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Bone morphogenetic protein and bone metastasis, implication and therapeutic potential

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TABLE OF CONTENTS

1. ABSTRACT

Bone metastasis is one of the most common and severe complications in advanced malignancies, particularly in the three leading cancers; breast cancer, prostate cancer and lung cancer. It is currently incurable and causes severe morbidities, including bone pain, hypercalcemia, pathological fracture, spinal cord compression and consequent paralysis. However, the mechanisms underlying the development of bone metastasis remain largely unknown. Bone morphogenetic proteins (BMPs) belong to the TGF-beta superfamily and are pluripotent factors involved in the regulation of embryonic development and postnatal homeostasis of various organs and tissues, by controlling cellular differentiation, proliferation and apoptosis. Since they are potent regulators for bone formation, there is an increasing interest to investigate BMPs and their roles in bone metastasis. BMPs have been implicated in various neoplasms, at both primary and secondary tumors,

particularly skeletal metastasis. Recently studies have also suggested that BMP signaling and their antagonists play pivotal roles in bone metastasis. In this review, we discuss the current knowledge of aberrations of BMPs which have been indicated in tumor progression, and particularly in the development of bone metastasis.

2. INTRODUCTION

Bone metastases are most commonly seen in prostate, breast and lung cancer, which are leading malignancies in female and/or male having the highest incidence and mortality rates (1-3). Bone metastasis usually leads to severe morbidities, which always persist until the death of patients, including bone pain, hypercalcemia, pathological fracture, spinal cord compression and consequent paralysis. In bone metastases, along with growth of cancer cells, oesteoblastic and oesteolytic activities are stimulated simultaneously or one occurs in predominance. Although osteoblastic lesions are commonly seen in prostate cancer whilst osteolytic lesions frequently occur in breast cancer, lung cancer and renal cancer, certain proportion of bone metastases have demonstrable mixtures of oesteoblastic and oestolytic lesions (4, 5). Since Dr. Paget proposed 'seeds' (metastatic cancer cells) and 'soil' (metastatic site) hypothesis for cancer metastasis in 1889, clinicians and scientists have spent immense effort to understand how the seeds and soil work together and subsequently develop metastases. As one of the most common metastatic sites, bone metastasis has been investigated extensively from molecular and histological characteristics to diagnosis and management. Knowledge about molecular mechanisms underlying osteoblast and osteolytic lesions has been expanded rapidly over last few decades. Parathyroid hormone-related protein (PTHrP), interleukin (IL)-11, IL-8, IL-6, and receptor activator of nuclear factor-κB ligand (RANKL) produced by metastatic cancer cells play critical roles in osteolytic bone metastases (6-9). On the other hand, endothelin-1, BMPs, prostaglandins and TNFα have been implicated in the development of osteoblastic lesions (10, 11). However, molecular mechanisms underlying the predisposition of the particular malignancies and subsequent colonisation and development of metastatic tumors remain largely unknown. As bone morphogenetic proteins (BMP) are potent regulators for bone formation, there is an increasing interest to investigate BMPs and their roles in bone metastasis. BMPs belong to the TGF-beta superfamily and are pluripotent factors involved in the regulation of embryonic development and postnatal homeostasis of various organs and tissues, by controlling cellular differentiation, proliferation and apoptosis. BMPs have been implicated in various neoplasms, at both primary and secondary tumors, particularly skeletal metastasis. Recently studies have also suggested pivotal roles played by BMP signaling and their antagonists in bone metastasis. In this review, we discuss the current knowledge of BMPs signaling, aberrations which have been indicated in tumor progression, and particularly in the development of bone metastasis.

3. BONE MORPHOGENETIC PROTEINS

Bone morphogenetic proteins (BMPs) belong to transforming growth factor-beta (TGF-β) superfamily, which were first named by Dr. Urist for their capacity to induce ectopic bone formation (12). BMP proteins were not purified and cloned until late 1980s (13-16), to date, more than 20 BMPs have been identified in humans (Table 1). In addition to their ability in facilitating intramembraneous/endochodral bone formation and formation of cartilage, BMPs play crucial roles in diverse developmental processes and homeostasis of various tissues and organs including tooth, kidney, prostate, breast, skin, hair, muscle, heart and neuron through coordinating cellular differentiation, proliferation, survival and apoptosis. Certain BMPs have also been shown to be involved in the maintenance of the metabolism of glucose and iron (Table 1).

3.1. Structural characteristics of BMPs

BMPs are synthesized as large precursor molecules, consisting of an amino-terminal (N-terminal) pro-region and a carboxy-terminal (C-terminal) ligand (13, 16, 17). Each BMP ligand has seven conserved cysteines at the C-terminal, in which six cysteines construct a cysteine knot, and the seventh cysteine contributes to the dimerisation (18). It has been shown that some of the proprotein convertases (PCs), such as furin, proprotein convertase subtilisin/kexin type 6 (PCSK6) and proprotein convertase subtilisin/kexin type 5 (PCSK5) which belong to a subtilisin-like proprotein convertase family, can proteolytically activate BMP precursors at the sequence of R-X-K/R-R or R-X-X-R (19-22). The pro-region of the precursor BMP protein controls the stability of the processed mature protein, and the amino acid motif adjacent to the cleavage site determines the efficiency of cleavage (20). Amongst the BMPs, growth differentiation factor-9 (GDF9) and BMP15 (GDF9B) may be an exception and have only six cysteines in the mature ligand which lacks the seventh cystein. This characteristic of BMP15 and GDF9 may help to define its ligand binding property to its receptors (23). The pro-region of some BMPs remains noncovalently associated with the mature ligand even after secretion from the cell, for example: GDF-8 and BMP9 (24, 25). Once processed and activated, BMP proteins are biologically active both as homodimer, and as heterodimer molecules, in which two chains are connected by disulfide bonds. Interestingly, the heterodimers of BMP4/7, BMP2/6, BMP2/7 and BMP7/GDF7 may be more effective than their respective homodimers (18, 26-28).

3.2. BMP receptors

BMP signals are mediated by receptors which are dedicated to TGF-β signaling, and include type I and type II serine/threonine kinase receptors. Seven type I and five type II receptors have been identified for TGF-β signaling in humans (Table 2). Six of the type I receptors and three of the type II receptors have been shown to mediate BMPs signaling (Figure 1) (29). BMPR1A, BMPR1B and BMPR2 are specific for the BMPs; whilst ACVRL1, ACVR1, ACVR1B, ACVR2B, and ACVR2A are also the receptors for activin; TGFBR1 (ALK5) is known as the type I receptor for TGF-β1, 2 and 3. Both receptor types are required for downstream signaling to occur; the type-I receptors are unable to bind their ligands

Table 1. Biological functions of BMPs

Official Name	Alternative Name	Gene Location	Year of identification	Key functions
BMP2	BMP2A	20p12	1988	Induces cartilage and bone formation; coordinates dorsoventral patterning and craniofacial development and heart development.
BMP3		4p14-q21	1988	Negatively regulates bone density, and inhibits osteogenic activities of certain BMPs
BMP4	ZYME; BMP2B; BMP2B1	14q22-q23	1988	Induces cartilage and bone formation; regulates formation of teeth, limbs, lung, eye, and bone from mesoderm; coordinates dorsoventral patterning and craniofacial development.
BMP5	MGC34244	6p	1990	Chondrogenesis
BMP6	VGR; VGR1	6p24-p23	1990	Osteogenesis and chondrogenesis. Involved in joint integrity
BMP7	OP-1	20q13	1990	Osteoblast differentiation, eye development, renal development/repair, and craniofacial development.
BMP8A		1p35-p32	2002	Unknown
BMP8B	BMP8; OP2	1p35-p32	1992	Osteogenesis, chondrogenesis, craniofacial development.
BMP10		2p13.3	1999	Trabeculation of embryonic heart
BMP15	GDF9B, ODG2	Xp11.2	1998	Oocyte and follicular development
GDF1		19p12	1991	left-right asymmetric organogenesis, including the heart and great vessels; neural development
GDF2	BMP-9; BMP9	10q11.22	1994	Induce bone formation; promotes chondrogenic differentiation; regulates angiogenesis; differentiating factor for cholinergic central nervous system neurons
GDF3		12p13.1	2000	Regulates adipose-tissue homeostasis; ocular and skeletal development
GDF5	BMP14; CDMP1	20q11.2	1994	Chondrogenesis, limb development, fracture healing, facilitates growth of tendon
GDF6		8q22.1	1999	Joint morphogenesis, facilitates growth of ligament, tendon
GDF7	BMP12	2p24-2p23	1998	Joint morphogenesis, facilitates growth of ligament, tendon
GDF8	MSTN, myostatin	2q32.1	1997	Regulates placental glucose homeostasis; negative regulator of skeletal muscle growth.
GDF9		5q23-5q33.1	1993	Regulates human folliculogenesis, directly affect oocyte growth and function.
GDF10	BMP-3b	10q11.22	1995	Osteogenesis inhibitor, dorsoventral patterning.
GDF11	BMP-11	12q13.13	1998	Mesodermal patterning and nervous system development
GDF15	PLAB, MIC-1, PDF, MIC1, NAG-1, PTGFB	19p13.1-13.2	1997	Inhibits differentiation into osteoclasts; regulates iron homeostasis; growth of granulocytes and macrophages

Biological functions of BMPs. Data collected from HGNC and Entrez Gene. Based on literature published BMP2(13); BMP3(13); BMP4(13); BMP5(14); BMP6(14); BMP7(16); BMP8A(313); BMP8B(314); BMP10(315); BMP15(316); GDF1(15, 317, 318); GDF2(319); GDF3(320-322); GDF5(323); GDF6(324); GDF7(325); GDF8(326); GDF9(320);GDF10(327); GDF11(328); GDF15(329-331).

Table 2. TGF-β and BMP receptors

Type I receptor	Type II receptor
ACVRL1 (ALK-1, ACVRLK1, ALK1, SKR3)	TGFBR2 (TGFR-2, TGFbeta-RII)
ACVR1 (ALK2, ACTRI, ACVRLK2, FOP, SKR1)	TGFBR3
BMPR1A (ALK3, ACVRLK3, CD292)	BMPR2 (BMPR-II, BMPR3, BMR2, BRK-3, T-ALK)
ACVR1B (ALK4, ACTRIB, ACVRLK4, SKR2)	ACVR2B (ActR-IIB)
TGFBR1 (ALK-5, ACVRLK4, SKR4, TGFR-1)	ACVR2A (ACTRII, ACVR2)
BMPR1B (ALK-6, ALK6, CDw293)	
ACVR1C (ALK7, ACVRLK7)	

without the presence of the type-II receptors, while the latter is incapable of signaling without their type-I counterparts (30).

Both types of BMP receptors consist of a N-terminal extracellular ligand binding domain, a single transmembrane region and a C-terminal serine/threonine kinase domain (31). The structure of the extracellular domain (ECD) of both receptors is similar. It has several conserved cysteines, which are important for the formation of characteristic three-dimensional structures. The ECD domain of type II receptors has three finger toxin folds, composed of three β-sheets and held together by a cluster of disulphide bonds to form the conserved scaffold (32, 33). The ECD domain of Type I receptor, for example BMPR-IA consists of two β-sheets and one α-helix which is different from the type II. This may be profound for their specific ligand binding (33). In addition to the conserved cysteine motif, the C-terminal of both type I and type II receptor ECDs have conserved clusters of hydrophobic residues, critical in ligand binding. The structure of this groove of residues is common in both types of receptors, but is less distinct in type-II receptors. In addition, all type-I receptors apart from ALK-1, have a large protruding hydrophobic residue on the core α-helix which fits into a hydrophobic pocket of the ligand. This process is known as the 'knob into hole' motif of type-I receptor ligand binding (33).

The intracellular region of the type I receptors, but not type II receptors, contains a highly conserved glycine and serine rich domain (GS domain), which is located in the intracellular juxtamembrane region of the receptor (31, 34). Type II receptors recruit type I receptors by phosphorylating the GS domain of type I receptor during signal transduction. The intracellular cytoplasmic region of both types of receptors consists of an enzymatic serine/threonine kinase domain critical in transducing the downstream signal of BMPs. This transphosphorylation is critical as it is required for activation of the type-I receptor, and hence downstream signaling. The type-II receptor kinase domain on the other hand, is constitutively active

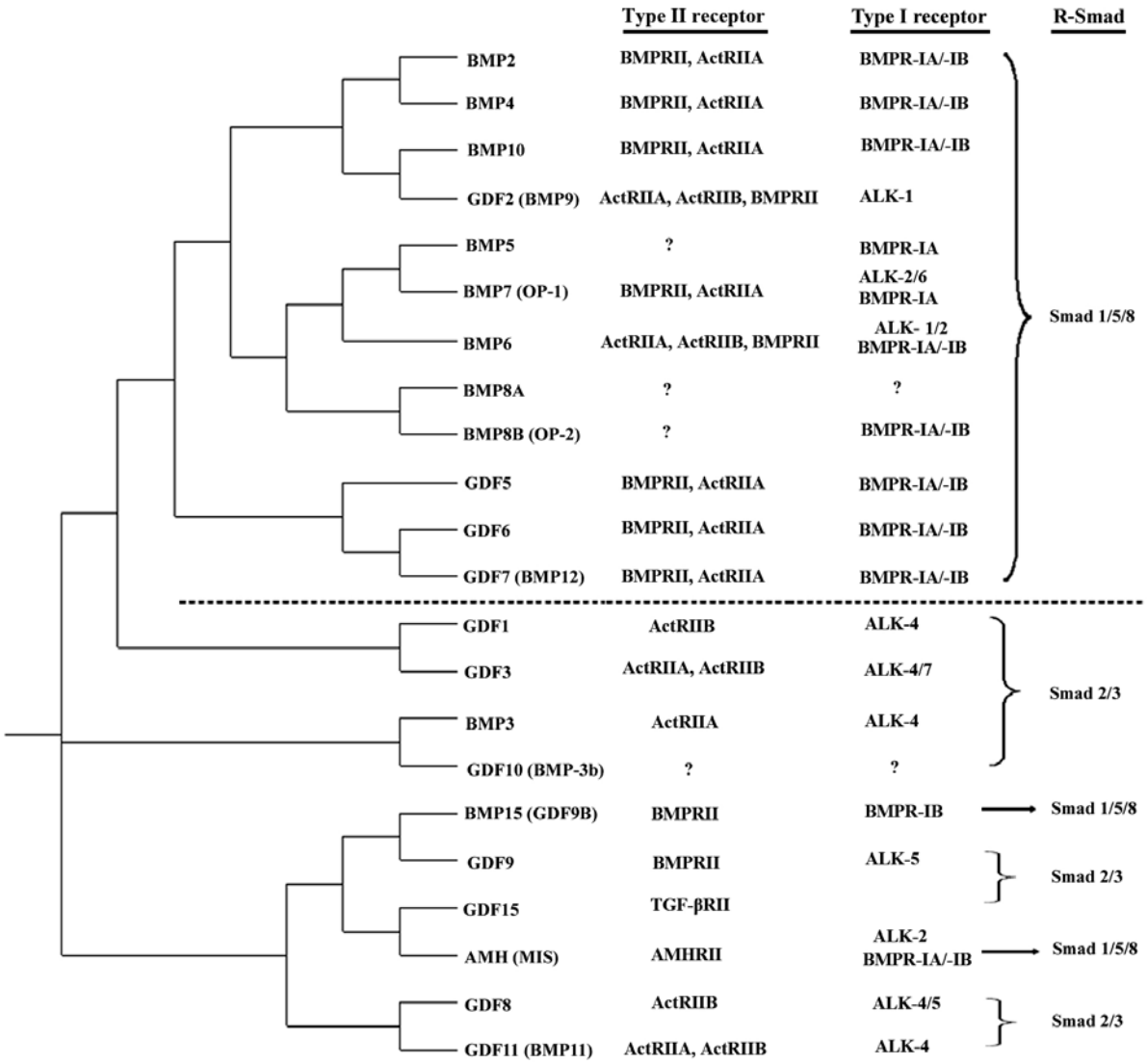

Figure 1. BMPs and their specific receptors and R-Smads. Dendrogram tree and key downstream signaling molecules of BMP/GDF. Phylogenetic analyses was performed using the ClustalW (http://www.ebi.ac.uk/clustalw/), and the dendrogram tree was drawn by using the Treeview (Version 1.6.6, http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). BMP2 (289-292); BMP3 (293); BMP4 (289, 290, 294-297); BMP5 (298, 299); BMP6 (297, 300, 301); BMP7 (292, 294, 296, 297); BMP10 (302); BMP15 (303); GDF1 (304); GDF2 (25, 305); GDF5 (67, 297, 306-308); GDF6 (302); GDF7 (302); GDF8 (309); GDF9 (23); GDF11 (310-312); GDF15 (150).

and hence does not require any activational phosphorylation event (35). In addition, type-II receptors have a short serine-threonine rich tail at the C-terminal of their kinase domains, not seen in type-I receptors (31).

3.3. Intracellular signal transduction

In absence of ligand binding, a small proportion of type I and type II receptors are present as preformed homodimers and heterodimers. Upon binding with ligands, oligomerisation of the receptors rapidly lead to conformational changes of the receptor complexes. This forms a ligand-receptor complex consisting of a homodimer/heterodimer of BMP ligands, Type-I and Type-II receptors. The Type-II receptors then transphosphorylates the GS domain of the Type-I receptors and leads to activation of downstream cascades (36). If the BMP ligand binds simultaneously to the preformed hetero-oligomeric complexes (PFC), this leads to activation of the Smad dependent pathway(37, 38). It includes recruitment of the pathway-restricted Smads (R-Smads, Smads1, 2, 3, 5 or 8), and regulates the transcription of target genes, this is known as the Smad dependent pathway. Unlike other members of TGF-β superfamily, BMPs have a higher affinity for the Type-I receptors, rather than the Type II receptors. Thus, BMP ligand can also bind to ALK3 or ALK6, and then recruits BMPRII into a hetero-oligomeric complex (BMP-induced signaling complexes, BISC), this leads to the activation of the Smad independent pathway

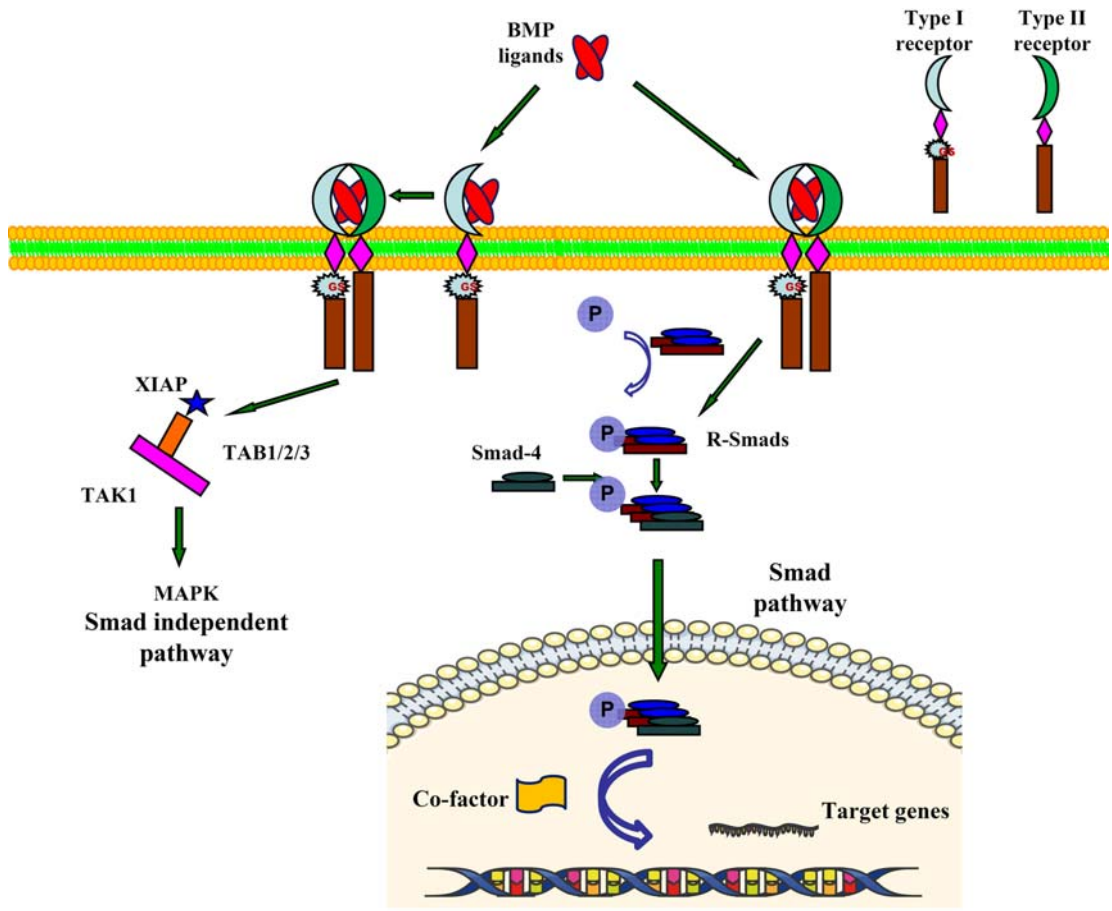

Figure 2. BMPs signaling pathways. BMPs ligands bind to type I and type II simultaneously, after the phosphorylation, type I receptors recruit R-Smads, and this leads to activation of the Smad-dependent pathway. Activation and translocation of R-Smads assisted by Co-Smad result in transcriptional regulation of target genes. If BMP ligands bind to the type I receptors first and then recruit the type II receptors, following phosphorylation by type II receptors, the type I receptors activate TAB1/2/3 through XIAP, which finally activate MAPK pathways, leading to the activation of Smad independent pathway.

(37, 38). Type I and type II BMP receptors are indispensable for both Smad dependent and independent pathways (Figure 2).

3.3.1. Smad dependent pathway

Smad proteins are important intracellular signaling molecules downstream of the BMP receptors. They are homologues of the originally identified Mad and Sma proteins, found in *Drosophila* and *C. Elegans*, respectively (39). To date, eight Smads have been identified in humans, and comprise three subgroups: pathway restricted Smads (receptor regulated Smads) (referred to as R-Smads which include Smad 1, 2, 3, 5 and 8), common mediator Smad (Co-Smad, Smad4), and inhibitory Smads (I-Smads, Smad 6 and 7) (38, 40). R-Smads 1, 5 and 8 are substrates of the type I receptors, including ALK-1, ALK-2, ALK-3 and ALK-6; whereas R-Smads 2 and 3 are substrates of the type I receptors including ALK-4, ALK-5 and ALK-7 (40-43). All Smad proteins share considerable homology in their primary sequences. In addition to a non-conserved proline rich linker region, R-Smads and Co-Smad contain two highly conserved Mad homology domains: the Mad homology 1 (MH1) domain in the amino-terminal part and the Mad homology 2 (MH2) domain at the carboxy-terminal. The MH1 domain can bind to specific DNA sequences, and the MH2 domains are responsible for homo- and heteromeric complex formation. The MH1 domain regulates transcription by interacting with other transcription factors, and contains a highly conserved β-hairpin eleven residues in length, which can directly bind to DNA through the major groove (44). In the case of the I-Smads, the MH1 domain is very short, with highly distinct sequences and is not able to bind DNA. Furthermore, the MH1 domain of inactive Smads acts as a repressor of the MH2 domain, by preventing it from forming a complex with Smad4. Phosphorylation of the C-terminal MH2 domain by the type I receptor appears to unfold the two domains and alleviates the inhibition of MH1 (39). In Smad3, basic helix 2 consists of a KKLKK sequence that acts as a nuclear localisation signal and hence is critical during Smad3 nuclear translocation (44). The MH2 domain is multifunctional and provides the Smads with their specificity and selectivity, as well as transcriptional

activity. In addition, the MH2 domain is critical in oligomerisation and when activated, Smads can form homo-oligomeric complexes by interacting through their MH2 domains (45). Finally, the MH2 domain is also responsible for specific interactions with type-I receptors. This is due to a pocket of basic residues that acts as a docking site for the phosphorylated GS domains of these receptors (45). Smad4 however does not interact with the receptors as it has an inserted element and instead Smad4 acts as a docking site for other R-Smads. The linker region of Smads meanwhile is variable in sequence and length and also contributes to the oligomerisation of Smads. In addition, phosphorylation of four PXS/TP motifs within the linker region by MAPK acts as a mechanism to prevent the accumulation of Smads in the nucleus (46). The proline rich PPXY (PY) sequence allows for interaction with WW motif containing proteins involved in Smad degradation (47). R-Smads contain the C-terminal Ser-Ser-X-Ser (SSXS) motif which is phosphorylated by the Type I receptor during signal transduction of BMPs (38). Of the R-Smads, Smads 2 and 3 are known as TGF-β/activin activated Smads, whereas Smads 1, 5 and 8 are the BMP activated Smads (48). Smad2/3 has been shown to colocalise to the cell membrane via a FYVE domain Smad interacting protein known as Smad-anchor for receptor activiation (SARA). During induction of TGF-β signaling, SARA acts to recruit Smad2 to its type-I receptor, resulting in its phosphorylation and dissociation from SARA, allowing it to complex with Co-Smad4 (49). Smad4 acts to form a heteromeric complex with the R-Smads and translocates them to the nucleus in order for them to regulate transcription of their target genes.

Following Smad-complex translocation into the nucleus, R-Smads interact with other proteins including transcriptional coactivators and repressors in order to bind to specific DNA sequences and regulate expression of target genes, inlcuding Id1-3, Smad6/7, type-I collagen, JunB and Mix.2. Smad1 binds with low affinity to SBEs and preferentially binds to GC-rich boxes with sequence GCCGNCGC (50). Smad2 and 3 in complex with Smad4 bind specifically via their MH1 domains to Smad binding elements (SBE) with AGAC/GTCT sequences found in target genes' promoter and enhancer (51).

3.3.2. Smad independent pathway

Unlike other members of TGF-β superfamily, BMPs have a higher affinity for the Type-I receptors, rather than the Type II receptors. Thus, BMP ligand can also bind to ALK3 or ALK6, and then recruits BMPRII into a hetero-oligomeric complex (BMP-induced signaling complexes, BISC), this leads to the activation of the Smad independent pathway (37, 38). During intracellular signal transduction, the X-linked inhibitor of apoptosis protein (XIAP) functions as an adaptor protein bridging between the Type I receptor and TGF-ß activated binding protein (TAB1/2/3), which is an activator of the MAPKKK TGF-ß activated tyrosine kinase 1 (TAK1) (52-54). The activation of TAK1 can lead to activation of p38, a mitogen-activated protein kinase (MAPK) (37, 55, 56). TAK1 can also activate Jun N-terminal kinases (JNKs), NF-kappaB (NF-kB) and Nemo-like kinase (NLK) (57-59). TAK1 normally induces apoptosis by activating JNK or P38 MAPK pathways. However, in Xenopus embryos, the BMP signal was shown to interact with XIAP to inhibit TAK1 associated apoptosis by impeding the action of caspases (53).

3.4 Regulatory system of BMP signaling

The regulation of BMP signaling may occur extracellularly during the process of ligand binding to the receptors, or intracellularly during signal relay or finally, affect regulation of their target genes (Figure 3). BMPs have also been shown to regulate their function through a negative feedback loop, in which the pseudoreceptor, Inhibitory Smads (Smad 6 and 7) antagonists of BMPs, appear to be involved (60).

3.4.1. Extracellular regulatory factors

3.4.1.1. BMP antagonists

The most important molecules to influence BMP signaling extracellularly are the BMP antagonists. BMP antagonists exert their influence over BMP and BMP receptors in two ways: direct competition between the antagonists and BMPs, and regulation of expression of the antagonists by BMPs themselves. The antagonists can bind to BMP receptors competitively, and block/inhibit the effect of the BMPs. For example, competition between Noggin and BMP4 regulates dorsalization during Xenopus development (61). On the other hand, noggin expression in osteoblasts can be induced by BMP2, 4 and 6. Therefore, the BMPs are able to modulate their effect via a negative feedback loop by upregulation of the expression of their antagonist (62). Recently, this feedback regulation of BMP antagonists by BMP7 has also been indicated in prostate cancer (63).

3.4.1.2. Pseudoreceptor

Besides the BMP antagonists, there are other mechanisms by which BMP signaling is regulated extracellularly, such as co-receptors and pseudoreceptors. BMP and activin membrane bound inhibitor (BAMBI) is a pseudoreceptor for serine-threonine kinase receptor. BAMBI has an extracelluar domain similar to that of the type I receptors, but lacks the intracelluar serine/threonine kinase domain. BAMBI binds to ligand competitively, and then inhibits signaling by BMPs and other TGF-β molecules (64). BMP4 can also induce the expression of BAMBI in mouse embryonic fibroblasts (65), in doing so, BMP4 creates a negative feedback loop to regulating BMP function. Some transmembrane tyrosine kinase receptors have been reported to interact with BMP receptors and regulate their signaling. TrkC, a neuronal tyrosine kinase receptor, has been shown to directly bind to BMPR-II, inhibiting its interaction with type I receptors and downstream signaling (66). Another tyrosine kinase receptor Ror2 forms heteromeric complex with BMPR-IB in a ligand independent manner leading to an inhibition of downstream Smad1/5 signaling by GDF-5 (67).

3.4.1.3. Co-receptors

Along with the negative regulators, like other members of the TGF-β superfamily, there are co-receptors for BMP ligands, which positively enhance their signaling. The repulsive guidance molecule family including RGMa,

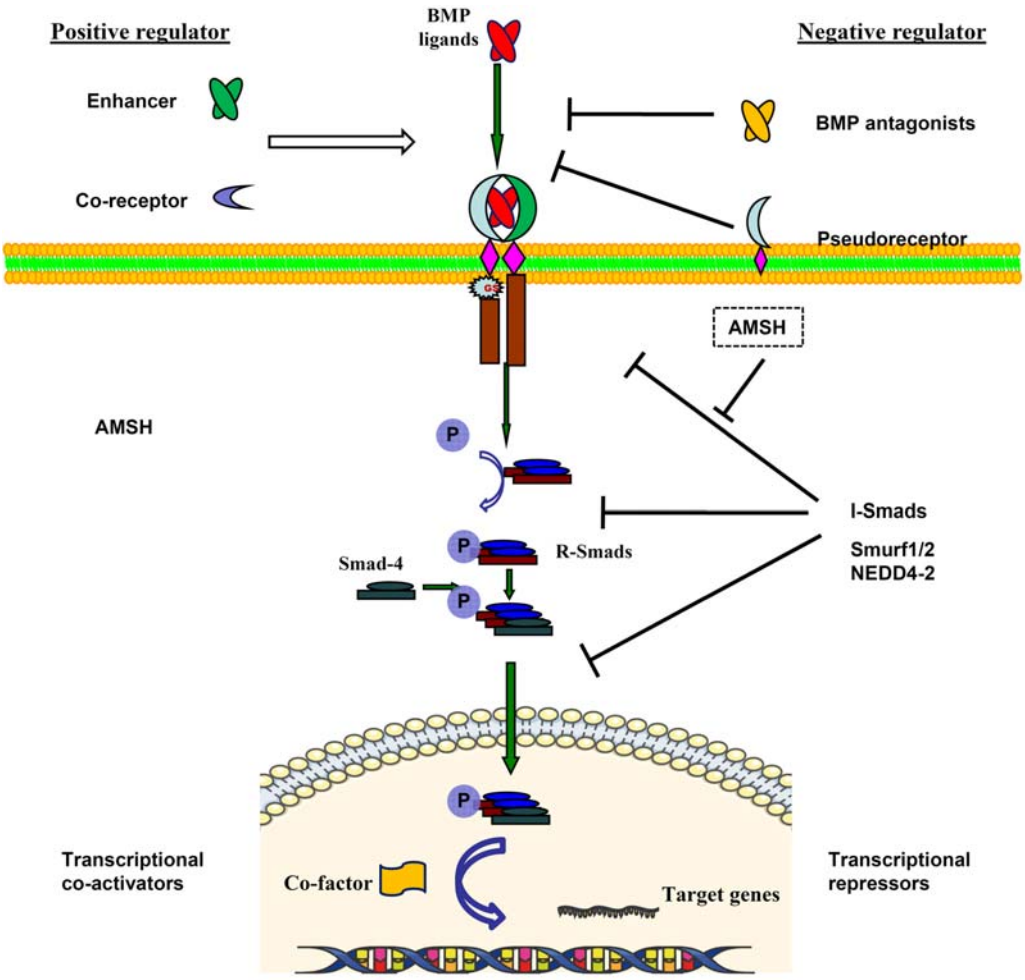

Figure 3. Regulatory factors on BMP signaling. Regulation of BMP signaling can occur during the process of ligand binding to receptor, and the intracellular signal transduction.

RGMb, and RGMc, are coreceptors for BMP2 and BMP4, and enhance their signaling (68-70). RGMb, also known as DRAGON, is the first co-receptor reported for BMP, and is a glycosylphosphatidylinositol-anchored member of the repulsive guidance molecule family. DRAGON binds directly to BMP2 and BMP4, but not BMP7 or other TGF-β ligands. The interaction between DRAGON and BMPs enhances the signaling and ultimately leads to a stronger biological response from the cell. Interestingly, this enhanced effect due to the DRAGON/BMP interaction can be reduced by the BMP2/4 antagonsit, Noggin (68). cGMP-dependent kinase I (cGKI) has also been shown to interact with and phosphorylate BMPR-II, leading to enhancement of BMP receptor signaling (71).

3.4.2. Intracellular regulatory factors for Smad signaling

Following the activation by the type I receptor, R-Smads relay the signal into the nucleus and subsequently regulate target genes. A number of proteins existing in the cytoplasm and nucleus interact with R-Smads and therefore can act to coordinate their signaling.

3.4.2.1. Inhibitory Smads (I-Smads)

I-Smads are structurally divergent from the rest of Smads. They act as negative regulators of TGF-β signaling by binding to type I receptors, thereby preventing R-Smad activation, and also compete with Smad4 for hetero-complex formation. Smad7 is responsible for inhibiting TGF-β/activin and BMP signaling, whereas Smad6 is specific for BMP signaling (72, 73). Smad6/7 inhibit signal transduction of BMPs, by inhibiting activation of Smad 1 and 5 by the BMP Type I receptor. Smad6/7 also inhibit the heterodimerization between Smad1/5 and Smad 4 (73, 74). The inhibition can be enhanced through a feedback up-regulation of Smad6/7 by BMPs stimulation (75, 76). However, the inhibitory effect on BMP signaling by I-Smads can also be regulated. The associated molecule with the SH3 domain of STAM (AMSH) is a direct binding partner for Smad6 and has been found to inhibit the interaction between Smad6 and the activated BMP type I receptor, thereby allowing more efficient BMP receptor-induced phosphorylation of R-Smads. In addition, AMSH was found to interfere with the interaction between Smad6 and the activated R-Smad.

Thus, AMSH promotes BMP signaling by negatively regulating the function of I-Smads (77).

3.4.2.2. Smad interacting proteins

As Smads generally bind SBEs with low affinity they need to interact with and be recruited by several other transcription factors. The first of these proteins to be identified was Forkhead box HI (FOXHI) which specifically recruits activated Smad2/4 to promoters (78). Others include P53 (79), Runx transcription factors (80), Smad interacting protein-1 (SIP-1) (81), ATF-2 (82) and YYI transcription factors which repress expression of genes including PAI-1 and Id-1 (83).

In addition, some transcriptional co-activators and repressors have also been reported to regulate Smad signaling by interacting with the MH2 binding domain of Smads. P300 and CREB-binding rotein (CBP) are histone acetyl transferase (HAT) proteins and interact with Smads to enhance transcription of their target genes by increasing the accessibility of the transcriptional machinery (84). Transcriptional corepressors include TG interacting factor 1 (TGIF1), TGIF2, ecotropic viral integration site-1 (Evi-1), Ski and Ski related novel gene (SnoN) which interact with Smad3/4 when they bind to the SBEs and recruit histone deacetylases (HDACs) to induce nucleosomal condensation and repress transcription of target genes (85-88). Sloan-Kettering retrovirus (Ski) binds Smad 1, 2, 3, 5 and 4 and inhibits BMP signaling (86, 89, 90). The transducer of ErbB-2 (Tob) is probably associated with the MH2 domain of Smad 1, 5, 6, 7 and 8 (91, 92). Induction of TGF-β signaling, results in Ski and SnoN degradation, allowing Smad3/4 to induce transcription (93-95).

3.4.2.3. Molecules that regulate degradation of the Smads

The concentration of available Smads in the intracellular pool is regulated by HECT type E3 ligases known as Smad Ubiquitination Regulatory Factors (Smurf) 1 and 2. The WW motifs of Smurfs interact with the PY domain in the linker region of R-Smads, inducing their degradation by the proteosome, which results in inhibition of TGF-β family signaling (96). Smurf 1 can directly interact with Smad 1/5, and facilitate their degradation (97). It can also indirectly interact with the BMP type I receptor through I-Smad 6 and 7, and induce ubiquitination and degradation of the receptors (98). TNF has been shown to inhibit osteoblastic bone formation through upregulation of Smurf 1 and 2 (99). In addition, Smurfs are responsible for the translocation of I-Smads from the nucleus into the cytoplasm, and enhance I-Smad interaction with the type-I receptors (100). This also results in Smurf-dependent ubiquitin degradation of the type-I receptors, leading to a down-regulation of cell surface receptor expression. A Ring type E3 ligase, Arkadia induces the ubiquitination of Smad7 but not type-I receptors, leading to an amplification of TGF-β signaling (101).

NEDD4-2 (neural precursor cell expressed, developmentally down-regulated 4-2) was recently found to be a direct binding partner of Smad7 (102). NEDD4-2 is structurally similar to Smurfs 1 and 2 (Smad ubiquitin regulatory factors). It can interact with Type I receptor via Smad 7, and induce its degradation. It can also bind to Smad 2 and 3 in the ligand-dependent manner, and degrade Smad 2, but not Smad 3. Overexpression of NEDD4-2 inhibits the transcriptional activity induced by TGF-β and BMPs. An ubiquitin C-terminal hydrolase (UCH37), is a deubiquitinating enzyme that can potentially reverse Smurf-mediated ubiquitination. It forms a stable complex with Smad 7, which deubiquitinates and stabilizes the type I TGF-β receptor (103).

4. BONE AND PREDISPOSITION OF METASTASIS TO BONE

Metastatic bone lesions are frequently occurring events in certain solid tumors, and the predominant type of metastasis in advanced diseases of breast cancer, prostate cancer and lung cancer. However it is still largely unknown, how the cancer cells (seeds) acquired capacities and predispositions, leading to the dissemination and development of bone metastasis. Recent studies from BMPs and their implication in malignancies may have shed light on this. It suggests that BMPs may play profound roles in assisting cancer cells to acquire certain capacities and preferentially disseminate towards bone.

4.1 Aberrant expression and signaling of BMPs in primary tumors and, their association with bone metastasis

BMPs and their receptors signaling have been implicated in development and progression of a variety of solid tumors, including prostate cancer, breast cancer and lung cancer etc (104, 105). Research currently focuses on BMP expression in prostate cancer and breast cancer. They are the most common malignancies with the highest incidence of bone metastasis, and are also representative for two distinct types of metastatic bone lesions; osteoblastic and osteolytic lesions.

4.1.1. Prostate cancer

An elevated level of BMP6 is associated with higher grade primary tumors and advanced prostate cancer with metastasis (106-109). BMP6 may contribute to the progression of prostate cancer independent of androgen stimulation (107, 109). In contrast to BMP6, BMP2, BMP4, BMP7, BMP9, BMP10 and GDF15 are expressed predominantly in normal prostate tissues, and their expression appear to be down-regulated or suppressed during the development and progression of prostate cancer (110-115).

The expression of BMPRIA, BMPRIB, and BMPRII in human prostate cancer tissues has also been investigated and was found to correlate with tumor grade. Using immunohistochemistry and Western blot analysis, it was shown that there was frequent loss of expression of these three receptors in high-grade prostate cancer. However, it appears that only the loss of expression of BMPRII has a correlation with poor prognosis in prostate cancer patients (116, 117). Loss of the expression of BMPR-IA, BMPR-IB and especially BMPR-II, in both prostate cancer tissues and cancer cell lines, has been shown to have an association with the progression of

prostate cancer (114, 116-118). Intracellular signaling molecules downstream of the BMP receptors have also been shown to have altered expression patterns in prostate cancer. The level of Smad 4 and Smad 8 in the nucleus is thought to be associated with the development of prostate cancer, and loss of Smad 4 is related to progression to a more aggressive phenotype (112). The pattern of expression of BMPs may be important in the pathogenesis of osteoblastic-type metastases in prostate cancer. It suggests that aberrant phenotype and function of BMPs are implicated in tumorigenesis and progression of prostate cancer, which may provide favorable characteristics to assist cancer cells dissemination to bone.

4.1.2. Breast cancer

Reduced expression of BMPs, including BMP2, BMP4, BMP6, BMP7, BMP12, BMP15 and GDF9a, have been revealed in a breast cancer cohort using both quantitative real time PCR and immunohistochemical methods. The decreased expression of BMP2, BMP7, GDF9a and BMP15 was associated with poor prognosis of the patients (119-121). A similar reduction of these BMPs has been demonstrated in other studies (122, 123). Decreased BMP7 expression in primary tumors was associated with bone metastasis (124). In contrast to these findings, elevated expression of BMPs, such as BMP2, BMP4, BMP5 and BMP7, has been demonstrated in other studies (119, 125-128). Although the expression of specific BMPs, such as BMP2, 4, 6 and 7 in breast cancer remains controversial, the abnormalities in their expression have indicated a role in the development and progression of breast cancer.

Meanwhile, investigations into the expression patterns of BMP receptors and intracellular signaling molecules have also been conducted, but to a rather limited extent. Elevated expression of BMPR-IB was associated with high tumor grade, high tumor proliferation, cytogenetic instability, and a poor prognosis in oestrogen receptor-positive carcinomas (129). This suggests that the expression of this type I receptor may associate with the ER status, and is regulated by estrogen. The results from the host lab showed a decreased level of BMPR-1B in breast cancer, which was associated with poor prognosis (130). Activation of the Smad pathway of BMPs (Smad1/5/8) and TGF-β (Smad2) was revealed in nuclei of breast cancer cells in both primary tumors and bone metastases, and similar involvements were also seen in an *in vivo* model. TGF-β3 and BMP2 could promote motility and invasiveness of breast cancer cells (MDA-231-D) *in vitro*. Moreover, expression of domain-negative receptors for TGF-β and/or BMPs in the MDA-231-D cells inhibited invasiveness *in vitro* and bone metastasis in the xenograft model. These results suggest that BMPs as well as TGF-ß promote invasion and bone metastasis of breast cancer (131). Although the phenotypic profile of BMPs needs to be clarified, there is no doubt that BMP and their receptor signaling play important roles in breast cancer, particularly the disease specific bone metastasis.

4.2 BMPs affect growth and survival of cancer cells

The notion that the BMPs may play a profound role in the progression of primary prostate tumors and the development of secondary tumors, especially bone metastasis, has been supported by lines of biological based investigations. However, the precise machinery underlying this connection is still unclear. The application of recombinant human BMPs (rh-BMPs) and artificial manipulation of the expression of BMPs or the signaling molecules makes it possible to investigate the biological functions of BMPs in cancer *in vitro*.

4.2.1. BMP and proliferation of cancer cells

Uncontrolled proliferation is one of the predominant features for cancer cells. As other members of TGF-β family, BMPs can inhibit proliferation of cancer cells. However, certain elements could divert the responses of cancer cell to BMPs, which include expression profile of androgen receptors, estrogen receptors, BMP receptors, intracellular signaling molecules and the specific BMPs.

BMP2 and 4 inhibit the growth of the androgen-sensitive prostate cancer cell line LNCaP, but not the androgen-insensitive PC-3 (132). The inhibitory effect of BMP2/4 on cell proliferation is related to the activation of Smad 1, up-regulation of the cyclin-dependent kinase inhibitor (CDKI) p21 (CIP1/WAF1), and phosphorylation of retinoblastoma (Rb) (132). Simliar, BMP2 inhibits estrodiol-induced proliferation of breast cancer cells, via up-regulation of cyclin kinase inhibitor, p21 which in turn inhibits the estradiol-induced cyclin D1-associated kinase activity (133). The up-regulation of p21 by BMP2 can also prevent EGF-induced proliferation of breast cancer cells (MDA-MB-231) (134). It is interesting to note that the regulation of p21 expression by BMP2 was mediated by Type-I receptors, Smad-1 and Smad-4. In MDA-MB-468 which only expresses Smad-1, BMP2 fails to induce p21 and inhibits the cellular proliferation (135). On the other hand, BMP6 and BMP7, are able to inhibit the proliferation of both androgen-sensitive and androgen-insensitive prostate cancer cells. BMP6 inhibits the proliferation of both LNCaP and DU-145 cells, by up-regulation of several cyclin-dependent kinase inhibitors such as p21/CIP, p18 and p19, which can be prevented by Noggin (136). Furthermore, BMP6 and BMP7 can also inhibit both oestrogen sensitive and insensitive breast cancer cells (137, 138). In contrast to the inhibitory effect, some BMPs may indirectly promote the proliferation of breast cancer cells, such as BMP4 which has a synergetic effect on the proliferation of breast cancer cells induced by fibroblast growth factor (FGF), epidermal growth factor (EGF) and hepatocyte growth factor (HGF) (139). This contrasting effect on proliferation of breast cancer cells was clearly demonstrated in a recently published study, where BMP7 could promote proliferation of MDA-MB-231 and BT-474 cells, but showed an inhibitory effect on the other breast cancer cell lines tested (140).

The nature of the diverse, sometimes contrasting effects of different BMPs is interesting, but the underlying mechanisms remain unclear. While BMPs themselves may hold some of the answers, BMP receptors are probably also determining factors in distinguishing between the pro- or anti-proliferation effects seen in prostate cancer cells. For example, Kim *et al* demonstrated that transfection of a domain negative BMP-RII (BMP-RIIDN) into PC-3 cells

(PC3M), resulted in a growth rate 10 time higher than that in control cells in a murine tumor model (117). Once the PC-3 cells express a constitutively active BMPRIB (c.a.-BMPRIB) in a tetracycline (Tet)-regulated manner, the Tet/doxycycline-regulated expression of the c.a.-BMPRIB results in the inhibition of both the *in vitro* cell proliferation and the tumor growth *in vivo* (141). The inhibition of prostate cancer cells mediated by BMPR-IB and BMPR-II were further evident in our recent study following knockdown of these receptors (114). However, recent studies have indicated that certain BMP receptors mediate contrasting effect in breast cancer cells. Over-expression of a domain negative BMPR-II in breast cancer cells is able to interfere with the phosphorylation of Smad-1 by BMPR-II, leading to an arrest of the cancer cells at the G1 phase of the cell cycle. This suggests that coupling between BMPs and BMPR-II has a significant role in controlling the proliferation and survival of breast cancer cells (142). A domain negative Type II TGF-β receptor (dnTbetaRII) could eliminate the anti-proliferative effect of BMP-2 in breast cancer cells by preventing the phosphorylation of Smad-1 (143). One of the Type I receptors, BMPR-IA (ALK-3) has been recently shown to be involved in the activated Smad pathway which contributes to development and progression of breast cancer at primary and secondary sites (131). While BMPR-IA and BMPR-II play positive roles for BMP induced proliferation and aggressiveness in breast cancer cells, another Type I receptor, BMPR-IB has been indicated as a negative regulator (144).

A biphasic effect on the proliferation of LNCaP can be induced by rh-BMP2 under appropriate hormonal conditions. A decrease in cell proliferation in response to rhBMP2 was elicited in the presence of an androgen, which was thought to be the result of up-regulation of BMPR-IB expression. Conversely, an increase in cell growth was seen in the absence of androgen (145). Similar biphasic effects were also revealed in LNCaP following exposure to rh-BMP7. BMP7 can also promote proliferation of LNCaP at lower concentrations (20ng/ml) in the absence of exogenous androgen, but inhibits proliferation at higher concentrations (80ng/ml) (141). It clearly indicates a possibility that sexual hormones and their receptors are able to regulate the response to BMPs.

4.3.2. BMPs and Apoptosis

Apoptosis or programmed cell death is crucial for physiological control of the cell population and regulation of tissue homeostasis. Aberration in apoptosis plays important roles during oncogenesis and subsequent progression. In addition to the pivotal role in the control of cell proliferation and growth, BMPs also play a profound role in regulating the apoptosis of cancer cells. For example, BMP4 induces apoptosis of myeloma cells through ALK3 and ALK6, BMP5 acts partially by ALK3, whereas BMP6 and BMP7 rely on ALK2 (146).

BMPs can regulate the transcription of genes in control of apoptosis via the Smad dependent pathway. For example, BMP9 induces prostate apoptosis response-4 (Par4) in prostate cancer cells and subsequently leads to apoptosis through Smad dependent pathway (114). The expression of the apoptosis mediators DRP-1 death kinase and ZIP kinase may be regulated by BMPs through the Type I receptor, as demonstrated by expressing constitutively active BMP type I receptors in the cells (147). Senescent cells, as the result of BMP4 treatment had lower ERK activation, VEGF expression, and Bcl2 expression than wild-type cells (148).

BMPs can also alter apoptosis through Smad independent pathways. BMP2 activates the p38 mitogen-activated protein kinase (MAPK) pathway in medulloblastoma cells leading to apoptosis, which can be prevented by Noggin (149). BMP10, a close member to BMP9 within the superfamily, has been shown to induce apoptosis in prostate cancer cell but contrastingly through Smad independent activation of MAPK pathway (115). Additionally, BMPs themselves may mediate apoptotic effect by other factors. For example, GDF-15 is indispensable for the pro-apoptotic activity of several apoptosis-inducing agents including the retinoid-related molecules (CD437 and ST1926) (150).

The apoptotic response to BMPs is dependent upon cell type and, within the same cell type, is dependent on phenotype, hormone and growth factors status, and survival condition. For example, BMP4 can inhibit DNA synthesis and induce apoptosis in two IL-6 dependent myeloma cells (OH-2 and IH-1), but not the IL-6 independent ANBL-6 cells (151). BMP7 can stablize the level of survivin in prostate cancer cells (LNCaP and C4-2B), and restore the activity of c-jun NH2-terminal kinase (JNK), both of which contribute to the anti-apoptotic activity of BMP7 (152, 153) BMP2 partially prevents an increase in caspase-3 mRNA levels in MCF-7 cells as a result of serum withdrawal in cell culture (serum free culture condition) (154).

Most interestingly, BMPs may have biphasic effects on apoptosis in cancer cells depending on their survival condition. For example, under routine culture conditions, BMP2 showed a pro-apoptotic effect in breast cancer cells (MCF-7) through regulating the expression and function of apoptosis related genes, such as protein kinase R (PKR) and eIF2α (155). Under deprivation of serum, BMP2 increases the resistance of MCF-7 cells to hypoxia induced apoptosis, via the activation of both the MAPK pathway and ID-1, and suppression of Caspase-3 (126, 154). The other example is BMP6, which can inhibit the proliferation of breast cancer cells (MDA-MB-231). Under deprivation of serum, BMP6 turns to protect these cancer cells from stress induced apoptosis through up-regulation of survivin via the Smad dependent pathway, and activation of p38 via the Smad independent pathway (137).

4.3. Epithelial-mesenchymal transition (EMT) and aggressive phenotypes acquired by metastatic cancer cells before dissemination from primary tumors

The most predominant characteristics acquired by cancer cells are invasiveness and motility, being fundamental for their dissemination and metastasis. Epithelial to mesenchymal transition (EMT) is a process involving a sequence of changes in gene-expression

patterns during which epithelial cells dissipate their epithelial features and acquire characteristics typical of mesenchymal cells. The key changes during EMT include a loss of E-cadherin expression and increases in N-cadherin, SNAI1, SLUG (SNAI2), TWIST, vimentin, fibronectin and accordingly many of these molecules have been shown to be deregulated in cancer (156). EMT has been shown to play an important role during tumor progression, leading to enhanced motile and adhesive capacities of cancer cells, and assisting them to spread and metastasize.

4.3.1. BMP and EMT

EMT regulated by BMPs has been implicated in foetal and postnatal development of different organs and tissues, and certain pathological processes including cancer. In normal development, BMP2 acts synergistically with TGF-β3 in the initiation of EMT during the generation of the endocardial cushion (157). The application of the BMP antagonist, Noggin, disrupts the EMT induced by BMPs during the development of the chicken heart (158). The EMT induced by BMP7 contributes to the repair of tubular injury in a fibrotic kidney (159, 160).

EMT not only causes a disruption of epithelial homeostasis which may lead to carcinogenesis, it can also transform the indolent tumor cells into a more aggressive colony, leading to metastasis. BMP4 can subvert the ability of mammary epithelial cells to form polarized lumen-containing structures, and also endows them with invasive properties (161). This supports the involvement of this BMP cytokine in the progression of breast cancer. In the bone metastasis-derived PC-3 prostate cancer cell line, BMP7 has been shown to induce epithelial-mesenchymal transdifferentiation with classical changes in morphology, and promote both motility and invasiveness in prostate cancer cells (152). However, in mammary epithelial cells (NMuMG), BMP7 was not able to induce EMT whereas TGF-β1 could (162). In contrast, some BMPs are able to reverse EMT and reduce the aggressive properties of tumor cells. For example, BMP6 restores E-cadherin-mediated cell-to-cell adhesion and prevents breast cancer metastasis through the down-regulation of δEF1. Higher level of δEF1 expression is associated with a more invasive phenotype of breast cancer cells (163). Another example is BMP7, which is able to increase cytokeratin expression and decrease vimentin in breast cancer cells *in vitro* and *in vivo*, leading to an epithelial-like phenotype (124).

Mechanisms underlying BMP induced EMT have been partially revealed recently. The induction of Inhibitor of differentiation factors (Id-1, Id-2 and Id-3), and activation of the proto-oncogene phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signaling pathway by some BMPs (BMP2 and BMP7) has been implicated in BMP-induced EMT, but further exploration is required (164-166).

4.3.2. Influence of BMPs on cellular motility and invasion

Invasion and metastasis are the major causes of cancer related mortality. The motility and invasiveness of cancer cells are amongst the main determining factors regarding the metastatic spread of a tumor. Recent evidence demonstrates that BMPs also regulate cellular motility and the invasiveness of some malignant cells, including lung cancer cells (A549 and H7249), malignant melanoma cells, and breast cancer cells (MCF-7) (167-169). BMP2 may contribute to the invasiveness of tumor cells via the induction of tenascin-W in the tumor surrounding stroma. Tenascin-W belongs to a family of extracellular matrix glycoproteins. It has been shown to be highly expressed in the stroma around breast carcinoma lesions, and has been linked to the aggressiveness of tumor cells via its interaction with a α8 integrin. HC11 cells derived from normal mammary epithelium do not express α8 integrin and fail to cross tenascin-W-coated filters. However, 4T1 mammary carcinoma cells do express α8 integrin and their migration is stimulated by tenascin-W. BMP2 can induce the expression of tenasin-W through the p38 MAPK and JNK pathway. This is in clear contrast to TGF-β1, which is a potent inducer of tenascin-C (170). Finally, up-regulation of Id-1 by BMP2 may be another contributing factor in BMP2 related aggressiveness of breast cancer cells (171).

In the case of prostate cancer cells, studies have revealed that the motility and invasiveness of prostate cancer cells can be increased by the BMPs. BMP2 and BMP7 promote the migration and invasion of osteoblastic prostate cancer cells (LAPC-4 and LAPC-9) in a dose-dependent manner, but BMP4 does not have this effect (172). BMP2 and BMP6 can increase the *in vitro* invasive ability of the prostate cancer cell lines C4-2B and LuCaP (173). BMP2 and, to a lesser extent, BMP4 will stimulate PC-3 cell migration and invasion in a dose-dependent fashion, an effect which Noggin can subsequently inhibit (174). On the other hand, some other BMPs may have an inhibitory effect on the aggressiveness of breast cancer cells. For example, forced expression of GDF-9a in breast cancer cells could reduce their invasiveness *in vitro* (120). BMP9 and BMP10 have been shown to inhibit motility and invasion of prostate cancer cells (114, 115). The expression of MMP-13 could be enhanced by TGF-β, but was inhibited by BMP2 (175). However, whether this is implicated in the invasiveness of breast cancer cells is still unknown.

4.4. Regulatory factors of BMP

A diversity of BMPs expression and signaling occurs in malignancies during their development and progression, which also reflects the complexity of both regulatory machinery for BMPs and their interactions with other factors. A number of hormones and growth factors have been indicated in the network with BMPs.

4.4.1. Sexual hormones

In prostate cancer, androgens play an important part in the carcinogenesis, progression and metastasis of the disease, and controlling the level of circulating androgens constitutes the only effective therapy in advanced disease. Androgens can induce the expression of some BMPs, BMP receptors and intracellular signaling molecules. With regard to the receptors, androgens induce the expression of BMPR-IB mRNA, but not the expression

of BMPR-IA and BMPR-II mRNAs in the androgen-sensitive human prostate cancer cell line LNCaP. As discussed above, rh-BMP2 induces a biphasic effect on the proliferation of LNCaP. In the presence of an androgen, there is a decrease in cell proliferation in response to rhBMP2. This is thought to be the result of up-regulation of BMPR-IB expression. Conversely, an increase in cell growth is seen in the absence of androgen. Thus, the induction of BMPR-IB expression by an androgen appears to convey an inhibition of cell proliferation in response to stimulation by BMPs (145). Turning to BMPs themselves, orchidectomy resulted in a decrease in the expression of BMP7 in a murine model and administration of testosterone or dihydrotestosterone caused an increase in the expression level (176). However, androgen deprivation appears to have no effect on BMP6 production in the normal rat prostate, suggesting an alternative and androgen-independent gene regulation for this particular protein (107).

The aberrations in the BMP phenotype and signaling in breast cancer may due to the ER status and self-adjustment by tumor cells themselves according to the needs for development and progression at different stages. Epigenetic regulation of BMPs and BMP receptors in breast cancer is associated with the ER status (177). Oestrogen can repress the expression of some BMP receptors, such as BMPR-IA, BMPR-IB, ACVR2A, and ACVR2B, but has no effect on the expression of ACVR1 and BMPR-II (138). In line with this observation, the expression of some BMPs and BMP receptors in breast cancer tissues has been shown to correlate with ER status. The expression of BMP7 has been found to highly correlate with the expression level of both estrogen receptor (ER) and progesterone receptor (178). Hypermethylation of BMP6 was observed in ER negative cases, indicating that BMP6 promoter methylation status correlated with ER status in breast cancer (177). Anti-oestrogen reagent raloxifene could increase the activity of the BMP4 promoter in U-2 OS osteoblast-like cells. ER-α, but not ER-β is thought to be indispensable for this effect on the BMP4 promoter. However, ER-β may synergetically enhance this activation of the BMP4 promoter by raloxifene (179). The role played by ER-β in the regulation of BMPs and BMP signaling by oestrogen in breast cancer cells remains unclear. In addition, oestrogen and BMPs can influence each other's function through interaction between their receptor and downstream signaling, such as ER and Smads (180, 181). However, the interaction between the oestrogen signaling pathway and the BMP pathway, and their implication in breast cancer still needs more exploration.

4.4.2 DNA methylation

Epigenetic regulation of genes has been involved in oncogenesis and disease progression. In term of BMPs, hypermethylation of BMP6 and thus reduced expression were observed in ER negative breast cancer tissues (177). Hypermethylation of the BMP and activin membrane-bound inhibitor (BAMBI) promoter lead to a decreased expression of the BAMBI gene, which resulted in an enhanced responsiveness to BMP signaling and thus the abnormal bone formation. (182). During cancer progression, besides the known inactivation of tumor suppressor genes by hypermethylation, activation of BMP6 by selective demethylation occurs and may also contribute to the shift to a more aggressive phenotype in prostate cancer (109). Aberrant methylation of BMP2 has also been recorded, and the resultant loss of BMP2 expression has been implicated in the carcinogenesis of gastric tumors (183).

4.4.3. Others

Several other factors and pathways have been indicated in the regulation of BMP expression and function. Nacamuli *et al* demonstrated that BMP3 expression can be controlled by recombinant human fibroblast growth factor in calvarial osteoblasts (184). EGF can also influence BMP expression. The expression of BMP6 has been shown to be reduced in breast cancer tissues, a reduction accompanied by a concurrent reduction in EGF receptor expression. The relation between BMP6 expression and EGF was further confirmed by the inductive expression of BMP6 in breast cancer cells (MCF-7) *in vitro* by EGF through EGF receptor activation (122). Retinoid induces expression of BMP2 in the retinoid-sensitive cell lines (149), and Rapamycin induced BMP4, and reduced follistatin expression in PC3 cells, which contributes to its anticancer effect (185). Our recent studies demonstrated that hepatocyte growth factor (HGF), a key regulator of metastasis and angiogenesis, could up-regulate the expression of BMP7 and BMP receptors in prostate cancer cells. This effect can be blocked by NK4, an antagonist of HGF (186, 187). It suggests that HGF participates in the change of BMP expression profile during the disease progression and metastasis. These studies collectively indicate that BMPs together with other growth factors, have a potential role to play during the development and progression of cancer, particularly in the disease specific bone metastases.

5. BMP AND COLONIZATION OF METASTATIC CANCER CELLS IN BONE

Following dissemination to bone through blood circulation, metastatic cancer cells need to survive and colonize with in bone tissue before fully establishing a metastatic lesion. During the colonisation, interactions amongst cancer cells, bone cells and bone matrix constitute a "vicious cycle" in favour of developing a bone metastasis (188-190). Within the vicious cycle, osteoinductive factors and osteolytic factors derived from cancer cells can act on osteoblasts and osteoclasts or their respective progenitor cells to stimulate their differentiation and function (Table 3), leading to corresponding osteoblastic and osteolytic lesions. The reciprocal promotions between osteoblasts and osteoclasts will occur after initial stimulation by tumor-derived factors, which can in turn to promote colonisation of metastatic cancer cells and their subsequent development. In addition, bone matrix provides a fertile 'soil' to cancer cells, which is enriched with growth factors and NCPs. These factors also help

Table 3. Tumor derived osteolytic and osteoblastic factors

Osteolytic factors	Osteoblastic factors
PTHrP	Endothelin 1(ET-1)
IL-6	TGFβ
IL-1	BMPs
TNFα	PDGF
Colony stimulating factors (CSF)	Prostaglandins
PDGF	TNFα
TGF-β	IL1
EGF	IGF
TNFα	FGF
Prostaglandins	VEGF
	WNT1
	PTHrP
	uPA
	PSA
	MDA-BF-1

cancer cells to survive and proliferate in the bone microenvironment (Figure 4).

5.1. Tumor cell derived osteoblastic factors

Bone metastasis has been characterised as either osteolytic or osteoblastic. This classification actually represents two extremes of a continuum in which dysregulation of the normal bone remodeling process occurs. Patients can have both osteolytic and osteoblastic metastasis or mixed lesions containing both elements. Most metastatic bone tumors from breast cancer have predominantly osteolytic lesions. In contrast, the metastatic lesions from prostate cancer are predominantly osteoblastic. During osteoblastic bone metastases, the balance between bone resorption and bone formation is tipped in favour of the latter. A number of factors produced by cancer cells, such as platelet-derived growth factor (PDGF), insulin-like growth factors (IGFs), fibroblast growth factors (FGFs), VEGF, Wingless and NT-1 (WNT1), parathyroid hornone related protein (PTHrP), urokinase-type plasminogen activator (uPA), prostate specific antigen (PSA), endothelin-1 (ET-1) and BMPs, have been implicated in osteoblastic lesions. These osteoblastic factors can either promote proliferation and function of osteoblasts, or induce osteoblast differentiation. BMPs, being the most potent to induce bone formation, their functions have been mainly discussed in this review. In following paragraphs, we focus on discussing other osteoblastic factors.

ET-1 is a well known vasoconstrictor, and also a mitogenic factor for osteoblasts (191). Serum level of ET-1 has been shown to be increased in patients with bone osteoblastic lesions (192). In advanced prostate cancer, its expression tends to be elevated in an androgen-independent manner (193). ET-1 mediates its effects on bone formation through the Endothelin A receptor (ET_AR). An ET_AR antagonist (atrasentan) has been shown to prevent osteoblastic bone metastases in a mouse model and reduce skeletal morbidity in men with bone metastases from prostate cancer (194, 195). Evidence also indicates that ET-1 increases osteoblast proliferation and new bone formation by activating the Wnt signaling pathway through suppression of the Wnt pathway inhibitor DKK1(196). In addition to directly affecting osteoblasts, ET-1 can also increase prostate cancer cell proliferation and enhance the mitogenic effect of other growth factors, including IGF-I, PDGF and EGF (197).

PDGF consists of subunit A and subunit B, which form AA, BB and AB isoforms. The BB isoform is a potent osteoinductive factor, which contributes to the osteoblastic lesions through promoting the migration and proliferation of osteoblasts (198, 199). Both acidic FGF (FGF-1) and basic FGF (FGF-2) increase the proliferation of osteoblasts, while FGF-2 is able to suppress the formation of osteoclasts (200). IGF system consists of two ligands, IGF-I and IGF-II, two receptors and seven binding proteins (IGFBPs). IGFs can elicit mitogenic stimulation of osteoblasts, increase bone matrix apposition and decrease the degradation of collagen. The osteoblast-stimulating factor, IGF-I has been implicated in the formation of metastasis from prostate cancer (201). Plasma IGFBP-3 levels were lowest in patients with bone metastases, while IGFBP-2 levels were elevated in prostate cancer patients (202, 203). Although high IGF-I levels and low IGFBP-3 levels may predict the risk of developing advanced-stage prostate cancer (203), evidence indicated that IGF-I was neither necessary nor sufficient for the osteoblastic response to the metastases of prostate cancer (204). The role of IGF system in bone metastasis still requires further investigation. VEGF has been shown to promote bone formation through directly activating the osteoblasts, and facilitating angiogenesis thus indirectly stimulating the process (205-207). The elevated level of VEGF has been implicated in the development of bone metastasis in prostate cancer (208-210).

WNT1 was elevated in the prostate cancer cells of advanced metastatic prostate carcinoma (211). Wnts produced by prostate cancer cells act in a paracrine fashion to induce osteoblastic activity in the bone metastases (212). WNT signaling can be inhibited by its WNT antagonist DKK1 (213). Inhibition of WNT signaling in osteoblasts can suppress osteoblast function and result in the osteolytic phenotype. DKK-1 production occurs early in the development of skeletal metastases, which results in the masking of osteogenic Wnts, thus favoring osteolysis at the metastatic site. As metastasis progresses, DKK-1 expression is decreased, thus allowing unmasking of Wnt's osteoblastic activity and ultimately resulting in osteosclerosis at the metastatic site (212).

PTHrP is an osteolytic factor, which has also been found to be abundant in bone metastases of prostate cancer. However, even in a metastatic tumor in which PTHrP is highly expressed, the osteoblastic lesions remain predominant. The explanation for this paradox is that NH2-terminal fragments of PTHrP share strong sequence homology with ET-1 and thus stimulate new bone formation by activating the ET_AR (214). The osteoblastic fragments of PTHrPs are products of the cleavage of PTHrPs by prostate-specific antigen (PSA). This provides a partial molecular explanation for the osteoblastic phenotype of PTHrP-positive prostate cancer bone metastases (215). uPA is also implicated in osteoblastic bone metastasis. uPA produced by prostate cancer cells has been shown to increase the osteoblastic bone metastases (216, 217). uPA can cleave and activate TGF-β which is produced in a latent form by osteoblasts. TGF-β regulates osteoblast and osteoclast differentiation but also regulates the growth of

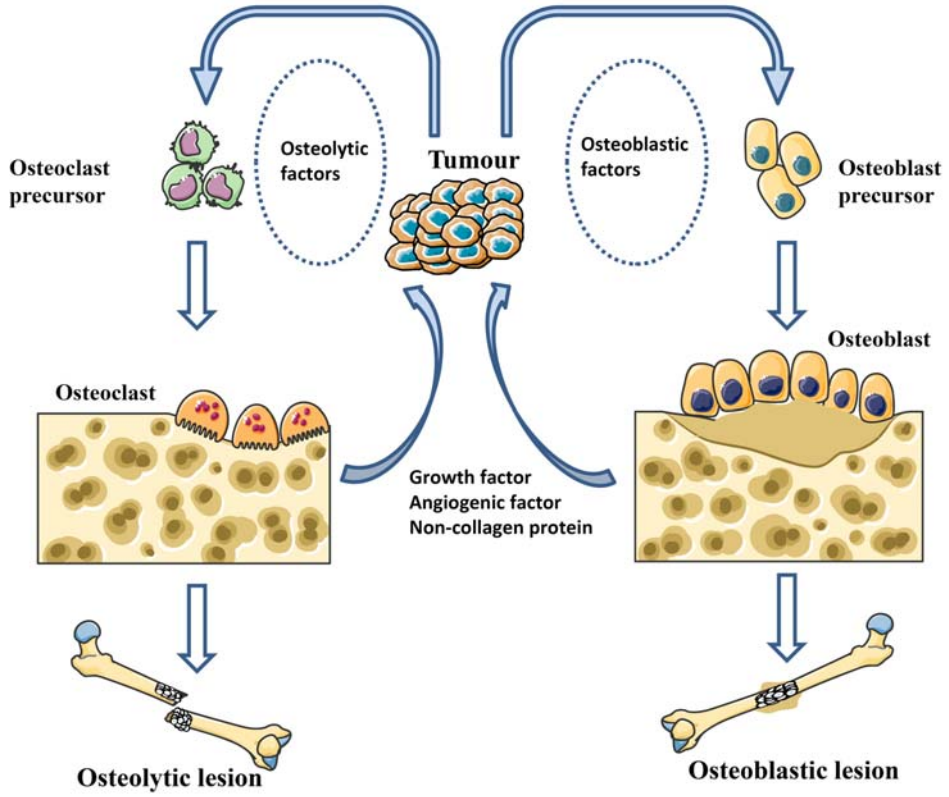

Figure 4. Vicious circle of bone metastasis. Interactions between metastatic cancer cells and bone microenvironment, which include tumor derived osteoblastic and osteolytic factors, osteoblasts, osteoclasts and bone matrix, contributing to the predominant osteoblastic lesions and osteolytic lesions respectively.

tumor cells themselves. uPA stimulated osteoblast proliferation may also due to the hydrolysing IGF-binding proteins and resulting increased level of free IGF (218). PSA is a kallikrein serine protease, which is secreted by prostate cancer cells and used routinely as a marker of prostate cancer progression. PSA not only can cleave PTHrP to release osteoblastic PTHrP fragments, it also activates osteoblast growth factors such as TGF-β (219). Like uPA, PSA can also cleave IGFBP3, thereby IGF-I is able to bind to its receptor and stimulate osteoblast proliferation (220, 221).

MDA-BF-1 is a secreted form of the ErbB3 growth factor receptor (222). Expression of MDA-BF-1 has been shown in the prostate cancer cells of bone metastases, but not in cancer cells from primary tumors of patients with localised disease (i.e., PCa confined to the prostate). Moreover, expression of MDA-BF-1 was not found in prostate cancer cells that metastasised to the liver, adrenal glands, or lungs. (223). It has been demonstrated that MDA-BF-1 mediated specific interactions between prostate cancer cells and bone and assisted in the osteoblast-mediated progression of the cancer in bone (189).

5.2. Osteolytic factors secreted from metastatic cancer cells

Osteolytic metastasis occurs in solid tumors including breast cancer, lung cancer, and renal cancer. In addition, multiple myeloma typically causes extensive bone destruction (224). The dominant feastures of osteolytic metastasis are lytic and destructive, although local bone formation response can also be observed. Most *in vivo* studies indicate that osteolysis is caused by osteoclast stimulation, not by the direct effects of cancer cells on bone. Osteolytic metastases are associated with increased osteoclast activity and reduced osteoblast activity. Metastatic cancer cells produce factors that stimulate osteoclastic bone resorption directly or indirectly. These factors include PTHrP, IL-1, IL-6, prostaglandin E2, TNF, and CSF-1.

PTHrP is one of the major mediators secreted by cancer cells which can induce osteolytic bone metastasis (2, 224). Expression of PTHrP in primary tumors of breast cancer are highly associated with bone metastases (225). These clinical observations have been confirmed by using a mouse model in which monoclonal antibodies directed against the 1–34 region of PTH-rP dramatically reduced the development and progression of bone metastasis (11). PTH-rP produced in breast cancer cells does not directly activate osteoclasts. It binds with PTH receptor on stromal cells/osteoblasts and increases the production of receptor activator of nuclear factor κB ligand (RANKL) that plays a central role in osteoclast differentiation and activation. RANKL then interacts with RANK expressed in the hematopoietic osteoclast precursors and promotes these precursors to differentiate into mature osteoclasts (226).

IL-6 is a potent stimulator of osteoclast formation and can enhance the effects of PTHrP on osteoclasts (227, 228). IL-6 is constitutively expressed by renal, bladder, prostate, cervical, glioblastoma and breast carcinoma cells (229-232).

Metastatic cancer cells may also produce IL-1, TNF and prostaglandins which increase RANKL expression and stimulate osteoclasts (233, 234). A recent study showed that breast cancer cells could stimulate osteoclastogenesis and prolong osteoclast survival by expressing and secreting CSF-1 (235, 236).

5.3. Abundant deposition of growth factors and molecules in bone contributes to the vicious circle

The question of why the bone is the most preferred metastatic site of prostate cancer has aroused intense interest for investigation. One would first contemplate the specific shortcut of blood circulation from primary sites to bone. For instance, a rich venous plexus surrounds the prostate and connects to the venous drainage of the spine: this collection of veins (Batson's plexus) is potentially one of the reasons why the lumbosacral spinal metastases are common in advanced prostate cancer (237). However, the anatomical explanation is not able to explain why the other axial skeleton, skull and ribs may also be involved in the bone metastasis from prostate cancer. A fertile 'soil' provided by bone on the other hand, may give some answer to the question why 'seeds' metastasise to bone.

The bone matrix synthesized by osteoblasts has a particular abundance of cytokines and non-collagen proteins, which may attract prostate cancer cells and allow them to survive and proliferate in the bone matrix. For example, BMPs and TGF-β enriched in bone matrix can facilitate the development of bone metatstasis. Osteonectin, osteopontin, osteocalcin, and bone sialoprotein can also modulate the properties of prostate cancer cells and facilitate their spreading and growth, including promoting their migration, invasion and proliferation (238-243).

Noncollagenous proteins (NCPs) released or synthesized through bone resorption and bone formation also help to generate a fertile 'soil'. NCPs include fibronectin, osteonectin, thrombospondin-2, βig-h3, bone gla protein (BGP, or osteocalcin), matrix gla protein (MGP), Small Integrin-Binding Ligand N-linked Glycoproteins (SIBLINGS) and small bone proteoglycans. One of the most abundant NCPs in bone matrix is fibronectin, which is accumulated extraceullarly at sites of osteogenesis and plays a profound role in the differentiation, proliferation and survival of osteoblasts (244-246). Osteonectin ('bone connector') was initially called 'bone-specific nucleator' of mineralization as it has high affinity for both collagen and mineral (247). It has been subsequently found to be present throughout the body, particularly at sites of tissue remodelling and matrix assembly. Evidence suggests that it is crucial in maintaining the bone turnover (248). Thrombospondin-2 is also abundant in bone, which may promote bone resorption and inhibit the bone formation through negatively controling the differentiation of bone cell precursors (249-251). Another abundant NCP βig-h3, which is induced by TGF-β, inhibits the differentiation of osteoblasts through interaction with the integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ (252, 253). Osteocalcin may inhibit bone formation (254), while MGP is a powerful inhibitor of mineralisation in arteries and cartilage (255). Members of the SIBLINGS family include: bone sialoprotein (BSP), osteopontin (OPN), dentin matrix protein (DMP), dentin sialophosphoprotein (DSPP) and matrix extracellular protein (MEPE). BSP has been suggested to be involved in hydroxyapatite nucleation (256), and to promote adhesion, differentiation and some other biological functions of osteoclasts (257). Osteopontin is crucially involved in anchoring osteoclasts to the mineral matrix of bone surface via the integrin $\alpha_V\beta_3$ (258, 259). Osteopontin is required and probably indispensable during the process of bone resorption (260, 261). Nine of 12 known Small Leucine-Rich Proteoglyans (SLRPs) have been found in skeletal tissue (262). The best characterized SLRP in bone is biglycan, which plays an important role in the differentiation of osteoblast precursors (263). It is also involved in the osteoblasts differentiation induced by BMP2/4 (264, 265).

Above all, both osteoblastic and osteolytic activities are indispensable for all types of bone metastases. In patients with osteoblastic lesions from prostate cancer, blood and urinary levels of bone resorption markers are often elevated (266). Blocking osteoclastic bone resorption can reduce related skeletal events in prostate cancer patients (267). Both osteoblasts and osteoclasts cooperate to drive the settlement and growth of cancer cells in bone.

5.4. Pivotal role of BMPs in bone metastasis

BMPs are the most powerful bone inductive factors enriched in bone matrix (97). BMPs are not only synthesised by osteoblasts and stored in bone matrix, they can also be secreted by cancer cells. BMPs secreted from the cancer cells can enhance their aggressiveness and also act on bone cells resulting bone lesions, in addition BMP released from bone matrix following abnormal osteolytic activities can act to coordinate the mutual reactions between cancer cells and bone environments. BMPs can also help to establish new blood vasculature in support of colonization and development of bone metastasis (Figure 5). BMPs are a group of key factors involved in the 'vicious circle' of bone metastasis.

5.4.1. Adaptable expression of BMPs in bone metastases

The aberrant expression of BMPs in cancer has been implicated in the progression of the disease. Primary prostate tumors and metastatic prostate tumors have a different phenotypic pattern of BMP expression and adopt different signaling pathways downstream of the BMP receptor. Most BMPs and BMP receptors are detectable at a relatively high level in normal prostate tissue. Their expression decreases in a manner that correlates with progression of the primary tumor, except BMP6 which shows an increase in this case. The expression of BMP7,

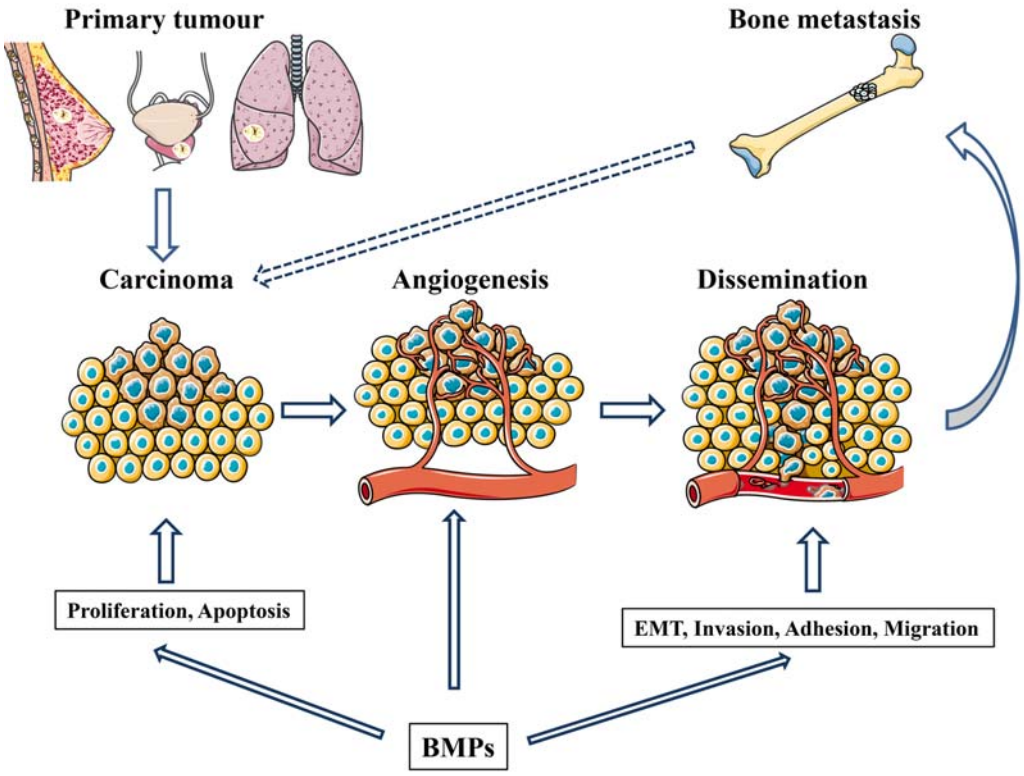

Figure 5. BMPs in bone metastasis. Down-regulation of most BMPs have been shown in primary tumors, which may be due to their inhibitory effect on cell proliferation and pro-apoptotic function. However, their impact on EMT and aggressive characteristics of cancer cells tends to be crucial in transforming the cancer cells to metastatic cells, leading to bone metastasis.

GDF15 and BMPR-IB can be induced by exposure to androgens in the androgen-sensitive prostate cancer cell lines and the 'normal' prostate epithelial cell lines. The same androgen inducible effects were not seen with BMP6 (109, 145, 176, 268). Aberrant expression of BMPs and BMP-associated molecules have also been shown to have a prognostic value (117). The pattern of BMP expression has a clear and close relationship with the development and progression of primary prostate tumors and also contributes to the onset and development of bone metastases. For example, BMP6 remains highly expressed in both primary prostate tumors and metastatic bone lesions. In contrast, BMP7 and GDF15, which are expressed at low levels in normal prostate and in primary prostate tumors, are re-expressed at a high level in skeletal metastatic lesions. This re-expression in metastatic bone lesion can be seen at a higher level than that seen in the normal bone tissues around the metastatic lesions (111, 269). BMPs that are normally enriched in the bone environment, not only promote the motility and invasion of cancer cells, they are also able to induce the expression of other growth factors, which enhances the vicious circle of bone-tumor-bone interactions. A few links have been documented in recent years. For example, BMP2 is able to stimulate a 2.7-fold increase in osteoprotegerin (OPG) expression in PC-3 cells which inhibits osteoclastogenesis (132), and BMP7 induces VEGF protein and mRNA expression in C4-2B cells, which contributes to the pro-osteoblastic activity of C4-2B cells. CM from breast cancer cells (MCF-7) or prostate cancer cells (LNCaP) could up-regulate osteopontin (OPN) in osteoblasts through the protein kinase C (PKC) pathway and mitogen-activated protein kinase (MAPK) pathway. This resulted in inhibition of proliferation and differentiation in osteoblastic cells (270).

From the moment a metastatic cell settles in the bone, there is constant interaction between the tumor cell and its residing microenvironment. Host factors from the bone environment and factors generated by the cancer cell exhibit a reciprocal influence over each other, the BMPs secreted by the cancer cells would certainly influence remodeling of the bone, including osteoblastic and osteoclastic activity. However, it remains unclear as to how exactly the local factors participate in the regulation of BMP expression in the prostate cancer cells. The aforementioned factors, such as sexual hormones, EGF and HGF may play a role in this reciprocal regulation and adaptable expression of BMPs in bone metastases.

5.4.2. BMPs and Angiogenesis

Angiogenesis is an important event during the development and progression of both primary and secondary tumors. In order for tumors to grow beyond a minute size, they need to induce the formation of new blood vessels (a process known as neovascularisation). In order for them to do this they need to promote angiogenesis, which has an activation phase where the endothelial cells proliferate and migrate, and a late phase

where the cells stop migrating, and stabilisation and maturation of the blood vessel occurs. It has been suggested that TGF-β1 signaling via ALK1/Smad1/-5 induced the activation phase of angiogenesis whereas TGF-β1 via ALK5/Smad2/-3 is responsible for promoting the late phase (271). Culturing on type-I collagen can promote spontaneous formation of tubular structures by endothelial cells, via up-regulated levels of BMPR-IB and BMPR-II expression. BMPR-II expression has been shown to be involved in the differentiation of endothelial cells, and suggested that the BMPRs, especially BMPR-II, may play a role during angiogenesis, and hence may be altered in human cancers (272). Smads are the transcriptional regulators of BMP target genes, including VEGF. Smad3 expression in rat proximal tubular cells resulted in an induction of endothelial cell proliferation, and an up-regulation of VEGF-A, while Smad2 induced expression of thrombospondin-1, suggesting that these two Smads have opposing roles during angiogenesis (273). In gastric cancer cells, Smad3 results in a down-regulation of VEGF expression, and smaller tumor nodules with decreased blood vessel formation (274). Unlike Smad2 and Samd3, Smad4 overexpression in pancreatic carcinomas can result in both decreased VEGF expression and an up-regulation of thrombospondin-1, leading to an inhibition of angiogenesis (275).

In addition to the direct stimulation of the aggressiveness of prostate cancer cells, some BMPs including BMP2, 4, 6, 7 and GDF5, are capable of inducing angiogenesis. This may be one of the ways in which they contribute to the process of bone formation (276-279). BMPs can not only directly regulate proliferation and migration of vascular endothelial cells, they can also promote angiogenesis indirectly through up-regulation of the expression of VEGF in both cancer cells and osteoblasts. Noggin, the BMP antagonist, produces the same effect as anti-VEGF antibody: it diminishes the pro-osteoblastic activity of osteoblast cells which are induced by conditioned medium from C4-2B cells (208, 278, 280). Also, the early stage of bone induction by rhBMP2 can be blocked by the anti-angiogenic agent (TNP-470) (277). This evidence indicates that the control of angiogenesis is, to some extent, integrated with the influence which BMPs have over osteoblastic activity. Dai *et al* have demonstrated that it is possible for BMP7 to promote osteosclerosis through VEGF in the skeletal metastases from prostate cancer (208). This angiogenesis induced by BMPs can also be synergized by basic fibroblast growth factor (bFGF) and TGF-β1(281), which suggests that the angiogenesis induced by BMPs is a vital event during the initial stage of bone metastasis development. In contrast to TGF-β1 and most BMPs, BMP9 has been shown to inhibit the proliferation of endothelial cells, as well as block VEGF mediated angiogenesis, via ALK-1 and BMPR-II and downstream Smad1/5 signaling (282).

5.4.3. Therapeutic potential of targeting BMPs

Both clinical and experimental studies suggest profound potential for targeting BMPs in treating bone metastasis. Decreased expression of BMP7 has been indicated in primary tumors in association with bone metastases. BMP7 is able to inhibit the growth of breast cancer tumors at primary sites and in bone *in vivo* (124). Orthotropic implant of tumors with silk scaffolds which were coupled with bone morphogenetic protein-2 (BMP2), and seeded with bone marrow stromal cells (BMSC), contributed to metastatic spread of breast cancer cells (283). These studies suggest that BMPs are involved in the bone metastasis of breast cancer. On the other hand, lack of BMP antagonists in breast cancer may contribute to the osteoblastic lesions of breast cancer. Conditioned medium (CM) from breast cancer cells (HT-39) could result in an up-regulation of bone sialoprotein mRNA expression in osteoprogenitor cells (MC3T3-E1 cells), and a promotion of their osteoblastic behaviour. This effect could be blocked through the addition of Noggin, a BMP antagonist (284). A more recent study also demonstrated that lack of Noggin expression in both breast and prostate cancer cells was associated with osteoblastic activities in bone metastases. Forced expression of Noggin in an osteo-inductive prostate cancer cell line (C4-2B) reduced *in vivo* osteoblastic responses induced by its intravenous xenografts, but had little or no influence on bone resorption and tumor growth (285). Unlike Noggin, another BMP antagonist, Gremlin, has been demonstrated to be over-expressed in some human cancers, including breast cancer (286). However, the roles that Gremlin and other BMP antagonists play in coordinating the osteoblastic and osteolytic activities in bone metastatic lesions are far from clear.

Meanwhile, increased expression of BMP receptors and activation of BMP signaling have also been implicated in breast cancer and the corresponding bone metastasis from the tumor. For example, BMPR-IB was up-regulated in oestrogen receptor-positive carcinomas and was associated with high tumor grade, high tumor proliferation, cytogenetic instability, and a poor prognosis (129). Activation of Smad pathway of BMPs (Smad1/5/8) and TGF-β (Smad2) was revealed in nuclei of breast cancer cells at both primary tumors and bone metastasis, an observation supported by studies using *in vivo* tumor models (287). Whether targeting R-Smads, using methods such as small inhibiting molecules is able to prevent bone metastasis, requires investigation.

BMPs are partly involved in the occasional osteolytic appearance in bone metastasis. The expression of BMP receptors in prostate cancer cells can also be influenced by stromal factors, such as hepatocyte growth factor (187). In an *in vivo* bone tumor model, exposure of tumor bearing subjects to Noggin, an antagonist of BMPs, reduces the size of bone lesions by a mechanism that involves both osteoblastic and osteolytic processes. The BMP antagonists, Noggin and follostatin, are also determining factors to the cells response to BMPs. Interestingly, the expression of these antagonists can be regulated by BMPs themselves probably through an autocrine or paracrine feedback loop. A good example is BMP7, whose endogenous expression is intimately linked to the levels of Noggin and follistatin in the same cell (288). These findings collectively indicate the value of BMPs and their antagonists in the management of bone

metastasis, and also highlight the complexity of the network which has to be clarified in future study.

6. CONCLUSIONS AND PERSPECTIVE

Aberrant expression of BMPs and BMP signaling molecules has been implicated in a variety of solid tumors and disease specific bone metastasis. The phenotypic profile of BMPs, BMP receptors and intracellular signaling molecules can be modified by sexual hormone and growth factors, in order to coordinate biological behaviors of cancer cells during the disease progression. Most BMPs elicit inhibitory effects on proliferation of cancer cells through their receptor signaling. At the primary site, expression of these BMPs is suppressed by hypermethylation or acquired growth independent of sexual hormone, which allows the corresponding cancer cells to grow and progress under reduced influence by the BMPs, such as BMP2, BMP4, BMP7, BMP9 and BMP10. Meanwhile expression of some BMPs, including BMP6 and BMP7, are up-regulated and are implicated in the EMT and enhanced cell invasion and motility, leading to a more aggressive phenotype and subsequent dissemination to secondary sites. This adaptable expression profile of BMPs may also occur in bone metastases, such as re-expression of BMP7 by prostate cancer cells assisting colonization of the cancer cells in bone. Involvement of BMP receptor signallling has also been clearly indicated in the bone metastases, particularly from prostate cancer and breast cancer. BMPs not only directly act on cancer cells to coordinate their abilities during disease progression and bone metastasis, they also indirectly contribute to bone metastasis through regulating tumor related angiogenesis. Together with osteoblastic factors, osteolytic factors, and bone microenvironment, BMPs and their receptors signaling form a vicious circle during bone metastasis.

More recent studies have demonstrated activation of BMP signaling in both breast primary tumors and bone metastases, which contribute to aggressiveness of tumor cells, and development of bone lesions. Lack of Noggin in both breast and prostate cancer cells correlates with their active osteoblastic feature. In the *in vivo* bone tumor model, Noggin, an antagonist of BMPs, has been shown to prevent bone metastasis by inhibiting both osteoblastic and osteolytic processes. These findings collectively indicate a promising therapeutic value for BMPs and their antagonists in the management of bone metastases.

In conclusions, BMPs and their signaling pathways play critical roles in the development, progression, and metastasis of various cancers. The protein and the receptors present important prognostic and therapeutic opportunities in cancers. Further investigations to elucidate the mechanisms underlying the involvement of BMPs in cancer are necessary, as is exploration on the therapeutic potential of these new targets.

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