (BMP) and growth and differentiation factor (GDF) signaling pathway and mutations causing connective tissue and skeletal disorders. The BMP signaling pathway is activated by extracellular ligands, such as BMPs and GDFs, through binding to type I (BMPRI) and type II (BMPRII) receptor complexes. Extracellular antagonists, such as noggin, can block ligand–receptor binding and pathway activation. Smad (shown) and non-Smad (not shown) mechanisms mediate BMP signal transduction to regulate transcriptional target genes, including sclerostin (SOST), a Wnt pathway inhibitor. Key components of the BMP pathway are shown to illustrate positions at which gene mutations act to cause specific human connective tissue and skeletal syndromes. Cross talk with the Wnt pathway is also shown. Specific disorders and their gene mutations (in parentheses), as discussed in text and Table 1, are shaded in yellow if the mutation enhances pathway signaling or in red if the pathway has diminished activation. White boxes indicate disorders for which the BMP pathway is either not directly affected or the functional consequence of the mutation is unknown. BDA2, Brachydactyly type A2; BDC, brachydactyly type C; AMDH, acromesomelic dysplasia Hunter–Thompson type; AMDG, acromesomelic dysplasia Grebe type; FOP, fibrodysplasia ossificans progressiva; VBD, Van Buchem disease.

to -7). The BMP type I receptors (BMPRIs), ALK-1 (also known as ACVRL1, activin A receptor-like 1), ALK-2 (ActRI, ACVR1, activin A receptor type I), BMPRIA (bone morphogenetic protein receptor type IA, also known as ALK-3), and BMPRIB (bone morphogenetic protein receptor type IB, also known as ALK-6), primarily signal by phosphorylation of Smad1, Smad5, and Smad8. ALK-4 (ActRIB, ACVR1B, activin A receptor type IB), TβRI (transforming growth factor-β receptor, type I, also known as ALK-5), and ALK-7 (ACVR1C, activin A receptor type IC), primarily signal by phosphorylation of Smad2 and Smad3. The type II receptors are ActRII (also known as ACVR2, activin A receptor type IIA), ActRIIB (ACVR2B, activin A receptor type IIB), TβRII (TGFBR2, transforming growth factor-β receptor type II),

BMPRII (bone morphogenetic protein receptor type II), and AMHRII (anti-Müllerian hormone receptor type II). The type III receptors, betaglycan (TGFBR3, transforming growth factor-β receptor type III) and endoglin (ENG, also known as CD105), and decoy receptors, such as BAMBI (BMP and activin membrane-bound inhibitor), do not signal independently but influence the signaling properties of receptor tetramers formed by type I and type II subunits.

The preferential pairing of each type II receptor with a specific type I receptor, the affinity of each cytokine for different receptor complexes, temporal and spatial coexpression of receptors, coreceptors, or decoy receptors, all vary and influence the probability that a certain complex will form and activate a specific signaling cascade. TGF-β1, -β2, and -β3 ligands are thought to primarily bind and activate tetramers composed of TβRI and TβRII subunits, resulting in phosphorylation of Smad2 and Smad3. Activins and some GDFs (including myostatin/GDF-8 and GDF-11) also signal through Smad2 and Smad3. Other GDFs and BMP ligands are thought to primarily function through receptor complexes that cause activation of Smad1, Smad5, and Smad8 through tetramers formed by BMPRIs and BMPRII, ActRII, or ActRIIB. Although specific combinations of tetramers are more likely to form in response to a given ligand, data suggest that a certain degree of promiscuous pairing occurs. For example, TGF-β-bound TβRII interacts and activates not only TβRI but also ALK-1 (Goumans et al. 2003), thus leading to activation of both Smad2 and Smad3 and Smad1, Smad5, and Smad8 in cells that express both types of type I receptors. In addition to activation of Smad-dependent pathways, TGF-β family ligands can also activate additional "noncanonical" pathways including the TRAF4–TRAF6 (TNF-receptor-associated factor 4 and/or 6)-TAK1 (TGF-β-activated kinase 1)-p38 MAPK pathway, the PI3K (phosphoinositide 3-kinase)-Akt pathway, the Rho-ROCK (Rho-associated protein kinase) pathway, and the JNK (c-Jun amino-terminal kinase), Erk (extracellular signal-regulated kinase) MAPK, and NF-κB (nuclear factor-κB) pathways (reviewed in Derynck and Zhang 2003). Activation of these noncanonical pathways is highly cell-specific and context-dependent.

EXTRACELLULAR MATRIX–DEPENDENT REGULATION OF TGF-β BIOAVAILABILITY

Connective tissue disorders are a family of diseases characterized by alterations in the composition, structure, or turnover of the extracellular matrix (ECM), a complex mixture of carbohydrates and proteins that includes collagens, proteoglycans, and glycoproteins (Mouw et al. 2014) that is secreted by cells in multicellular organisms. The specific properties of each type of connective tissue (i.e., cartilage, bone, ligaments, skin, muscle, and vessel walls) are dependent on the tissue-specific molecular composition of the ECM. In addition to providing physical support to cells, tissues, and organs, the ECM participates in regulating complex cellular behaviors including proliferation, migration, apoptosis, and differentiation. Dynamic remodeling of the ECM through regulated deposition, assembly, and degradation is critical for all ECM functions. ECM exerts its influence on surrounding cells through its interaction with matrix-sensing receptors on the cell surface, such as integrins (Campbell and Humphries 2011), and by regulation of the bioavailability of signaling molecules through regulated sequestration, release, and activation (Hynes 2009). The importance of the ECM to normal development and homeostasis is highlighted by the number of disorders that are either caused or exacerbated by abnormal ECM function (reviewed in Bateman et al. 2009; Bonnans et al. 2014).

TGF-β ligands (TGF-β1, TGF-β2, and TGF-β3) are secreted as biologically inactive latent complexes that are converted into an active form through a tightly regulated process that depends on interactions with components of the ECM (Munger and Sheppard 2011; Robertson and Rifkin 2016). TGF-β latent complexes (referred to as the small latent complex, or SLC) are comprised of TGF-β dimers and latency-associated peptide (LAP) dimers. LAPs are

translated as prodomains from the same genes and mRNAs encoding the corresponding TGF-βs and are referred to as β1-LAP for TGF-β1, β2-LAP for TGF-β2, and β3-LAP for TGF-β3. LAP prodomains are cleaved during posttranslational processing and remain associated with the corresponding TGF-β molecule through noncovalent interactions (Munger and Sheppard 2011). Disruption of noncovalent interactions between TGF-β and LAP molecules, through LAP degradation (Lyons et al. 1988; Yu and Stamenkovic 2000), chemical modification, such as that caused by reactive oxygen species or low pH (Lyons et al. 1988; Barcellos-Hoff and Dix 1996), and/or through binding-induced conformational change (Munger et al. 1997; Shi et al. 2011), results in conversion of latent TGF-β into active TGF-β. TGF-β–LAP dimers (SLC) covalently bind to one of the latent TGF-β binding proteins (LTBP-1, -3, and -4) through disulfide bonds formed between LAP and LTBPs (Todorovic and Rifkin 2012), resulting in the large latent complex, or LLC. Most cell-types do not secrete TGF-β as a SLC but rather already in complex with LTBP proteins. Interactions between LTBPs and ECM components, in particular with fibrillin microfibrils, are thought to be important for proper localization, concentration, and activation of latent TGF-β (Ramirez et al. 2007). Importantly, integrins (and in particular αvβ6, αvβ8, and αvβ1) have been shown to be necessary for activation of TGF-β1 and TGF-β3 through their interaction with arginine-glycine-aspartic acid (RGD) amino acid domains present in β1-LAP and β3-LAP (but not β2-LAP) (Munger et al. 1998; Mu et al. 2002; Dong et al. 2014; also reviewed in Munger and Sheppard 2011). Although prodomains similar to LAPs are present in other members of the TGF-β family and associate noncovalently with their corresponding ligands, they are not known, with the exception of myostatin, to be associated with latency. These prodomains might, however, facilitate binding to the ECM through interactions with fibrillin microfibrils. For example, fibrillin has been shown by surface plasmon resonance to bind BMP-2, BMP-4, BMP-7, BMP-10, and GDF-5 (Sengle et al. 2008). In particular, the interaction between fibrillin-1 and BMP-4 has been shown to be important for regulating BMP-4 bioavailability (Nistala et al. 2010). Although latent TGF-β can also be found in association with glycoprotein-A repetitions predominant protein (GARP) (Stockis et al. 2009; Wang et al. 2012), expression of GARP appears to be limited to platelets and regulatory T lymphocytes (Macaulay et al. 2007; Tran et al. 2009), suggesting that this regulatory molecule is not a major regulator of TGF-β bioavailability in the ECM (Stockis et al. 2009; Tran et al. 2009; Wang et al. 2012).

ROLES OF TGF-β AND BMP SIGNALING IN BONE FORMATION, SKELETAL DEVELOPMENT, AND PATTERNING

Bone is described as a hard connective tissue because the terminally differentiated osteoblasts that form much of the bone tissue produce an ECM that mineralizes, and provides bone with its rigid structure. Bone tissue forms through two distinct mechanisms that either depend exclusively on osteoblasts or additionally on chondrocytes (Yang 2013). Most flat bones, such as craniofacial bones and part of the clavicle, are intramembranous, developing by direct differentiation of mesenchymal progenitors into osteoblasts (Soltanoff et al. 2009). The entire appendicular and most of the axial skeleton have an endochondral origin, forming through a cartilage template that is subsequently replaced by osteoblasts and bone (Soltanoff et al. 2009). TGF-β and BMP signaling are crucial for the development of both bone types (Chen et al. 2012) and for regulating chondrogenesis and osteogenesis (Kulkarni et al. 1993; Mishina et al. 1995; Winnier et al. 1995; Zhang and Bradley 1996; Sanford et al. 1997; Sirard et al. 1998; Gu et al. 1999; Beppu et al. 2000; Dunker and Krieglstein 2002; Wang et al. 2014; Salazar et al. 2016; Wu et al. 2016).

Although BMP signaling promotes differentiation and maturation of osteoblasts, TGF-β exerts a dual role during osteogenesis by inducing commitment to the osteoblast lineage while at the same time preventing terminal differentiation (Moses and Serra 1996; Serra et al.

1999; Alliston et al. 2001; Derynck and Akhurst 2007). R-Smads physically interact with and regulate the transcriptional activity of runt-related transcription factor 2 (Runx2, also known as Cbfa1), a master regulator of mesenchymal differentiation toward the osteoblastic cell lineage (Komori et al. 1997; Otto et al. 1997; Leboy et al. 2001; Miyazono et al. 2004; Hecht et al. 2007; Javed et al. 2008, 2009; reviewed in Lian et al. 2004; Schroeder et al. 2005). Runx2 function is repressed by TGF-β and Smad2 and Smad3 signaling, and enhanced by BMPs and Smad1, Smad5, and Smad8 signaling (Cohen 2009; van der Kraan et al. 2009).

Activin A, another member of the TGF-β family of ligands, is also expressed in bone and cartilage, and activates the Smad2- and Smad3-dependent pathway with potential overlap with TGF-β functions (Ogawa et al. 1992; Massagué and Gomis 2006; Eijken et al. 2007; Tsuchida et al. 2009; Pauklin and Vallier 2015; Salazar et al. 2016).

Regulation of Chondrogenesis and Longitudinal Bone Growth by TGF-β and BMP Signaling

In addition to its dual role in osteoblastogenesis, TGF-β signaling exerts a similar dual role in chondrogenesis by inducing the condensation of undifferentiated mesenchymal cells, stimulating their commitment to the chondrogenic lineage, and promoting proliferation of chondrocytes and deposition of cartilage-specific ECM, but also inhibiting terminal differentiation of chondrocytes (Leonard et al. 1991; Ballock et al. 1993; Mackay et al. 1998; Worster et al. 2000; Yang et al. 2001; Tuli et al. 2003; Zhang et al. 2004; Song et al. 2007; Mueller and Tuan 2008; Furumatsu et al. 2009). BMP signaling, in contrast, is dispensable for the initial step of mesenchymal condensation but required for commitment to the chondrogenic lineage, proliferation, differentiation, and maturation of chondrocytes (Li et al. 2003; Horiki et al. 2004; Yoon et al. 2005; Retting et al. 2009; Culbert et al. 2014). BMP-mediated chondrogenic induction is achieved through the direct transcriptional activation of the transcription factor Sex determining region Y-box 9 (Sox9), which is the master regulator of chondrogenesis (Zehentner et al. 1999; Akiyama 2008; Pan et al. 2008). Genetic disruption of Sox9 results in perturbed skeletogenesis throughout the body (Akiyama et al. 2002; Mori-Akiyama et al. 2003). Differential regulation of Runx2 by TGF-β and BMP signaling, as discussed above, also participates in regulation of chondrocyte maturation. TGF-β and BMP signaling regulate chondrogenesis not only during embryonic skeletogenesis but also during postnatal bone growth. Longitudinal growth of long bones is mediated by proliferation and differentiation of chondrocytes within the growth plate, where morphologically distinct zones are recognized, each containing chondrocytes at different stages of differentiation and producing stage-specific ECM. Growth plates, located between the epiphysis and diaphysis at the ends of long bones, are normally spared from ossification until the end of longitudinal growth (Kronenberg 2003). TGF-β and BMP signaling are both required for growth plate progression and maintenance (Brunet et al. 1998; Yang et al. 2001; Zhang et al. 2003, 2005; Yoon et al. 2005; Pogue and Lyons 2006; Seo and Serra 2007; Keller et al. 2011; Jing et al. 2013).

BMP signaling occurs throughout the various zones of the growth plate indicating multiple roles in growth plate development and maintenance (Solloway et al. 1998; reviewed in Pogue and Lyons 2006). Mice deficient for the BMP-specific antagonist, noggin, show overgrowth of skeletal elements (Brunet et al. 1998) supporting that BMP signaling promotes cell proliferation, differentiation, and maturation within the growth plate. Conversely, chondrocyte-specific overexpression of noggin results in gross reduction of cartilaginous elements (Tsumaki et al. 2002). A balance between positive and negative regulators of the BMP pathway is essential for proper bone growth, as shown by dysregulated bone development when the BMP pathway is over- or underactivated in genetically modified mouse models. Both genetically enhanced and genetically ablated BMP signaling result in impaired bone growth, which is caused by either premature onset of chondrocyte maturation toward hypertrophy or impaired proliferation

within the growth plate (Francis-West et al. 1999; Mikic 2004; Kobayashi et al. 2005; Yoon et al. 2005; Pogue and Lyons 2006; Jing et al. 2013).

TGF-β signaling in the growth plate occurs in proliferating and hypertrophic chondrocytes and in the periosteum, suggesting a role in maintenance of these tissues (Ellingsworth et al. 1986; Sandberg et al. 1988; Gatherer et al. 1990; Pelton et al. 1990; Millan et al. 1991; Morales 1991; Serra et al. 2002; Ivkovic et al. 2003; Serra and Chang 2003; Minina et al. 2005). TGF-β signaling controls longitudinal bone growth through inhibition of chondrocyte terminal differentiation and maintenance of the proliferating chondrocyte pool (Serra et al. 1997; Alvarez et al. 2001; Yang et al. 2001; Alvarez and Serra 2004). Consequently, conditional inactivation of *Tgfbr2* or *Tgfbr1*, which leads to ablation of TGF-β signaling, results in severe shortening of the limbs among other skeletal manifestations (Baffi et al. 2004; Seo and Serra 2007; Matsunobu et al. 2009; Hiramatsu et al. 2011).

Growth Plate Defects Caused by Altered TGF-β and BMP Signaling

Acromesomelic dysplasias (AMDs) are characterized by brachydactyly (BD) and short stature (Table 1). Various forms of AMD are caused by mutations in the gene coding for GDF-5, also known as cartilage-derived morphogenetic protein 1 (CDMP-1), and impaired BMP signaling. Growth retardation and the decreased size of appendicular skeletal elements are a result of premature growth plate closure (Thomas et al. 1996). Distal skeletal elements are more severely affected by size reduction than proximal elements. Clinical manifestations in acromesomelic dysplasia Hunter–Thompson type (AMDH), Grebe type (AMDG), and Du Pan syndrome are overlapping with the most severe growth retardation in AMDG and with specific fibula malformation in Du Pan syndrome (Langer and Garrett 1980; Langer et al. 1989; Costa et al. 1998; Faiyaz-Ul-Haque et al. 2002; Szczaluba et al. 2005).

AMD-associated *GDF5* mutations result in loss of GDF-5 protein function. The AMDG-specific *GDF5* mutation, C400Y, results in an inactive protein that is not secreted, and acts in a dominant-negative manner by preventing the secretion of other BMP molecules, consistent with the severe phenotype in patients carrying this mutation (Thomas et al. 1996, 1997). Although homozygous carriers of AMDG mutations display severe autopod (digit) malformation with reduction of fingers and toes to ball-shaped structures, heterozygous carriers are only mildly affected, indicating a dose-dependent effect of GDF-5 on growth plates. The phenotypic finding of AMD is recapitulated in mice deficient for *Gdf5*, including severe size reduction of long bones and abnormal joint development (Storm et al. 1994).

Roles of TGF-β and BMP Signaling in Limb Formation and Digit Patterning

During embryogenesis, the development of the appendicular skeleton first becomes evident by the outgrowth of limb buds. Limb bud outgrowth and patterning is a multistep process that is orchestrated by "signaling centers" within the limb field that provide positional and instructive information to the surrounding cells through gradients of growth factors, including BMPs, fibroblast growth factors (FGFs), Sonic hedgehog (SHH), and Wnts (Johnson and Tabin 1997; Martin 1998). BMPs are widely expressed in a dynamic pattern during limb development and have a range of diverse functions (Bandyopadhyay et al. 2006; Robert 2007). During limb bud initiation, BMP signaling is crucial for the induction of the apical ectodermal ridge (AER), a specialized epithelial structure located at the distal tip of the developing limb that controls formation of the proximal–distal axis from body center toward tips of appendages (Johnson and Tabin 1997; Martin 1998; Ahn et al. 2001). Early ablation of the AER or conditional inactivation of BMP signaling in the ectoderm leads to limb truncation (Saunders 1948; Summerbell et al. 1973; Rowe et al. 1982; Ahn et al. 2001). The AER functions to initiate and maintain normal gene expression patterns in the progress zone (PZ), an area of undifferentiated cells in the mesoderm directly underlying the AER, and is furthermore re-

quired for the maintenance of SHH expression within the zone of polarizing activity (ZPA), a crucial signaling center located at the posterior margin of the limb that controls anterior-posterior pattering. Together, the AER and ZPA coordinate gradients of growth factors to regulate formation, size, shape, and location of future bone and joints in the appendicular skeleton (reviewed in Johnson and Tabin 1997; Galloway and Tabin 2008). Interlinked feedback loops between AER, ZPA, and mesenchymal BMP signaling regulate initiation, propagation, and termination of the limb signaling system (reviewed in Benazet et al. 2009; Zeller et al. 2009). Disruption of these feedback loops result in impaired progression of limb bud development and specification of digit identity (Kawakami et al. 1996; Ahn et al. 2001; Khokha et al. 2003; Ovchinnikov et al. 2006; Montero et al. 2008; Suzuki et al. 2008; Benazet et al. 2009).

BMP signaling controls patterning of digits via a crescent-like structure located at the distal tip of each digit ray, called the phalanx-forming region (PFR). The PFR is marked by high levels of BMP activity and is formed and maintained by continuous recruitment of cells from the PZ. Cells recruited from the PZ aggregate distal to metatarsal and metacarpal condensations, commit to chondrogenic fate, and are eventually integrated into the condensing underlying digital ray. PFR-ablation or inhibition of BMP signaling via noggin results in truncation of the digit (Montero et al. 2008; Suzuki et al. 2008). TGF-β and activin signaling are also needed to establish the PFR and to induce Sox9 expression (Chimal-Monroy et al. 2011).

To form joints, the initially continuous digital rays become segmented. Many studies showed the importance of a balanced interplay among multiple factors for both joint formation and homeostasis (Storm and Kingsley 1996; Brunet et al. 1998; Rountree et al. 2004; Koyama et al. 2007). Location of a future synovial joint is initially indicated by cell condensations within an area of the digit ray called the interzone (reviewed in Archer et al. 2003; Decker et al. 2014). Cells in this region do not undergo chondrogenic differentiation but adopt a flattened morphology. The joint interzone later divides into three discrete layers. Cells within the medial layer express BMP-2 and subsequently undergo cell death, thereby forming the joint cavity, whereas the remaining outer layers will give rise to the articular cartilage (reviewed in Pacifici et al. 2005). During joint formation, noggin is expressed within the intermedial layer of the interzone (Brunet et al. 1998; Capdevila and Johnson 1998; Merino et al. 1998; Goumans and Mummery 2000; Pizette and Niswander 2000; Seemann et al. 2005; Lorda-Diez et al. 2013), where it counteracts the effect of GDF-5, the key molecule required for defining the future joint interzone within the unpatterned digital ray. Loss of GDF-5 function leads to abnormal joint development with complete or partial fusion of consecutive skeletal elements and structural changes in autopods, along with other skeletal defects, whereas loss of noggin activity results in failure to initiate joint formation and thus symphalangism (Storm and Kingsley 1996, 1999; Brunet et al. 1998).

Digit Malformations Caused by Disruption of TGF-β and BMP Signaling

Depending on the specific perturbation, disruption in the BMP signaling pathway can cause various types of BD, all characterized by reduction or absence of phalanges in one or more digits and resulting in shortened toes and/or fingers (Bell 1951; Stricker and Mundlos 2011). Although BD can occur as an isolated malformation, more commonly it is one of several features of syndromes caused by mutations that impair BMP ligand or receptor function, for example, in AMDs (see previous section; Table 1).

Depending on the affected digit, BD is classified into groups A to E (Table 2) (Fitch 1979). Inactivating mutations in genes coding for the BMPRIB receptor or its ligand GDF-5 (Nishitoh et al. 1996; Lehmann et al. 2003, 2006) cause BDA2, which is characterized by short middle phalanges in the second and fifth digits. A microduplication in a downstream regulatory element of BMP-2, which is thought to affect limb-specific expression, was also found in some patients with BDA2 (Dathe et al. 2009). *GDF5* mutations also can cause BDC, characterized

Table 2. Summary of brachydactyly types and associated clinical manifestations

Brachydactyly type (OMIM #)	Gene mutation	Clinical manifestation in the skeletal system
A1 (112500)	*IHH*	Hypoplasia/aplasia of all middle phalanges
A2 (112600)	*GDF5, BMP2*	Hypoplasia/aplasia middle phalanges of digit 2 and 5
B1 (113000)	*ROR2*	Hypoplasia/aplasia of distal phalanges of digits 2 to 5, fusion of distal interphalangeal joints; duplication of distal phalange in digit 1
B2 (611377)	*NOG*	Hypoplasia/aplasia of distal and middle phalanges, syndactyly (variable)
C (113100)	*GDF5*	Shortening of metacarpal 1, shortening or absence of middle phalanges of digit 2, 3, and 5, hypersegmentation of digit 2 and/or 3
E1 (113300)	*HOXD13*	Shortening metacarpal 4; sometimes shortening of metatarsal 4
E2 (613382)	*PTHLH*	Shortening of metacarpals (variable in affected digits but most common in digit 4 and 5)

by short or absent middle phalanges in second, third, and fifth fingers, short first metacarpal and hypersegmentation of the second and/or third finger (Polinkovsky et al. 1997; Robin et al. 1997; Galjaard et al. 2001; Everman et al. 2002; Savarirayan et al. 2003; Holder-Espinasse et al. 2004; Schwabe et al. 2004). These BD phenotypes are recapitulated in vivo by ablation of *Bmpr1b* or *Gdf5* in mice, which results in impaired proliferation of mesenchymal progenitors and increased apoptosis in phalanges, with preservation of interdigital mesenchyme (Baur et al. 2000; Yi et al. 2000). That impaired signaling through BMPRIB or GDF-5 in the developing autopod results in very specific malformations and does not affect all phalanges or digit identities suggests that other BMP ligands or pathways compensate for this specific deficiency during limb development.

Although reduced GDF-5 and BMPRIB signaling causes BDA2, excessive BMP signaling activity through GDF-5 and BMPRIA leads to proximal symphalangism (SYM1), multiple synostosis syndromes (SYNS1 and SYNS2), and BDB2. SYM1, SYNS1, and SYNS2 syndromes are characterized by multiple joint fusions that are restricted to proximal interphalangeal joints of the hands and feet and carpal and tarsal bones; SYNS1 and SYNS2 patients additionally display multiple joint fusions in vertebrae and the hip (da-Silva et al. 1984; Gaal et al. 1987; Gong et al. 1999; Takahashi et al. 2001; Mangino et al. 2002). SYM1 is caused by gain-of-function mutations in *GDF5* that strongly increase ligand affinity for the BMPRIA receptor and result in loss of binding specificity for BMPRIB (Nickel et al. 2005; Seemann et al. 2005). Increased BMP signaling activity also follows from *GDF5* mutations that render GDF-5 resistant to noggin and cause SYNS2 (Schwaerzer et al. 2012; Degenkolbe et al. 2013). SYNS1 and SYM1 can also result from heterozygous missense mutations in the gene coding for the BMP inhibitor noggin. Although secretion of noggin is only reduced in SYM1, it is completely abolished in SYNS1, explaining the more severe SYNS1 phenotype and pointing toward a dosage-dependent effect of this inhibitor during skeletogenesis (Marcelino et al. 2001).

The importance of balanced levels of BMP signaling during digit development is further emphasized by other congenital syndromes, in which mutations, such as those found in BDB2, induce a shift in this tightly regulated process. Short distal phalanges in combination with symphalangism, fusion of carpal and tarsal bones and partial cutaneous syndactyly are characteristic skeletal malformations in BDB2 patients. Point mutations within the *NOGGIN (NOG)* gene cause reduced binding capacity to BMP ligands and result in reduced sequestration of BMP ligand by the mutant protein, which shifts the state of the BMP signaling pathway balance toward activation (Lehmann et al. 2007) and results in disruption of digit patterning.

Roles of TGF-β and BMP Signaling in Craniofacial Development

TGF-β and BMP signaling are required for proper formation of craniofacial structures, and in particular for proper development of cranial bones and palate (Rigueur et al. 2015). During craniofacial development, plates of calvarial bone interconnected via fibrous sutures envelop the brain. The fibrous sutures serve as the sites of bone expansion during postnatal growth and provide plasticity to the skull in response to the growing brain (reviewed in Opperman 2000; Warren et al. 2001). Alterations in both TGF-β and BMP signaling have been associated with premature closure of sutures and craniosynostosis. Increased Smad-dependent BMP signaling induced in cranial neural crest cells by a constitutively active BMPRIA receptor causes premature suture closure in mice, which can be rescued by genetically or chemically inhibiting this pathway (Komatsu et al. 2013). During calvarial development, a major role of FGF is to suppress expression of the BMP inhibitor noggin via Erk1 and Erk2 MAPK signaling and thus induce suture closure by increasing BMP signaling (Warren et al. 2003). TGF-β ligands are also expressed in the developing skull and most likely involved in regulating suture closure. TGF-β1 and TGF-β2 are expressed in sutures undergoing fusion, whereas TGF-β3 expression is restricted to patent sutures, possibly suggesting that TGF-β1 and TGF-β2 promote, whereas TGF-β3 prevents, closure (Opperman et al. 1997; Most et al. 1998; Chong et al. 2003). In accordance with these observations, TGF-β2 has been shown to induce suture closure in an Erk1 and Erk2 MAPK-dependent manner in an ex vivo murine calvaria model (Opperman et al. 2006).

In addition to their role in calvaria fusion, BMP and TGF-β signaling are also essential for development and fusion of the palatal shelves (Bush and Jiang 2012). Fusion between the shelves requires first formation and then disintegration of the midline epithelial seam (MES). This leads to mesenchymal fusion of the palate, and subsequent bone formation, which occurs through intramembranous ossification (Baek et al. 2011). The main function of TGF-β in palate development is to promote fusion of the shelves. Before palatal shelf elevation, epithelial expression of TGF-β1 and TGF-β3 is induced within the future adhesion site, and then diminishes during disintegration of the MES (Cui et al. 1998; Cui and Shuler 2000; Yang and Kaartinen 2007; Bush and Jiang 2012). Global ablation of TGF-β2 or TGF-β3 ligands or cranial neural crest specific knockout of *Tgfbr2* or *Tgfbr1* causes cleft palate, highlighting the importance of this signaling pathway in palatogenesis (Kaartinen et al. 1995; Proetzel et al. 1995; Sanford et al. 1997; Ito et al. 2003; Dudas et al. 2006; Xu et al. 2006). In *Tgfb3*-null mutants, palatal shelf adhesion occurs, but impaired apoptosis causes persistence of the MES resulting in non-union of the palatal mesenchyme. Treatment of these mice with recombinant TGF-β3 or overexpression of Smad2 rescues palate fusion (Kaartinen et al. 1997; Taya et al. 1999; Cui et al. 2005; Dudas et al. 2006; Xu et al. 2006). BMP signaling regulates specification and migration of cranial neural crest cells to the facial primordia (reviewed in Nie et al. 2006), and also controls mesenchymal cell proliferation. Tissue-specific inhibition of this signaling pathway by conditional inactivating mutations in BMP receptors or overexpression of the antagonist noggin causes a reduced proliferation rate within the palatal mesenchyme and results in palate clefting (Dudas et al. 2004; Liu et al. 2005; He et al. 2010; Baek et al. 2011).

Craniofacial Defects Caused by Altered TGF-β or BMP Signaling

Cleft palate with or without involvement of the lip is a common congenital defect in humans (Parada and Chai 2012) that has been associated with loss-of-function mutations in *BMP4* (orofacial cleft 11; OFC11) and *BMP7* (Suzuki et al. 2009; Wyatt et al. 2010). Although the functional consequences of these mutations are not fully understood, some observations support that they alter posttranslational processing to reduce protein activity in a tissue-dependent manner (Akiyama et al. 2012). In addition, both cleft palate and craniosynostosis are associated with

Loeys–Dietz syndrome, which is caused by mutations in critical mediators and regulators of TGF-β signaling, and is discussed below in greater detail.

TGF-β SIGNALING AND VESSEL WALL HOMEOSTASIS

Structure of the Arterial Vessel Wall

The vessel wall of major arteries is composed of three connective tissue layers: an inner layer (tunica intima), a middle layer (tunica media), and an outer layer (tunica adventitia) (Wagenseil and Mecham 2009), each composed of different types of cells and ECM components (Fig. 4). Endothelial cells line the lumen of the vessel and are anchored to a basement membrane that separates these cells from the tunica intima. The internal elastic lamina separates the tunica intima from the tunica media, which is populated by vascular smooth muscle cells (VSMCs) and contains elastic fibers, complex structures that contain both elastin and fibrillin-containing microfibrils, collagen fibers, and proteoglycans (Wagenseil and Mecham 2009). Although the majority of VSMCs reside in the tunica media, some VSMCs or VSMC-like cells are also present in the tunica intima and/or can be recruited to the intima in response to vascular injury (Shanahan and Weissberg 1998; Owens et al. 2004; Fukuda and Aikawa 2010; Iwata et al. 2010). The tunica intima, which is generally much thicker in human arteries compared with other mammals, does not contain elastic fibers. The outer layer or tunica adventitia is populated by adventitial fibroblasts, is rich in collagen, does not contain elastic fibers, and is

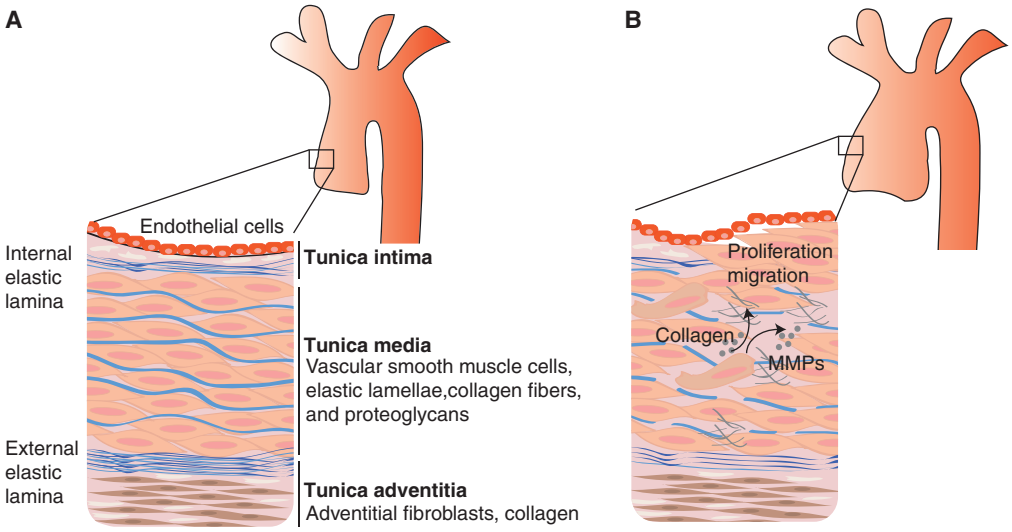

Figure 4. Schematic representation of the vessel wall of major arteries. (*A*) The vessel wall of arteries is organized in layers, which are referred to as tunica intima, media, and adventitia. Endothelial cells line the lumen and are attached to a basement membrane. The connective tissue immediately below the endothelial cell layer is referred to as tunica intima. The internal elastic lamina separates the tunica intima from the tunica media, which contains vascular smooth muscle cells (VSMCs) and elastic fibers, organized in fenestrated sheets (also called lamellae). The external elastic lamina separates the tunica media from the tunica adventitia, which contains mostly fibroblasts and collagen, but no elastic fibers. (*B*) Arterial wall remodeling associated with aneurysm development include increased VSMC migration and proliferation (and associated loss of contractile function), increased secretion of matrix-degrading enzymes (such as matrix metalloproteinases [MMPs]), and increased deposition of collagen and fragmentation of elastic fibers. The ultimate effect of these processes is the replacement of the ordered and layered architecture of elastic lamellae and vascular smooth muscle cells with disorganized and weak connective tissue.

separated by the tunica media by the external elastic lamina (reviewed in Wagenseil and Mecham 2009). Some studies have suggested that the adventitial layer contains progenitors that *trans*-differentiate into VSMCs (Sartore et al. 2001; Hoofnagle et al. 2004; Hu et al. 2004; Passman et al. 2008). The composition of the ECM secreted by vascular cell types, and by VSMCs in particular, varies depending on the type of blood vessel and in response to developmental, physiological, and pathological stimuli (Kelleher et al. 2004; Xu and Shi 2014). For example, a study of the type of ECM components secreted by VSMCs at various stages of mouse development identified a critical period from embryonic day E14 to ~2 weeks after birth during which expression of matrix proteins, such as elastin and fibrillar collagens, is very pronounced, and which is followed by a period of low-expression that continues into adulthood (Kelleher et al. 2004; Wagenseil and Mecham 2009). Under physiological circumstances, elastin is thought to only be secreted and productively deposited by VSMCs during the fetal and neonatal periods (Davis 1993; Kelleher et al. 2004).

Both mechanical stimuli and biochemical signals regulate the interaction between the ECM and vascular cells (Owens et al. 2004; Mack 2011; Humphrey et al. 2015). Changes in the ECM are sensed by vascular cells through matrix-binding receptors, such as integrins, and influence cell migration, proliferation, and survival (Moiseeva 2001); in turn, vascular cells can modify the composition and structure of the ECM by increasing or decreasing secretion of matrix proteins and matrix-remodeling enzymes (Leung et al. 1976; O'Callaghan and Williams 2000).

TGF-β Signaling in Vascular Homeostasis

The importance of TGF-β signaling regulation in the complex relationship between ECM and vascular cells is highlighted by the number of vascular disorders that result from failed ECM-dependent regulation of TGF-β signaling or from mutations in critical mediators of TGF-β signal transduction (reviewed in Goumans and Mummery 2000; Goumans et al. 2009). Of particular importance to the focus of this review are the effects of altered TGF-β signaling on the development of aneurysm, a pathological dilation of a blood vessel that can result in fatal tear and rupture.

The effects of TGF-β and BMP signaling on the biology of the vessel wall vary depending on developmental stage, cellular context, and presence of other ligands or stimuli. Prenatally, TGF-β and BMP signaling are key regulators of both development and morphogenesis of the vascular system (Goumans and Mummery 2000; Goumans et al. 2009). Early during embryonic development, TGF-β signaling is required for formation of the vascular plexus and differentiation of both endothelial cells and VSMCs (Dickson et al. 1995; Oshima et al. 1996; Goumans and Mummery 2000; Larsson et al. 2001; Carvalho et al. 2007; Goumans et al. 2009). TGF-β signaling is also required for morphogenesis of the cardiac outflow tract and VSMC-dependent deposition of elastic fibers (Wurdak et al. 2005; Choudhary et al. 2006).

Prenatal or perinatal ablation of TGF-β signaling in mouse models by deletion of *Tgfbr2* in VSMCs with a VSMC-specific *Cre* (Langlois et al. 2010), neural crest- and mesoderm-specific *Cre* (Choudhary et al. 2009), or an inducible VSMC-specific *Cre* (Li et al. 2014) results in defective elastogenesis, vessel wall dilation, aneurysm, and dissection. However, deletion of *Tgfbr2* in VSMCs after ~8 weeks of age has no apparent consequence on VSMC function and vessel wall homeostasis (Li et al. 2014), suggesting that complete loss of TGF-β signaling in adult VSMCs does not cause major pathology in the adult vessel wall. The early developmental sensitivity to TGF-β signaling ablation in VSMCs might relate to the timing of physiological elastin deposition, which is complete once animals reach adulthood, at ~4–8 weeks after birth (Davis 1993; Kelleher et al. 2004; Wagenseil and Mecham 2009). The expression of other determinants of vessel wall homeostasis might also require TGF-β signaling during this critical time window, but then become irrelevant, redundant, or independent of TGF-β signaling later in life.

In the adult aorta, excessive TGF-β signaling, rather than impaired TGF-β signaling, has been linked to maladaptive remodeling of the vascular ECM, VSMC dysfunction, and development of thoracic aortic aneurysm (TAA), a common form of aneurysm often associated with genetic syndromes (Chen et al. 2013). TGF-β-dependent changes during aneurysm development include increased expression and/or activities of matrix-degrading enzymes such as metalloproteinases, degradation of elastic fibers, enhanced proliferation and migration of VSMCs, and excessive collagen secretion and deposition (reviewed in Jones et al. 2009). Overall, the effects of these alterations, which target both matrix degradation and matrix deposition, weaken the aortic wall rendering it more prone to dilatation, dissection, and rupture.

Marfan Syndrome

The importance of TGF-β signaling in the context of ECM homeostasis is well illustrated by the case of Marfan syndrome (MFS). MFS is an autosomal dominant disorder caused by heterozygous mutations in the gene that encodes fibrillin-1 (*FBN1*), the main component of extracellular microfibrils (Dietz et al. 1991). It is characterized by cardiovascular, ocular, and musculoskeletal defects (Table 1), including a high risk for aortic root aneurysm and dissection (Judge and Dietz 2005). MFS pathology was initially attributed to a structural deficit caused by defective fibrillin-1 assembly into microfibrils. However, simple structural changes could not explain some MFS manifestations, such as craniofacial dysmorphism, bone overgrowth, myxomatous degeneration of the mitral valve, and low-muscle mass and fat stores, all of which pointed to a more complex relationship between the ECM and developmental signals. This observation led to the hypothesis that dysregulation of TGF-β signaling contributed to MFS pathology. It is now recognized that most manifestations of MFS can be understood as a failure in the ECM-dependent control of TGF-β signaling (Neptune et al. 2003). Under physiological circumstances, extracellular microfibrils serve complex and somewhat divergent functions in regulating TGF-β signaling. They serve both as "positive" regulators of TGF-β signaling, by concentrating latent TGF-β at sites of intended function, and as "negative" regulators of TGF-β signaling, by sequestering latent TGF-β and preventing its conversion to the active form (Ramirez et al. 2004; Robertson et al. 2015).

Despite these two contrasting roles, several studies examining a diverse set of MFS phenotypes, both in patients and mouse models, have shown that a signature consistent with increased activation of the TGF-β signaling pathway is associated with MFS pathology, and that pharmacological antagonism of TGF-β with neutralizing antibodies attenuates or prevents disease progression (Neptune et al. 2003; Ng et al. 2004; Habashi et al. 2006; Cohn et al. 2007). Studies conducted in MFS mouse models have suggested that effects of TGF-β signaling attenuation with neutralizing antibodies are dependent on the developmental stage of the vessel wall, with early TGF-β inhibition, between three and six weeks of age, being deleterious, and late TGF-β inhibition, after 6 weeks of age, being beneficial (Cook et al. 2015). These observations are consistent with the studies discussed above suggesting that TGF-β signaling has a protective role prenatally and perinatally, when it is required to complete vessel wall development and elastogenesis, and a pathogenic role after such events are completed.

Importantly for clinical management of MFS patients, the Food and Drug Administration (FDA)-approved angiotensin II type 1 receptor (AT1) blocker losartan, an antihypertensive drug, has been shown to reduce TGF-β signaling and ameliorate many manifestations of MFS in mouse models (Neptune et al. 2003; Ng et al. 2004; Habashi et al. 2006; Cohn et al. 2007; Lacro et al. 2007). Additionally, in a randomized clinical trial of pediatric MFS patients, losartan, at the FDA-recommended dose for hypertension, was shown to be equally effective as atenolol used at a dose far above the FDA-recommended daily dose, with both drugs leading to a significant decline in Z-score over time (body size-indexed aortic root dimension over time) (Lacro et al. 2014; Mallat and Daugherty 2015). A trial in adults with MFS showed that

use of losartan was associated with a significant reduction in aortic root growth rate and also attenuated dilatation of the more distal ascending aorta in patients that had undergone aortic root replacement (Groenink et al. 2013).

Although several studies have shown that antagonism of angiotensin II signaling results in decreased TGF-β signaling in a variety of tissues, including kidney, lung, skeletal muscle, heart, and aorta (Shihab et al. 1997; Sun et al. 1998; Lavoie et al. 2005; Habashi et al. 2006; Yao et al. 2006; Cohn et al. 2007; Podowski et al. 2012), the exact mechanism by which this occurs is not fully understood (Gibbons et al. 1992; Stouffer and Owens 1992; Wolf et al. 1993, 1999; Kagami et al. 1994; Lee et al. 1995; Campbell and Katwa 1997; Fukuda et al. 2000; Boffa et al. 2003; Naito et al. 2004; Rodriguez-Vita et al. 2005; Zhou et al. 2006; Chen et al. 2013). Suppression of excessive Erk1 and Erk2 MAPK activation with losartan or an inhibitor of MAPK kinase (MAPKK, also known as MEK) has been shown to normalize aortic architecture and aneurysm pathology in MFS mouse models (Habashi et al. 2011), indicating that Erk MAPK activation is critical to aneurysm progression. Although not fully elucidated, the pathogenic effects of Erk1 and Erk2 MAPK signaling might relate to induced expression of metalloproteinases (Ghosh et al. 2012) or TGF-β1 ligand (Hamaguchi et al. 1999).

Perturbed sequestration of TGF-β ligands in bone and cartilage might also explain the skeletal features of MFS. MFS patients show increased length of appendicular skeletal elements, especially in phalanges. Although the TGF-β bioavailability in skeletal tissue of MFS patients has not been evaluated, TGF-β ligands are abundantly expressed in growth plates and bone (Pedrozo et al. 1998; Minina et al. 2005). Osteoblasts derived from fibrillin (*Fbn1* or *Fbn2*)-deficient mice show abnormal activation of TGF-β signaling (Nistala et al. 2010). The stimulatory effect of TGF-β on cell proliferation and chondrogenesis in the growth plate and its inhibition of chondrocyte maturation suggest that bone overgrowth in MFS may result from growth plate enlargement. In addition to perturbed TGF-β bioavailability, MFS mutations might also alter binding of fibrillin-1 to BMPs (Arteaga-Solis et al. 2001; Sengle et al. 2008; Nistala et al. 2010), suggesting that skeletal abnormalities in MFS might result from altered, and perhaps misbalanced, BMP and TGF-β signaling.

Loeys–Dietz Syndrome

Loeys–Dietz syndrome (LDS) is an autosomal dominant Mendelian disorder with widespread phenotypic overlap with MFS, including bone overgrowth, skeletal deformity, and a strong predisposition for aortic root aneurysm and dissection (Loeys et al. 2005). When compared with MFS, LDS also shows many unique features (Table 1) in the craniofacial, skeletal, cutaneous, and cardiovascular systems (MacCarrick et al. 2014). Patients with LDS can also show evidence of immunologic dysregulation including asthma, eczema, and gastrointestinal inflammation that associates with increased numbers of T-regulatory cells that inappropriately secrete proinflammatory T-helper 2 cytokines (Frischmeyer-Guerrerio et al. 2013). Inactivating mutations in the genes encoding TGF-β receptors (*TGFBR1* or *TGFBR2*) (Loeys et al. 2005), intracellular mediators of TGF-β signaling (*SMAD3* and *SMAD2*) (van de Laar et al. 2011; Micha et al. 2015), or TGF-β ligands (*TGFB2* and *TGFB3*) (Boileau et al. 2012; Lindsay et al. 2012; Bertoli-Avella et al. 2015), have all been shown to cause LDS or LDS-like syndromes. In contrast, mutations in genes coding for TGF-β family receptors primarily expressed in endothelial cells, such as the *ENG* (McAllister et al. 1994) and *ACVRL1* (Lesca et al. 2004) genes, cause hereditary hemorrhagic telangiectasia type 1 and type 2, syndromes characterized by dilated small blood vessels (telangiectasias) and improper connections between arteries and veins (arteriovenous malformation, or AVMs), but typically without aortic aneurysm or widespread connective tissue involvement (and as such not the focus of this review). The phenotypic similarities between MFS and LDS suggest that these syndromes share a common pathogenic mechanism. However, although the role of enhanced TGF-β signaling is well established

in MFS, the mechanisms by which LDS mutations cause disease and a tissue signature for increased TGF-β signaling during aneurysm progression remain unclear. In vitro experiments consistently show that mutant receptors are expressed, traffic to the cell surface, and bind ligand but fail to phosphorylate Smad2 in response to exogenous TGF-β (Mizuguchi et al. 2004; Horbelt et al. 2010; Gallo et al. 2014). However, histological and biochemical assessment of aortic tissue derived from patients affected by LDS, or mouse models, show a paradoxical signature of high TGF-β signaling (van de Laar et al. 2011; Lindsay et al. 2012; Gallo et al. 2014). These contrasting observations have generated controversy in the field regarding the specific role of TGF-β in the initiation and progression of aneurysm (Lavoie et al. 2005; Dietz 2010; Mallat and Daugherty 2015). Various mechanisms have been proposed to explain how LDS mutations can paradoxically result in increased TGF-β signaling in vivo, including the possibility that angiotensin II receptor signaling, rather than TGF-β signaling, might be responsible for the observed increase in Smad2 and Smad3 phosphorylation and upregulation of TGF-β-driven gene products (Davis et al. 2014), or that different intrinsic susceptibility to LDS-causing mutations in cells of different type or embryonic origin might result in paracrine signaling overdrive caused by excessive secretion of TGF-β1 ligand (Lindsay and Dietz 2011). However, none of these mechanisms has been validated to date. A possible paracrine mechanism is suggested by the observation that expression of TGF-β1 is increased in tissues derived from LDS-3 and LDS-4 patients and in LDS mouse models (van de Laar et al. 2011; Lindsay et al. 2012; Gallo et al. 2014), and that treatment of LDS mouse models with losartan, which normalizes aortic root growth and aortic wall architecture in these animals, is associated with reduction of excessive TGF-β1 expression and Smad2 and Erk MAPK activation in LDS mice (Gallo et al. 2014).

Perturbations in TGF-β signaling in LDS are also associated with cleft palate (Table 1). In LDS patients, defective TGF-β signaling during palate development might result in failed mesenchymal fusion of palatal shelves (Yang and Kaartinen 2007) and thus in cleft palate. An alternative, yet controversial, explanation (Iwata et al. 2012) is that in *Tgfbr2*-null mice, cleft palate might result from an imbalance between Smad and non-Smad TGF-β signaling caused by "noncanonical" pairing of TβRI and TβRIII/betaglycan, and overactivation of the TRAF6–TAK1–p38 signaling pathway, which leads to a proliferation defect responsible for nonunion of the palatal shelves (Iwata et al. 2012). This idea was supported by the observation that concomitant deletion of the *Tgfbr1* gene rescued the cleft palate phenotype in cranial neural crest-specific *Tgfbr2*$^{-/-}$ mice. TβRI or TβRII receptors with LDS mutations, which traffic to the cell surface and can bind ligand, might in a similar fashion increase signaling by favoring "noncanonical" pairing of the remaining functional receptors in the developing palate of LDS mouse models and patients.

Shprintzen–Goldberg Syndrome

Shprintzen–Goldberg syndrome (SGS) is characterized by virtually all the craniofacial, skeletal, cutaneous and cardiovascular manifestations found in LDS and MFS, with the addition of highly penetrant intellectual disability. It is caused by heterozygous loss-of-function mutations in the Sloan Kettering Institute proto-oncogene (*SKI*) (Carmignac et al. 2012; Doyle et al. 2012), a potent inhibitor of TGF-β-induced Smad signaling. Ski-dependent inhibition of TGF-β signaling occurs through suppression of Smad2 and Smad3 phosphorylation and nuclear translocation, by displacement of positive regulators of TGF-β-induced transcription, such as p300 and CREB-binding protein (CBP), and recruitment of negative regulators such as histone deacetylases (Akiyoshi et al. 1999; Nomura et al. 1999; Prunier et al. 2003). All mutations causing SGS occur in amino-terminal domains of Ski that participate in Smad2 and Smad3 binding, and cultured fibroblasts from patients with SGS show increased Smad- and non-Smad-mediated TGF-β signaling and expression of TGF-β-driven genes (Doyle et al. 2012). The fact that mutations in *SKI* that cause

SGS increase TGF-β signaling and fully phenocopy LDS supports the notion that enhanced TGF-β signaling contributes to the pathogenesis of both conditions.

INTEGRIN–FIBRILLIN INTERACTIONS AND OVERACTIVATION OF TGF-β SIGNALING

As discussed above, fibrillin-1 regulates the bioavailability of TGF-β by both concentrating and sequestering latent TGF-β in the ECM, and mutations that interfere with this functions cause MFS. Interestingly, a specific subset of fibrillin-1 mutations causes stiff skin syndrome (SSS), a condition characterized by perinatal onset of dense dermal fibrosis and joint contracture, in the absence of any features of MFS. Skin derived from SSS patients shows large macroaggregates of disorganized microfibrils that concentrate latent TGF-β in the dermis and increased levels of Smad2 phosphorylation, indicative of overactivation of TGF-β signaling (Loeys et al. 2010).

SSS-causing mutations all cluster in the only fibrillin-1 domain that harbors an RGD domain used to mediate cell–matrix interactions via binding to integrin receptors, suggesting that disruption of integrin–fibrillin-1 interactions contributes to the pathogenesis of this disorder. Fibrillin-1 fragments with SSS mutations fail to support attachment and spreading of cells that express integrins αvβ3 and/or α5β1, which are the integrins that were reported to bind the RGD domain in fibrillin-1 (Loeys et al. 2010). In addition, mice heterozygous for an RGD to RGE encoding mutation in *Fbn1*, predicted to cause an obligate loss of integrin binding, recapitulate the dermal sclerosis found in patients and mice heterozygous for SSS mutations (Gerber et al. 2013). Taken together, these data indicate that failed integrin binding to fibrillin-1 initiates and sustains a sclerotic phenotype in the skin.

A unique population of cells that express high levels of integrins αvβ3 and/or α5β1 is present in the dermis of SSS mouse models (Gerber et al. 2013), suggesting that disruption of the interaction between fibrillin-1 and integrins results, directly or indirectly, in increased expression of these integrins and/or recruitment of cells that naturally express high levels of αvβ3 and/or α5β1. Treatments that mimic engagement of β1 integrin, such as administration of a β1 integrin-activating antibody, ameliorate skin stiffness and normalize expression of both αvβ3 and/or α5β1 in the dermis (Gerber et al. 2013). Similar results are observed by inactivating the gene encoding β3 integrin, treatment with TGF-β neutralizing antibody, or inhibition of the Erk MAPK pathway, suggesting cross-talk between integrins and TGF-β signaling pathways in SSS (Gerber et al. 2013). Increased TGF-β signaling in SSS skin might be caused by excessive integrin-mediated TGF-β activation and/or increased TGF-β bioavailability and activation of Erk1 and Erk2 MAPK in a β3 integrin-dependent manner, with the bulk of current evidence favoring the latter (Scaffidi et al. 2004; Munger and Sheppard 2011; Gerber et al. 2013).

SSS mice recapitulate multiple aspects of systemic sclerosis (SSc), including tightening and hardening of the skin, a tissue signature for excessive TGF-β signaling, and a propensity to develop autoantibodies and other markers of autoinflammation. In this light, it is notable that SSS mice show excessive concentration and activation of plasmacytoid dendritic cells (pDCs) in the dermis. pDCs normally perform a surveillance function for viral pathogens (Swiecki and Colonna 2010). On activation, pDCs express high levels of interferon-α, interleukin (IL)-6, and TGF-β; this can lead to autoantibody production and T-helper cell skewing toward proinflammatory subsets (as seen in both SSc and mouse models of SSS). Wild-type pre-pDCs showed increased adherence and activation when plated on ECM produced by SSS fibroblasts, when compared with wild-type cell-derived ECM, suggesting that the altered matrix environment is sufficient to initiate this process. The current view is that the altered matrix in SSS increases integrin expression by pDCs and the local concentration and activity of TGF-β, known to induce pDCs to produce even more TGF-β. The resulting increased concentrations of profibrotic and proinflammatory cytokines in the skin provide a plausible mechanism by which point mutations in a connective tissue protein can pheno-

copy autoimmune scleroderma (SSc), a condition that also shows microfibrillar macroaggregates and increased dermal concentration of TGF-β, albeit through an unknown initiating event (Loeys et al. 2010).

ROLE OF TGF-β AND BMP SIGNALING IN BONE HOMEOSTASIS

In addition to their crucial functions in skeletal development, it has become clear over the last few years that balanced BMP and TGF-β signaling is necessary for postnatal bone homeostasis (Fig. 5). Bone remodeling maintains bone quality and stability, and occurs through continuous cycles of bone resorption by osteoclasts and new bone formation by osteoblasts and osteocytes (Teitelbaum and Ross 2003; Capulli et al. 2014). Osteoblasts exert key regulatory roles in bone homeostasis by controlling the differentiation of osteoclasts from the hematopoietic cell lineage and by regulating osteoclast activity and survival. Osteoblasts secrete RANKL (receptor activator of NF-κB ligand, also known as OPGL, TRANCE, ODF) and osteoprotegerin (OPG, also known as OCIF, TNFSF11B), the antagonist of RANKL. Binding of RANKL to its cognate surface receptor RANK on preosteoclasts induces osteoclast differentiation and bone resorption. OPG protects the skeleton from excessive bone loss by sequestering and thus neutralizing RANKL by inhibiting binding to its receptor. Balanced levels of RANK, RANKL, and OPG are required to maintain bone ho-

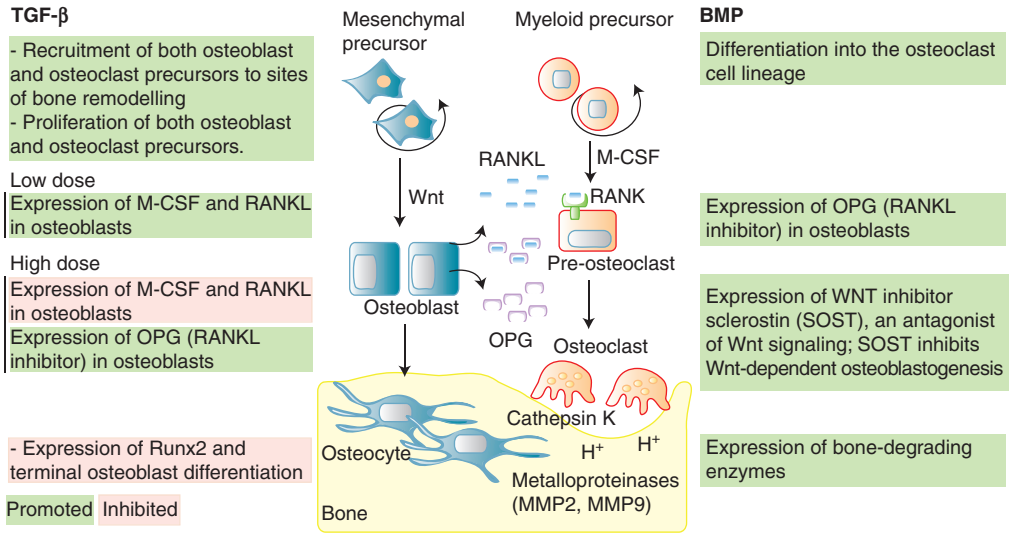

Figure 5. Roles of transforming growth factor β (TGF-β) and bone morphogenetic protein (BMP) signaling in bone homeostasis. Bone homeostasis is maintained through continuous cycles of bone resorption by osteoclasts and new bone formation by osteoblasts and osteocytes. Both TGF-β and BMP signaling participate in regulating this process. TGF-β can act as a chemoattractant and recruit both preosteoblast and preosteoclasts to areas of bone remodeling and also promotes proliferation, differentiation, and survival of osteoclasts. At low doses, TGF-β increases secretion of RANKL (receptor activator of NF-κB ligand) and suppresses expression of the inhibitor OPG (osteoprotegerin), thus promoting osteoclastogenesis by activating RANK signaling in preosteoclasts. At high doses, it suppresses RANKL and increases OPG expression and thus inhibits osteoclastogenesis. This biphasic effect limits excessive bone degradation in the presence of high levels of active TGF-β derived from conversion of latent TGF-β by proteases and acid secreted by osteoclasts during bone degradation. BMP signaling suppresses osteoclast differentiation by increasing expression of OPG in osteoblasts. It can, however, also promote bone resorption by increasing the expression of sclerostin (SOST), an inhibitor of Wnt signaling which is a pathway that normally promotes osteoblastogenesis and bone formation. M-CSF, Macrophage-colony stimulating factor.

meostasis, and imbalance results in excess resorption, as found in osteoporosis, or excess bone formation, as found in sclerosing bone dysplasias and osteopetrosis. Osteoblasts, furthermore, promote osteoclast proliferation, function and survival by secretion of macrophage colony-stimulating factor (M-CSF, also known as CSF1) that binds to its cognate surface receptor CSFR1 (aliases C-FMS, CD115) on the membrane of osteoclasts (Sherr et al. 1988; Suda et al. 1999). Targeted inactivation of the gene encoding M-CSF in mouse models leads to an osteopetrotic phenotype because of complete absence of osteoclasts (Wiktor-Jedrzejczak et al. 1990; Marks et al. 1992). OPG expression in osteoblasts is controlled by multiple bone metabolic regulators, including vitamin D, prostaglandin E2, tumor necrosis factors (TNFs), and IL-1, and also by TGF-βs and BMPs (Hofbauer et al. 1998; Murakami et al. 1998; Takai et al. 1998; Thirunavukkarasu et al. 2001; Wan et al. 2001; Sato et al. 2009). TGF-β signaling has both positive and negative effects on osteoclastogenesis, which initially led to controversial reports on the role of TGF-β in bone homeostasis. It is now understood that TGF-β acts as a chemoattractant and recruits both preosteoblast mesenchymal stromal cells and preosteoclasts to areas of bone remodeling (Pfeilschifter et al. 1990; Pilkington et al. 2001; Tang et al. 2009). TGF-β can promote bone resorption by directly regulating proliferation, differentiation, and survival of osteoclasts (Tashjian et al. 1985; Dieudonne et al. 1991; Kaneda et al. 2000) or indirectly by regulating the expression and secretion of pro-osteoclastic proteins in osteoblasts (Quinn et al. 2001; Mohammad et al. 2009; Tang and Alliston 2013). The TGF-β effects on osteoblasts are dose-dependent, with low doses increasing secretion of RANKL and suppressing expression of OPG to promote osteoclastogenesis, and high doses suppressing RANKL and increasing OPG to inhibit osteoclastogenesis (Karst et al. 2004). This mechanism functions to limit bone resorption when vast amounts of latent TGF-β are released from the bone matrix during high osteoclast activity. Excessive bone resorption is also prevented by TGF-β's action as chemoattractant for preosteoblast mesenchymal stromal cells, thus coupling bone resorption with new bone formation.

BMP signaling is well characterized for its bone-protective functions both in vitro and in vivo. This effect is in part mediated by the BMP-dependent increase of OPG expression in osteoblasts, and consequent suppression of osteoclast differentiation (Kaneko et al. 2000; Wan et al. 2001; Jensen et al. 2010; Kamiya and Mishina 2011). However, BMP signaling also regulates bone resorption, as is apparent by the high bone mass phenotype when *Bmpr1a* expression is specifically inactivated in osteoblasts, which results in failed osteoclastogenesis (Kamiya et al. 2008). This effect appears to be mediated by BMP-dependent effects on the Wnt signaling pathway. Wnt signaling controls commitment of mesenchymal progenitors to the osteoblastic lineage and stimulates the expression of the osteoclast inhibitor OPG (Glass et al. 2005; Hill et al. 2005; Kennell and MacDougald 2005; Rodda and McMahon 2006; Kamiya and Mishina 2011; Baron and Kneissel 2013). Wnt activating gene mutations cause high bone mass, whereas inactivating mutations cause osteopenia characterized by severely reduced bone mineral density (Gong et al. 2001; Boyden et al. 2002; Little et al. 2002; Patel and Karsenty 2002; Babij et al. 2003; Winkler et al. 2003). The high-bone-mass phenotype in mice with osteoblast-specific *Bmpr1a* silencing was found to be triggered by impaired expression of sclerostin (SOST) (Kamiya et al. 2008), an antagonist of Wnt signaling that acts by sequestering the co-receptor low-density lipoprotein receptor-related protein 5 (LRP5) into inactive complexes (van Bezooijen et al. 2007; Kamiya et al. 2010; Krause et al. 2010), as also discussed below in the context of sclerosteosis and Van Buchem disease. In addition, treatment of osteoclastic precursors with BMP induces differentiation along the osteoclast cell lineage, and further stimulates expression of key enzymes for bone degradation (Kaneko et al. 2000; Jensen et al. 2010). The observation that BMP can regulate both bone formation and bone resorption is consistent with the notion that, with the exception of fibrodysplasia ossificans progressiva (FOP), mutations in

BMP signaling components do not generally cause anabolic bone diseases.

Heterotopic Ossification Caused by Dysregulation of BMP Signaling

Postnatal bone formation is normally restricted to the skeleton during fracture healing; however, heterotopic ossification (HO) can occur at extraskeletal sites, frequently in response to severe tissue trauma (Kaplan et al. 2004; McCarthy and Sundaram 2005; Evans et al. 2014). In a rare genetic form of HO, FOP, ectopic endochondral ossification forms episodically and progressively at extraskeletal sites within soft connective tissue, including skeletal muscle, fascia, tendon, and ligaments, resulting in severe disability (Kaplan et al. 2008a; Shore and Kaplan 2008, 2010). At birth, only minor skeletal abnormalities occur in FOP patients, with congenital big toe malformation as the most characteristic feature. Most cases of FOP are caused by a c.617G>A point mutation in the *ACVR1* gene, which encodes the ACVR1/ALK-2 type I receptor and confers a moderate gain-of-function in this BMP type I receptor (Shafritz et al. 1996; de la Pena et al. 2005; Fiori et al. 2006; Shore et al. 2006; Billings et al. 2008; Fukuda et al. 2009; Kaplan et al. 2009; Shen et al. 2009; Chaikuad et al. 2012; Culbert et al. 2014). Signaling through the ALK-2 receptor occurs in chondrocytes and osteoblasts, and is required for early stages of chondrogenesis (Culbert et al. 2014). Because surgical removal of ectopic bone frequently leads to recurrent or even exacerbation of HO shortly after surgery in FOP patients, treatment has been limited to pain management that is associated with the bone formation process by counteracting the inflammatory reaction using high doses of glucocorticoids (Kaplan et al. 2008b; Pignolo et al. 2013). The discovery of the genetic cause of FOP is leading to the development of alternative therapeutic strategies to directly prevent HO formation (Kaplan et al. 2013). Palovarotene, a retinoic acid receptor γ (RARγ) agonist, has emerged as a potent candidate for treatment of HO. Pretreatment with palovarotene renders cells unresponsive to BMP-2 and decreases BMP pathway-specific R-Smad phosphorylation in vitro; it also successfully blocks HO in transgenic mice expressing a constitutively active form of the ALK-2 receptor not found in individuals with FOP (Shimono et al. 2011) and in a knockin mouse with the FOP *ALK2* c.617G>A (R206H) mutation (Chakkalakal et al. 2016). The efficacy of palovarotene is currently tested in a phase II clinical trial (ClinicalTrials.gov, NCT02190747). Moreover, a monoclonal antibody against activin A was reported to impair HO in a mouse model and in iPS cells with the FOP mutation (Hatsell et al. 2015; Hino et al. 2015); although the cellular mechanism for this mode of action has not been determined, the data suggest that activin A may be a therapeutic target for FOP.

In progressive osseous heteroplasia (POH), a second genetic form of HO, ectopic bone forms by intramembraneous ossification and is caused by inactivating mutations in the guanine nucleotide binding protein alpha stimulating (GNAS) gene (Kaplan and Shore 2000; Shore et al. 2002; Shore and Kaplan 2010). Although a detailed mechanism for HO formation in POH remains to be elucidated, data suggest that the mutations lead to overactivation of the hedgehog and BMP pathways and failure to inhibit osteogenic differentiation (Bowler et al. 1998; Wang and Wong 2009; Zhang et al. 2012; Regard et al. 2013). Currently, there is no effective treatment for POH other than surgical intervention (Pignolo et al. 2015).

Gain of Bone Mass in Camurati–Engelmann Disease

Sclerosing bone dysplasias are human syndromes characterized by abnormal accumulation of bone within the skeleton. There is a wide range of clinical manifestations, severity of bone accumulation, and genetic causes. In Camurati–Engelmann disease (CED), one form of sclerosing bone dysplasia, progressive diaphyseal cortical thickening occurs in long bones (Janssens et al. 2006), with first manifestations usually occurring in femur and tibia, and then expanding progressively toward distal areas of the limb, accompanied by chronic

pain (Ribbing 1949; Janssens et al. 2006). Calvarial and pelvic bone thickening are also common (Janssens et al. 2006). CED is caused by *TGFB1* missense mutations in the LAP region or signal peptide that destabilize the disulfide binding needed for homodimerization of latent TGF-β1, resulting in increased TGF-β signaling through either premature activation or intracellular retention of the protein and intracrine signal transmission (Saito et al. 2001; Janssens et al. 2003, 2006). Clinical manifestations do not include fibrosis or aneurysm, and no evidence for increased TGF-β signaling has been described in the aortic wall or other tissues, suggesting that these mutations have tissue-specific effects on TGF-β activity. It appears that patients with CED have increased rates of bone resorption as well as bone formation, with the overall balance shifted toward bone formation, possibly because of increased proliferation of osteoblasts (Hernandez et al. 1997; Saito et al. 2001; McGowan et al. 2003). CED can be successfully treated with glucocorticosteroids, which decrease proliferation, maturation and ECM synthesis in osteoblasts and osteocytes, and also increase the rate of apoptosis of these cells, thus resulting in a net decrease in bone mass (Low et al. 1985; Naveh et al. 1985).

High-Bone-Mass Disorders, Sclerosteosis and Van Buchem Disease, Caused by Mutations in Sclerostin (SOST)

Autosomal recessive loss-of-function mutations in the *SOST* gene, a direct target of BMP signaling, cause sclerosteosis (SOST1) (Fig. 2; Table 1) (Beighton 1988; Balemans et al. 2001; Brunkow et al. 2001). SOST1 is characterized by progressive skeletal overgrowth and increased bone mass and strength (Brunkow et al. 2001). Syndactyly and gigantism are also frequently found in patients with SOST1, and the appearance of a square-shaped jaw is a common facial feature (Beighton 1988). Heterozygous carriers are only mildly affected and display age-related thickening of the calvaria, mandible, ribs, clavicles, and long bones with increased bone mineral density in otherwise clinically inconspicuous individuals (Beighton et al. 1977; Stein et al. 1983; Gardner et al. 2005).

Deletion of a long-range regulatory element distal to the *SOST* gene results in decreased expression of sclerostin, the protein product of the *SOST* gene, and causes a form of sclerosing bone dysplasia known as Van Buchem disease (VBD) (Wergedal et al. 2003; Loots et al. 2005; van Lierop et al. 2013). Patients with VBD develop progressive increased thickness in calvaria, ribs, diaphysis of long bones, and tubular bones of hand and feet accompanied by hearing impairment and facial palsy (Van Buchem et al. 1962; Fryns and Van den Berghe 1988; Van Hul et al. 1998; Brunkow et al. 2001; Balemans et al. 2002; Staehling-Hampton et al. 2002; Wergedal et al. 2003; van Lierop et al. 2013).

The overall VBD phenotype is similar to SOST1, but less severe and without the appearance of hand malformation or gigantism. Deletion of an evolutionarily conserved region required for sclerostin expression in osteoblast-like cells and skeletal anlagen is able to recapitulate the VBD phenotype in mouse models (Loots et al. 2005). Sclerostin-deficient mice or mice expressing a dominant missense mutation in the *LRP5* gene (encoding a Wnt receptor component) that leads to reduced sclerostin binding show a high-bone-mass phenotype with increased bone strength, thereby recapitulating the key features of SOST1 and VBD (Semenov and He 2006; Li et al. 2008). Conversely, sclerostin overexpression in mice shows a reciprocal phenotype—that is, low bone mass and decreased bone strength—emphasizing the negative regulatory function of sclerostin on bone formation (Winkler et al. 2003).

Analysis of serum from patients with SOST1 and VBD revealed an inverse relationship between levels of the bone formation marker procollagen I amino-terminal propeptide (PINP) and sclerostin. PINP is the larger precursor of collagen type I that, when cleaved to its mature form, constitutes ~90% of all collagens in bone (Parfitt et al. 1987; Prockop and Kivirikko 1995; Young 2003). Serum from VBD patients contains a low level of sclerostin coupled with a significant increase in PINP, whereas heterozygous carriers display sclerostin and PINP levels

intermediate between those in unaffected controls and homozygous patients (van Lierop et al. 2014). Sclerostin protein levels are undetectable in SOST1 patients, explaining the more severe phenotype in this disease in comparison to VBD (van Lierop et al. 2011).

Knowledge gained from both genetic diseases, SOST1 and VBD, has led to the development of novel treatment strategies against osteoporosis by using humanized antibodies against sclerostin to counteract the osteocatabolic effect of sclerostin (Kim et al. 2013; Clarke 2014). Romosozumab is currently in phase II clinical trial, whereas a phase I trial of blosozumab is completed. Current treatment of SOST1 and VBD, however, is limited to either surgical removal of excess bone, which harbors a high risk (du Plessis 1993; Tholpady et al. 2014) or treatment with the glucocorticoid prednisone, which can successfully counteract bone accumulation as well as bone resorption in a VBD patient (van Lierop et al. 2010).

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

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

Arthritis is a chronic, destructive disease affecting articular cartilage and subchondral bone leading to loss of joint function and pain. Osteoarthritis (OA) is the most common form with increased prevalence in the aged population and characterized by progressive degeneration of articular cartilage, aberrant chondrocyte maturation, and osteophyte formation in subchondral bone, resulting in remodeling and functional loss of the joint (Zhang and Jordan 2010). Proinflammatory cytokines (TNF-α, IL-1) and matrix metalloproteinases (MMPs) play central roles in OA (Lories and Luyten 2005; Wojdasiewicz et al. 2014). Elevated BMP-2 expression was found in articular chondrocytes of OA mouse models (Blaney Davidson et al. 2006). In cartilage, BMP-2 has both anabolic and catabolic effects by stimulating expression of ECM proteins, such as collagen type II, and matrix degrading enzymes, such as MMPs (Blaney Davidson et al. 2007; van der Kraan et al. 2010). Single-nucleotide polymorphisms (SNPs) in *BMP2* were found in OA patients but their functional relevance is unknown (Valdes et al. 2004, 2006). The expression levels of *BMP7* decline with increasing age and with progression of OA (Chubinskaya et al. 2002), and the expression domains of *GDF5* and *GDF6* expand in adult human OA articular cartilage (Erlacher et al. 1998). BMP-7 possesses cartilage-protective properties by enhancing the expression of cartilage ECM and counteracting catabolic enzyme synthesis (Huch et al. 1997; Im et al. 2003). Clinical application of BMP-7 as a treatment for OA is promising with greater symptomatic improvement in comparison to placebo treated group (Hunter et al. 2010; Matthews and Hunter 2011).

The role of TGF-β signaling in human OA pathology is not well understood, but increasing evidence supports its function in maintaining articular cartilage. Age-related loss of TβRI expression, which may shift signal transduction from TβRI and Smad2 and Smad3 to ALK-1 and Smad1, Smad5 and Smad8, was found in two independent OA mouse models (van der Kraan et al. 2012). This resulted in the formation of transcriptionally active Runx2–Smad1 or Runx2–Smad5 complexes, which in turn activated terminal chondrocyte maturation and MMP13 expression (Blaney Davidson et al. 2009). MMP13 is the main collagenase that cleaves type II collagen in articular cartilage leading to OA (Reboul et al. 1996; Billinghurst et al. 1997). Expression of a truncated form of TβRII (Serra et al. 1997) or silencing *Smad3* expression (Yang et al. 1999) also results in an OA phenotype in mice, further showing the importance of TGF-β signaling in the maintenance of joint homeostasis. OA is observed in all characterized etiologies of LDS (mutations in *TGFBR1*, *TGFBR2*, *SMAD3*, and *TGFB2*), but appears to be particularly frequent in pa-

tients with *SMAD3* mutations (van de Laar et al. 2011). Although all of these alterations lead to impaired TGF-β signaling in vitro, all also associate with paradoxically increased signaling in vivo, precluding a definitive conclusion regarding the precise role of TGF-β in this process.

The TGF-β family member GDF-8, also known as myostatin, was reported to be highly expressed in human rheumatoid arthritis (RA) synovial tissues (Dankbar et al. 2015). Myostatin was found to increase RANKL-mediated osteoclast differentiation through Smad2 activation and regulation of nuclear factor of activated T cells (NFAT) c1. The absence of myostatin in genetically null ($Mstn^{-/-}$) mice or depletion of myostatin by myostatin-specific neutralizing antibodies decreases osteoclast formation and bone destruction at joints in a RA mouse model (Dankbar et al. 2015), suggesting a potential new therapeutic target for RA.

Osteoporosis occurs when bone remodeling is unbalanced and bone loss exceeds bone formation, either by insufficient new bone formation or by excessive resorption, and results in increased fracture risk (Raisz 2005). The prevalence of this disease increases with age. Many candidate genes are associated with osteoporosis, including multiple *TGFB1* polymorphisms that may reduce the secretion of TGF-β1 (Yamada et al. 1998; Langdahl et al. 2003). SNPs in *TGFBR1* and *TGFBR2*, *SMAD2*, *SMAD3*, and *SMAD4*/*DPC4* genes, as well as *SMAD7* were correlated with osteoporosis, but their relevance needs to be confirmed (Watanabe et al. 2002). Polymorphisms in *BMP2* and *BMP4* are also linked to osteoporosis, low bone mineral density, and fragility fracture risk (Styrkarsdottir et al. 2003; Ramesh Babu et al. 2005), although a correlation between a SNP in *BMP2* and osteoporosis remains to be confirmed (Medici et al. 2006). Osteoporosis, which presents with a high risk of pathologic fractures and delayed bone healing, is also a feature of LDS, and appears to be particularly frequent and most severe in patients with heterozygous missense mutations in *TGFBR1* or *TGFBR2* (Kirmani et al. 2010; Tan et al. 2013). Given the dual roles of TGF-β signaling in bone formation and resorption, and the paradoxical effects of LDS mutations on TGF-β signaling in vivo, the mechanisms leading to osteoporosis in LDS remain unclear. That the osteoporotic skeletal phenotype in LDS patients is recapitulated in LDS mouse models with heterozygous missense mutations in *TGFBR2* (Dewan et al. 2015) suggests that this mouse model will provide informative mechanistic insight into the bone pathology of LDS and osteoporosis.

Osteogenesis imperfecta (OI) is an inherited disorder characterized by brittle bones and fractures due to aberrant connective tissue matrix that is caused by mutations in collagen type I (dominant OI) or in posttranslational modifiers of collagen I (recessive OI) (Van Dijk and Sillence 2014). Increased TGF-β signaling was shown in mouse models for each form of OI, and the OI phenotype could be rescued by TGF-β inhibition, consistent with aberrant TGF-β activity causing the underlying OI bone pathology (Grafe et al. 2014). These results emphasize the sensitivity of the TGF-β-mediated balance between bone resorption and deposition; however, further investigations are needed to fully understand the roles of BMP and TGF-β in bone homeostasis and quality.

CONCLUDING REMARKS

Numerous human syndromes are caused by mutations in the TGF-β and BMP pathways, reflecting the fundamental functions of these signaling molecules and their pathways in development, patterning, and homeostasis in many tissues. Both pathways have synergistic and overlapping effects but also possess discrete functions that are revealed in pathway-specific human genetic diseases of connective tissues. Mutations affecting the functions of the BMP pathway are predominantly associated with skeletal tissue diseases, whereas those affecting TGF-β-specific components are more diverse in their manifestation, including cardiovascular pathologies, along with a range of connective tissue effects.

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