

Bone Morphogenetic Protein–Based Therapeutic Approaches

Jonathan W. Lowery¹ and Vicki Rosen²

¹Division of Biomedical Science, Marian University College of Osteopathic Medicine, Indianapolis, Indiana 46222

²Department of Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts 02115

Correspondence: jlowery@marian.edu; vicki_rosen@hsdm.harvard.edu

Bone morphogenetic proteins (BMPs) constitute the largest subdivision of the transforming growth factor (TGF)- β family of ligands and exert most of their effects through the canonical effectors Smad1, 5, and 8. Appropriate regulation of BMP signaling is critical for the development and homeostasis of numerous human organ systems. Aberrations in BMP pathways or their regulation are increasingly associated with diverse human pathologies, and there is an urgent and growing need to develop effective approaches to modulate BMP signaling in the clinic. In this review, we provide a wide perspective on diseases and/or conditions associated with dysregulated BMP signal transduction, outline the current strategies available to modulate BMP pathways, highlight emerging second-generation technologies, and postulate prospective avenues for future investigation.

SIGNAL TRANSDUCTION IN THE BONE MORPHOGENETIC PROTEIN PATHWAY

Bone morphogenetic proteins (BMPs) constitute the largest subdivision of the transforming growth factor (TGF)- β family of ligands with nearly thirty distinct human proteins bearing the BMP name. Important differences exist among these molecules with regard to pathway mechanics and effects on cell behavior. Two of the first BMPs to be cloned, BMP-1 and BMP-3, are not signaling molecules in the classical sense; BMP-1 is a metalloprotease that promotes BMP signaling (Kessler et al. 1996; Li et al. 1996), whereas BMP-3 is a nonsignaling receptor antagonist (Gamer et al. 2005). The nomenclature that accompanied the discovery

of BMPs is most often based on sequence homology and may be confusing when discussing BMP effects. Clarification comes, however, by focusing on the downstream pathways activated by each BMP ligand. For instance, as will be discussed below, it is now known that the intracellular signaling effectors Smad1, Smad5, and Smad8 actuate autoinduction of bone at extra-skeletal sites, which is the original function attributed to the BMP pathway (Urist 1965; Wozney et al. 1988). We contend, then, that proteins that elicit activation of Smads 1, 5, and 8 are bona fide components of the canonical BMP signaling cascade. We use this narrow definition of BMP signaling in this review and, on this basis, identify approximately 12 bona fide BMP ligands in humans (Table 1).

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Table 1. Components of the canonical bone morphogenetic protein (BMP)-induced Smad signaling pathway

Ligands	BMP-2 (BMP-2A, BDA-2A) BMP-4 (BMP-2B, BMP-2B1, MCOPS6, OFC11, ZYME) BMP-5 BMP-6 (VGR, VGR1) BMP-7 (OP-1) BMP-8A BMP-8B (OP-2) BMP-9 (GDF-2, HHT5) BMP-10 GDF-5 (BMP-14, OS5, LAP4, BDA1C, CDMP1, SYM1B, SYNS2) GDF-6 (BMP-13, KFM, KFS, KFS1, KFSL, SGM1, CDMP2, LCA17, MCOP4, SCDO4, MCOPCB6) GDF-7 (BMP-12)
Type I receptors	ALK-1 (ACVRL1) ALK-2 (ACVR1, ActRI) ALK-3 (BMPRIA) ALK-6 (BMPRIB)
Type II receptors	BMPRII ActRII (ActRIIA, ACVR2, ACVR2A) ActRIIB (ACVR2B)
R-Smad	Smad1 Smad5 Smad8 (Smad9)
Co-Smad	Smad4

Alternative names are in parentheses.

BMP ligands are generally portrayed as homodimers of two identical subunits that are related by twofold rotational symmetry around the intermolecular disulfide bond through a cysteine knot, a hallmark of this ligand family (Hinck 2012). BMPs are synthesized as large precursor molecules, consisting of a signal peptide, a large prodomain, and a carboxy-terminal region of 100 to 125 amino acids and upon secretion from the cell are further processed to their mature forms. The ability of BMPs to form heterodimers with each other has been established through in vitro studies and genetic studies in model organisms, and multiple BMPs are often coexpressed in tissues, suggesting heterodimer formation may occur in vivo. However, to date, only homodimeric BMPs have been purified from harvested human tissue. Interest in the formation of heterodimers continues as it represents a fairly simple way to alter the functionality of BMP ligands. For example, producing heterodimers of BMP-2/7, BMP-2/6, and

BMP-4/7 leads to enhanced activity (Aono et al. 1995; Israel et al. 1996; Xu et al. 2009; Isaacs et al. 2010; Valera et al. 2010; Buijs et al. 2012; Zheng et al. 2012; Bi et al. 2013; Krase et al. 2014; Dang et al. 2015; Morimoto et al. 2015; Neugebauer et al. 2015), although the reason for this remains to be determined.

BMP ligands activate signaling by complexing with receptor kinases with dual specificity that are present at the cell surface (Fig. 1). These receptors are classified into type I and II receptors, of which there are seven and five, respectively. Four type I receptors (i.e., ALK-1, ALK-2, ALK-3/BMPRIA, and ALK-6/BMPRIB) and three type II receptors (i.e., BMPRII, ActRII, and ActRIIB) serve as BMP signal transducers (Table 1). In the classical (i.e., the canonical) Smad pathway, ligand binding brings a pair of constitutively active type II receptors into close proximity with a pair of type I receptors, allowing receptor trans-phosphorylation to occur. The activated type I receptors phosphorylate

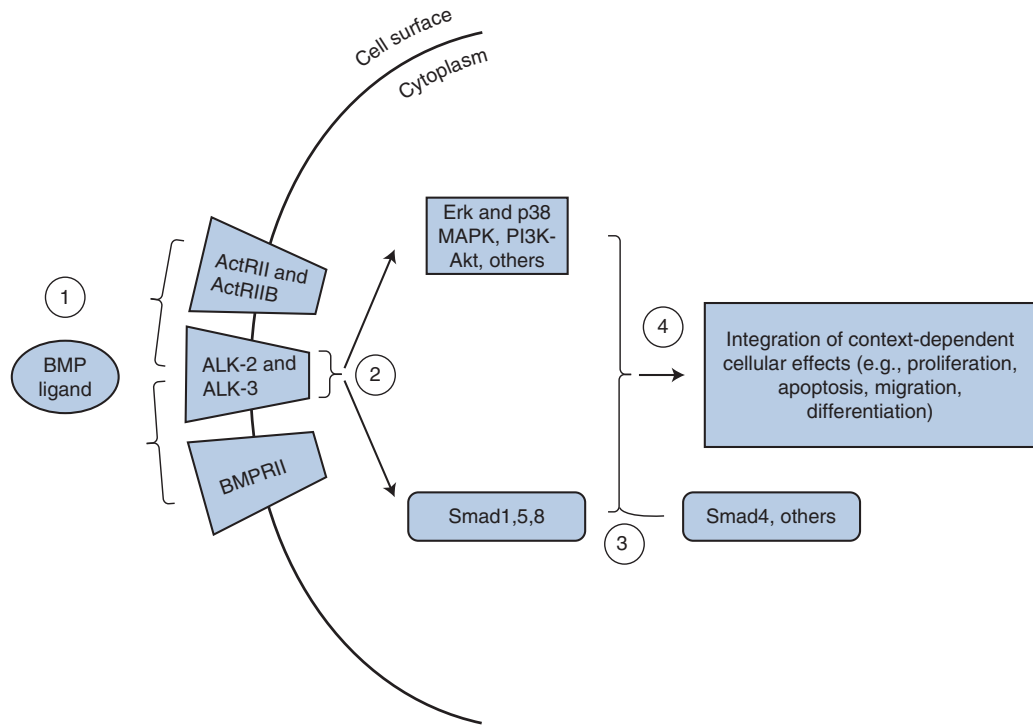


Figure 1. The bone morphogenetic protein (BMP) pathway and potential strategies for therapeutic modulation. (1) Activation of the BMP pathway occurs via interaction between dimeric BMP ligands and complexes of type I (e.g., ALK-2 and ALK-3) and type II receptors (BMPRII, ActRII, or ActRIIB). This step may be inhibited by delivery of extracellular ligand traps such as naturally occurring antagonists, receptor decoys, or neutralizing antibodies. Alternatively, BMP ligand availability may be enhanced through delivery of exogenous ligands or inhibiting endogenous extracellular BMP antagonists by neutralizing antibodies or small molecules. (2) Ligand binding leads to activation of the type I receptors by type II receptors and subsequent phosphorylation of the receptor-activated Smads 1, 5, and 8 (R-Smads) along with other pathways including extracellular signal-regulated kinase (Erk) and p38 mitogen-activated protein kinase (MAPK), and PI3K-Akt. The kinase activities of the type I receptors may be blocked by small molecule inhibitors such as LDN-193189. The BMP pathway inhibitors FKBP12 and casein kinase 2 endogenously limit the activities of the type I receptors and may be inactivated by delivery of FK506 and CK2.3, respectively, to increase BMP signal transduction. (3) R-Smads may perform Smad4-independent activities such as regulating microRNA processing or associate with Smad4 or other transcription factors to control gene regulatory networks. Persistence of BMP signaling may be modulated by regulating the Smurf1-mediated ubiquitylation of Smad effector proteins by disrupting Smurf1 interaction with R-Smads by small molecule inhibitors or by increasing Smurf1 protein levels. (4) R-Smad-dependent and R-Smad-independent events are integrated in a context-dependent manner to control cellular activity.

the carboxyl termini of the aforementioned Smads 1, 5, and 8, thus activating them (Fig. 1). The receptor-activated Smads, or R-Smads, can then form complexes with the transcription factor Smad4 and translocate into the nucleus to influence gene regulation (Katagiri and Watabe 2016).

It should be noted that Smad4-independent BMP activities have also been reported (Fig. 1),

consistent with the finding that several noncanonical signaling pathways such as p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (Erk), and Akt, and microRNA processing (Davis et al. 2008; Zhang 2009) are also regulated by BMP ligands. In fact, a proteomic study indicates that the phosphorylation status of nearly 400 proteins changes within thirty minutes of stimulation by BMP-



2, suggesting that these modifications do not depend on Smad activation and Smad-mediated signaling and transcription (Kim et al. 2009). One proposed mechanism for how Smad versus non-Smad signaling occurs stems from biochemical analyses that show the presence of preformed BMP receptor oligomers on the cell surface before ligand binding. In this model, preformed receptor complexes containing one type I BMP receptor and one type II BMP receptor are proposed to participate in canonical BMP signaling, whereas ligand-induced receptor complex formation between homodimeric type I and type II receptors are proposed to segregate with noncanonical p38 MAPK BMP signaling (Nohe et al. 2002; Hassel et al. 2003). More recently, a number of high-resolution microscopy techniques have refined this idea (Guzman et al. 2012). It appears that preformed heteromeric BMP receptor complexes are highly dynamic and transient in the absence of ligand, and, once ligand is added to the complex, canonical signaling is quickly initiated and completed. In contrast, noncanonical BMP pathway activation requires greater stability to initiate and complete signaling and takes place in specialized membrane microdomains that enhance type I and type II receptor interactions after ligand association via cytoskeletal elements and membrane scaffolding proteins. It is important to point out that the exact biological significance of distinctions in receptor complex formation has yet to be determined in vivo.

BMP pathway activity is regulated at many levels (Walsh et al. 2010; Huang and Chen 2012). Extracellular antagonists, such as noggin and gremlin, function to sequester ligands upstream of receptor binding, preventing pathway activation. The inhibitory Smads, Smad6 and Smad7, block R-Smad activation at the type I receptor level, and prevent R-Smad interaction with Smad4. R-Smad and receptor degradation is promoted by E3 ubiquitin ligases such as Smurf1 (Smad ubiquitination regulatory factor 1). Additionally, transcriptional regulation by Smads can be blocked by interaction with inhibitory proteins such as c-Ski or the c-Ski-like proto-oncogene product SnoN (SKIL) (Hill 2016; Miyazawa and Miyazono 2017).

CURRENT STRATEGIES TO MODULATE BMP PATHWAY ACTIVITY

Aberrations in BMP signal transduction—both overactivation and underactivation—are implicated in a variety of clinically relevant settings. A later section of this review will detail the rationale for and provide evidence of successful BMP-based therapeutics. Here, we wish to provide a brief outline of strategies currently available to modulate the BMP pathway in vivo, starting from upstream of receptor engagement and moving downstream from receptor and effector activity (Table 2). A greatly expanded discussion of this topic with detailed applications has been published recently (Lowery et al. 2016).

Modulation of BMP Activity in the Extracellular Environment

The United States Food and Drug Administration (FDA) has approved the use of recombinant BMP-2 and BMP-7, which are marketed as InFuse Bone Graft and OP-1, respectively, in several orthopedic and oral and maxillofacial applications. Significant off-label use of these products has been noted, however, and ongoing or upcoming clinical trials seek to evaluate the usefulness of recombinant BMP-2 and/or BMP-7 in additional orthopedic or dental applications. Along with naturally occurring BMP ligands, numerous engineered biomimetic versions have been generated for optimized expression in *Escherichia coli* (Saito et al. 2003; Seol et al. 2006; He et al. 2008; Lee et al. 2008, 2009; Bergeron et al. 2009; Lin et al. 2010; Zouani et al. 2010; Allendorph et al. 2011; Sugimoto et al. 2012; Tang et al. 2012; Kang et al. 2013; Suarez-Gonzalez et al. 2014; Kuo et al. 2014; Lauzon et al. 2014; Beauvais et al. 2015; Falcigno et al. 2015; Liu et al. 2015; Ma et al. 2015; Zhang et al. 2015; Zhou et al. 2015). Additionally, several BMP-inspired ligands have been developed with enhanced signaling ability compared with naturally occurring BMP ligands (Table 3). Specific applications involve engineered BMP ligands and are highlighted in a recent review (Lowery et al. 2016). Delivery of cDNAs encoding these natural or engineered BMP ligands for

Table 2. General overview of current strategies to modulate bone morphogenetic protein (BMP) signal transduction

Extracellular	Overexpression of ligand via gene transfer	Overexpression of extracellular antagonist via gene transfer
	Delivery of recombinant ligand	Delivery of recombinant extracellular antagonist or decoy receptor
	Neutralization of extracellular antagonists by antibody, decoy ligand, or small molecule	Neutralization of ligand by antibody
	RNA interference–mediated silencing of endogenous expression of extracellular antagonists or microRNAs	RNA interference–mediated silencing of expression of endogenous ligands
Intracellular	Overexpression or induced expression of BMP receptors by gene transfer, pharmacological agent, or RNA interference–mediated silencing of microRNAs	RNA interference–mediated silencing of expression of receptors or effectors
	Delivery of CK2.3 peptide or FK506 to alleviate BMP receptor inhibition	Delivery of BMP receptor kinase inhibitors
	Stabilizing effector turnover by RNA interference–mediated silencing or small molecule inhibition of Smurf1	

synthesis *in vivo* has also been achieved in numerous settings (Lowery et al. 2016). Additionally, several FDA approved drugs regulate expression of BMP ligands or potentiate the BMP pathway, including the statin drugs lovastatin and simvastatin (Sugiyama et al. 2000; Maeda et al. 2001; Song et al. 2003; Bradley et al. 2007; Kodach et al. 2007; Zhang and Lin 2008), the Rho-kinase inhibitor fasudil (Kanazawa et al. 2009, 2010), and the phosphodiesterase inhibitors pentoxifylline, rolipram, and sildenafil (Horiuchi et al. 2001; Horiuchi et al. 2002; Rondelet et al. 2010; Tokuhara et al. 2010; Yen et al. 2010; Munisso et al. 2012; Yang et al. 2013b).

BMP signal transduction can be blocked by preventing ligands in the extracellular space from interacting with receptors embedded in the plasma membrane. One method to achieve this blockade is through the use of soluble decoy receptors, composed of the ligand-binding domains of individual BMP receptors. This approach takes advantage of defined ligand affinities for particular receptors. A successful example of this strategy is an ALK-1 decoy, marketed as dalantercept, which preferentially sequesters BMP-9 and BMP-10 (Cunha et al. 2010; Mitchell et al. 2010; Larrivee et al. 2012; Ricard et al. 2012; Bendell et al. 2014; Hawinkels et al. 2016). This molecule is currently in

clinical trials as an anti-angiogenic cancer therapy (see clinicaltrials.org registry numbers NCT01458392, NCT01642082, NCT01720173, NCT01727336, and NCT02024087). This approach parallels BMP regulation *in vivo* as BMP ligands are sequestered upstream of receptor engagement by a large number of naturally occurring soluble antagonists (Walsh et al. 2010). Several of these, most notably noggin and gremlin, either delivered as recombinant proteins or synthesized *in vivo* by gene transfer, have shown efficacy in modulating BMP signaling (Lowery et al. 2016). Conversely, several neutralizing antibodies have been developed to block the activity of naturally occurring BMP antagonists in the extracellular environment (Hampton et al. 2007a,b; Ciucan et al. 2013). It is possible that the interaction between noggin and its target BMP ligand could be disrupted by small molecules (Ahmed et al. 2010).

Modulating Receptor and Effector Activities

BMP signal transduction may be blunted by small molecules that block the BMP receptor protein kinase pocket. The first such compound was dorsomorphin (Yu et al. 2008b), which served as a guide for subsequent generations of analogs, such as LDN-193189, with enhanced

Table 3. Engineered bone morphogenetic protein (BMP) ligands

Category	Name	Comment(s)	Selected references
BMP-2-based	B2A (B2A2-K-NS)	BMP-2-based peptide with heparin-binding domain that augments the activity of BMP-2 but has no intrinsic signaling ability	Lin et al. 2005, 2007, 2012; Smucker et al. 2008; Cunningham et al. 2009; Liu et al. 2012; Sardar et al. 2015
	BMP2-L51P	BMP-2 mutant that augments the activity of BMP-2 but has no intrinsic signaling ability	Albers et al. 2012; Sebaldo et al. 2012; Khattab et al. 2014
	BMP2_108 mBMP	BMP-2-based peptide; mimics the activity of BMP-2 BMP-2-based peptide with mineral-binding domain; mimics the activity of BMP-2	Zhang et al. 2015 Suarez-Gonzalez et al. 2014
	OPD	BMP-2-based peptide; mimics or presumed to mimic the activity of BMP-2	Lee et al. 2009
	P1		Lee et al. 2008
	P2		
	P24		Lin et al. 2010; Tang et al. 2012
	PEP7		Kang et al. 2013
	Unnamed		Saito et al. 2003; Seol et al. 2006; He et al. 2008; Zouani et al. 2010; Falcigno et al. 2015; Liu et al. 2015; Ma et al. 2015; Zhou et al. 2015
BMP-2/activin A chimerae	AB204	Segmental chimera of BMP-2 and activin A with enhanced activity over BMP-2; noggin-resistant	Allendorph et al. 2011; Ahn et al. 2014; Yoon et al. 2014, 2015a,b; Kim et al. 2015
	AB204-I103Y	Variant of AB204; enhanced activity over BMP-2 and AB204	Yoon et al. 2014
	AB211	Segmental chimera of BMP-2 and activin A with enhanced activity over BMP-2; noggin-resistant	Allendorph et al. 2011
	AB215		Allendorph et al. 2011; Jung et al. 2014

Continued

Table 3. Continued

Category	Name	Comment(s)	Selected references
BMP-2/BMP-9 chimera	BB29	Segmental chimera of BMP-2 and BMP-9 with enhanced folding when produced in <i>Escherichia coli</i>	Allendorph et al. 2011
BMP-6/BMP-7 chimera	80-1	Segmental chimera of BMP-6 and BMP-7 with reduced noggin binding when compared with BMP-7	Schwaerzer et al. 2012
BMP-7-based	BMP7-E60K	BMP-6-informed mutant with reduced noggin binding	Schwaerzer et al. 2012
	THR-123	BMP-7-based peptide	Sugimoto et al. 2012
	Unnamed	BMP-7-based peptide; mimics activity of BMP-7	Zouani et al. 2010
BMP-9-based	MB109	BMP-9-based peptide optimized for production in <i>E. coli</i>	Kuo et al. 2014
	pBMP9	BMP-9-based peptide with enhanced activity over BMP-9	Bergeron et al. 2009; Lauzon et al. 2014; Beauvais et al. 2015
	SpBMP9	BMP-9-based peptide with enhanced activity over BMP-9	Beauvais et al. 2015
	Unnamed	BMP-9-based peptide; mimics activity of BMP-9	Zouani et al. 2010
GDF-5-based	GDF5-S94N	Naturally occurring mutant with enhanced activity due to decreased inhibition by noggin	Schwaerzer et al. 2012
	GDF5-N445K		Seemann et al. 2009
	GDF5-N445T		Seemann et al. 2009; Degenkolbe et al. 2015
	GDF5-V453/V456	BMP-2-informed variant of GDF-5; enhanced activity over GDF-5 and BMP-2	Kasten et al. 2010; Kleinschmidt et al. 2014
BMP heterodimers	BMP-2/6	Heterodimer with enhanced activity over BMP-2 and BMP-6	Isaacs et al. 2010; Valera et al. 2010
	BMP-2/7	Heterodimer with enhanced activity over BMP-2 and BMP-7	Xu et al. 2009; Buijs et al. 2012; Zheng et al. 2012; Bi et al. 2013; Dang et al. 2015; Morimoto et al. 2015
	BMP-4/7	Heterodimer with enhanced activity over BMP-4 and BMP-7	Aono et al. 1995; Krase et al. 2014; Neugebauer et al. 2015

specificity and efficacy (Table 4). Specific applications involving engineered BMP ligands are summarized in a recent review (Lowery et al. 2016). Conversely, the peptide CK2.3 leads to increased BMP signal transduction by disrupting the inhibitory interaction between casein kinase (CK) 2 and BMP type I receptors (Akkiraju et al. 2015), whereas the FDA approved immunosuppressant FK506 activates BMP signaling by inhibiting FKBP12. The BMP pathway can also be modulated downstream from receptor activity by stabilizing Smads 1, 5, and 8 through silencing the expression of the E3 ubiquitin ligase Smurf1 or, potentially, by preventing Smurf1 interaction with these Smads (Okada et al. 2009; Kato et al. 2011; Cao et al. 2014).

CLINICAL RELEVANCE OF BMP-BASED THERAPEUTICS

Orthopedic and Craniofacial Settings

More than 50 million people in the United States alone have osteoporosis or osteopenia (Wright et al. 2014), and this number is expected to increase as the population ages. Thus, understanding the mechanisms that regulate bone growth and remodeling is an important goal. It is widely accepted that BMP signaling is required for normal skeletal development and patterning (Salazar et al. 2016). However, in comparison to the information available regarding the embryonic role of BMP signaling in skeletogenesis, relatively little is known about the roles of the BMP pathway in the post-natal skeleton, and many of the available data are merely correlative. For instance, although BMP signaling levels correlate with bone mineral density (Szwercas et al. 2002; Yan et al. 2009; Nallamshetty et al. 2013; Shen et al. 2013; Guemes et al. 2014; Kureel et al. 2014), and aberrations in the expression of BMP pathway components or BMP-induced effects are observed in bone marrow stromal cells (BMSCs) from aged (Moerman et al. 2004) or osteoporotic (Prall et al. 2013; Haasters et al. 2014) subjects, respectively. These correlative findings raise the possibility of a causative relationship. Other results are controversial and/or inconsis-

tent between studies. Two studies have linked a single nucleotide polymorphism (SNP) in the *BMP2* gene (rs2273073, c.109T>G, Ser37Ala) with lumbar spine bone mineral density and osteoporotic fractures in an international cohort and an Icelandic cohort, respectively (Reneland et al. 2005; Styrkarsdottir et al. 2003). However, this SNP is not associated with bone parameters in studies of Dutch (Medici et al. 2006), Swedish (McGuigan et al. 2007), or American Caucasian populations (Ichikawa et al. 2006). Similarly, a SNP in *BMP4* (rs17563, c.538C>T, Val147Ala) is linked to bone mineral density in Australian Caucasian women (Ramesh Babu et al. 2005) and possibly Taiwanese women (Lin et al. 2008) but not in Italian women (Semprini et al. 2000). It should be noted that associations between bone mineral density and two other SNPs in *BMP2* or one SNP in *BMP4* were found in Korean males (Choi et al. 2006), and we are not aware of reports corroborating or contradicting these findings.

Beyond these correlative findings, several studies show that systemic administration of recombinant BMP-2, BMP-6, or BMP-7 or alleviating inhibition of the BMP receptor ALK-3 using a synthetic peptide improve bone mass and associated parameters (Turgeman et al. 2002; Simic et al. 2006; Domic-Cule et al. 2014; Akkiraju et al. 2015). These anabolic effects are likely due to increased osteoblastogenesis and/or an enhanced bone formation rate in vivo, which is supported by the high-bone-mass phenotype seen by 4 months of age in transgenic mice with constitutively activated canonical BMP signaling in osteoblasts (Zhang et al. 2009). These studies suggest that augmenting BMP signaling in individuals with low bone mass may hold therapeutic benefit, and a phase II clinical trial is examining this possibility through injection of recombinant BMP-2 into the hip (NCT00752557). Although results are not yet available for this study, the rationale is reminiscent of the numerous reports detailing the ability of recombinant BMP ligands to promote local bone growth in maxillofacial applications, including the FDA approved use for recombinant BMP-2 in sinus lift and alveolar ridge augmentation procedures. Building on

Table 4. Bone morphogenetic protein (BMP) receptor kinase inhibitors

Name	Comment(s)	Selected references
ILWY	Dramatically enhanced selectivity for ALK-2 versus other type I BMP receptors (approximate order of selectivity: ALK-2 > ALK-3 > ALK-6); greatly reduced off-target effects compared with DM and LDN	Tsugawa et al. 2014
DMH1	Pan-type I BMP receptor inhibitor (approximate order of selectivity: ALK-3 > ALK-1 > ALK-6 > ALK-2); reduced off-target effects compared with DM and LDN	Hao et al. 2010; Ao et al. 2012; Engers et al. 2013; Mohedas et al. 2013; Sheng et al. 2014; Alsamarah et al. 2015; Owens et al. 2015; Lin et al. 2016; Sanders et al. 2016
DMH2	Pan-type I BMP receptor inhibitor (approximate order selectivity: ALK-6 > ALK-3 > ALK-2); notable off-target effects, including BMPRII, TβRII, ALK-4, ALK-5, AMPK, VEGFR2)	Hao et al. 2010; Langenfeld et al. 2013; Tsugawa et al. 2014
DMH3	Presumed pan-type I BMP receptor inhibitor; reduced off-target effects compared with DM and LDN	Hao et al. 2010
Dorsomorphin (DM)	Pan-type I BMP receptor inhibitor (approximate order of selectivity: ALK-2 > ALK-3 > ALK-1 > ALK-6); notable off-target effects, including BMPRII, ActRII, ActRIIB, TβRII, ALK-5, AMPK, VEGFR2, and PDGFRβ	Hao et al. 2008, 2010; Yu et al. 2008b; Bai et al. 2010; Boergermann et al. 2010; Kim et al. 2010; Vogt et al. 2011; Ao et al. 2012; Hamasaki et al. 2012; Engers et al. 2013; Mohedas et al. 2013; Shanmugam and Cherayil 2013; Chang et al. 2014; Garulli et al. 2014; Horbelt et al. 2015
K02288	Modestly enhanced selectivity for ALK-1 and ALK-2 versus other type I BMP receptors (approximate order of selectivity: ALK-2 > ALK-1 > ALK-6 > ALK-3); reduced off-target effects compared with DM and LDN	Mohedas et al. 2013; Sanvitale et al. 2013; Kerr et al. 2015
LDN-193189 (LDN)	Pan-type I BMP receptor inhibitor (approximate order of selectivity: ALK-1 ~ ALK-2 > ALK-3 > ALK-6); notable off-target effects, including BMPRII, ActRII, ActRIIB, TβRII, ALK-5, AMPK, VEGFR2, and PDGFRβ	Cuny et al. 2008; Yu et al. 2008a; Boergermann et al. 2010; Lee et al. 2011; Steinbicker et al. 2011; Vogt et al. 2011; Hamasaki et al. 2012; Saeed et al. 2012; Balboni et al. 2013; Engers et al. 2013; Helbing et al. 2013; Komatsu et al. 2013; Mohedas et al. 2013; Sanvitale et al. 2013; Peterson et al. 2014; Tsugawa et al. 2014; Horbelt et al. 2015; Kajimoto et al. 2015; Malhotra et al. 2015; Mayeur et al. 2015

Continued

Table 4. Continued

Name	Comment(s)	Selected references
LDN-212854	Significantly enhanced selectivity for ALK-1 and ALK-2 versus other type I BMP receptors (approximate order of selectivity: ALK-2 > ALK-1 > ALK-3); reduced off-target effects compared with DM and LDN-193189	Mohedas et al. 2013; Dey et al. 2016
LDN-214117	Dramatically enhanced selectivity for ALK-2 versus other type I BMP receptors (approximate order of selectivity: ALK-1, ALK-2 > ALK-3); greatly reduced off-target effects compared with DM and LDN-193189	Mohedas et al. 2014
ML-347	Dramatically enhanced selectivity for ALK-1 and ALK-2 versus other type I BMP receptors (approximate order of selectivity: ALK-2 > ALK-1 > > ALK-3); reduced off-target effects compared with DM and LDN-193189	Engers et al. 2010, 2013
VU5350	Pan-type I BMP receptor inhibitor (approximate order selectivity: ALK3 > ALK2 > ALK6); notable off-target effects, including BMPRII, TβRII, AMPK, VEGFR2)	Tsugawa et al. 2014

these results, an ongoing clinical trial examines the possible benefit of coating dental implants with recombinant BMP-2 (NCT00422279). Augmenting BMP signaling has also been used with considerable success in the healing of recalcitrant fractures. Although most fractures heal without intervention, ~10% result in non-unions, increasing patient morbidity owing to infection and requiring increased hospital stay. To date, recombinant BMP-2 and BMP-7 have received FDA approval as adjunct therapies for the treatment of nonunion fractures, in which the benefits of treatment include accelerated healing and lower infection rates (Ali and Brazil 2014). Augmenting BMP signaling by recombinant ligand administration has also shown efficacy in procedures that require bone grafts, such as skeletal defects resulting from severe trauma, tumor resection, pathological degeneration, and congenital malformation. In these circumstances, BMPs can be combined with autografts that are harvested from the patient's own skeleton, allografts harvested from cadavers, or synthetic bone substitutes. The best examples of success in using exogenous BMPs are in the area of spine fusion surgery, where BMP-2 and BMP-7 have shown efficacy equal to that of using autograft for establishing bone union (Burkus et al. 2005). Concerns relating to the dose of exogenous BMP required for healing, the mode of BMP delivery and the potential for unwanted heterotopic ossification (HO) at neighboring sites have led to the ongoing development of novel BMP molecules that show greater potency and would be predicted to have enhanced efficacy and safety when delivered at lower doses (Cahill et al. 2015). Alternatively, the use of agents that can modulate the production of endogenous BMPs would offer substantial benefit although the clinical usage of this approach remains to be uncovered.

Heterotopic Ossification

The studies described above show that BMP signaling is a potent inducer of de novo bone formation. Thus, it is not altogether surprising that BMP signaling is implicated in the pathogenesis of HO, a common acquired disorder in

which bone forms at extraskeletal sites, and, once formed, may impair mobility and cause chronic pain. HO is often associated with the soft tissue trauma during joint replacement or other major reconstructive surgeries. HO is also an unfortunate and troublesome complication seen in severely wounded soldiers, amputees, or paralyzed individuals. Current treatments for nongenetic forms of HO include nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit the production of prostaglandins at the injury site, and local irradiation to block the recruitment of skeletal stem cells to the site of injury. Although both can be somewhat effective in dampening the HO response, each treatment has severe side effects. NSAID use has been associated with gastrointestinal distress, renal toxicity, and reduced platelet function. Radiation, although preventing HO, destroys healthy tissue. In severe cases, surgical removal of the HO has been performed, but this practice often increases hospital stay, and there remains the potential for recurrence of HO because of the trauma induced by tissue resection. A new treatment option that has shown promise in animal models is the delivery of synthetic retinoid agonists that potently inhibit the early BMP-mediated chondrogenic stage of HO (Sinha et al. 2016). Retinoid agonist therapy is currently being examined in patients with a rare, genetic form of HO (see below). BMP receptor kinase inhibitors may also have utility in treating HO. Combinatorial approaches that combine retinoid agonists with BMP receptor kinase antagonists have also been suggested as potential therapies as they might be able to be tailored to the patient.

Unlike acquired HO, fibrodysplasia ossificans progressiva (FOP) is a rare and highly disabling skeletal disease characterized by seemingly spontaneous episodes of HO that often begin in early childhood. The crippling accumulation of extraskeletal bone tissue in FOP results in skeletal deformities, chronic pain, and joint ankylosis, and eventually encompasses much of the body (Huning and Gillessen-Kaesbach 2014). FOP is caused by missense mutations in the *ACVR1/ALK2* gene that alter the tertiary structure of this type I BMP receptor

and enable activins, ligands that do not normally trigger BMP signaling, to induce BMP signals (Shore et al. 2006; Hatsell et al. 2015; Hino et al. 2015). Current treatments for FOP, such as steroidal and nonsteroidal NSAIDs, are palliative but do not prevent the progression of HO. Clinical trials with synthetic retinoid agonists are ongoing (NCT02190747, EudraCT 2014-001453-17, EudraCT 2014-002496-28), based on the notion that FOP and HO have common pathogenic mechanisms that converge on activation of BMP signaling. Unlike HO, blocking activin signaling may be an effective treatment for FOP patients. Systemic blockade of activins has been shown to ameliorate cancer-induced cachexia, raising the possibility that similar agents might successfully control the BMP signaling caused by activins in patients with FOP (Zhou et al. 2010). As apparent from the Regeneron Pharmaceuticals website, clinical trials examining safety of this approach have begun. Additionally, given that a single, recurrent mutation underlies most FOP cases, strategies such as allele-specific RNA interference may prove useful in reducing expression of mutant ALK-2 through gene therapy approaches (Lowery and Rosen 2012).

Vascular Disease

Related to the notion that aberrant BMP pathway activation leads to HO, evidence also indicates that elevated BMP signaling plays a major role in vascular calcification (Cai et al. 2012; Garcia de Vinuesa et al. 2015; Morrell et al. 2016). For instance, genetic loss of the BMP pathway antagonists matrix-Gla protein, which binds to and inhibits BMP ligands (Zebboudj et al. 2002; Yao et al. 2006), or Smad6 leads to widespread vascular calcification in mice (Luo et al. 1997; Galvin et al. 2000). As such, strategies designed to reduce BMP pathway activation, including RNA interference of individual BMPs and delivery of recombinant BMP antagonists or small molecule inhibitors of BMP signaling, diminish vascular inflammation and reactive oxygen species formation, and/or limit the degree of vessel calcification (Derwall et al. 2012; Saeed et al. 2012; Koga et al. 2013; Zhang

et al. 2014; Kajimoto et al. 2015; Malhotra et al. 2015). Together, these studies suggest that therapies aimed at reducing BMP signaling in the vasculature may be beneficial in patients at high risk for calcification such as those with end-stage renal disease; however, we are unaware of completed or ongoing clinical trials examining this possibility.

In contrast to the logical connection between BMP-induced extracellular matrix formation and vascular calcification, little was known about the critical role of BMP signaling in maintaining integrity of the pulmonary vasculature before the finding that the vast majority of patients with heritable pulmonary arterial hypertension (HPAH) carry heterozygous mutations in the *BMPR2* gene (Deng et al. 2000; Lane et al. 2000). HPAH is a rare form of the relatively common disease pulmonary hypertension (PH), in which the small, resistance-level arterioles of the lung undergo structural remodeling to become thicker and less compliant. These changes increase the load on the right ventricle and lead to right ventricular hypertrophy and, ultimately, right-sided heart failure (Simonneau et al. 2009). Given that the pathology in all forms of pulmonary hypertension (PH) show strikingly similar pathology, that ~20% of patients with sporadic pulmonary arterial hypertension (PAH) also carry *BMPR2* mutations, and that BMP signal transduction and pathway components are down-regulated in the lungs of PH patients (Lowery and de Caestecker 2010), it is likely that adequate levels of BMP signaling are required for proper regulation of the pulmonary vasculature. However, the specific roles of BMP signaling in this context remain unclear. Numerous mechanisms have been proposed for the dysregulated BMP signaling with varying degrees of experimental support, including anti-inflammatory and/or antimutagenic effects, direct deregulation of vascular tone, deregulation of endothelial cell apoptosis and integrity of the tunica intima, and anti-oxidant actions by repressing reactive oxygen species formation (Lowery and de Caestecker 2010).

Clearly, an increased understanding of the endogenous actions of BMP signaling in the

pulmonary vasculature could contribute to developing targeted therapies in the future. Even without a detailed understanding of the downstream actions, animal models suggest that several strategies aimed at generally increasing BMP signal transduction in the pulmonary vasculature may be beneficial in PAH. Indeed, the phosphodiesterase-5 inhibitor sildenafil, which is FDA approved for the treatment of PAH, prevents disease development in a toxin-induced model of PAH in rats and this is associated with increased BMP signaling in the lung (Yang et al. 2013b). Similarly, the FDA approved antimalarial chloroquine attenuates PAH development in rats with an associated increase in BMPRII expression (Long et al. 2013). More direct evidence comes from the observations that increasing BMPRII expression using direct gene transfer or by repressing the action of miR-20a reduces the severity of PAH development (Reynolds et al. 2007; Brock et al. 2014). Moreover, increasing BMPRII expression has been reported to reverse the pathological changes associated with PAH in mice (Reynolds et al. 2012; Feng et al. 2016), suggesting that increasing the availability of BMPRII alone may be sufficient to provide therapeutic benefit in PAH, although this has not been observed in every study (McMurtry et al. 2007). Alternatively, beneficial outcomes are apparent when BMP signal transduction is increased in established PAH by neutralizing the action of gremlin (Ciucan et al. 2013), administering recombinant BMP-9 (Long et al. 2015), or alleviating the FKBP12-mediated inhibition of BMP type I receptors using the FDA approved small molecule FK506 (Spiekerkoetter et al. 2013). Of note, a clinical trial examining the safety and efficacy of FK506 in patients with sporadic or heritable PAH (NCT01647945) was initiated but then terminated because of limited funding and/or slow recruitment of subjects.

It is striking to point out that the vascular abnormalities characteristic of PAH have generally been considered restricted to the pulmonary vascular bed (Fares 2014). This is especially intriguing when considering that several BMP ligands are present in human and rodent serum at biologically active concentrations (David

et al. 2008; Herrera and Inman 2009) and that BMP pathway components are expressed by vascular cells derived from other locations in the body (Lowery and de Caestecker 2010). Indeed, emerging evidence suggests that PAH patients likely experience vascular manifestations in the systemic circulation, including endothelial dysfunction and/or structural anomalies of capillaries (Fares 2014). The latter is consistent with genetic studies in animals that reveal the developmental requirement of BMP signaling in normal embryonic angiogenesis and vessel maturation (Lowery and de Caestecker 2010). Furthermore, most patients with hereditary hemorrhagic telangiectasia (HHT), which is characterized by structurally weak arteriovenous malformations (AVMs) that may appear in numerous vascular beds, inherit mutations in genes encoding the BMP receptors ALK-1 or endoglin (Cai et al. 2012; Garcia de Vinuesa et al. 2015). Treatment for AVMs varies by location and suspected severity, but generally involves coagulation therapy, surgical removal, or occlusion. It is unclear at present if BMP pathway modulation will be useful in the treatment of HHT and we are not aware of any clinical trials evaluating this possibility.

Tissue Fibrosis

Given that increased BMP pathway activation is implicated in calcification and ossification of soft tissue, it is somewhat surprising that activating the BMP pathway has been identified as a potential therapy for tissue fibrosis. In particular, the ability of BMP signaling to oppose TGF- β -induced fibrosis and promote tissue recovery has been shown in several clinically relevant contexts (Hudnall et al. 2016). For example, endogenous BMP signaling plays a critical role in recovery after obstructive uropathies and treatment of mice with exogenous BMP-7 enhances renal recovery after unilateral ureteral obstruction, in which TGF- β promotes glomerular fibrosis (Manson et al. 2011b). Activation of BMP signaling also has beneficial effects on TGF- β -induce fibrosis of cardiomyocytes (Wang et al. 2012b), ocular burn injuries (Saika et al. 2006), and silica-induced or aller-



gen-induced pulmonary fibrosis (Myllarniemi et al. 2008; Yang et al. 2013a, 2016; Stumm et al. 2014). These studies serve as substantial proof-of-concept for the notion that activation of BMP signaling may hold broad therapeutic benefit in other contexts of fibrosis. Support for this hypothesis comes from preclinical studies examining the utility of recombinant BMP-7 or BMP-7-based gene therapy in bone marrow fibrosis (Gonzalez et al. 2002), corneal fibrosis (Saika et al. 2005; Tandon et al. 2013), hepatic fibrosis (Kinoshita et al. 2007; Hao et al. 2012; Zhong et al. 2013; Wang et al. 2014), lens fibrosis (Saika et al. 2006), prosthesis-related fibrosis (Tan et al. 2013), cardiac fibrosis (Zeisberg et al. 2007; Urbina and Singla 2014), and numerous models of renal fibrosis (Vukicevic et al. 1998; Klahr and Morrissey 2003; Zeisberg et al. 2003; Sugimoto et al. 2007; Manson et al. 2011a,b; Zhen-Qiang et al. 2012; Li et al. 2015). Although much of the research thus far has focused on BMP-7, it should be noted that other strategies aimed at generally increasing BMP signal transduction have been reported to be beneficial in models of tissue fibrosis. These include administration of recombinant BMP-2 or gene therapy-based BMP-2 expression (Yang et al. 2009; Wang et al. 2012b), administration of a BMP-related peptide (Sugimoto et al. 2012) or the small molecule FK506 (Qi et al. 2014), reducing expression of the BMP antagonist gremlin using siRNA (Zhang et al. 2010), or delivery of the downstream BMP target genes *Id1* and *Id3* (Saika et al. 2006).

Hemochromatosis and Iron Deficiency Anemia

The body has a complex system to regulate iron homeostasis (Gangat and Wolanskyj 2013). Iron is essential to make the hemoglobin necessary for red blood cells to carry oxygen; anemia occurs when iron levels are inadequate. Iron excess, however, is toxic, and as there is no known mechanism for regulated iron excretion, systemic iron homeostasis must be maintained by tightly balancing intestinal iron absorption and iron release by macrophages and hepatocytes (Babitt et al. 2007). Iron release into the

circulation occurs through the iron exporter ferroportin. This export process is regulated by the iron regulator protein hepcidin, a 25-amino-acid peptide produced by the liver (Zhao et al. 2013). Hepcidin levels are sensitive to the iron status in the body through mechanisms that involve canonical BMP–Smad signaling (Parrow and Fleming 2014). Hepatocyte-specific deletion of *Smad4* produces mice with a severe iron overload phenotype, whereas mutations in the *HFE2* gene, which encodes a BMP coreceptor hemojuvelin, result in juvenile hemochromatosis, a disease characterized by severe iron overload (Babitt et al. 2006). Further evidence of the importance of BMP signaling in iron homeostasis comes from analysis of the physiological role of BMP-6. *Bmp6* mRNA expression correlates with body iron stores in mice, and mice lacking BMP-6 show low hepcidin expression and severe iron overload, which can be corrected by increasing BMP-6 levels (Andriopoulos et al. 2009). Most recently, BMP-2 has been implicated in iron homeostasis with effects independent of BMP-6 (Koch et al. 2017).

These and additional experimental data support the notion that decreasing BMP might be beneficial in treating disorders of iron deficiency. For instance, neutralizing antibodies targeting BMP-6 have been shown to increase serum iron levels in mice (Andriopoulos et al. 2009; Meynard et al. 2011; Wang et al. 2012a). It should be noted that, although this provides compelling evidence implicating BMP-6 as the predominant BMP ligand in iron homeostasis *in vivo*, it does not rule out strategies targeting the BMP–Smad signaling pathway in general. Consistent with this, reducing BMP signaling through several means, including ALK-3 and hemojuvelin decoys, or the kinase inhibitors dorsomorphin and LDN-193189, increase serum iron levels in models of iron deficiency anemia (Babitt et al. 2007; Yu et al. 2008b; Andriopoulos et al. 2009; Steinbicker et al. 2011; Theurl et al. 2011; Wang et al. 2012a). Data from completed or ongoing clinical trials examining these strategies in humans are not yet available, but the possible use of LDN-193189 for treating anemia of inflammation is a focus

of the Bridging Interventional Gaps (BrIDGs) Program of the National Institutes of Health (NIH) National Center for Advancing Translational Sciences.

Central Nervous System Ischemia-Related Injury

Bmp7 expression increases following ischemic injury to the cerebrum (Chang et al. 2003), raising the possibility that BMP signaling exerts protective or reparative actions in this tissue, and that augmenting its effect may be beneficial in the treatment of stroke. Support for this assertion comes from reports that administration of recombinant BMP-7 in a rodent model of transient ischemia before or at the onset of reperfusion leads to neuroprotection and decreased cerebral apoptosis through reduced activation of NF- κ B, caspase 3, caspase 8, and caspase 9 (Pei et al. 2013; Xu et al. 2013). The translational potential of this strategy was further advanced by the showing that intracisternal administration of recombinant BMP-7 one day after reperfusion leads to rapid improvement of neurological function (Liu et al. 2001). This finding was accompanied by increased glucose usage and local cerebral blood flow in the affected region (Liu et al. 2001). The therapeutic window for BMP pathway activation appears to be early as BMP-7 administration 2 weeks following ischemia-reperfusion did not lead to functional improvement (Shin et al. 2014).

Collectively, the results discussed above suggest that exogenous activation of BMP signaling may exert protective effects following stroke. It should be noted that administration of the BMP inhibitor noggin has also been reported to promote functional recovery following stroke injury. For example, recombinant noggin delivered by intraventricular implantation of an osmotic pump 2 weeks after ischemia-reperfusion promotes functional recovery and tissue repair (Shin et al. 2014). The timing of noggin delivery in this study leaves open the possibility that the protective actions of endogenous BMP signaling occur early after ischemia-reperfusion injury. That said, transplantation of noggin-expressing BMSCs 6 hours following reperfusion

also leads to improved neurological function (Chen et al. 2011). We suggest that these seemingly conflicting results may be attributed to the difference in augmenting endogenous repair mechanisms versus actuating artificial, exogenous repair mechanisms that introduce numerous unknown variables to the injury site. Head-to-head comparison of these different therapeutic strategies is required to determine which is more appropriate in the clinical setting; however, we are not aware of any completed or ongoing clinical trial evaluating modulation of BMP signaling in stroke.

Spinal Cord Injury

In vitro and in vivo evidence point to a role for BMP signaling in regulating neural lineage determination, with a seeming predilection for astrocytic differentiation over neuronal or oligodendrocytic fates (Mabie et al. 1997; Setoguchi et al. 2004; Hampton et al. 2007a; Xiao et al. 2010). Several groups have attempted to promote regeneration of the central nervous system following injury by modulating BMP signaling. For instance, expression of BMP-2 and BMP-7 increase rapidly with compressive injury of the spinal cord in rodents (Matsuura et al. 2008; Xiao et al. 2010) and intrathecal administration of recombinant noggin improves locomotor function 10 weeks after injury (Matsuura et al. 2008). Increased regrowth of the corticospinal tract is also observed with noggin administration in this model (Matsuura et al. 2008). These findings suggest that inhibition of endogenous BMP signaling may be beneficial in improving motor function caused by spinal cord injury (SCI), and this conclusion is further supported by results obtained when neural precursor cells expressing noggin are delivered to the site of injury (Setoguchi et al. 2004). However, it should be noted that, in a different rodent SCI model involving severing of the spinal cord, administration of a neutralizing antibody against noggin also increases axonal sprouting and neural plasticity (Hampton et al. 2007a,b). The impact of noggin inhibition on motor function following SCI was not reported in the short follow-up period of these

studies. We are not aware of any completed or ongoing clinical trials evaluating BMP pathway modulation for treatment of SCI.

Myocardial Infarction

Loss of cardiomyocytes and cardiac function is seen in several kinds of myocardial injury including myocardial infarction, viral myocarditis, and ischemia-reperfusion. Current treatments are not effective at replenishing cardiomyocyte numbers and strategies to reduce apoptosis or increase proliferation may improve cardiac function. In vitro studies indicate that BMP-2 treatment reduces cardiomyocyte apoptosis in response to hypoxia or oxidative stress (Ebelt et al. 2013), and systemic administration of a single dose of recombinant BMP-2 limits infarct size and reduces cardiomyocyte apoptosis after acute myocardial ischemia in mice (Ebelt et al. 2013); however, no long-term benefit on cardiac function is observed during the follow-up period of 3 weeks postinfarction. It is possible that benefit could occur with a lengthier follow-up period. This is, in fact, the case when recombinant BMP-10 is released from an implanted sponge; cardiac function improves beginning ~6 weeks postinfarction and continues for at least 12 weeks postinfarction (Sun et al. 2014). BMP-10 administration is also associated with increased cardiomyocyte proliferation and reduced infarct size (Sun et al. 2014). These findings must be balanced, however, with results showing that infarct size is substantially reduced by heterozygous loss of *Bmp4* when ischemia is followed by reperfusion (Pachori et al. 2010). Consistent with this, administration of recombinant noggin or dorsomorphin thirty minutes before reperfusion reduces infarct size (Pachori et al. 2010). In addition, administration of recombinant follistatin-like 1 during reperfusion reduces the activation of Smad1, 5, and 8, and improves cardiac function at an early time point of 24 hours postinjury (Ogura et al. 2012).

These collective findings raise the possibility that BMP signaling exerts biphasic effects in the injured myocardium. BMP signaling may promote cardiomyocyte apoptosis immediately

following injury, and cardiomyocyte proliferation and function after a lag period. This complex picture of BMP action creates uncertainty for the therapeutic rationale of modulating the BMP pathway as a means to improve cardiac function following myocardial infarction. Characterizing the currently unknown mechanism that mediates this shift is an important area for future investigation. We are not aware of any clinical trials evaluating the ability of BMP signaling to improve cardiac function following myocardial injury.

Other Pathologies

In addition to the applications above that have substantial experimental and clinical underpinnings, preclinical studies also suggest that increasing BMP pathway activation may be beneficial in other clinically relevant scenarios, such as retinal injury, in which recombinant BMP-4 treatment reduces retinal ganglion cells death (Ueki and Reh 2012), strabismus, in which recombinant BMP-4 reduces force generation and size of the superior rectus muscle (Anderson et al. 2011), infertility, in which administration of recombinant BMP-6 or BMP-7 in vivo improves oocyte quality and folliculogenesis (Lee et al. 2001; Park et al. 2012), and diet-induced obesity, in which recombinant BMP-7 treatment improves blood lipids and hyperglycemia (Boon et al. 2013). Additionally, two simultaneous and independent reports suggest that augmenting BMP signaling may be beneficial in promoting skeletal muscle growth and inhibiting muscle wasting by opposing the effects of myostatin (also known as growth and differentiation factor 8, GDF-8) and related molecules (Sartori et al. 2013; Winbanks et al. 2013). The ligand GDF-5 appears to be predominantly responsible for the endogenous antagonism of Smad2/3-mediated effects in this context (Sartori et al. 2013), although artificially increasing BMP-7 expression can exert the same or similar effects (Winbanks et al. 2013). It is worth noting that direct delivery of BMP ligands to the skeletal muscle environment is well documented to promote ectopic bone formation and is the defining characteristic for bona fide BMP ligands.

Thus, capitalizing on findings that suggest that increasing local BMP activity improves muscle repair will likely be challenging and requires clever strategies to activate BMP signaling using intracellular means. Indeed, both landmark studies show that adenoviral-based expression of a constitutively active version of ALK-3 leads to substantial myofiber hypertrophy (Sartori et al. 2013; Winbanks et al. 2013).

Conversely, preclinical studies suggest that decreasing BMP pathway activation may be beneficial in varied clinically relevant scenarios, such as Duchenne's muscular dystrophy, in which *Noggin* gene delivery improves skeletal muscle histology and markers of myogenesis (Shi et al. 2011), intraventricular hemorrhage of the brain, in which recombinant noggin treatment restores cellular morphology, myelination, and motor function (Dummula et al. 2011), regeneration of the liver, in which small molecule BMP type I receptor inhibitors, including LDN-193189, DMH2, or VU5350, increase hepatocyte proliferation following partial hepatectomy and LDN-193189 restores liver mass (Tsugawa et al. 2014), acute lung injury, in which LDN-193189 preserves epithelial barrier integrity after bleomycin-induced injury (Helbing et al. 2013), and rhytid, in which an ALK-3 decoy reduces wrinkle formation (Yoon et al. 2013).

FINAL PERSPECTIVES

The evidence discussed above strongly indicates that modulation of BMP signaling may be beneficial in treating human disease and improving patient quality of life. It is striking, then, how few tools are currently available to target this pathway in the clinical setting. The only FDA approved use at present is that of recombinant ligands delivered in relatively few clinical scenarios, such as open or nonunion fractures, vertebral fusion, and maxillofacial bone augmentation. The large quantities of ligand that must be used in these settings have created major concerns over both the cost and the potential for adverse events such as inflammation and HO. We are encouraged by the significant advances that have been made in addressing both

of these limitations. For instance, numerous groups have generated short BMP-inspired biomimetic peptides that can be synthesized and are less expensive to generate than recombinant proteins, and, in some cases, have enhanced activity over the naturally occurring BMP ligands (Table 3). Two promising examples of this approach are the BMP-9-based peptide pBMP-9 (Bergeron et al. 2009; Lauzon et al. 2014; Beauvais et al. 2015) and the chimeric activin A/BMP-2 protein (AB204) (Allendorph et al. 2011; Ahn et al. 2014; Yoon et al. 2014; Kim et al. 2015; Yoon et al. 2015a,b). It is possible that drugs aimed at blocking the action of BMP antagonists could further reduce the quantity of ligand or peptide required for clinical benefit (Okada et al. 2009; Ahmed et al. 2010; Kato et al. 2011; Cao et al. 2014). Additionally, considerable advancement has been made toward the development of a carrier system that stabilizes BMP ligands at the site of implantation, and reducing the diffusion of BMP ligands from the delivery site, which would improve the ability to enact local activation of BMP signaling while reducing the likelihood of adverse events at distant sites (Agrawal and Sinha 2016). Although we are unaware of completed or ongoing clinical trials examining these next-generation BMP signaling activation strategies, recent progress allows us to envision a future in which augmentation of the BMP pathway can be accomplished using extremely small quantities of material.

Some disease conditions or particular patient populations, however, may not be well suited for broad BMP signaling activation strategies. For instance, although preclinical models suggest that correcting deficient BMP signaling by ligand delivery might help in treating PAH (Long et al. 2015), the ability of BMP signaling to promote vascular calcification raises concern regarding the systemic administration of BMP signaling activators. Such scenarios may call for more nuanced strategies, such as the one built on the observation that the BMP antagonist gremlin is elevated in PAH and that delivery of an antigremlin neutralizing antibody improves disease pathology (Ciuclan et al. 2013). Extension of this methodology to other disease states

may reveal new, targeted strategies that modulate BMP signaling in defined physiological contexts by inhibiting aberrantly expressed antagonists or ligands.

Given that BMP signaling typically controls a large network of genes and cellular outcomes, it is highly likely that only a small portion of these directly relates to disease pathology, and that partial restoration would provide clinical benefit. Again using PAH as an example, defects in BMPRII-dependent signaling lead to changes in numerous signaling pathways in the pulmonary vasculature (Lowery and de Caestecker 2010), only one of which is impaired nitric oxide signaling (Frank et al. 2008). However, drugs that potentiate or promote nitric oxide action such as sildenafil are effective in improving pulmonary hemodynamics (Abrams et al. 2000; Prasad et al. 2000; Galie et al. 2005). To us, this signifies that the future of BMP-based therapeutics must take into account the downstream events that are controlled by BMP signaling in any given pathological context, thus allowing for targeted therapies—perhaps increasing the expression of even a single BMP target gene like *Id1* or *Id3* (Saika et al. 2006).

Finally, we wish to draw attention to the need for drugs that discriminate between individual type I BMP receptors. As discussed above, altered activity of the type I BMP receptor ALK-2 causes FOP (Shore et al. 2006), and pharmacological inhibition of BMP signaling reduces ectopic bone formation in preclinical models (Yu et al. 2008a; Peterson et al. 2014). Although compelling and groundbreaking, the drug used in these studies, LDN-193189, also potently inhibits other type I BMP receptors in addition to ALK-2 and has notable off-target effects on AMP kinase, vascular endothelial growth factor (VEGF) receptor 2 and platelet-derived growth factor (PDGF) receptor β , which must be taken into consideration (Lowery et al. 2016). As outlined in Table 4, this has prompted several groups to develop new type I BMP receptor inhibitors with dramatically enhanced selectivity for ALK-2 (and the closely related ALK-1) over the other type I receptors (Engers et al. 2013; Mohedas et al. 2013, 2014; Tsugawa et al. 2014). We are unaware of com-

pleted or ongoing clinical trials data comparing the safety or efficacy of these compounds, although one of these, LDN-212854, has been shown to block ectopic bone formation in a mouse model of BMP-dependent HO (Mohedas et al. 2013). The significant interest in drugs for treating FOP may also be translatable to another human disease, diffuse intrinsic pontine glioma (DIPG), which is also caused by activating mutations in the *ACVR1/ALK2* gene (Buczakowicz et al. 2014; Fontebasso et al. 2014; Taylor et al. 2014; Wu et al. 2014). Furthermore, the intensity of this line of investigation may develop a toolbox to help delineate the effects of specific type I receptors in vivo; for example, differential type I receptor targeting proved beneficial in building an understanding of how BMP signaling promotes liver regeneration in a rodent model (Tsugawa et al. 2014).

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