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Review

Bone morphogenetic protein signaling in bone homeostasis

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ABSTRACT

Bone morphogenetic proteins (BMPs) are cytokines belonging to the transforming growth factor-β (TGF-β) superfamily. They play multiple functions during development and tissue homeostasis, including regulation of the bone homeostasis. The BMP signaling pathway consists in a well-orchestrated manner of ligands, membrane receptors, co-receptors and intracellular mediators, that regulate the expression of genes controlling the normal functioning of the bone tissues. Interestingly, BMP signaling perturbation is associated to a variety of low and high bone mass diseases, including osteoporosis, bone fracture disorders and heterotopic ossification. Consistent with these findings, in vitro and in vivo studies have shown that BMPs have potent effects on the activity of cells regulating bone function, suggesting that manipulation of the BMP signaling pathway may be employed as a therapeutic approach to treat bone diseases. Here we review the recent advances on BMP signaling and bone homeostasis, and how this knowledge may be used towards improved diagnosis and development of novel treatment modalities. **This article is part of a Special Issue entitled "Muscle Bone Interactions".**

Contents

Abbreviations: ALK, Activin receptor-like kinase; BMP, bone morphogenetic protein; EndoMT, endothelial-to-mesenchymal transition; HO, heterotopic ossification; MAPK, mitogen activated protein kinase; MMP, matrix metalloproteinase; OB, osteoblast; OC, osteoclast; RANK, receptor activator of nuclear factor-κB (NF-κB); RANKL, receptor activator of NF-κB ligand; SMAD, homolog of the *Drosophila* protein, mothers against decapentaplegic (MAD) the *Caenorhabditis elegans* protein SMA; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

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Introduction

Bone remodeling, resorption and formation

Bone remodeling occurs throughout life and involves the coordination between bone resorption and new bone formation. Interestingly, resorption and formation are regulated by systemic and local release of cytokines and growth factors, including the bone morphogenetic proteins (BMPs). As we will describe throughout this review article, BMPs have been unveiled as crucial regulators of bone homeostasis. BMPs coordinate anabolic and catabolic processes by affecting the differentiation and activity of osteoblasts (OBs) and osteoclasts (OCs), which are the main cell types responsible for bone formation and resorption, respectively.

BMPs and other growth factors such as transforming growth factor-β (TGF-β) and insulin growth factor (IGF) are released from the digested bone matrix as well as delivered from OC-like cells to activate osteogenesis, which is initiated with the recruitment of mesenchymal OB precursors. Next, differentiated chondrocytes and active OBs generate the organic bone matrix that will be further maturated with the incorporation of inorganic salts. Finally, mature OBs embedded in the mineralized bone undergo apoptosis or differentiate into quiescent osteocytes [1]. Such anabolic and catabolic activities are coupled through *basic multicellular units* (BMU) (Fig. 1), which resemble anatomical microenvironments where bone remodeling takes place. It consists of OBs and OCs, but also osteocytes, bone lining cells and capillaries [2]. This whole multicellular assembly moves in three dimensions throughout the bone matrix to rebuild the bone architecture. Interestingly, the cells in the BMU are not directly in contact with the bone marrow, but covered by a *canopy* of bone lining/OB-like cells intimately associated with capillaries, comprising the *bone remodeling compartment* (BRC). This structure is thought to be of crucial importance since it facilitates the release of certain molecules (such as thyroid hormone, estrogen, vitamin D, parathyroid hormone and circulating BMPs) from the blood vessels [3]. Interestingly, disruption of the *canopy* leads to disorders in bone homeostasis [4], partially due to a decrease in the recruitment and differentiation of OCs and OBs.

Bone remodeling requires the formation of new blood vessels or *angiogenesis*, which provides the bone remodeling site with osteogenic precursors, as well as cytokines and growth factors that regulate the activity of OBs and OCs [5–9]. Capillaries also facilitate the diffusion of the growth factors and minerals delivered from the degraded bone matrix [10] to the peripheral blood. In fact, disruption in angiogenesis usually leads to defects in bone remodeling and repair [11]. Noteworthy, OBs, osteocytes and OCs secrete molecules such as vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) to modulate the angiogenic response [3], suggesting that there is a bilateral communication between blood vessels and bone tissues to regulate each other. Finally, although not directly involved in bone remodeling, the bone tissue is continuously invaded by immune cells, that modulate the activity

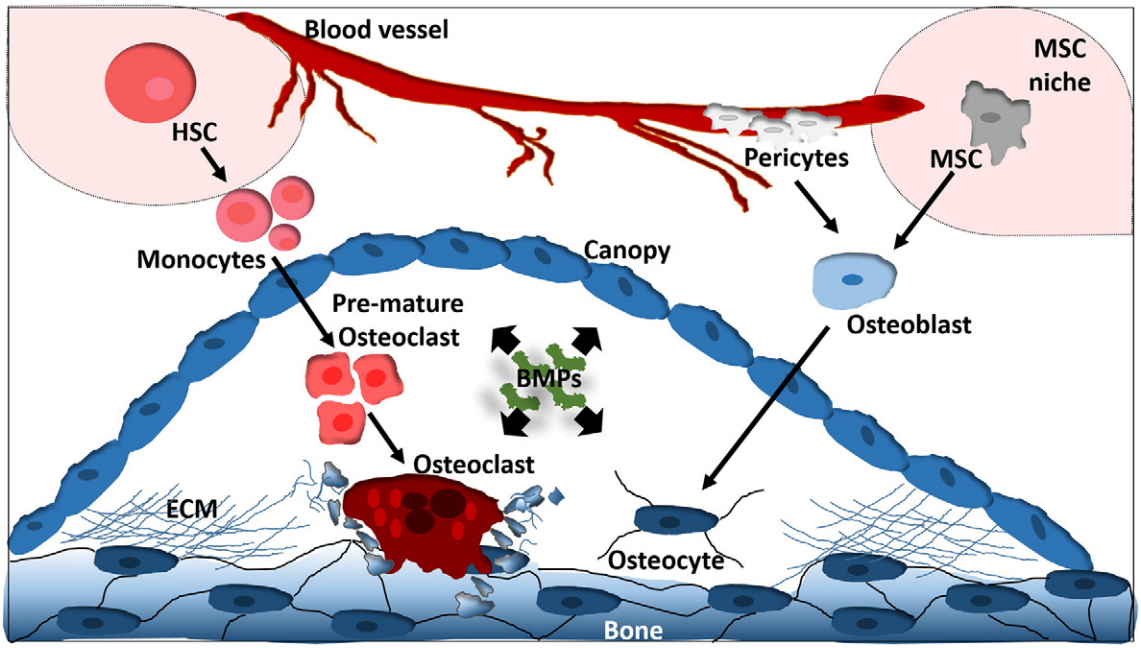

Fig. 1. Basic multicellular units (BMU). BMU represent functional bone remodeling entities where the main cell types involved in bone remodeling (i.e., mesenchymal progenitors that will differentiate into osteoblasts and osteocytes, osteoclasts and osteoclast progenitors and blood vessels consisting of endothelial cells and pericytes) are represented. A canopy made of mesenchymal cells (in humans) or osteomacs (in mice) facilitates the interchange of molecules between the bone tissue and the vasculature. HSC, hematopoeitic stem cell; ECM, extra-cellular matrix; MSC, mesenchymal stem cell.

and proliferation of OBs and OCs. Such crosstalk is so important, that aberrant activation of the immune system often leads to abnormalities in bone homeostasis [12].

In summary, even though a broad variety of cell types have been described to contribute to bone remodeling, in this review we will mostly focus on OB and OC activities and their responses to bone morphogenetic proteins (BMPs) to rebuild the bone architecture. The role of other cell populations in bone remodeling has been previously described elsewhere [11,13–15].

Cell types in bone tissue: osteoblasts and osteoclasts

As it has been indicated above, basically two cell types are responsible to perform bone remodeling: OBs and OCs. This process and the activity of these cell types should be regulated very tightly, since a misbalance in their activity usually provokes bone diseases in humans.

Osteoblasts (OB)

Briefly, OB cells are in charge of the generation of the organic extracellular matrix (ECM). The OB lineage includes mesenchymal progenitors, pre-OB, mature OB, bone-lining cells and osteocytes. Traditionally, it has been considered that they derive from mesenchymal stem cells that differentiate into pre-OBs [16]. Nevertheless, such mesenchymal progenitors can be provided from the vasculature, surrounding soft tissues or from the bone tissue itself. Unfortunately, thus far lineage tracing experiments performed in mice have not been able to determine their exact origin [13]. Recently it has been suggested that other cell types, such as endothelial cells, may give rise to OB-like cells in particular conditions such as heterotopic ossification (HO) [17], through a mechanism named endothelial-to-mesenchymal transition (EndoMT) which is dependent on TGF-β and BMPs.

Osteoclasts (OCs)

OCs are the primary cells in charge of bone resorption. These cells secrete a cocktail of enzymes to dissolve and process the organic matrix using an acidic medium. OCs are originated from the fusion of hematopoietic stem cells at the bone surface in a process denominated osteoclastogenesis [18,19]. Of note, while OBs and chondrocytes are derived from a common mesenchymal progenitor, OCs differentiate from the monocyte lineage and, consequently, many of the intracellular proteins regulating their maturation and activation are common to immune activation cascades. Using co-culture systems, it was suggested that paracrine signals secreted by stromal cells (and OB progenitors) regulated the differentiation of OCs [20]. Nowadays, it is accepted that osteoclastogenesis results from the interplay between receptor activator of nuclear factor-κB (NF-κB) (RANK) ligand (RANKL), macrophage colony-stimulating factor M-CSF and osteoprotegerin (OPG), a member of the tumor necrosis factor (TNF) receptor superfamily.

BMP signaling

BMPs are pleiotropic cytokines belonging to the TGF-β superfamily, which also includes TGF-βs and Activins. Their isolation from bone extracts and further gene identification occurred in the 1980s, based on the previous findings by Marshall R. Urist two decades before [21], when he suggested the presence of osteoinductive molecules in demineralized bone matrix extracts.

In general, binding of TGF-β ligands to specific receptors at the cell membrane triggers the recruitment of signaling complexes responsible to transduce the signal into the cytoplasm and eventually to regulate the expression of genes, many of them in charge of maintaining the bone homeostasis. Noteworthy, among all members comprising the TGF-β superfamily, almost exclusively BMPs display osteogenic properties [22].

Ligands

BMP ligands have been classified into 4 different subfamilies according to their sequence similarity and functions [23]: (i) BMP-2 and -4; (ii) BMP-5, -6, -7, -8a and -8b; (iii) BMP-9 and -10; and (iv) BMP-3, -3b, -13, -11, -12, -14, -15, and -16. Interestingly, the group (iv) does not possess osteogenic properties and, in some cases, even inhibits BMP function (i.e., BMP-3 and BMP-13 [24,25]). Moreover, the osteoinductive capabilities of the BMP ligands have been demonstrated to be cell type specific [26], indicating that BMP signal transduction is tightly regulated at diverse checkpoints, from the extracellular space and cell membrane towards the nucleus. In bone, BMPs are produced by a variety of cell types, including endothelial cells, OBs and chondrocytes. Nevertheless, some particular BMPs were found in serum samples, suggesting that they may cycle in the blood stream to act also at systemic levels and not exclusively in a local manner [27–29].

Like other members of the TGF-β superfamily, BMP ligands are initially synthesized as large dimers containing a secretion signal peptide in the N-terminus domain (called *prodomain*) and a cysteine-knot mature motif in the C-terminus [30]. TGF-β ligands are usually secreted attached to a prodomain named LAP (*latent associated peptide*), which regulates the folding of the molecule and prevents the interaction with the receptor at the cell membrane [31]. In contrast, BMPs do not exhibit such latency [32] and are usually cleaved by cellular serine endoproteases to release the active forms before secretion [33]. In addition, further posttranslational modifications are associated to BMP ligands. For example, BMP dimers are susceptible of being N- and O-glycosylated in order to prolong their half-life and modulate receptor binding [34–36].

The active signaling molecule is usually formed by homodimerization. For most of the ligands, covalent dimerization occurs through a disulfide bond, requiring a seventh conserved cysteine, outside of the cysteine-knot motif, within each monomer [37]. Moreover, in particular experimental settings heterodimers have been shown to be more potent than the corresponding homodimers (i.e., BMP-2/-5; BMP-2/-6; BMP-2/-7) [38,39].

Once secreted, the availability of BMPs is modulated by ligand binding proteins at the extracellular space. A number of molecules have been described to sequester BMP ligands and impair their interaction with receptor complexes, thereby inhibiting their osteogenic ability. Some of these secreted antagonists have been reviewed elsewhere [40–42]: twisted gastrulation (Tsg); Noggin; Chordin; the CAN family, which includes Gremlin and Cerberus, differential screening-selected gene aberrative in neuroblastoma (DAN), protein related to DAN and Cerberus (PRDC); Coco [43]; uterine sensitization-associated gene-2 (USAG-1) [44], Follistatin [45] and Sclerostin [46]. Interestingly, matrix Gla protein (MGP) interferes with BMP signaling through sequestering BMP-2 and BMP-4, although other mechanisms of action have been attributed to MGP as well [47]. In addition to these antagonists, some other molecules have been described to increase BMP activity. Among them, KCP/Kielin increases the binding of BMP-7 to the BMP type I receptor [48]. Crossveinless 2 (CV2, also called BMP endothelial cell precursor derived regulator, BMPER) may either promote or interfere with BMP activity depending on the context [49].

Receptors and co-receptors (BMP receptors and other BMP binding proteins)

TGF-β superfamily ligands bind as dimers to transmembrane receptor complexes at the cell surface (Fig. 2) [50]. Such complexes are comprised of two types of serine/threonine kinase receptors, which are known as type I receptors (containing a glycine and serine rich domain, termed GS domain) or type II receptors. This domain is located between the transmembrane and the kinase domain and is phosphorylated by the type II receptor upon ligand binding [51]. In humans seven type I (ALK1–7) and five type II (ActR-IIa, ActR-IIb, AMHR-II, BMPR-II

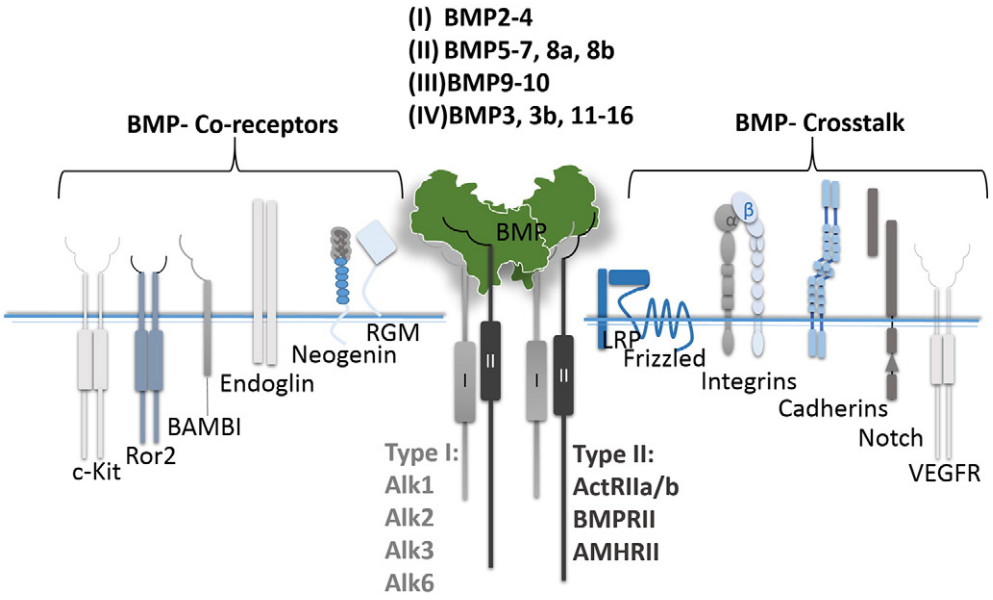

Fig. 2. BMP ligands, receptors, co-receptors and interacting receptors. BMP signal transduction involves a number of ligands, type I and type II serine/threonine kinase receptors, and co-receptors, which regulate the activation of intracellular mediators upon interaction with extracellular stimuli. BMPs are classified into 4 different (I–IV) subfamilies according to sequence homology. Importantly, BMP families I–III elicit osteogenic properties, while IV does not. In addition, the BMP signaling interplays with cellular cascades initiated by a variety of membrane receptors, resulting in a crosstalk that provides fine-tuning. LRP, lipoprotein receptor-related protein; Ror, receptor tyrosine kinase-like orphan receptor; VEGFR, vascular endothelial growth factor receptor.

and TGF-βR-II) receptors have been identified so far [52,53], which are needed for signaling. Type I and type II receptors are able to interact with a variety of ligands. One clear example of such receptor promiscuity is the Activin type II receptors ActR-IIa and ActR-IIb that bind BMPs and Activins [54]. Binding of a BMP ligand dimer to a receptor provokes the assembly of type I and type II receptors in a hetero-oligomeric complex.

Using complementary biophysical approaches including single particle tracking microscopy (SPTM) [55] and patch fluorescence recovery after photo bleaching (FRAP) analyses [56,57], it was shown that hetero-oligomeric BMP receptor complexes assemble by two distinct modes: (i) pre-formed-complexes (PFCs) comprising pre-assembled BMP type I and type II receptors bind BMPs to transduce canonical SMAD signaling; and (ii) BMP-induced signaling complexes (BISCs) are assembled upon ligand-induced recruitment of the BMP type II receptor towards the BMP-bound high affinity type I receptors and induce activation of MAPK/p38 signaling.

Irrespective of the receptor assembly mode, the type II receptor kinase is thought to be constitutively active even in the absence of ligand and, upon BMP-induction, phosphorylates the GS domain of the type I receptor to transduce the signaling cascade. Binding of the intra-cellular FK506-binding protein (FKBP)12 (FKBP12) acts as a gatekeeper mechanism, setting a threshold for type I receptor activation in the absence of ligand [58,59]. Moreover, disruption of the GS domain by substitution of a conserved asparagine residue led to constitutive active receptors [60,61]. BMPs bind the type I receptors ALK1/2/3/6 and the type II receptors ActR-IIa, ActR-IIb and BMPR-II [62,63]. Importantly, BMPs display a different affinity for BMP type I receptors: BMP-2/-4, BMP-5/-6/-7 and BMP-12/-13/-14 bind to ALK3/6, while BMP-5/-6/-7 can also bind ALK2. ALK2 can also interact with BMP-9/-10, which preferentially bind ALK1 [64]. Among the three type II receptors, BMP-9 has been suggested to have higher affinity for BMPR-II [65]. In addition to BMPR-II, BMPs signal through ActR-IIa and ActR-IIb [66]. While the type I receptors ALK2/3/6 and the type II receptors have a broad expression pattern, the type I receptor ALK1 is more selectively expressed, including for example endothelial cells.

BMP binding to type I and type II receptors can be facilitated (or modulated) by so-called co-receptors that lack an intrinsic signaling motif:

(i) Betaglycan or type III TGF-β receptor (TβRIII) [67] and Endoglin (CD105) [68]. Betaglycan is a transmembrane co-receptor that increases ligand–receptor affinity, thus intracellular responses. It has been shown that, apart from interacting with other TGF-β ligands, it is capable of binding BMPs [69]. On the other hand, Endoglin acts as a co-receptor for ALK1 and they engage in a complex that drives TGF-β and BMP-9/-10 responses [70,71].
(ii) EGF–CFC family (including Cripto [72]). These are GPI-anchored membrane proteins essential for Nodal, vegetal (Vg)1 and growth and differentiation factor (GDF)1 signaling.
(iii) the glycosyl-phosphatidylinositol-linked family of repulsive guidance molecules (RGM) [73,74]. This family includes RGMa, RGMb or DRAGON, RGMc or hemojuvelin (HJV) and RGMd that are proteins formed by a N-terminal signal peptide, an intermediate von Willebrand factor type D partial domain and a C-terminal glycosyl-phosphatidylinisotol (GPI) anchor [75]. These co-receptors bind with high affinity to the type I receptors and alter their ability to partner with type II receptors, thus increasing cell sensitivity to BMPs.

In addition to modifying ligand's affinity, it has been suggested that binding of co-receptors may influence the orientation of the receptor complexes upon binding with different ligands, thereby determining the phosphorylation of their down-stream substrates. Moreover, the binding kinetics and the stability of the complex formed between ligand and receptor may influence the potency of the signal transduction by the receptor kinase [30]. BMP signaling diversity is enhanced by crosstalk to other transmembrane receptor pathways such as, receptor tyrosine kinase (RTK) [76,77], c-Kit [78], receptor tyrosine kinase-like orphan receptor (Ror)2 (Ror2) [79] or G-protein coupled receptor (GPCR) [80]. Such crosstalk can be in synergy by feed-forward

mechanisms [81] or counteract each other for a negative fine tuning [39,82,83]. Crosstalk to other cascades is mediated via either protein–protein interactions or transcriptional feedbacks [81,84]. Protein–protein crosstalk interactions occur directly on the plasma-membrane through interactions between BMP receptors and other transmembrane co-receptors, such as c-Kit [78], but also in the cytosol, where SMADs have been shown to associate with effector molecules activated by non-BMP ligands, such as Jak/Stat [85,86], Rho-GTPases [87], Dll4/Notch [88,89] and VEGF pathways [90,91] as well as IGF [92]. The interplay between BMP and Wnt represents a good example of the multitude of crosstalk mechanisms possible. BMP-Wnt crosstalk was shown in bone forming [93] and bone resorbing cells [94,95]. BMP and Wnt signaling are tightly linked by transcriptional feedbacks such as BMP-induced expression [96] or repression [97] of the Wnt ligands, Wnt-induced expression of BMP ligands [98–100] and BMP-induced expression of dual BMP and Wnt antagonists [101]. The multitude of interactions illustrates why the Wnt and BMP signaling cascades seem to act synergistically, but also antagonize each other in a cellular context and differentiation stage dependent fashion [102]. Interestingly, during endochondral ossification, TGF-β attenuates Wnt signaling while BMP-2 supports it, suggesting a differential effect among all TGF-β family members on Wnt signaling [103]. Besides transcriptional links, BMP and Wnt pathways share effector signaling proteins (e.g. GSK3β [104–106], β-catenin [107–109] and Akt [110]), which act as molecular bridges between both.

Furthermore, there are a number of molecules preventing the interaction between ligands and BMP receptors, which have been termed pseudoreceptors. BAMBI (BMP and Activin membrane-bound inhibitor) conserves the structure of a BMP type I receptor, though it lacks the intracellular kinase domain. In this sense, BAMBI is thought to compete in the formation of BMP receptor complexes with actual receptor kinases, inhibiting the signal transduction [111]. Cysteine-rich motor neuron 1 protein (CRIM1) is a glycosylated transmembrane protein that belongs to the Chordin family. CRIM1 acts as an antagonist by down-regulating the production and maturation of BMPs and affecting the proper secretion and release of BMPs to the extra-cellular space [112]. Neogenin has been recently postulated as a BMP receptor. It has been shown to bind BMP-2, -4, -6 and -7 and somehow suppresses intracellular activation induced by BMPs by activating the RhoA pathway [113].

Additionally, BMP bioavailability is tightly regulated by low or high affinity binding to ECM components such as collagen [114,115], fibrillin [116,117] and possibly also fibronectin [118] (Fig. 3). Those BMP–ECM interactions steer and shape BMP gradients and bioavailability during development [119,120] and may also define extracellular BMP concentrations within the bone micro-environment. Current tissue-engineering strategies include a combination of ECM molecules with recombinant BMPs to modulate release kinetics and increase bone forming capacity of BMP delivery systems [121,122]. BMPs can become released from ECM e.g. upon matrix metalloprotease (MMP) activation [123,124]. Interestingly it seems that MMP activation is involved in HO processes (see below), suggesting that MMP activation contributes to regulate the bioavailability of BMPs in disease [125].

Canonical SMAD signaling

As we have described above, TGF-β receptors form a hetero-oligomeric complex with type I and type II receptors, and co-receptors may participate of this complex. Signal transduction initiated upon BMP stimulation is summarized in Fig. 4. Once the constitutively active type II receptor phosphorylates the GS domain in the type I receptor, the latter phosphorylates and activates a group of transcription factors known as R-SMAD (receptor regulated) proteins [126]. The subset of R-SMADs targeted by the type I receptor differs between TGF-β (SMAD2/3) and BMP ligands (SMAD1/5/8). BMP SMADs share a common structure consisting of a DNA binding domain at the N-terminus and a protein–protein interaction domain at the C-terminus domain, connected through a linker domain [22]. Upon phosphorylation in the C-terminus domain, these factors hetero-oligomerize with the common mediator SMAD4 (Co-SMAD) [127] and translocate into the nucleus to regulate the expression of BMP responsive genes. In this sense, BMP responsive elements (BRE) have been already characterized in gene promoters such as *Inhibitor differentiation (Id)-1*, *Id-3* and others [128–130].

Phosphorylation of R-SMADs can be also modulated by intracellular mediators. For example, protein phosphatase magnesium dependent 1A (PPM1A) has been shown to inhibit BMP-induced SMAD transcriptional activity [131,132]. SCP-1 and SCP-2 (small C-terminal domain phosphatases 1 and 2) mediate dephosphorylation of SMADs, not only at the C-terminus, but also at the linker domain, suppressing their transcriptional activity [133]. Interestingly, whereas the R-SMAD C-terminus domain is usually phosphorylated by type I kinase receptors, the linker region is often a target of other cellular kinases, such as MAPK and GSK3-β, leading to their de-activation through proteasomal-mediated degradation and competition with canonical phosphorylation [104,134]. Therefore, whether SCPs potentiate or block SMAD function depends on a balance between linker and C-terminus phosphorylation. Nevertheless, the role of the linker

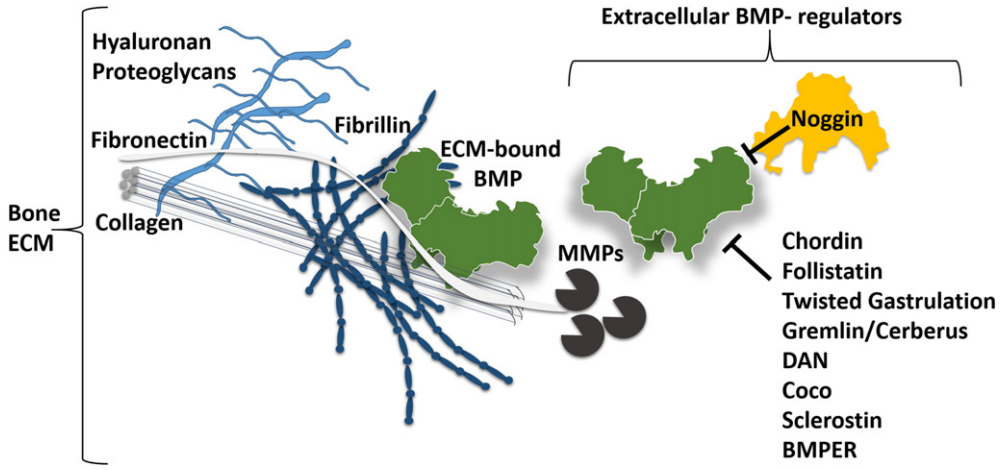

Fig. 3. BMP signaling is modulated by the extracellular space. The ability of BMPs to interact with membrane receptor complexes is influenced by components of the extracellular matrix (ECM), such as collagen, fibronectin, fibrillin, as well as BMP-interacting proteins (for example, Noggin, Chordin, etc.), which sequester BMP ligands, thereby establishing BMP concentration gradients and different BMP release kinetics.

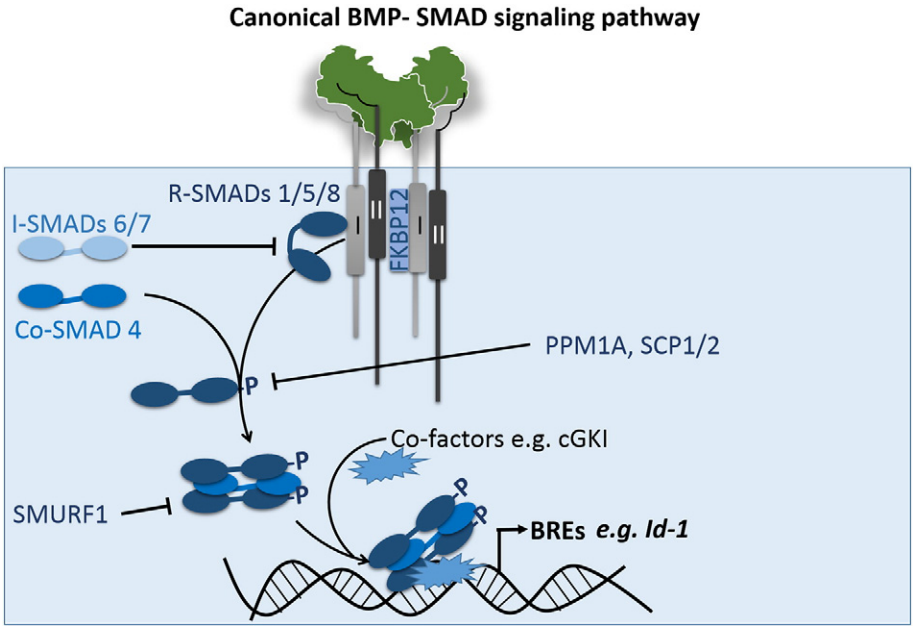

Fig. 4. Canonical SMAD signaling pathway. Upon receptor hetero-oligomerization, the BMP receptors trigger the so-called canonical SMAD pathway, which involves the phosphorylation of the BMP SMADs 1/5/8, which translocate into the nucleus in a complex with the Co-SMAD (SMAD4) and regulate the expression of BMP responsive genes. FKBP12 is a negative regulator of BMP receptor signaling; when bound to BMPRI, it inhibits transphosphorylation of BMPRI by BMPRII kinase. In response to BMP challenge, FKBP12 dissociates from the BMPRI, which then becomes more signaling active. BRE, BMP response element; cGKI, cGMP-dependent protein kinase; Co-SMAD, common mediator SMAD; FKBP12, FK506 binding protein 12; Id, inhibitor differentiation; I-SMAD, inhibitory SMAD; R-SMAD, receptor regulated SMAD.

phosphorylation is still in debate, since Alarcón et al. demonstrated a potentiation of SMAD1/5/8 transcriptional activity upon linker phosphorylation by Cyclin-dependent kinase 8/9 (CDK8/9) [135]. Additionally, the recruitment of the BMP type II receptor associated protein cGMP-dependent protein kinase I (cGK1) to SMAD1 enhances its transcriptional activity acting as a transcriptional co-factor [84]. In summary, it seems that recruitment of additional co-factors and the timing of SMAD linker phosphorylation may influence the function and transcriptional activity of SMADs.

Complementarily, SMAD activity is also regulated by ubiquitination. Although this was first described for TGF-β SMADs [136,137], a number of E3 ubiquitin ligases were later reported to ubiquitinate BMP SMADs

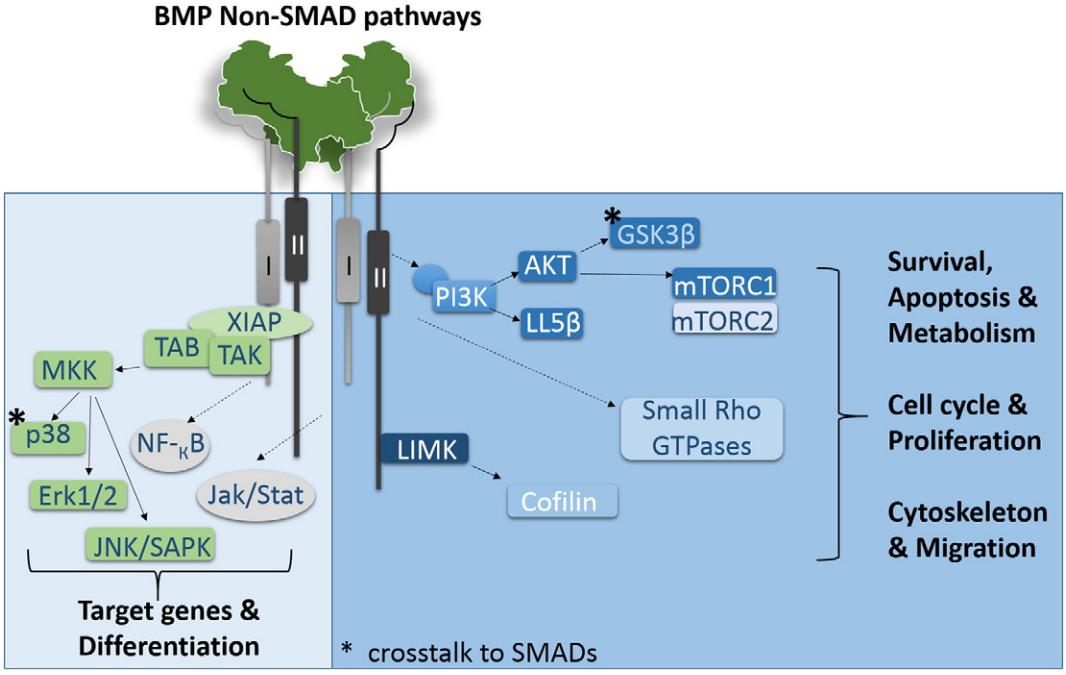

Fig. 5. BMP non-SMAD signaling pathways. Besides canonical SMAD signaling, BMP receptor complexes modulate a number of parallel signaling cascades, as described for MAPK (i.e., p38, ERK and JNK) and NF-κB, Jak/Stat, LIMK, PI3K, mTORC and RhoGTPases. Such non-canonical signaling cascades increase the number of genes, and subsequent cellular responses, which expression is regulated by BMPs. Moreover, p38, JNK and GSK3β kinases have been reported to exert a negative feedback on canonical BMP signaling, which results in a tight regulation of gene expression activated by BMPs.

[138]. For example, SMAD specific E3 ubiquitin protein ligase (SMURF)1 (SMURF1) has been shown to target SMAD1/5 [139,140] while SMURF2 and CHIP can mediate ubiquitination of SMAD1 only [141,142]. Ubiquitination of substrates can occur in different ways. For example, lysine-48 poly-ubiquitination acts as a mark for proteasomal-mediated degradation, thus repressing signal transduction, whereas poly-ubiquitin chains at lysine-63 lead to protein aggregate formation and regulate a myriad of cellular processes [143]. In addition, mono-ubiquitination has been described as a negative regulator of R-SMADs [144,145]. Poly-ubiquitin labeling of SMADs has been usually associated with linker phosphorylation, thus inhibiting the BMP pathway [133]. During the last decade a complex system of (de)ubiquitinating enzymes has been discovered associated to TGF-β signaling, affecting not only R-SMADs, but also other components of the pathway, such as receptors, Co-SMAD and inhibitory SMADs (I-SMADs) [146]. I-SMADs, i.e. SMAD6/7 antagonize the activation of signal transducing R-SMADs and Co-SMAD. SMAD6 has been shown to mitigate BMP signaling by competing with SMAD4 for complex formation with SMAD1 [147]. Arginine methylation of SMAD6 by receptor recruited protein arginine methyltransferase 1 (PRMT1) was recently shown as an additional signaling step upstream of R-SMAD phosphorylation [148]. SMAD7 can be recruited to the receptor and interacts with SMURF1/2 to induce the degradation of the type I receptor kinase [149,150].

Non-SMAD signaling

Besides canonical SMAD signaling, non-SMAD signaling pathways (Fig. 5) attract increasing attention to transduce BMP signals. These pathways are able to promote or fine tune canonical SMAD signaling (such as shown for cGK1) [84], by modulating SMAD signaling durations and intensities (such as shown for protein phosphatase PP2A or the stem cell factor (SCF)–c-Kit pathway) [78,151] or by acting irrespectively of SMAD signaling at the level of gene transcription (such as shown for the activation of MAPK pathways, including p38, ERK and JNK) [152]. Some of these pathways include the induction of cellular response independent on gene transcription, such as the orchestration of cytoskeletal rearrangements, which is required for cell polarity and migration. A number of pathways, including LIMK/Cofilin [153], PI3K/Akt [154], and the RhoGTPases [155] have been shown to modulate these cytoskeletal rearrangements in response to BMPs. Moreover, even though the effect of BMPs on non-SMAD signaling may not be so determinant by itself, it definitely affects the way that these pathways are regulated by their canonical stimuli, such as inflammation, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, interferon (IFN-Y), Notch, Hedgehog and Wnt [156]. A fairly less understood crosstalk is the link between inflammatory pathways and osteoinductive BMP-signaling. It was shown that TNF-α-activated NF-κB pathway in bone progenitors inhibits the BMP-2-induced osteoblastic differentiation interfering with SMAD signaling, suggesting that this mechanism might be contributing to osteoporosis [157]. *In vitro*, TNF-α potently suppressed TGF-β and BMP-2-induced SMAD signaling, as well as BMP-2-induced *Runx2* expression, and thereby inhibiting osteoblast mineralization [158].

Role of BMPs as sensors of mechanical stress

As the most rigid structural system in the human body, the skeleton is continuously undergoing mechanical stimulation such as tension, strain, compression and bending which one can summarily refer to as bone "mechanical loading" [91]. In general, regular moderate physical activity and modest mechanical stimulation were shown to have a positive effect on bone homeostasis and the quality of bone [159–163]. Using experimental bioreactor systems, these observations have been further supported by the demonstration that mechanical loading of bone progenitor cells indeed cooperates with BMP signaling to induce a faster and more pronounced osteogenic differentiation response [159, 164–166]. Cells undergoing mechanical stimulation show a higher BMP responsiveness and a quicker activation of canonical SMAD signaling [167] while others reported that mechanical loading decreases the expression of BMP antagonists [168]. Some genes where shown to carry mechanical stress-response elements [169] and a transcriptional up-regulation of *BMP-7*, *BMP-2*, *alkaline phosphatase (ALP)*, and *collagen (COL)-I* mRNA in OB-like cells was shown upon mechanical stimulation [170,171]. Interestingly, this effect seems to be cell-type specific [172]. The molecular mechanisms responsible for this mechano-BMP crosstalk are not fully understood today and many ongoing investigations will shed light on its molecular basis and physiological relevance in different cell types.

Regulation of osteoblasts by BMPs

TGF-β superfamily ligands have been extensively demonstrated to regulate bone formation and, in particular, the generation and activation of OBs during development. In general, TGF-β ligands block the proliferation and mineralization of OBs [173]. Importantly, the expression of both TGF-β type I and type II receptors in murine, rat, and human OBs is decreased during OB differentiation, which may imply that OBs are less sensitive to TGF-β in the late phase of their differentiation [174]. On the contrary, BMPs potently regulate the later stages of OB differentiation. BMPs play important roles in skeletal development and physiology but are also involved in the development of many organs. This is illustrated by the fact that the majority of knock-out mice for BMP and BMP receptors (BMPRs) are lethal at early embryonic stages, or exhibit abnormalities in skeletal patterning [13,175,176]. In addition, some BMP family members may have redundant roles in specific tissues/organs. In contrast to BMP-2 knock-out animals, which were predisposed to suffer bone fractures [177], no apparent skeletal abnormalities during the development of long bones were found in conditional knock-out models for BMP-2, BMP-7 and BMP-4. Nevertheless, the double knock-out BMP-2/BMP-4 exhibited improper long bone development, suggesting that there is a broad redundancy among BMPs [178].

Transcription factors regulating osteoblast differentiation

As said before, mature OBs derive from mesenchymal progenitors. A number of transcription factors have been demonstrated to take part in the OB differentiation (Fig. 6A). As such, *runt-related transcription factor 2* (*Runx2*) and *Osterix* (*Osx*) are required for OB differentiation and, indeed, animal models where their expression has been impaired exhibit a lack of mature OBs [179–182]. Other factors, such as *Msx2*, *Sox9*, *β-catenin*, *peroxisome proliferator-activated receptor gamma* (*PPARγ*), *ATF4* and the *activator protein* (AP)-1 family members have been shown to modulate the differentiation and activation of mature OBs, by either interacting with Runx2/Osx or by functioning independently [183–192].

Regulation of osteoblast differentiation by BMPs

According to multiple in vitro evidence, stimulation of the BMP signaling pathway has been shown to promote osteoblastogenesis. As such, BMP–SMAD signaling up-regulates the expression of *Runx2* [193], which interacts directly with SMAD1/5/8 to activate the transcription of down-stream genes [194,195], thereby favoring OB differentiation. In this regard, Matsubara et al. demonstrated that BMP canonical signaling activates the expression of *Osterix* through a mechanism involving *Runx2* and *Msx2* [196]. In contrast, TGF-β induced-SMAD3 blocks the function of Runx2 through direct physical interaction [197]. Surprisingly, while in vivo Runx2 is necessary to induce the expression of *Osterix* by BMP-2, such ligand is able to activate the transcription of *Osterix* in *Runx2* knock-out mesenchymal cells in vitro [182].

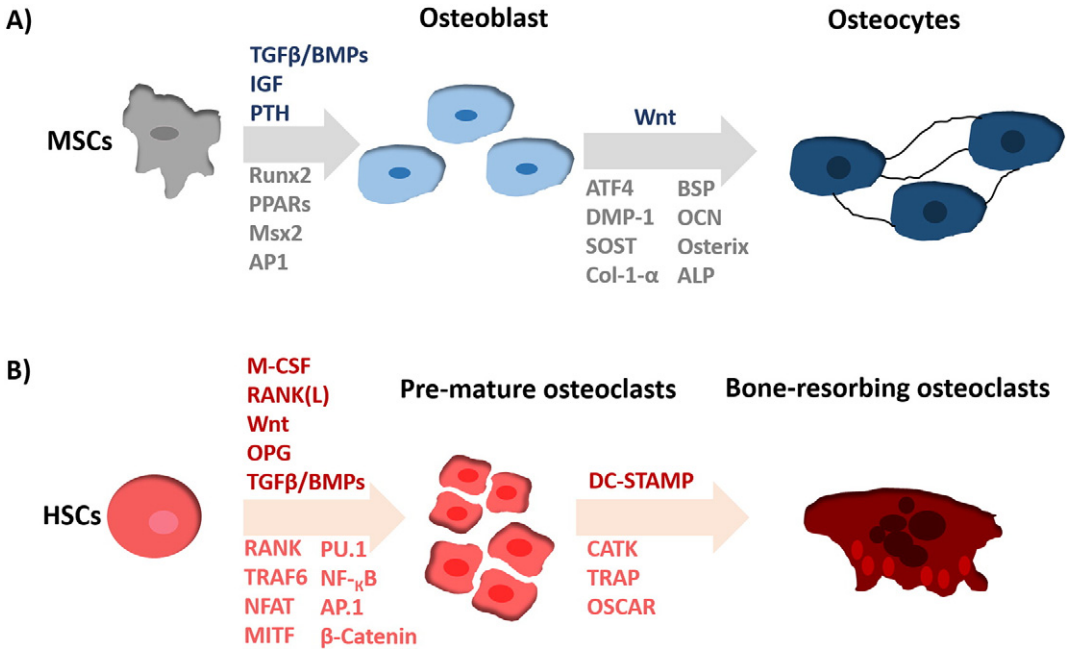

Fig. 6. BMPs modulate osteoblast and osteoclast differentiation and activity. Research during the last decades has resulted into the identification of specific cellular factors, which regulate particular steps in the differentiation of osteoblasts and osteoclasts from their respective progenitor cells. A) Osteoblasts and osteocytes are derived from mesenchymal stem cells (MSCs) in a process that is mainly directed by the transcription factor *Runx2* in the early stages, and potently stimulated by BMPs. B) Active osteoclasts are obtained from hematopoietic stem cells (HSCs) through a mechanism called osteoclastogenesis that is tightly regulated by inflammatory transcription factors including NFAT, NF-κB and AP-1. BMPs mostly act on osteoclastogenesis in a non-direct manner, by inducing the secretion of regulatory molecules in other cells, which will eventually affect osteoclasts.

Consequent with these findings, specific regulators of the BMP signaling cascade have a negative effect on OB differentiation. For example, it has been mentioned that BMP-3 interferes with BMP signaling, which correlates the inhibitory effect of BMP-3 on BMP-2 and BMP-4-induced OB differentiation [198]. Furthermore, the E3 ligase SMURF1 targets BMP–SMADs and receptors for proteasomal degradation [199,200]. Consequently, SMURF1 blocks the early differentiation of OBs and chondrocytes, via targeting of Runx2 [201]. Moreover, the inhibitory SMADs (SMAD6 and SMAD7) down-regulate osteoblastogenesis. SMAD6 overexpression inhibits BMP-2-induced OB differentiation of C2C12 [202]. Importantly, BMP-2 and BMP-7 up-regulate the expression of SMAD6, in a negative feedback mechanism [203]. The same type of balancing mechanism involves the transcription factor CCAAT-enhancer-binding protein homologous protein (CHOP), which is up-regulated in response to BMP-2 to inhibit osteoblastogenesis via interfering with Runx2 [204]. On the other hand, *SMAD7* knock-out mice exhibit impaired osteoblastogenesis. Moreover, OC activity was up-regulated in these mice, suggesting that SMAD7 interferes with bone formation and promotes bone resorption [205].

Finally, BMPs modulate non-SMAD signaling cascades in order to promote OB differentiation. For example, induction of cyclooxygenase-2 (COX-2) has been shown to be relevant for the osteogenic activity of BMP-9 in mesenchymal cells [206]. Other factors, such as AP-1, are activated by BMP-2 [207] and TGF-β [208] to promote bone formation.

Despite all these findings on BMPs as key players in bone formation, results from knock-out animal models showed no skeletal disorders when BMP-2, BMP-4 or BMP-7 was genetically impaired [178]. Moreover, specific disruption of the BMP type I receptor ALK3 in OBs led to normal amount of OBs and increased bone mass, since OCs became less active [209]. These results should be interpreted very carefully, since compensation by other receptors or ligands has been reported. In addition, it is likely that negative targeting of BMP signaling pathway components will affect other signaling cascades involved in bone formation, that might compensate a bone promoting effect of BMPs. In the next section we will discuss how BMPs modulate bone formation by mechanisms other than canonical SMAD signaling.

Regulation of osteoclasts by BMPs

OCs are derived from a population of bone marrow mononuclear cells constituting the hematopoietic stem cells. Basically osteoclastogenesis is activated by RANK, RANKL, osteoprotegerin (OPG) and macrophage stimulating factor (M-CSF) [210]. OC maturation is characterized by the fusion of mononuclear OC precursors into giant multinuclear cells (Fig. 6B).

Transcription factors regulating osteoclast differentiation

In contrast to OBs, the signaling pathways and cellular factors involved in the generation of new OCs are mainly involved in inflammatory cascades. In fact, probably the best-described factor driving osteoclastogenesis is *nuclear factor of activated T cells* (*NFAT*) [211]. NFAT regulates the expression of several OC genes, such as the *AP-1* family, *PU.1* and microphthalmia associated factor (MITF) [212]. Disturbances in the expression or activity of these factors have been demonstrated to interfere with osteoclastogenesis *in vivo* [213–216]. Another transcription factor traditionally linked to inflammation that plays an important role in OCs is *nuclear factor kappa-B* (NF-κB). Activated by the up-stream kinase TNF-receptor associated factor (TRAF)-6 (which also targets NFAT [217]), NF-κB is released in the cytoplasm and translocated into the nucleus to regulate specific genes. Inhibition of NF-κB transcriptional activity impairs osteoclastogenesis [218,219]. Finally, the Wnt pathway modulates the generation of OCs, although its activity is not so well defined as in OB formation. A number of studies have shown that the influence of Wnt on OCs formation depends on a tight balance between canonical and non-canonical Wnt signaling, which can promote either bone formation or resorption by different mechanisms [220–222].

Regulation of osteoclast differentiation by BMPs

Compared to the effect of BMPs on osteoblastogenesis, the role of BMPs modulating the differentiation of OCs is not so well reported and much of the information that we have nowadays comes from association studies based on cohorts of patients with bone defects. Moreover, clinical interventions using BMP-based therapy have surprisingly resulted in increased bone resorption, rather than bone formation, suggesting that BMPs potentiate OC differentiation and activation. One example of this is the prospective study on 77 patients undergoing interbody fusion with allograft containing rhBMP-2. After two years follow-up, patients carrying rhBMP-2 allografts displayed an increased rate of end-plate erosion and allograft resorption with subsidence, in comparison with DBM allografts [223]. A similar result was obtained using rhBMP-2 femoral ring allografts to treat patients undergoing anterior lumbar interbody fusion, which resulted in an increased number of non-unions, no significant improvement in the fusion rate and early aggressive bone resorption [224]. Supporting this finding, delivery of rhBMP-2 in non-human primates using collagen implants in metaphyseal bone to treat bone defects resulted in transient bone resorption and increased amount of OCs [225]. Other BMP ligands, such as BMP-7 provoked a similar response. Concretely, bone resorption and no significant increase in bone mass were observed when treating spinal fractures in 5 patients with BMP-7 [226].

Interestingly, using *in vitro* and *in vivo* models it has been suggested that BMPs potentiate OC activity in an indirect manner. In fact, BMPs activate OBs that subsequently induce osteoclastogenesis through the RANKL–OPG pathway [227–231]. Specific knock-out of the BMP type I receptor *ALK3* in OBs decreased bone resorption, thereby increasing bone mass [209]. Recently, it has been shown that over-expression in C2C12 of a constitutive receptor ALK2–R206H associated to FOP provokes an increase in the expression of TGF-β. TGF-β was responsible of enhancing osteoclastogenesis in surrounding tissues or when co-cultured with the pre-OC cell line Raw 264.7 [232]. Increased OC activity was also detected in transgenic mice lacking the BMP antagonist twisted gastrulation (Twsg1) [233]. This was restored by adenoviral-mediated transduction of *Twsg1* in primary OCs or addition of ectopic BMP-2. Finally, *in vitro* stimulation of mature OCs and pre-OC cells with recombinant BMPs or over-expression of components of the BMP pathway has demonstrated that BMPs induce osteoclastogenesis [234]. For example, this was shown by Zheng et al. using the heterodimer BMP-2/-7, which displayed a higher activity than other ligands tested [235].

BMPs in low and high bone mass diseases

BMP-related bone diseases may be classified into two phenotypical outcomes, the loss (low bone mass) or the gain (high bone mass) of bone respectively. During loss of bone, BMP signaling is usually impaired in bone forming cells while BMPs increase the activity of bone resorbing cells. On the other hand, a gain in bone mass is characterized by a BMP dependent increase in the activity of bone forming cells which exceeds normal levels. We will give examples of the BMP related molecular events underlying both low and high bone mass diseases in the following sections (Fig. 7).

Osteoporosis

The most prominent and prevalent low bone mass disease is osteoporosis, a systemic disease affecting more than 75 million people in the United States, Europe and Japan [236]. Osteoporosis is characterized by a decrease in bone mass and a disturbed micro-architecture of bone and its ECM. It leads to severe bone fragility and an increased risk in bone fracture. Osteoporosis mostly affects elderly people and postmenopausal women displaying low serum levels of hormones regulating bone remodeling (such as PTH and Estrogen), and often requires pharmacological or surgical intervention.

Probably the key factor associated to osteoporosis is the low activity of bone forming cells, while the activity of bone resorbing cells is increased. This results in misbalanced turnover rate and ratio between bone formation and resorption during remodeling. Since osteoporosis is a systemic disease, there are various local and systemic causes suggested. An overview on them is subject to several excellent reviews [237,238].

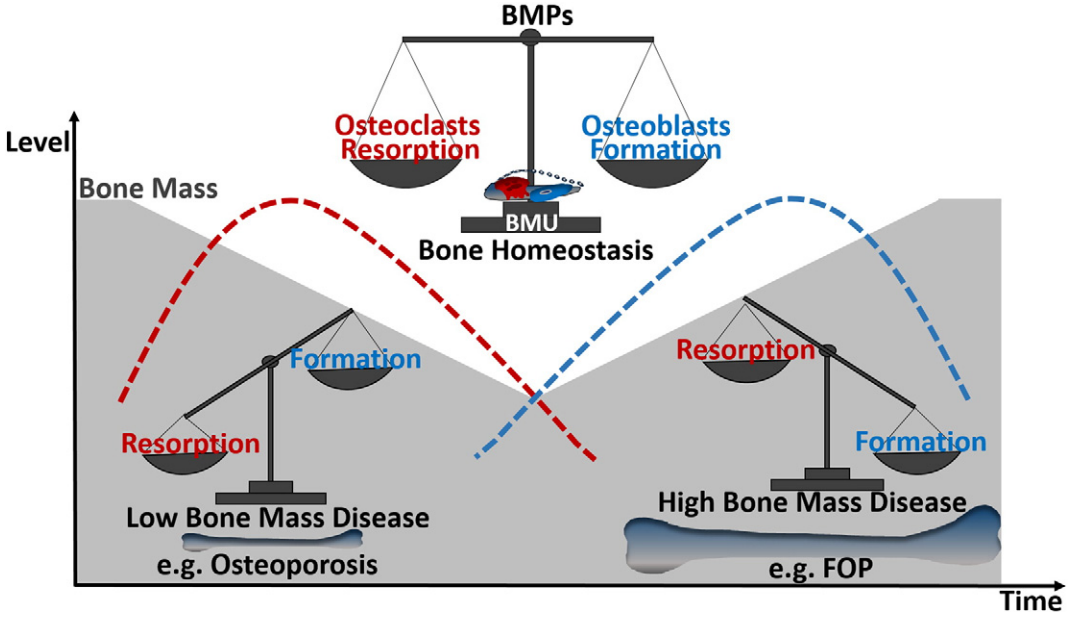

Fig. 7. Perturbation in BMP signaling is associated to bone diseases. Since BMPs modulate the activity of the main cell types regulating bone remodeling, hyper-activation or disruption of the BMP signaling cascade in bone has been linked to a number of skeletal diseases. As such, loss of BMP activity in osteoblasts tilts the balance towards osteoclast-mediated bone resorption, which eventually results in low bone diseases such as osteoporosis. On the contrary, hyper-activation of BMP signaling in mesenchymal progenitors and osteoblasts leads to heterotopic ossifications (e.g. FOP), where the over-stimulated activity of osteoblasts cannot be compensated by bone resorption mechanisms.

Osteoporosis, BMP signaling in bone forming cells

Deregulation of BMP signaling associated to osteoporosis has been observed at several levels. In osteoporotic bone tissue from patients and animal models, extracellular bioavailability of BMPs was reduced [239,240]. This could be traced back to the lack of systemic BMP-inductive hormones such as parathyroid hormone (PTH) or estrogen which both induce BMP expression in bone tissue [241,242]. The use of anabolic hormones to increase local BMP bioavailability thus appears as a promising strategy to treat osteoporosis [27,239,243,244]. As such, PTH treatment enhanced phosphorylation of SMAD1 and increased commitment of MSCs to the OB lineage [240]. Moreover, PTH treatment was able to significantly suppress BMP-induced inhibitory SMAD6 expression [245] and to restore bone-forming abilities of BMPs in aged rats [246]. PTH treatment also suppressed the expression of the BMP antagonists Sclerostin, Chordin and Dickkopf1 [247–250]. The regulation of the *SOST* gene, encoding for the dual Wnt and BMP antagonist Sclerostin, seems to play a leading role in osteoporosis, suggesting a tight crosstalk between Wnt and BMP [251,252]. These findings were confirmed by gene targeting in animal models: as such, loss of *Bmp2* leads to a phenotype similar to osteoporosis in mice [177]. Furthermore, overexpression of BMP antagonists such as Noggin or Gremlin caused osteopenia and spontaneous fracture similar to what is observed in osteoporosis [253].

On the level of BMP receptors, it has been suggested that also heterooligomeric BMP receptor complexes comprising type I and type II receptors do not assemble properly in the plasma membrane of osteoprogenitor cells isolated from mice with low bone mass [254]. By performing transcriptional profiling of osteoporotic samples from human bone, it was shown that also several BMP intracellular transcriptional inhibitors such as MAB21L2 were upregulated [255]. However, it is not clear to date, whether natural occurring genetic alterations in BMP signaling components cause osteoporosis directly [256–258]. In this sense, some studies suggested that single nucleotide polymorphisms (SNPs) of new candidate genes (e.g. BMP15) can predispose patients to osteoporosis [259].

Controversial results exist with respect to intracellular pathway dysregulation. It was shown that in human osteoporotic bone progenitors the canonical SMAD signaling behaved normally while non-SMAD MAPK pathways displayed an impaired BMP responsiveness [260]. Consistent with these findings, it was shown that in MSCs, BMP-induced expression of OB-associated genes (e.g. *ALP* and *osteopontin*) requires activation of MAPK and PI3K [76,261–263]. Interestingly, bone progenitors from osteoporotic patients were shown to display an impaired migratory behavior upon BMP stimulation, suggesting that both BMP-induced differentiation and recruitment may play a role [264]. Some years ago, another study suggested that impaired BMP signaling in osteoporosis is due to the presence of B-cell synthesized humoral anti-BMP2 antibodies [265]. As such, osteoporosis might be considered also as an auto-immune disorder causing a high anti-BMP2 titer, which prevents osteogenic differentiation of bone progenitors.

Taken together, there exists a strong correlation between increased age, lack of systemic hormones and the likelihood to develop osteoporosis due to impaired BMP signaling in bone progenitor cells. To fully understand the molecular basis, more research is required focusing on BMP-signaling in bone progenitors from osteoporotic animal models and patients.

Osteoporosis, BMP signaling in bone resorbing cells

As said before, in addition to reduced OB function, osteoporosis is characterized by exacerbated OC activity. For a long time, BMP activity in bone was thought to be restricted to bone forming cells with mesenchymal origin such as mesenchymal stem cells, OBs or osteocytes. Nevertheless, recent research showed that also hematopoietic cells such as OCs respond to BMPs. This is making BMPs to act as central regulators mediating bone remodeling at the level of OB and OC activities. In that respect it was shown that BMPR-IA and BMPR-II as well as SMAD1 and SMAD5 are expressed in isolated OCs. It was therefore suggested that BMPs directly stimulate OC function via BMPR-IA and BMPR-II [234]. Moreover BMP-2 was able to directly stimulate bone resorption by induction of OC formation [266–268]. Genetic ablation of *Bmpr1a* in differentiated OCs increased osteoblastic bone formation in mice [269]. On the other hand, genetic ablation of *Tsg*, an extracellular BMP antagonist, showed a striking enhancement of osteoclastogenesis caused by increased cell fusion, differentiation, and function of OCs [270]. In fact, the current picture suggests that signaling induced by BMP-2 and BMP-4 is a prerequisite for osteoclastogenesis [227]. Interestingly, the BMP-induced SMAD and non-SMAD MAPK pathways are utilized during osteoclastogenesis at different stages [271]. While BMP-induced p38 signaling was required for early OC pre-fusion, the canonical SMAD pathway was a pre-requisite for late OC fusion. It was also suggested, that BMP-9 protects OCs from apoptosis via induction of SMAD and non-SMAD pathways [272]. In contrast to this, BMP-7 was shown to inhibit the differentiation of human $CD14^+$ monocytes to OCs [273]. It is likely, that different BMP family members exert very different and even opposing effects on osteoclastogenesis. Moreover spatiotemporal appearance of distinct BMP family members over the course of bone remodeling may be important to maintain the balance between bone formation and resorption. Current pharmacological attempts to treat osteoporosis focus on targeting OC activity via the RANK–RANKL–OPG pathway [237,274], representing the major OC activation pathway (see above). A specific inhibition of OC BMP signaling, not affecting the positive effects on OB differentiation, may be a future challenge in developing new strategies to treat osteoporosis.

Bone fractures

Endogenous fracture healing is a multistep mechanism coordinated by BMPs. Two independent mechanisms responsible for fracture healing are i) endochondral bone formation and ii) intramembranous bone healing. Whereas flat bones such as bones from the skull, clavicle and trabecular bones heal via intramembranous ossification, diaphyseal fractures heal by endochondral mechanisms, forming a cartilaginous callus intermediate. This callus requires further remodeling and maturation to become finally replaced by bone [275,276]. Endochondral bone healing initiates by inflammation. White blood cells infiltrate the fracture and secrete inflammatory cytokines such as TNF-α and interleukins [277]. Inflammation teams up with hypoxic conditions and changes in the mechanical properties of the fracture critically influencing repair [278]. Maturation of the callus is driven by Wnt signaling regulating the proliferation of chondrogenic progenitors and their differentiation into mature chondrocytes through condensation [279–281]. At this point, BMPs were shown to support chondrocyte maturation [282,283]. An angiogenic switch within the avascular hypoxic callus involves the expression of vascular endothelial growth factor (VEGF) leading to blood vessel recruitment. The callus vascularization initiates remodeling and eventual resorption. Callus resorption goes hence with recruitment of osteoprogenitors which differentiate into osteoblasts and finally, mature osteocytes. Recent data show that mesenchymal progenitors respond to BMPs by directional migration *in vitro* [284]. Much better understood is their osteogenic differentiation driven by canonical–SMAD signaling. It may be therefore that the positive effects of BMPs on fracture healing are twofold, i) recruitment of progenitors via induction of cell migration and possibly chemotaxis and ii) induction of their differentiation [91]. Some severe fractures also termed “critical size defects” fail to heal without intervention [285]. Therapeutic application of BMPs to support critical size defect repair is performed since many years. However, application of recombinant BMPs to fracture sites in some cases failed to improve bone healing as expected [286]. Responsible may be the local feedback of BMP antagonists as they critically balance BMP action in fracture healing [287]. Currently, cell type specific actions of the different BMP ligands in fracture healing are under investigation [288]. In focus are the molecular BMP-signaling mechanisms on the three main cell types important for fracture

repair: chondrocytes, endothelial cells and osteoprogenitors as well as investigating redundant or exclusive functions of individual BMP ligands in fracture repair.

Heterotopic bone formation

An outstanding example of a disease characterized by increase in bone mass is fibrodysplasia (myositis) ossificans progressiva (FOP), a rare autosomal dominant disorder characterized by progressive heterotopic chondrogenesis and HO of the soft connective tissues [289]. FOP patients are heavily "skeletalized" with bone growing at extraskeletal sites. The molecular aspects of FOP and its dramatic phenotype were itself subject to a number of excellent reviews [290–293]. Work on FOP has revealed that canonical SMAD and non-SMAD signaling pathways are overactive in cells from FOP patients [294–296] which could be traced back to a heterozygous mutation in chromosome 2q23–24. This mutation found in nearly all sporadic and familial cases of classic FOP leads to a single amino acid exchange (617G→A; R206H) within the GS activation domain of *ACVR1*, the BMP type I receptor also known as ALK2. Exchange of arginine 206 for histidine leads to a hyper-activation of ALK2 [297], which is considered the underlying molecular cause of the enhanced BMP signaling and thus ectopic chondrogenesis, osteogenesis and joint fusions during the development of FOP [298,299]. In some other FOP patients, atypical ALK2 mutations have been reported, also resulting in a hypersensitive activation of the ALK2 receptor [300–302]. Interestingly, the GS domain located R206H mutation impairs the inactivation of the receptor by preventing the interaction with FKBP12, which function has been afore described [299,303,304]. Lack of FKBP12 binding to mutant ALK2 therefore promotes its gain of function with respect to signal transduction.

Moreover, recent studies have shown that not only the deregulated BMP signaling in bone progenitors drives FOP but other events are required to stimulate the HO including activation of sensory neurons, mast cell degranulation, lymphocyte infiltration, skeletal myocyte cell death, and endothelial–mesenchymal transition (EndoMT) [305]. It was shown that ECs expressing the mutant ALK2 R206H receptor undergo a dedifferentiation process which results in mesenchymal like stem cells. These pluripotent cells may be able to further differentiate into active OBs, thereby contributing to HO [17]. It may be therefore, that the cellular origin of ectopic bone in FOP may not be the classical MSC derived bone progenitor but ECs undergoing EndoMT [306,307].

Importantly, HO was first observed in wounded soldiers, where soft tissue trauma is associated with HO [308]. Subsequently, animal models demonstrated that muscle trauma increased the expression of *BMP-2*, *BMP-4*, *Sox9* and *Runx2* and that BMP-2 sensitizes the muscle to undergo trauma induced HO [309]. In the case of FOP patients, who display abnormally elevated BMP signaling, physical trauma induced by surgical intervention triggers HO [310]. These findings suggested that local inflammatory responses were acting in combination with the overactive BMP signaling in order to promote ectopic bone formation and, indeed anti-inflammatory drugs constitute the main treatment for FOP [293]. Additionally, the constitutive active ALK2-R206H receptor was shown to be druggable by BMPRI specific small molecule inhibitors such as dorsomorphin or LDN-193189 which are currently further characterized and optimized with respect to specificity, pharmacokinetics and dynamics [311,312]. Understanding the molecular mechanisms of FOP and HO may also be of advantage to foster and amplify cell responses to BMPs during bone repair. A promising strategy seems to use FK506 (tacrolimus), a clinically approved immunomodulatory compound which binds FKBP12. FK506 interacts with FKBP12 and is able to dissociate it from BMP type I receptors, e.g., ALK1, ALK2, and ALK3 which mimics the effect of the ALK2-R206H receptor FOP mutation. *In vitro* FK506 was able to induce BMP-dependent SMADs in the absence of BMPs [313] and to rescue defective BMP receptor activity [314]. FK506 like compounds, such as rapamycin, were sufficient to promote SMAD signaling and induce chondrogenic and osteogenic differentiation [313,315,316] by induction of BMP type I receptor signaling.

Using BMP receptor specific inhibitors on one hand and drugs which specifically target BMP signaling inhibitors on the other may therefore open novel roads to treat overactive BMP signaling in FOP but also to facilitate cellular responses to BMPs to stimulate repair.

Conclusions: therapeutic potential of modulating the BMP pathway

BMPs have been extensively demonstrated to regulate bone formation during development. Therefore this characteristic has focused the development of BMP-based strategies to promote bone regeneration and tissue engineering. As such, probably the most intended use of BMPs consists in the treatment of bone pathologies, such as bone fractures. Non-union fractures are usually treated using autograft bone implants, where bone is collected from the iliac crest. This has been of particular interest in dentistry [317]. Nevertheless, this approach is not always possible and many factors influence the clinical outcome of such procedures [318]. Therefore, establishing a standardized therapy has boosted the optimization of treatments based on BMPs. To date, BMP-2 and BMP-7 (rhOP-1) are clinically approved by the FDA and other BMPs have undergone or are currently undergoing clinical trials [319–321]. Besides application for critical size defects, many successful off label usages e.g. for spine fusion and oral and maxillofacial reconstruction were reported [322,323].

Unfortunately, direct delivery of BMPs to reconstruct bone defects has often resulted in undesirable tissue responses, such as bone resorption, pseudarthrosis, and local inflammation [324,325]. Moreover, the BMP doses currently tested are relatively high, which results in high costs and side effects [326]. In this sense, current efforts are based on improving the efficacy of BMP treatments by direct modification of the BMP components (for example, making them resistant to their own natural antagonists [327–331]), synergistic combination of BMPs with other bone inducing factors (such as IGF, VEGF and platelet-derived growth factor (PDGF) [332,333]), but also the development of new scaffolds and carrier systems to deliver BMP ligands in a more effective manner [121,122,334–339].

In summary, in this review we have described current evidence highlighting the role of components of the BMP signal transduction pathway maintaining the homeostasis of the bone tissue. The continuous development of specific antagonists of BMP signaling, as well as the research performed on methodologies to improve the bioavailability and activity of BMP ligands has led to the identification of novel BMP regulators and improved our understanding about the cellular and molecular determinants underlining several bone diseases. Nevertheless, using this knowledge further research is still necessary to develop new therapeutic approaches that contribute to find a cure for a broad spectrum of bone diseases.

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