Bone Reports 10 (2019) 100207

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

Bone morphogenetic proteins: Their role in regulating osteoclast differentiation

Raphael Huntley[a], Eric Jensen[a], Rajaram Gopalakrishnan[a], Kim C. Mansky[b,*]

[a] Diagnostic and Biological Sciences, School of Dentistry, University of Minnesota, Minneapolis, MN 55455, United States of America
[b] Developmental and Surgical Science, School of Dentistry, University of Minnesota, Minneapolis, MN 55455, United States of America

ARTICLE INFO

Keywords:
BMP
Osteoclasts
SMAD1/5
MAPK
Coupling
Receptors

ABSTRACT

The ability to create recombinant bone morphogenetic proteins (BMPs) in recent years has led to their rise as a common clinical adjuvant. Their application varies, from spinal fixation to repairing palatal clefts, to coating implants for osseointegration. In recent years questions have been raised as to the efficacy of BMPs in several of these procedures. These questions are due to the unwanted side effect of BMPs on other cell types, such as osteoclasts which can resorb bone at the graft/implant site. However, most BMP research focuses on the anabolic osteoinductive effects of BMPs on osteoblasts rather than its counterpart- stimulation of the osteoclasts, which are cells responsible for resorbing bone. In this review, we discuss the data available from multiple *in-vitro* and *in-vivo* BMP-related knockout models to elucidate the different functions BMPs have on osteoclast differentiation and activity.

1. Introduction

In 1889, Senn inadvertently found that bone rudiments initiate bone formation (N S, 1889). However, it appears that, Senn had been beaten by about 300 years as the Dutch surgeon, Job van Meekeren recorded the use of dog bone to repair a human cranial defect in 1668 (Meekeren, 1668). More recently, the work of Urist in 1965 is credited for the discovery that a specific component of bone, which he called BMP or bone morphogenetic protein, induced ectopic bone formation (Celeste et al., 1990; Urist, 1965). His work implanting decalcified bone in muscle pouches led him to prove the osteoinductive properties of BMPs, and hence the derivation of the name. Throughout the 1970's Dr. Urist's laboratory demonstrated the role of BMPs in bone formation. In the 1980 and early 1990's, BMPs were sequenced and cloned which paved the way for their use in clinical applications (Wozney et al., 1988). It is now known that not all BMP ligands induce ectopic bone formation, and various highly homologous BMPs have varying effects on osteogenesis. Some BMPs, such as BMP 3, inhibit bone formation (Kokabu et al., 2012), while others, termed osteogenic BMPs (BMPs 2, 4, 5, 6, 7, and 9), are able to induce ectopic bone (Luu et al., 2007).

1.1. Clinical use and complications of BMPs

In 1988 the first sequences of human BMP ligands were published, and the sequences of other BMP ligands followed soon after. This lead to the clinical use of recombinant BMPs to be approved for acute bone fractures, non-unions and spinal fusions (Boden et al., 2000; Burkus et al., 2005; Friedlaender et al., 2001; Govender et al., 2002; Kleeman et al., 2001). Both BMP2 and BMP7 may cause early osteolysis resulting in implant dislodgment in patients following spinal fusion surgery (Mroz et al., 2010; Simmonds et al., 2013). These effects may be due to the stimulation of BMP2 on osteoclast differentiation and activity in addition to their osteogenic effects through osteoblasts. To date, most of the research has focused on the effect of BMPs on osteoblasts and bone deposition rather than osteoclasts. In this review, we focus on some of the *in-vitro* and *in-vivo* results available from our lab and others on the regulation of osteoclasts by BMPs.

1.2. Osteoclast biology

The bone is comprised of multiple cell types, including osteoblasts, mesenchymal-derived cells responsible for synthesizing bone and bone degrading osteoclasts, which arise from hematopoietic derived stem cells (Teitelbaum, 2000). Bone homeostasis and normal skeletal function result from the proper balance of osteoblasts and osteoclasts. To maintain this balance in the adult skeleton, osteoblasts and/or osteocytes, terminally differentiated osteoblasts, produce two cytokines required to promote osteoclast differentiation, macrophage colony

* Corresponding author at: 16-262B Moos Tower, 515 Delaware St SE, Minneapolis, MN 55455, United States of America.
E-mail address: kmansky@umn.edu (K.C. Mansky).

https://doi.org/10.1016/j.bonr.2019.100207
Received 6 September 2018; Received in revised form 9 April 2019; Accepted 2 May 2019
Available online 05 May 2019
2352-1872/ © 2019 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).

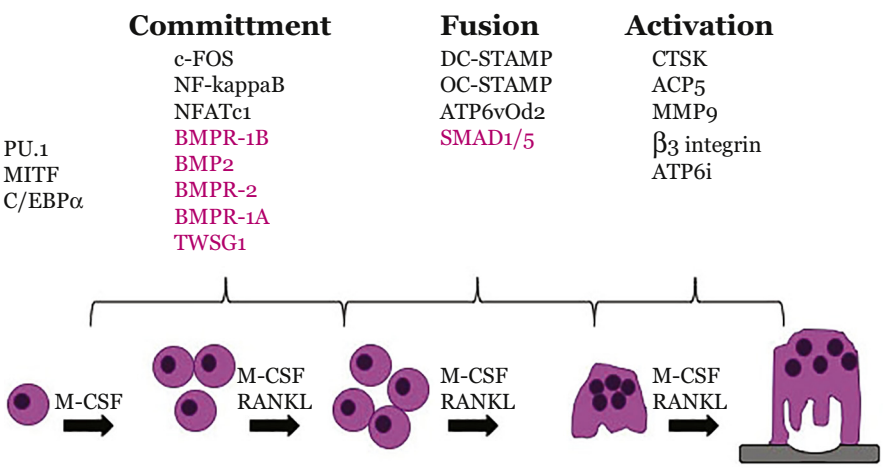

Fig. 1. Osteoclast differentiation. Drawing depicts osteoclast differentiation and proteins involved in commitment, fusion and activity. Names of proteins under each heading are involved in the process described by the heading but does not indicate time of expression during osteoclast differentiation. Proteins involved in BMP signaling are highlighted in pink and based on data presented in Jensen et al. (2010) and Rodriguez et al. (2009). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL) (Xiong et al., 2011).

Osteoclasts are derived from the monocyte/macrophage lineage. The transcription factor PU.1 which induces expression of c-FMS, the receptor for the cytokine M-CSF, is necessary for commitment to the monocyte/macrophage lineage (Tondravi et al., 1997). RANKL stimulation induces expression of the master regulator of osteoclast differentiation, NFATc1, through both c-Fos and NF-κB pathways (Grigoriadis et al., 1994; Iotsova et al., 1997; Takayanagi et al., 2002). M-CSF stimulation has been shown to promote osteoclast precursor proliferation while RANKL stimulates osteoclast precursors to exit the cell cycle and terminally differentiate (Ross, 2006).

As part of terminal differentiation, osteoclast precursors undergo fusion to become multinuclear cells (Fig. 1). In recent years, proteins necessary for osteoclast fusion have been identified in mouse models. One such protein is DC-STAMP or the "master fusigen" and the related protein OC-STAMP (Mensah et al., 2010; Yagi et al., 2005). Recent work by Verma et al. suggests that one role of DC-STAMP in the fusion process is the exposure of phosphatidylserines on the surface of osteoclasts, and the exposure of these phosphatidylserines regulates the activity of several proteins including annexins, S100A4 and syncytin1 (Verma et al., 2018). In osteoclasts null for ATP6v0d2, another protein that has been demonstrated to be necessary for osteoclast fusion, there is decreased expression of ADAM 8 and 12 (Lee et al., 2006). ADAMs cleave extracellular regions of transmembrane proteins and are involved in cell-cell and cell-matrix adhesion providing a possible mechanism by which ATP6v0d2 regulates osteoclast fusion (Lee et al., 2006).

Small GTPases such as Rho, Rac and Cdc42 have been shown to be involved in osteoclast differentiation, resorption and survival (as reviewed in Weivoda and Oursler, 2014). Guanine nucleotide exchange factors (GEFs) such as Vav3 which link small GTPases with integrins have also been shown to be essential for osteoclast activity (Faccio et al., 2005). For bone resorption to begin, multinuclear osteoclasts must attach to the bone surface. This attachment which occurs through activation of integrins and c-Src, a RANKL activated kinase, results in the cytoskeleton being rearranged to allow for fusion and resorption (Boyce and Xing, 2008; Teitelbaum, 2000; Teitelbaum, 2006). Osteoclasts express the integrin αvβ3 which recognizes the arginine-glycine-aspartic acid (RGD) peptide expressed in bone matrix proteins (Teitelbaum, 2006). Besides allowing for the attachment of osteoclasts to bone matrix proteins, αvβ3 along with actin binding proteins such as vinculin and talin surround an F-actin core to form numerous podosomes, a structure essential for osteoclast resorption (Fukunaga et al., 2014; Zou et al., 2013). Podosomes condense into a thick ring of actin which allows for the attachment to the bone surface to form a sealing zone where acidified cytoplasmic vesicles are transported (Jurdic et al., 2006; Pfaff and Jurdic, 2001; Saltel et al., 2008; Saltel et al., 2004; Stenbeck, 2002). Besides attachment, the sealing zone allows for the "sealing off" of the resorptive pit into which osteoclasts secrete proteases such as cathepsin K as well as H^+ and Cl^- (Seeman, 2009). Optimal bone resorption is a cycle consisting of cell migration to a resorption site, resorption of the underlying bone matrix, detachment and migration to next resorption site. Integrins are necessary for all the steps in this process (Georgess et al., 2014). During bone resorption by the osteoclast, growth factors embedded in the bone matrix are released and either recruit osteoblasts to the areas of resorption or help stimulate osteoblast activity (Charles and Aliprantis, 2014).

1.3. BMP structure and function

BMPs are a large family of growth factors that have been demonstrated to regulate development and/or maintenance of adipose, neurological, ophthalmic, cardiovascular, pulmonary, gastrointestinal, urinary and musculoskeletal systems (Abe, 2006; Carreira et al., 2014; Wang et al., 2014). The BMP family resides within the larger TGF-β super-family, which is defined by its ability to bind to serine or threonine in cell surface receptors. In cell types other than osteoclasts, BMPs have been shown to initiate chemotaxis, cell growth, differentiation, and apoptosis; however, in osteoclasts BMPs have been shown to enhance differentiation, activity, and inhibit apoptosis (Broege et al., 2013; Fong et al., 2013; Jensen et al., 2010; Rodriguez et al., 2009; Wang et al., 2014). BMPs usually function by short-range diffusion, although they can create a gradient by binding to extracellular BMP binding proteins such as noggin, chordin, gremlin, and twisted gastrulation. The subfamily of BMPs is large with 30 known ligands, and they comprise one third of the known TGF-β superfamily ligands (Abe, 2006; Bragdon et al., 2011; Wang et al., 2014).

BMP ligands are produced as precursor proteins that are dimerized in the cytoplasm before being cleaved and secreted in their mature form. They function by binding to BMP receptors, which induces formation of a hetero-tetrameric receptor unit consisting of two type 1 receptors and two type 2 receptors (Miyazono et al., 2010; Wang et al., 2014). Both type 1 and type 2 receptors are single pass transmembrane proteins with an extracellular domain and a cytoplasmic domain with serine/threonine kinase function (Miyazono et al., 2010). There are three type 1 receptors capable of binding to BMP ligands (BMPR-1A, BMPR-1B, ActR-1A); and three type 2 receptors also with the ability to bind to BMP ligands (BMPR-2, ActR-2A, ActR-2B) (Miyazono et al., 2010). BMP ligand-receptor complex affinity vary by both ligand and receptor type (Mueller and Nickel, 2012). BMPs can also bind to a preformed receptor complex; in this case constitutively active BMPR-2 is already linked to BMPR-1 for BMP binding. BMPR-2 phosphorylates BMPR-1 which in turn leads to activation of SMAD 1/5/9 pathway.

SMAD 1/5/9 is known as the canonical BMP signaling pathway (reviewed in Miyazono et al., 2010). A second mechanism for BMP ligands to activate BMP signaling is when BMP receptors are not dimerized but binding of BMP ligand to BMPR-1A recruits BMPR-2 into a complex, which is internalized *via* caveolae (Miyazono et al., 2010). This signaling complex has been shown to initiate non-SMAD pathways or non-canonical signaling pathways such as MAPK signaling (Miyazono et al., 2010).

2. BMP ligands expression in osteoclast

2.1. Indirect regulation of osteoclasts by BMP

As will be described in the following sections, multiple groups have shown that BMPs can directly regulate osteoclast differentiation. However, earlier studies demonstrated that BMPs regulate osteoclast differentiation indirectly through actions on chondrocytes, osteoblasts and osteocytes. As early as 1995, cultures of rat bone marrow cells treated with OP-1 were demonstrated to enhance the ability of vitamin D_3 to induce formation of TRAP positive osteoclasts *in vitro* (Hentunen et al., 1995). Both murine and chicken chondrocytes have been shown to express *RankL* mRNA and protein which was significantly enhanced by BMP2/SMAD1 activation (Usui et al., 2008). It has been suggested that RANKL protein expression from chondrocytes can regulate osteoclast differentiation at growth plates to remove calcified matrix through BMP induction (Usui et al., 2008). Kanatani et al. were the first to demonstrate that BMP2 could stimulate mature osteoclasts in the presence but not absence of stromal cells (Kanatani et al., 1995). Other studies have shown that BMP2 stimulates bone resorption by osteoclasts through modulation of *Rankl* mRNA expression by stromal cells (Granholm et al., 2013; Tachi et al., 2010). Other groups have demonstrated that BMP2 can modulate the expression of *Opg* mRNA by osteoblasts (Hofbauer et al., 1998). BMP2 in combination with IL-1α was shown to upregulate expression of *Rankl* mRNA by osteoblasts and indirectly enhance osteoclast differentiation hinting that BMP2 may enhance bone resorption during conditions resulting in inflammation (Koide et al., 1999). Additionally, PGE_2 treatment of osteoblast/osteoclast cultures increase the *RankL* to *Opg* mRNA ratio and thereby indirectly regulates osteoclast differentiation (Blackwell et al., 2009). Even BMP inhibitors such as Noggin have been shown to affect osteoclast differentiation through Noggin's effect on stromal/osteoblast differentiation (Abe et al., 2000). Noggin's direct effect on osteoclast differentiation will be discussed in a later section of this review labelled “BMP inhibitors”. Analysis of a mouse model with deletion of *Bmpr1a* in osteoblasts demonstrates an increase in bone mass due to disruption in the ratio of RANKL to OPG protein resulting in a decrease in osteoclast differentiation and activity (Kamiya et al., 2008). Until more recently, the critical role of osteocytes in regulating skeletal development were not well understood. In a mouse model using *Dmp1-Cre* to disrupt *Bmpr1a* in osteocytes, the authors determined that the expression of *Opg* bone mRNA was increased and *RankL* bone mRNA was decreased, leading to a sharp reduction in osteoclast differentiation and activity (Kamiya et al., 2016). These results are similar to the observations noted in mice null for *Bmpr1a* in osteoblasts. These data collectively suggest that BMPs can act on other skeletal cells and indirectly regulate osteoclast differentiation and activity.

2.2. Direct regulation of osteoclasts by BMP

In recent years, it has become more evident that BMPs also have direct effects on osteoclast differentiation. Further elucidation on the mechanisms by which different ligands induce responses is necessary to fully understanding the role of BMPs in the skeleton. With the increasing use of BMPs in the clinic for osteogenic purposes, clinicians should possess a solid understanding of the effects of BMPs on osteoclasts which may negate the desired effect. By immunohistochemical staining, osteoclasts were demonstrated to express BMP ligands 2, 4, 6 and 7 and later confirmed by others through RT-qPCR and western blot (Garimella et al., 2008; Jensen et al., 2010; Kaneko et al., 2000; McCullough et al., 2007; Okamoto et al., 2006). In the following sections, we will discuss what is known about individual BMP ligands, and their effects on osteoclast differentiation and activity.

2.3. BMP1

Originally BMP1 was incorrectly categorized as a member of TGF-β superfamily but in reality, it is a zinc dependent metalloproteinase shown to cleave the C-terminus of procollagen type I, II and III (Kessler et al., 1996; Sarras Jr, 1996). BMP1 is a protease important in the activation of TGF-β and BMP2/4 and is involved in processes such as morphogenesis, tissue repair and tumor progression (reviewed in Vadon-Le Goff et al., 2015). Anderson et al. demonstrated that rat and human osteoclasts express BMP1 protein in their cytoplasm; however, the function of BMP1 in osteoclasts is still unknown (Anderson et al., 2000).

2.4. BMP2

Multiple groups of researchers have demonstrated that osteoclasts express *Bmp2* mRNA in both early lineage cells (bone marrow macrophages) and mature osteoclasts (Itoh et al., 2001; Jensen et al., 2010). In early hematopoietic stem cells, BMP2 can increase or decrease cell proliferation, depending on concentration, but BMP stimulation did not affect cell lineage commitment (Bhatia et al., 1999). This differential response to BMP2 may be a method of regulating maintenance or cell expansion of early lineage cells. In lineage committed mononuclear osteoclast precursors, BMP2 acts to enhance survival and proliferation, and its phenotypic effect can be potentiated by IL-1α, prostaglandin, vitamin D3, and PTH (Abe et al., 2000; Itoh et al., 2001; Koide et al., 1999).

We are currently investigating the phenotype of mice null for *Bmp2* expression in osteoclasts. In our analysis of the *in vitro* cultures of osteoclasts from *Bmp2*$^{fl/fl}$*;LysM-Cre* mice, the cells are smaller but more numerous than in the wild type littermates (Mansky/Gopalakrishnan, unpublished results). These data support our studies and the studies of others that BMP2 enhances osteoclast size but is not necessary for osteoclast differentiation.

2.5. BMP3

BMP3, also known as osteogenin, or a “non-canonical” BMP, is the most abundant BMP in the bone matrix, embedded by osteoblasts and osteocytes (> 65% of BMP stored in bone (Daluiski et al., 2001)). It acts as a negative regulator of bone density by inhibiting osteoblast differentiation (Daluiski et al., 2001). However, BMP3 expression has not yet been reported in osteoclasts and cartilage and is thought to be expressed primarily by mature osteoblasts (Kokabu et al., 2012).

2.6. BMP4

BMP4 is closely related to BMP2, and both share common osteogenic functions. Global knockouts for BMP4 are embryonically lethal before the onset of skeletogenesis (Winnier et al., 1995). In mice with a conditional deletion of *Bmp2* and *Bmp4* using *Prx1-Cre*, there is a delay in endochondral process partially due to a delay in osteoclast recruitment to the mineralized cartilage (Bandyopadhyay et al., 2006). Both BMP2 and 4 protein is highly expressed by osteoclasts at fracture sites (Spector et al., 2001). Overexpression of BMP4 in bone (*Col1a1-Bmp4*) leads to osteopenia due to increased osteoclast number (Okamoto et al., 2006). These mice however, die shortly after birth making it difficult to study skeletal development (Okamoto et al., 2006). BMP4 was shown to upregulate OPG in ST2 cell line suggesting that BMP4 could indirectly

regulate osteoclast differentiation through modulation of the RANKL/OPG ratio (Tazoe et al., 2003).

2.7. BMP5

Due to their interesting phenotype, BMP5 knockout mice have been studied for almost 100 years, although at the time, and for about 75 years after, researchers did not know that the mice were BMP5 deficient (King et al., 1994). Later BMP5 studies were able to clarify its role in bone homeostasis. BMP5 is able to stimulate osteoclast formation in a biphasic mode, although stimulation requires higher doses than BMP2 (Wutzl et al., 2006). Combinations of BMP2, 5 and 6 can stimulate osteoblastogenesis but had no additive effect on osteoclast differentiation (Wutzl et al., 2006). Lastly, giant cell tumors have been shown to express BMP5 as well as other BMP ligands (Kudo et al., 2009).

2.8. BMP6

Although most closely related to BMP5, BMP6 acts in an opposing manner to BMP5 in osteoclasts. In co-cultures with osteoclasts and osteoblasts, BMP6 inhibits osteoclastogenesis (Simic et al., 2006). This effect was shown to be indirect, working through osteoblasts. BMP6 mRNA and protein expression increases during osteoclast differentiation (Pederson et al., 2008). BMP6 expressed by osteoclasts may also regulate bone formation. Conditioned media from mature osteoclasts stimulated mesenchymal cell nodule formation and was attenuated by a BMP6 neutralizing antibody (Pederson et al., 2008).

2.9. BMP7

BMP7, also called OP1, was discovered in 1990 (Ozkaynak et al., 1990). In human osteoclast cultures, BMP7 inhibited CD14+ monocytes differentiation into osteoclasts (Maurer et al., 2012). *c-Fos* and *Nfatc1* protein expression were upregulated but were not stable in the presence of BMP7, and *MafB*, an inhibitor of osteoclast differentiation, was not downregulated in the presence of BMP7 (Maurer et al., 2012).

2.10. BMP8

BMP8 is expressed in developing skeletal tissues but does not seem to play an important role in osteoclastogenesis (DiLeone et al., 1997; Ozkaynak et al., 1992). There is no research to date to suggest that BMP8 regulates osteoclast differentiation or activity.

2.11. BMP9

BMP9 increases osteoclast resorption and survival by activation of the SMAD pathway (Fong et al., 2013). Recently BMP9 has been shown to enhance osteoclast differentiation by upregulating ALK1 receptor and inhibiting activation of the ERK1/2 signaling pathway (Li et al., 2016).

3. BMP inhibitors

BMP signaling is tightly regulated by multiple extracellular binding proteins, which modulate the diffusion from a source and control BMP signaling (Rosen, 2006). Multiple extracellular BMP regulators exist and can both regulate the function of BMPs but can also regulate each other (Rosen, 2006). Mice with a global knockout of the BMP inhibitor *Twisted gastrulation* (*Twsg1*) demonstrated increased numbers, size and activity of osteoclasts but no detectable change in osteoblast activity (Rodriguez et al., 2009). Osteoclasts from the *Twsg1* knockout mice also have increased phosphorylated SMAD1/5/8 expression (Rodriguez et al., 2009). Additionally, osteoclast differentiation was inhibited by overexpression of *Twsg1* (Pham et al., 2010). Mice overexpressing Noggin, another extracellular BMP modulator, showed increased bone volume due to decreased bone formation and resorption (Okamoto et al., 2006). Co-culture experiments with osteoblasts from *noggin* overexpressing mice demonstrated that Noggin inhibits osteoclast formation by attenuating BMP activities in osteoclasts as osteoclast differentiation could be rescued by addition of BMP2 (Abe et al., 2000; Okamoto et al., 2006). Jensen et al. demonstrated that addition of recombinant Noggin to osteoclast cultures before day 3 inhibited osteoclast fusion and differentiation while Noggin addition after day 3 resulted in no significant change in osteoclast differentiation (Jensen et al., 2010). Lastly, a group of researchers demonstrated that osteoclasts express *sclerostin* which can inhibit BMP6 and BMP7 but not BMP2 or BMP4 (Kusu et al., 2003). They demonstrated that *sclerostin* is secreted only by bone resorbing osteoclasts, and therefore, may be a mechanism by which bone apposition is inhibited in the vicinity of bone resorption (Kusu et al., 2003). However, expression of *sclerostin* by osteoclasts has not been reported by others and remains controversial.

4. BMP receptors

Multiple studies have demonstrated that osteoclasts express BMP receptors *Bmpr-1a*, *Bmpr-1b* and *Bmpr-2* mRNA and protein (Broege et al., 2013; Garimella et al., 2008; Jensen et al., 2010; Kaneko et al., 2000; Okamoto et al., 2011; Shi et al., 2016; Shi et al., 2017). The mechanism(s) in osteoclasts that regulate expression of BMP receptors is unknown. However, in unpublished studies from our lab, we did not measure any change in expression of BMP receptors by RT-qPCR in TWSG1 null osteoclasts, suggesting that BMP signaling itself may not regulate expression of BMP receptors in osteoclasts.

4.1. BMPR-2

Our group deleted *Bmpr-2* from all cells in the myeloid lineage including osteoclasts, and the conditional knockout mice were osteopetrotic due to decreased osteoclast differentiation (Broege et al., 2013). *Bmpr-2* conditional knockout mice had changes in the noncanonical signaling pathway (MAPK) and no changes in the canonical (SMAD1/5/8) signaling in the knockout mice compared to wild type (Broege et al., 2013). Data from other studies suggest that in the *Bmpr-2* conditional mouse model the amino-terminus of the BMPR-2 receptor may still be expressed and able to continue to activate the SMAD signaling pathway (Yu et al., 2005). Due to the defective osteoclast differentiation, these mice are osteopetrotic, displaying increased bone volume and increased trabeculae (Broege et al., 2013). Interestingly, in this animal model, it was not the osteoclast number on the bone surface that was altered, but the activity of these osteoclasts *in-vivo* (Broege et al., 2013).

4.2. BMPR-1A

Mice expressing a conditional deletion of *Bmpr1a* in mature osteoclasts (*Bmpr1a*$^{fl/fl}$*;Ctsk-Cre*) demonstrated increased bone resorption, suggesting that activation of BMPR-1A in the late stages of osteoclast differentiation negatively regulates osteoclast activity (Okamoto et al., 2011). Additionally, mice with a deletion of *Bmpr-1a* in osteoclasts measured increase bone formation, suggesting that BMPR-1A signaling in osteoclasts regulates osteoclast and osteoblast coupling (Okamoto et al., 2011). Recently, *Bmpr-1a* was conditionally deleted from osteoclasts with *LysM-Cre* which would decrease BMPR-1A protein expression in all myeloid cells. The osteoclasts from these mice were decreased in their ability to form multinuclear TRAP positive cells with decreased expression of SMAD1/5/8 protein; however, even though mRNA for *Mitf* and *Pu.1* were increased, *Nfatc1* and other late osteoclast genes were decreased (Li et al., 2017).

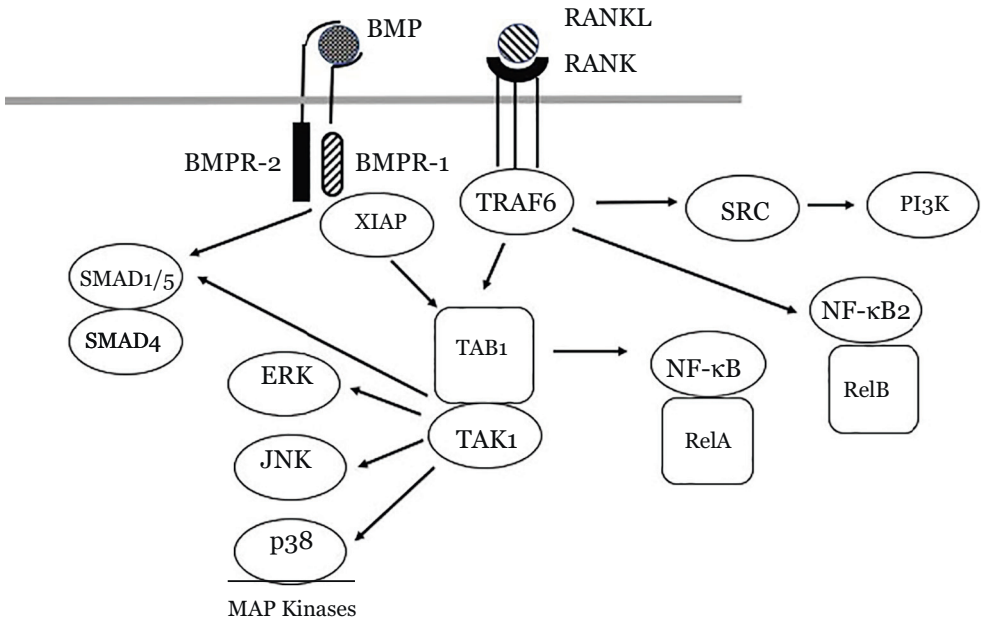

Fig. 2. Intersection of BMP and RANKL signaling pathways. Cartoon depicts downstream proteins activated by BMP and/or RANKL signaling pathways. Both pathways are able to activate TAK1 kinase which allows for activation of MAP kinase, SMAD1/5 and NF-kB (Qi et al., 2014).

4.3. BMPR-1B

Global knockouts of *Bmpr-1b* have an osteopenic phenotype (Shi et al., 2016). *In-vivo* analysis of bone activity markers indicates that neither bone formation nor resorption is changed in the global *Bmpr-1b* mice (Shi et al., 2016). *In-vitro* cultures of osteoclasts from BMPR-1B null mice revealed increased proliferation of osteoclast precursors, more osteoclast differentiation, reduced apoptosis but surprisingly also reduced resorption (Shi et al., 2016).

5. BMP signaling

As stated earlier, BMPs can signal through both a noncanonical signaling pathway, or SMAD-independent signaling pathway, and canonical signaling pathway, which is the SMAD-dependent signaling pathway (Fig. 2).

5.1. Noncanonical signaling

While not known in osteoclasts, BMP signaling has been shown to activate PI3K/Akt, P/kC and Rho-GTPases (reviewed in Zhang, 2009). All of these kinases have been shown to regulate osteoclast differentiation and activation (as reviewed in Mellis et al., 2011). It has been shown that mitogen activated protein kinases or MAPKs are activated by BMP signaling in osteoclasts. Mice null for TAK1 expression, a MAPKKK, in mature osteoclasts led to a decrease in SMAD1/5/9 signaling in osteoclasts (Qi et al., 2014). This data suggests that BMPs can activate both noncanonical and canonical signaling *via* TAK1 in osteoclasts. BMP2 stimulation has been shown to activate MAPKs, ERK1/2, JNK and p38 in osteoclasts (Broege et al., 2013). Itoh et al. demonstrated that BMP2 cannot activate NF-κB in osteoclasts but rather it inhibits NF-κB activation by RANKL (Itoh et al., 2001). In osteoclasts, BMP9 stimulation has been shown to activate both SMAD1/5/8 and ERK1/2 (Fong et al., 2013). BMP9 was shown to activate ERK1/2 through BMPR-2, as loss of BMPR-2 expression led to loss of ERK1/2 but not SMAD activation (Fong et al., 2013).

5.2. Canonical signaling

The R-Smads are phosphorylated and associate with a common Smad4 (Wang et al., 2014). The R-Smad/Smad4 complex translocate to the nucleus to regulate gene expression (Wang et al., 2014). Multiple research groups have demonstrated that osteoclasts express phosphorylated R-SMADs as well as SMAD4 (Jensen et al., 2010; Morita et al., 2016; Tasca et al., 2015).

5.2.1. SMAD1/5/9

In our initial study of the necessity of *Smad1/5/9* expression during osteoclast differentiation, we first established by RT-qPCR that osteoclasts express only detectable levels of *Smad1* and *Smad5* but not *Smad9* (Tasca et al., 2015). Osteoclast precursors isolated from the femurs of $Smad1/5^{fl/fl}$ mice infected with an adenovirus expressing CRE were inhibited in their ability to differentiate and resorb a calcium phosphate substrate (Tasca et al., 2015). We detected no change in *c-Fos* and *Nfatc1* mRNA expression but did measure a decrease in *Dc-stamp* and *Ctsk* mRNA expression (Tasca et al., 2015). To further characterize the role of SMAD1/5 during osteoclast differentiation, we created *Smad1/5* conditional knockouts in osteoclasts (Tasca et al., 2018). Loss of *Smad1/5* expression in the myeloid lineage had no significant effect on the skeleton; however, loss of Smad1/5 expression in macrophage/osteoclasts and mature osteoclasts enhanced osteoclast differentiation (Tasca et al., 2018). Surprisingly, we measured increased cortical thickness and an increase in osteoblast activity by P1NP ELISA (Tasca et al., 2018). Lastly, conditioned media from SMAD1/5 null osteoclasts increased mineralization of MC3T3 cells compared to wild type conditioned media, and expression of known osteoclast/osteoblast coupling factors were increased in SMAD1/5 null osteoclasts (Tasca et al., 2018).

5.2.2. SMAD4

In-vitro cultures of $Smad4^{fl/fl}$ infected with CRE expressing adenovirus demonstrated that loss of SMAD4 expression during the early stages of osteoclast differentiation results in the loss of osteoclast differentiation (Tasca et al., 2015). We measured less expression of *Nfatc1*, *Dc-stamp* and *Ctsk* by RT-qPCR as well as decrease in pSMAD2/3 expression by western blot (Tasca et al., 2015). However, conditional

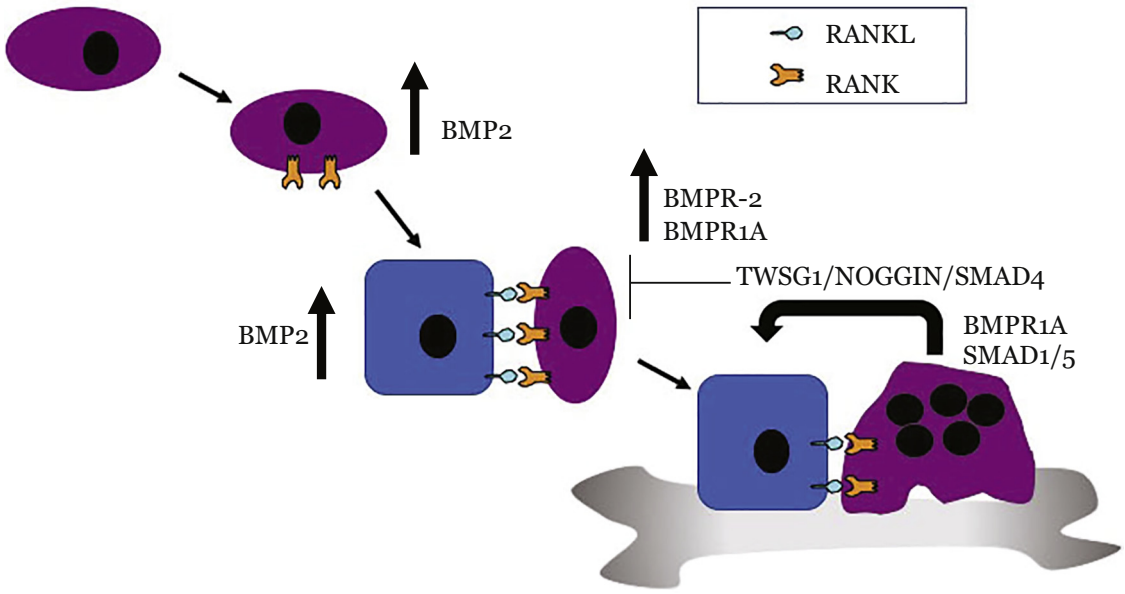

Fig. 3. Role of BMPs in regulating osteoclast differentiation. "Up" arrows indicate that BMP2 positively regulates osteoclast proliferation and RANKL expression. Loss of BMPR-2 or BMPR-1 in myeloid cells leads to loss of osteoclast differentiation. TWSG1, Noggin or SMAD4 expression leads to inhibition of osteoclast differentiation. Lastly BMPR-1A and SMAD1/5 have been shown in mature osteoclasts to regulate osteoclast-osteoblast coupling signals.

Table 1
Table of BMP/osteoclast mouse models.

Genotype	Skeletal phenotype	Osteoclast phenotype	Reference
Twsg1	Osteopenic	Enhanced osteoclasts Increased SMAD1/5 activation	(Rodriguez et al., 2009)
Bmpr-2;LysM-Cre	Osteopetrotic	Decreased, smaller osteoclasts, Changes in noncanonical, no change in SMAD1/5 signaling	(Broege et al., 2013)
Smad1/5;Cfms-Cre	Osteopenic	Increased osteoclast activity and bone formation	(Tasca et al., 2015)
Bmpr-1A;Ctsk-Cre	Osteopetrotic	Increased bone formation	(Okamoto et al., 2011)
Bmpr-1A;LysM-Cre	Osteopenic	Decreased fusion	(Li et al., 2017)
Smad4;Ctsk-Cre	Osteopenic	Increased osteoclast differentiation due to changes in TGF-β signaling	(Morita et al., 2016)

deletion of *Smad4* in mature osteoclasts (*Smad4*$^{fl/fl}$*;Ctsk-Cre*) results in osteopenia due to increased osteoclast differentiation (Morita et al., 2016). The changes in osteoclast differentiation were not due to changes in BMP signaling but due to changes in the sensitivity to TGF-β signaling (Morita et al., 2016). Morita et al. demonstrated that TGF-β1 signaling blocks expression of *Prdm1* mRNA, a repressor of osteoclast differentiation (Morita et al., 2016). Loss of *Prdm1* expression upregulates expression of *Bcl-6* and *Irf8* RNA, both of which are repressors of osteoclast differentiation (Morita et al., 2016). Summary of BMP activities during osteoclast differentiation is shown in Fig. 3 and Table 1.

6. Coupling of bone formation and resorption by BMPs

As stated in the beginning of this review, BMPs indirectly regulate osteoclast differentiation and activity through modulation of RANKL and OPG. With the creation of mice null for components of the BMP signaling pathway in osteoclast lineage cells, it has become clear that BMPs also regulate expression of osteoclast factors that couple bone resorption and bone formation. Mice null for *Bmpr1a* in mature osteoclasts demonstrate changes in bone formation which led to the conclusion that BMP signaling in osteoclasts negatively regulates osteoblast mineralization (Shi et al., 2017). Additionally, the increase in bone formation in BMPR1A null mice suggests that BMPR1A signaling in osteoclasts regulates osteoclast and osteoblast coupling (Okamoto et al., 2011). Osteoclasts are able to recruit osteoblasts to sites of bone remodeling through osteoclast-osteoblast signaling (Pederson et al., 2008; Shi et al., 2017; Weivoda et al., 2016). There have been numerous potential coupling factors discovered as of recent. Expression of CX43/GJA1 was increased in *Bmpr1a*-null osteoclasts and was demonstrated to be a downstream target of BMPR1A signaling in osteoclasts and can mediate osteoclast-osteoblast communication during remodeling (Shi et al., 2017). As stated above in our SMAD1/5 cKO mice, we measured changes in bone formation, an increase in *Wnt1*, *Gja1* and *Sphk1* mRNA expression and conditioned media from SMAD1/5 null osteoclasts enhanced the ability of MC3T3 cells to mineralize. Future studies will confirm which changes are responsible for the increase in bone formation and what other coupling factors are regulated by BMP (Fig. 4).

7. Conclusions

BMP plays a critical and complex role in bone development and homeostasis. The ability to culture osteoclasts without supporting cells *in-vitro* has allowed multiple groups to determine the direct effects of individual BMP ligands in regulating osteoclast differentiation. Additionally, the ability to create knockout mice in cells of the osteoclast lineage has and will continue to provide insight into the necessity for BMP ligands and receptors in regulating osteoclast differentiation and activity. These fundamental studies are necessary to understand the function of BMPs in bone, and understand their physiological effects, from signaling process, and cell-cell interactions that BMPs activate when used in the clinical setting.

Transparency document

The Transparency document associated with this article can be found, in online version.

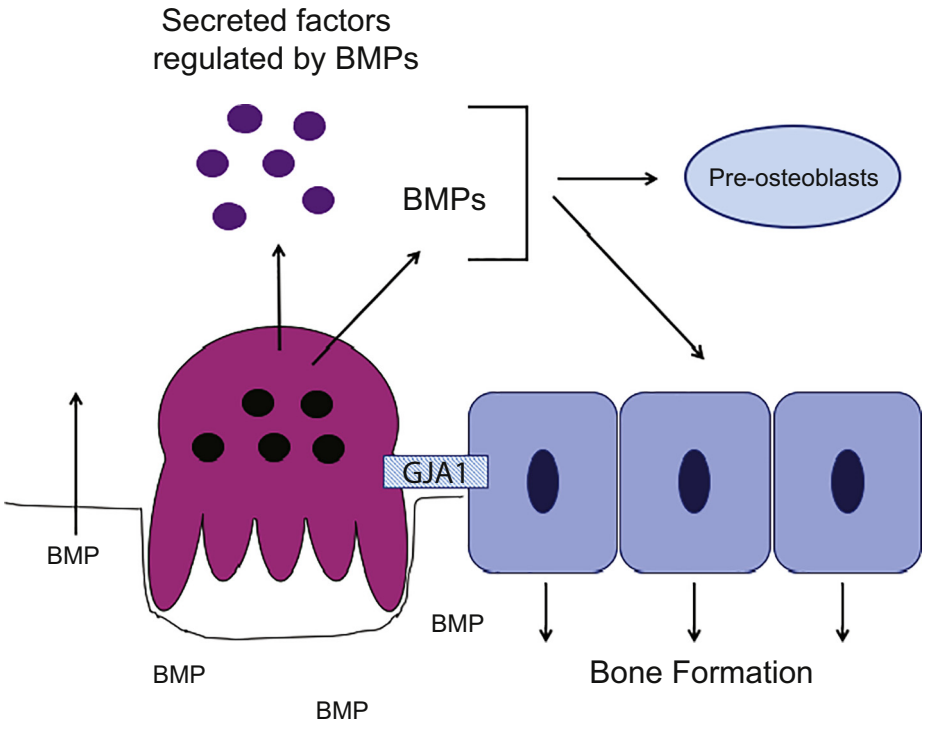

Fig. 4. BMP signaling regulates osteoclast-osteoblast coupling. Osteoclasts secrete BMPs such as BMP6 or other factors such as Wnts regulated by BMP signaling to activate pre-osteoblasts' differentiation or bone formation by mature osteoblasts. During bone resorption osteoclasts release BMPs stored in the bone matrix. BMP signaling has also been shown to regulate expression of CX43/GJA1 in osteoclasts which interacts with osteoblast to regulate mineralization (Shi et al., 2017).

Acknowledgements

We would like to acknowledge the help and support of the other members of the Mansky/Gopalakrishnan lab in writing this review (T90DE022732 (R.H.) and AR056642 (R.G.)).

Conflict of interests

Authors have nothing to disclose.

References

Abe, E., 2006. Function of BMPs and BMP antagonists in adult bone. Ann. N. Y. Acad. Sci. 1068, 41–53.
Abe, E., Yamamoto, M., Taguchi, Y., Lecka-Czernik, B., O'Brien, C.A., Economides, A.N., Stahl, N., Jilka, R.L., Manolagas, S.C., 2000. Essential requirement of BMPs-2/4 for both osteoblast and osteoclast formation in murine bone marrow cultures from adult mice: antagonism by noggin. J. Bone Miner. Res. 15, 663–673.
Anderson, H.C., Hodges, P.T., Aguilera, X.M., Missana, L., Moylan, P.E., 2000. Bone morphogenetic protein (BMP) localization in developing human and rat growth plate, metaphysis, epiphysis, and articular cartilage. J. Histochem. Cytochem. 48, 1493–1502.
Bandyopadhyay, A., Tsuji, K., Cox, K., Harfe, B.D., Rosen, V., Tabin, C.J., 2006. Genetic analysis of the roles of BMP2, BMP4, and BMP7 in limb patterning and skeletogenesis. PLoS Genet. 2, e216.
Bhatia, M., Bonnet, D., Wu, D., Murdoch, B., Wrana, J., Gallacher, L., Dick, J.E., 1999. Bone morphogenetic proteins regulate the developmental program of human hematopoietic stem cells. J. Exp. Med. 189, 1139–1148.
Blackwell, K.A., Hortschansky, P., Sanovic, S., Choudhary, S., Raisz, L.G., Pilbeam, C.C., 2009. Bone morphogenetic protein 2 enhances PGE(2)-stimulated osteoclast formation in murine bone marrow cultures. Prostaglandins Other Lipid Mediat. 90, 76–80.
Boden, S.D., Zdeblick, T.A., Sandhu, H.S., Heim, S.E., 2000. The use of rhBMP-2 in interbody fusion cages. Definitive evidence of osteoinduction in humans: a preliminary report. Spine (Phila Pa 1976) 25, 376–381.
Boyce, B.F., Xing, L., 2008. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch. Biochem. Biophys. 473, 139–146.
Bragdon, B., Moseychuk, O., Saldanha, S., King, D., Julian, J., Nohe, A., 2011. Bone morphogenetic proteins: a critical review. Cell. Signal. 23, 609–620.
Broege, A., Pham, L., Jensen, E.D., Emery, A., Huang, T.H., Stemig, M., Beppu, H., Petryk, A., O'Connor, M., Mansky, K., Gopalakrishnan, R., 2013. Bone morphogenetic proteins signal via SMAD and mitogen-activated protein (MAP) kinase pathways at distinct times during osteoclastogenesis. J. Biol. Chem. 288, 37230–37240.
Burkus, J.K., Sandhu, H.S., Gornet, M.F., Longley, M.C., 2005. Use of rhBMP-2 in combination with structural cortical allografts: clinical and radiographic outcomes in anterior lumbar spinal surgery. J. Bone Joint Surg. Am. 87, 1205–1212.
Carreira, A.C., Lojudice, F.H., Halcsik, E., Navarro, R.D., Sogayar, M.C., Granjeiro, J.M., 2014. Bone morphogenetic proteins: facts, challenges, and future perspectives. J. Dent. Res. 93, 335–345.
Celeste, A.J., Iannazzi, J.A., Taylor, R.C., Hewick, R.M., Rosen, V., Wang, E.A., Wozney, J.M., 1990. Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. Proc. Natl. Acad. Sci. U. S. A. 87, 9843–9847.
Charles, J.F., Aliprantis, A.O., 2014. Osteoclasts: more than 'bone eaters'. Trends Mol. Med. 20, 449–459.
Daluiski, A., Engstrand, T., Bahamonde, M.E., Gamer, L.W., Agius, E., Stevenson, S.L., Cox, K., Rosen, V., Lyons, K.M., 2001. Bone morphogenetic protein-3 is a negative regulator of bone density. Nat. Genet. 27, 84–88.
DiLeone, R.J., King, J.A., Storm, E.E., Copeland, N.G., Jenkins, N.A., Kingsley, D.M., 1997. The Bmp8 gene is expressed in developing skeletal tissue and maps near the achondroplasia locus on mouse chromosome 4. Genomics 40, 196–198.
Faccio, R., Teitelbaum, S.L., Fujikawa, K., Chappel, J., Zallone, A., Tybulewicz, V.L., Ross, F.P., Swat, W., 2005. Vav3 regulates osteoclast function and bone mass. Nat. Med. 11, 284–290.
Fong, D., Bisson, M., Laberge, G., McManus, S., Grenier, G., Faucheux, N., Roux, S., 2013. Bone morphogenetic protein-9 activates Smad and ERK pathways and supports human osteoclast function and survival in vitro. Cell. Signal. 25, 717–728.
Friedlaender, G.E., Perry, C.R., Cole, J.D., Cook, S.D., Cierny, G., Muschler, G.F., Zych, G.A., Calhoun, J.H., LaForte, A.J., Yin, S., 2001. Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. J. Bone Joint Surg. Am. 83-A (Suppl. 1), S151–S158.
Fukunaga, T., Zou, W., Warren, J.T., Teitelbaum, S.L., 2014. Vinculin regulates osteoclast function. J. Biol. Chem. 289, 13554–13564.
Garimella, R., Tague, S.E., Zhang, J., Belibi, F., Nahar, N., Sun, B.H., Insogna, K., Wang, J., Anderson, H.C., 2008. Expression and synthesis of bone morphogenetic proteins by osteoclasts: a possible path to anabolic bone remodeling. J. Histochem. Cytochem. 56, 569–577.
Georgess, D., Machuca-Gayet, I., Blangy, A., Jurdic, P., 2014. Podosome organization drives osteoclast-mediated bone resorption. Cell Adhes. Migr. 8, 191–204.
Govender, S., Csimma, C., Genant, H.K., Valentin-Opran, A., Amit, Y., Arbel, R., Aro, H., Atar, D., Bishay, M., Borner, M.G., Chiron, P., Choong, P., Cinats, J., Courtenay, B., Feibel, R., Geulette, B., Gravel, C., Haas, N., Raschke, M., Hammacher, E., van der Velde, D., Hardy, P., Holt, M., Josten, C., Ketterl, R.L., Lindeque, B., Lob, G., Mathevon, H., McCoy, G., Marsh, D., Miller, R., Munting, E., Oevre, S., Nordsletten, L., Patel, A., Pohl, A., Rennie, W., Reynders, P., Rommens, P.M., Rondia, J., Rossouw, W.C., Daneel, P.J., Ruff, S., Ruter, A., Santavirta, S., Schildhauer, T.A., Gekle, C., Schnettler, R., Segal, D., Seiler, H., Snowdowne, R.B., Stapert, J., Taglang, G., Verdonk, R., Vogels, L., Weckbach, A., Wentzensen, A., Wisniewski, T., Group BMPEiSfTTS, 2002. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. J. Bone Joint Surg. Am. 84-A, 2123–2134.
Granholm, S., Henning, P., Lindholm, C., Lerner, U.H., 2013. Osteoclast progenitor cells present in significant amounts in mouse calvarial osteoblast isolations and osteoclastogenesis increased by BMP-2. Bone 52, 83–92.
Grigoriadis, A.E., Wang, Z.Q., Cecchini, M.G., Hofstetter, W., Felix, R., Fleisch, H.A.,

Wagner, E.F., 1994. c-Fos: a key regulator of osteoclast-macrophage lineage determination and bone remodeling. Science 266, 443–448.
Hentunen, T.A., Lakkakorpi, P.T., Tuukkanen, J., Lehenkari, P.P., Sampath, T.K., Vaananen, H.K., 1995. Effects of recombinant human osteogenic protein-1 on the differentiation of osteoclast-like cells and bone resorption. Biochem. Biophys. Res. Commun. 209, 433–443.
Hofbauer, L.C., Dunstan, C.R., Spelsberg, T.C., Riggs, B.L., Khosla, S., 1998. Osteoprotegerin production by human osteoblast lineage cells is stimulated by vitamin D, bone morphogenetic protein-2, and cytokines. Biochem. Biophys. Res. Commun. 250, 776–781.
Iotsova, V., Caamano, J., Loy, J., Yang, Y., Lewin, A., Bravo, R., 1997. Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. Nat. Med. 3, 1285–1289.
Itoh, K., Udagawa, N., Katagiri, T., Iemura, S., Ueno, N., Yasuda, H., Higashio, K., Quinn, J.M., Gillespie, M.T., Martin, T.J., Suda, T., Takahashi, N., 2001. Bone morphogenetic protein 2 stimulates osteoclast differentiation and survival supported by receptor activator of nuclear factor-kappaB ligand. Endocrinology 142, 3656–3662.
Jensen, E.D., Pham, L., B Jr., C.J., Espe, K., Carlson, A.E., Westendorf, J.J., Petryk, A., Gopalakrishnan, R., Mansky, K.C., 2010. Bone morphogenetic protein 2 directly enhances differentiation of murine osteoclast precursors. J. Cell. Biochem. 109, 672–682.
Jurdic, P., Saltel, F., Chabadel, A., Destaing, O., 2006. Podosome and sealing zone: specificity of the osteoclast model. Eur. J. Cell Biol. 85, 195–202.
Kamiya, N., Ye, L., Kobayashi, T., Lucas, D.J., Mochida, Y., Yamauchi, M., Kronenberg, H.M., Feng, J.Q., Mishina, Y., 2008. Disruption of BMP signaling in osteoblasts through type IA receptor (BMPRIA) increases bone mass. J. Bone Miner. Res. 23, 2007–2017.
Kamiya, N., Shuxian, L., Yamaguchi, R., Phipps, M., Aruwajoye, O., Adapala, N.S., Yuan, H., Kim, H.K., Feng, J.Q., 2016. Targeted disruption of BMP signaling through type IA receptor (BMPR1A) in osteocyte suppresses SOST and RANKL, leading to dramatic increase in bone mass, bone mineral density and mechanical strength. Bone 91, 53–63.
Kanatani, M., Sugimoto, T., Kaji, H., Kobayashi, T., Nishiyama, K., Fukase, M., Kumegawa, M., Chihara, K., 1995. Stimulatory effect of bone morphogenetic protein-2 on osteoclast-like cell formation and bone-resorbing activity. J. Bone Miner. Res. 10, 1681–1690.
Kaneko, H., Arakawa, T., Mano, H., Kaneda, T., Ogasawara, A., Nakagawa, M., Toyama, Y., Yabe, Y., Kumegawa, M., Hakeda, Y., 2000. Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. Bone 27, 479–486.
Kessler, E., Takahara, K., Biniaminov, L., Brusel, M., Greenspan, D.S., 1996. Bone morphogenetic protein-1: the type I procollagen C-proteinase. Science 271, 360–362.
King, J.A., Marker, P.C., Seung, K.J., Kingsley, D.M., 1994. BMP5 and the molecular, skeletal, and soft-tissue alterations in short ear mice. Dev. Biol. 166, 112–122.
Kleeman, T.J., Ahn, U.M., Talbot-Kleeman, A., 2001. Laparoscopic anterior lumbar interbody fusion with rhBMP-2: a prospective study of clinical and radiographic outcomes. Spine (Phila Pa 1976) 26, 2751–2756.
Koide, M., Murase, Y., Yamato, K., Noguchi, T., Okahashi, N., Nishihara, T., 1999. Bone morphogenetic protein-2 enhances osteoclast formation mediated by interleukin-1alpha through upregulation of osteoclast differentiation factor and cyclooxygenase-2. Biochem. Biophys. Res. Commun. 259, 97–102.
Kokabu, S., Gamer, L., Cox, K., Lowery, J., Tsuji, K., Raz, R., Economides, A., Katagiri, T., Rosen, V., 2012. BMP3 suppresses osteoblast differentiation of bone marrow stromal cells via interaction with Acvr2b. Mol. Endocrinol. 26, 87–94.
Kudo, N., Ogose, A., Ariizumi, T., Kawashima, H., Hotta, T., Hatano, H., Morita, T., Nagata, M., Siki, Y., Kawai, A., Hotta, Y., Hoshino, M., Endo, N., 2009. Expression of bone morphogenetic proteins in giant cell tumor of bone. Anticancer Res. 29, 2219–2225.
Kusu, N., Laurikkala, J., Imanishi, M., Usui, H., Konishi, M., Miyake, A., Thesleff, I., Itoh, N., 2003. Sclerostin is a novel secreted osteoclast-derived bone morphogenetic protein antagonist with unique ligand specificity. J. Biol. Chem. 278, 24113–24117.
Lee, S.H., Rho, J., Jeong, D., Sul, J.Y., Kim, T., Kim, N., Kang, J.S., Miyamoto, T., Suda, T., Lee, S.K., Pignolo, R.J., Koczon-Jaremko, B., Lorenzo, J., Choi, Y., 2006. v-ATPase V0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. Nat. Med. 12, 1403–1409.
Li, H., Zhao, D., Wang, S., Ding, J., Zhao, L., 2016. Bone morphogenetic protein9 promotes the differentiation of mouse spleen macrophages into osteoclasts via the ALK1 receptor and ERK 1/2 pathways in vitro. Mol. Med. Rep. 14, 4545–4550.
Li, A., Cong, Q., Xia, X., Leong, W.F., Yeh, J., Miao, D., Mishina, Y., Liu, H., Li, B., 2017. Pharmacologic calcitriol inhibits osteoclast lineage commitment via the BMP-Smad1 and IkappaB-NF-kappaB pathways. J. Bone Miner. Res. 32, 1406–1420.
Luu, H.H., Song, W.X., Luo, X., Manning, D., Luo, J., Deng, Z.L., Sharff, K.A., Montag, A.G., Haydon, R.C., He, T.C., 2007. Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. J. Orthop. Res. 25, 665–677.
Maurer, T., Zimmermann, G., Maurer, S., Stegmaier, S., Wagner, C., Hansch, G.M., 2012. Inhibition of osteoclast generation: a novel function of the bone morphogenetic protein 7/osteogenic protein 1. Mediat. Inflamm. 2012, 171209.
McCullough, K.A., Waits, C.A., Garimella, R., Tague, S.E., Sipe, J.B., Anderson, H.C., 2007. Immunohistochemical localization of bone morphogenetic proteins (BMPs) 2, 4, 6, and 7 during induced heterotopic bone formation. J. Orthop. Res. 25, 465–472.
Meekeren, Jv, 1668. Heel- en Geneeskonstige Aanmerkingen. Commelijn Amsterdam.
Mellis, D.J., Itzstein, C., Helfrich, M.H., Crockett, J.C., 2011. The skeleton: a multifunctional complex organ: the role of key signalling pathways in osteoclast differentiation and in bone resorption. J. Endocrinol. 211, 131–143.
Mensah, K.A., Ritchlin, C.T., Schwarz, E.M., 2010. RANKL induces heterogeneous DC-STAMP(lo) and DC-STAMP(hi) osteoclast precursors of which the DC-STAMP(lo) precursors are the master fusogens. J. Cell. Physiol. 223, 76–83.
Miyazono, K., Kamiya, Y., Morikawa, M., 2010. Bone morphogenetic protein receptors and signal transduction. J. Biochem. 147, 35–51.
Morita, M., Yoshida, S., Iwasaki, R., Yasui, T., Sato, Y., Kobayashi, T., Watanabe, R., Oike, T., Miyamoto, K., Takami, M., Ozato, K., Deng, C.X., Aburatani, H., Tanaka, S., Yoshimura, A., Toyama, Y., Matsumoto, M., Nakamura, M., Kawana, H., Nakagawa, T., Miyamoto, T., 2016. Smad4 is required to inhibit osteoclastogenesis and maintain bone mass. Sci. Rep. 6, 35221.
Mroz, T.E., Wang, J.C., Hashimoto, R., Norvell, D.C., 2010. Complications related to osteobiologics use in spine surgery: a systematic review. Spine (Phila Pa 1976) 35, S86–104.
Mueller, T.D., Nickel, J., 2012. Promiscuity and specificity in BMP receptor activation. FEBS Lett. 586, 1846–1859.
N S, 1889. On the healing of aseptic bone cavities by implantation of antiseptic decalcified bone. Am J Med Sci 98, 219–243.
Okamoto, M., Murai, J., Yoshikawa, H., Tsumaki, N., 2006. Bone morphogenetic proteins in bone stimulate osteoclasts and osteoblasts during bone development. J. Bone Miner. Res. 21, 1022–1033.
Okamoto, M., Murai, J., Imai, Y., Ikegami, D., Kamiya, N., Kato, S., Mishina, Y., Yoshikawa, H., Tsumaki, N., 2011. Conditional deletion of Bmpr1a in differentiated osteoclasts increases osteoblastic bone formation, increasing volume of remodeling bone in mice. J. Bone Miner. Res. 26, 2511–2522.
Ozkaynak, E., Rueger, D.C., Drier, E.A., Corbett, C., Ridge, R.J., Sampath, T.K., Oppermann, H., 1990. OP-1 cDNA encodes an osteogenic protein in the TGF-beta family. EMBO J. 9, 2085–2093.
Ozkaynak, E., Schnegelsberg, P.N., Jin, D.F., Clifford, G.M., Warren, F.D., Drier, E.A., Oppermann, H., 1992. Osteogenic protein-2. A new member of the transforming growth factor-beta superfamily expressed early in embryogenesis. J. Biol. Chem. 267, 25220–25227.
Pederson, L., Ruan, M., Westendorf, J.J., Khosla, S., Oursler, M.J., 2008. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. Proc. Natl. Acad. Sci. U. S. A. 105, 20764–20769.
Pfaff, M., Jurdic, P., 2001. Podosomes in osteoclast-like cells: structural analysis and cooperative roles of paxillin, proline-rich tyrosine kinase 2 (Pyk2) and integrin alphaVbeta3. J. Cell Sci. 114, 2775–2786.
Pham, L., Beyer, K., Jensen, E.D., Rodriguez, J.S., Davydova, J., Yamamoto, M., Petryk, A., Gopalakrishnan, R., Mansky, K., 2010. Bone morphogenetic protein 2 signaling in osteoclasts is negatively regulated by BMP antagonist, twisted gastrulation. J. Cell. Biochem. 112, 793–803.
Qi, B., Cong, Q., Li, P., Ma, G., Guo, X., Yeh, J., Xie, M., Schneider, M.D., Liu, H., Li, B., 2014. Ablation of Tak1 in osteoclast progenitor leads to defects in skeletal growth and bone remodeling in mice. Sci. Rep. 4, 7158.
Rodriguez, J.S., Mansky, K.C., Jensen, E.D., Carlson, A.E., Schwarz, T., Pham, L., Mackenzie, B., Prasad, H., Rohrer, M.D., Petryk, A., Gopalakrishnan, R., 2009. Enhanced osteoclastogenesis causes osteopenia in twisted gastrulation-deficient mice through increased BMP signaling. J. Bone Miner. Res. 24, 1917–1926.
Rosen, V., 2006. BMP and BMP inhibitors in bone. Ann. N. Y. Acad. Sci. 1068, 19–25.
Ross, F.P., 2006. M-CSF, c-Fms, and signaling in osteoclasts and their precursors. Ann. N. Y. Acad. Sci. 1068, 110–116.
Saltel, F., Destaing, O., Bard, F., Eichert, D., Jurdic, P., 2004. Apatite-mediated actin dynamics in resorbing osteoclasts. Mol. Biol. Cell 15, 5231–5241.
Saltel, F., Chabadel, A., Bonnelye, E., Jurdic, P., 2008. Actin cytoskeletal organisation in osteoclasts: a model to decipher transmigration and matrix degradation. Eur. J. Cell Biol. 87, 459–468.
Sarras Jr., M.P., 1996. BMP-1 and the astacin family of metalloproteinases: a potential link between the extracellular matrix, growth factors and pattern formation. Bioessays 18, 439–442.
Seeman, E., 2009. Bone modeling and remodeling. Crit. Rev. Eukaryot. Gene Expr. 19, 219–233.
Shi, C., Iura, A., Terajima, M., Liu, F., Lyons, K., Pan, H., Zhang, H., Yamauchi, M., Mishina, Y., Sun, H., 2016. Deletion of BMP receptor type IB decreased bone mass in association with compromised osteoblastic differentiation of bone marrow mesenchymal progenitors. Sci. Rep. 6, 24256.
Shi, C., Zhang, H., Louie, K., Mishina, Y., Sun, H., 2017. BMP signaling mediated by BMPR1A in osteoclasts negatively regulates osteoblast mineralization through suppression of Cx43. J. Cell. Biochem. 118, 605–614.
Simic, P., Culej, J.B., Orlic, I., Grgurevic, L., Draca, N., Spaventi, R., Vukicevic, S., 2006. Systemically administered bone morphogenetic protein-6 restores bone in aged ovariectomized rats by increasing bone formation and suppressing bone resorption. J. Biol. Chem. 281, 25509–25521.
Simmonds, M.C., Brown, J.V., Heirs, M.K., Higgins, J.P., Mannion, R.J., Rodgers, M.A., Stewart, L.A., 2013. Safety and effectiveness of recombinant human bone morphogenetic protein-2 for spinal fusion: a meta-analysis of individual-participant data. Ann. Intern. Med. 158, 877–889.
Spector, J.A., Luchs, J.S., Mehrara, B.J., Greenwald, J.A., Smith, L.P., Longaker, M.T., 2001. Expression of bone morphogenetic proteins during membranous bone healing. Plast. Reconstr. Surg. 107, 124–134.
Stenbeck, G., 2002. Formation and function of the ruffled border in osteoclasts. Semin. Cell Dev. Biol. 13, 285–292.
Tachi, K., Takami, M., Zhao, B., Mochizuki, A., Yamada, A., Miyamoto, Y., Inoue, T., Baba, K., Kamijo, R., 2010. Bone morphogenetic protein 2 enhances mouse osteoclast differentiation via increased levels of receptor activator of NF-kappaB ligand expression in osteoblasts. Cell Tissue Res. 342, 213–220.
Takayanagi, H., Kim, S., Koga, T., Nishina, H., Isshiki, M., Yoshida, H., Saiura, A., Isobe, M., Yokochi, T., Inoue, J., Wagner, E.F., Mak, T.W., Kodama, T., Taniguchi, T., 2002. Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev. Cell 3, 889–901.

Tasca, A., Stemig, M., Broege, A., Huang, B., Davydova, J., Zwijsen, A., Umans, L., Jensen, E.D., Gopalakrishnan, R., Mansky, K.C., 2015. Smad1/5 and smad4 expression are important for osteoclast differentiation. J. Cell. Biochem. 116, 1350–1360.
Tasca, A., Astleford, K., Blixt, N.C., Jensen, E.D., Gopalakrishnan, R., Mansky, K.C., 2018. SMAD1/5 signaling in osteoclasts regulates bone formation via coupling factors. PLoS One 13, e0203404.
Tazoe, M., Mogi, M., Goto, S., Togari, A., 2003. Involvement of p38MAP kinase in bone morphogenetic protein-4-induced osteoprotegerin in mouse bone-marrow-derived stromal cells. Arch. Oral Biol. 48, 615–619.
Teitelbaum, S.L., 2000. Bone resorption by osteoclasts. Science 289, 1504–1508.
Teitelbaum, S.L., 2006. Osteoclasts and integrins. Ann. N. Y. Acad. Sci. 1068, 95–99.
Tondravi, M.M., McKercher, S.R., Anderson, K., Erdmann, J.M., Quiroz, M., Maki, R., Teitelbaum, S.L., 1997. Osteopetrosis in mice lacking haematopoietic transcription factor PU.1. Nature 386, 81–84.
Urist, M.R., 1965. Bone: formation by autoinduction. Science 150, 893–899.
Usui, M., Xing, L., Drissi, H., Zuscik, M., O'Keefe, R., Chen, D., Boyce, B.F., 2008. Murine and chicken chondrocytes regulate osteoclastogenesis by producing RANKL in response to BMP2. J. Bone Miner. Res. 23, 314–325.
Vadon-Le Goff, S., Hulmes, D.J., Moali, C., 2015. BMP-1/tolloid-like proteinases synchronize matrix assembly with growth factor activation to promote morphogenesis and tissue remodeling. Matrix Biol. 44-46, 14–23.
Verma, S.K., Leikina, E., Melikov, K., Gebert, C., Kram, V., Young, M.F., Uygur, B., Chernomordik, L.V., 2018. Cell-surface phosphatidylserine regulates osteoclast precursor fusion. J. Biol. Chem. 293, 254–270.
Wang, R.N., Green, J., Wang, Z., Deng, Y., Qiao, M., Peabody, M., Zhang, Q., Ye, J., Yan, Z., Denduluri, S., Idowu, O., Li, M., Shen, C., Hu, A., Haydon, R.C., Kang, R., Mok, J., Lee, M.J., Luu, H.L., Shi, L.L., 2014. Bone morphogenetic protein (BMP) signaling in development and human diseases. Genes Dis. 1, 87–105.
Weivoda, M.M., Oursler, M.J., 2014. The roles of small GTPases in osteoclast biology. Orthop. Muscular Syst. 3.
Weivoda, M.M., Ruan, M., Pederson, L., Hachfeld, C., Davey, R.A., Zajac, J.D., Westendorf, J.J., Khosla, S., Oursler, M.J., 2016. Osteoclast TGF-beta receptor signaling induces Wnt1 secretion and couples bone resorption to bone formation. J. Bone Miner. Res. 31, 76–85.
Winnier, G., Blessing, M., Labosky, P.A., Hogan, B.L., 1995. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev. 9, 2105–2116.
Wozney, J.M., Rosen, V., Celeste, A.J., Mitsock, L.M., Whitters, M.J., Kriz, R.W., Hewick, R.M., Wang, E.A., 1988. Novel regulators of bone formation: molecular clones and activities. Science 242, 1528–1534.
Wutzl, A., Brozek, W., Lernbass, I., Rauner, M., Hofbauer, G., Schopper, C., Watzinger, F., Peterlik, M., Pietschmann, P., 2006. Bone morphogenetic proteins 5 and 6 stimulate osteoclast generation. J. Biomed. Mater. Res. A 77, 75–83.
Xiong, J., Onal, M., Jilka, R.L., Weinstein, R.S., Manolagas, S.C., O'Brien, C.A., 2011. Matrix-embedded cells control osteoclast formation. Nat. Med. 17, 1235–1241.
Yagi, M., Miyamoto, T., Sawatani, Y., Iwamoto, K., Hosogane, N., Fujita, N., Morita, K., Ninomiya, K., Suzuki, T., Miyamoto, K., Oike, Y., Takeya, M., Toyama, Y., Suda, T., 2005. DC-STAMP is essential for cell-cell fusion in osteoclasts and foreign body giant cells. J. Exp. Med. 202, 345–351.
Yu, P.B., Beppu, H., Kawai, N., Li, E., Bloch, K.D., 2005. Bone morphogenetic protein (BMP) type II receptor deletion reveals BMP ligand-specific gain of signaling in pulmonary artery smooth muscle cells. J. Biol. Chem. 280, 24443–24450.
Zhang, Y.E., 2009. Non-Smad pathways in TGF-beta signaling. Cell Res. 19, 128–139.
Zou, W., Izawa, T., Zhu, T., Chappel, J., Otero, K., Monkley, S.J., Critchley, D.R., Petrich, B.G., Morozov, A., Ginsberg, M.H., Teitelbaum, S.L., 2013. Talin1 and Rap1 are critical for osteoclast function. Mol. Cell. Biol. 33, 830–844.