Bone morphogenetic proteins, breast cancer, and bone metastases: striking the right balance

Catherine Zabkiewicz, Jeyna Resaul, Rachel Hargest, Wen Guo Jiang and **Lin Ye**

Cardiff China Medical Research Collaborative, Cardiff University School of Medicine, Cardiff, UK

Correspondence should be addressed to L Ye
Email
YeL@Cardiff.ac.uk

Abstract

Bone morphogenetic proteins (BMPs) belong to the TGF-β super family, and are essential for the regulation of foetal development, tissue differentiation and homeostasis and a multitude of cellular functions. Naturally, this has led to the exploration of aberrance in this highly regulated system as a key factor in tumourigenesis. Originally identified for their role in osteogenesis and bone turnover, attention has been turned to the potential role of BMPs in tumour metastases to, and progression within, the bone niche. This is particularly pertinent to breast cancer, which commonly metastasises to bone, and in which studies have revealed aberrations of both BMP expression and signalling, which correlate clinically with breast cancer progression. Ultimately a BMP profile could provide new prognostic disease markers. As the evidence suggests a role for BMPs in regulating breast tumour cellular function, in particular interactions with tumour stroma and the bone metastatic microenvironment, there may be novel therapeutic potential in targeting BMP signalling in breast cancer. This review provides an update on the current knowledge of BMP abnormalities and their implication in the development and progression of breast cancer, particularly in the disease-specific bone metastasis.

Introduction

Breast cancer is the most common cancer in women worldwide (Ferlay *et al.* 2015). In developed countries, it receives media coverage and research funding above all other cancers (Konfortion *et al.* 2014, American Cancer Society 2016) and thus continual progress is made in understanding tumour biology, developing diagnostics and improved therapeutics. Despite progress, 15% of patients diagnosed with metastatic breast cancer survive 5 years, compared to 99% of stage I breast cancers (Cancer Research UK 2017). Even for those treated at an early stage, there is still a significant risk of relapse, often several years later. This is particularly true of oestrogen receptor-positive breast cancers, which are at a particular risk of late relapse (Yamashita *et al.* 2016). The leading metastatic site is bone, which holds the majority of tumour burden at death (Awolaran *et al.* 2016). Osteolytic lesions lead to bone pain, fractures, spinal cord compression and hypercalcaemia, reducing quality and length of life for the patient. Symptomatic management includes inhibition of osteoclast activity, swinging the balance of bone turnover away from osteolysis.

Known since 1965 as a key regulator of bone development and turnover, bone morphogenetic proteins (BMPs) have more recently been implicated in bone metastasis of many solid tumours (Ye *et al.* 2007b, Davis *et al.* 2016). Given the propensity for breast cancer to metastasise to bone makes BMPs of particular interest in this area. Their influence on breast tumour biology may

also extend well beyond the bone microenvironment, opening avenues for targeted therapies that could reduce metastatic potential. Here, we review the current knowledge regarding BMPs role in breast cancer progression, metastasis and relapse.

BMP signalling aberrations in breast cancer

BMP signalling pathway

BMPs are members of the TGF-β super family, which regulate cellular differentiation, proliferation, apoptosis and motility, particularly in embryonic development and tissue homeostasis (Nohe et al. 2004, Ye et al. 2009, Davis et al. 2016). Binding to a complex of serine-threonine kinase transmembrane receptors comprising Type I and Type II receptors induces intracellular signalling through the pathway-restricted Smads (R-Smads-Smads 1, 5 and 8) and Smad-4, which assists the translocation into nucleus, thus regulating BMP responsive genes in association with transcriptional co-activators or co-repressors. This pathway is known as the Smad dependent or canonical

pathway. In noncanonical BMP signalling, the receptor complex is instead recruited as a result of ligand binding, triggering a Smad-independent pathway, which involves various branches of the mitogen-activated protein kinase (MAPK) pathway, RAS pathways, PI3K/Akt pathways, P/KC pathways and Rho-GTPases pathways, dependant on both the BMP ligand and receptors recruited (Derynck & Feng 1997, Nohe et al. 2004, Ye et al. 2009, Bragdon et al. 2011, Davis et al. 2016). As a vital embryonic pathway, several layers of inhibition and control are important for normal tissue development and add further to the great plasticity of BMP signalling (Fig. 1).

Regulation of BMP signalling

BAMBI (BMP and activin membrane bound inhibitor) is a pseudoreceptor related to type I receptors, which limits BMP function. It is present in breast cancer cell lines and expression has been noted as upregulated in cancers, but as it also abrogates TGF-β signalling, the pro-oncogenic effect may not be specific to BMPs (Wang 2015). I-Smads (inhibitory Smads) such as Smad 6 and 7
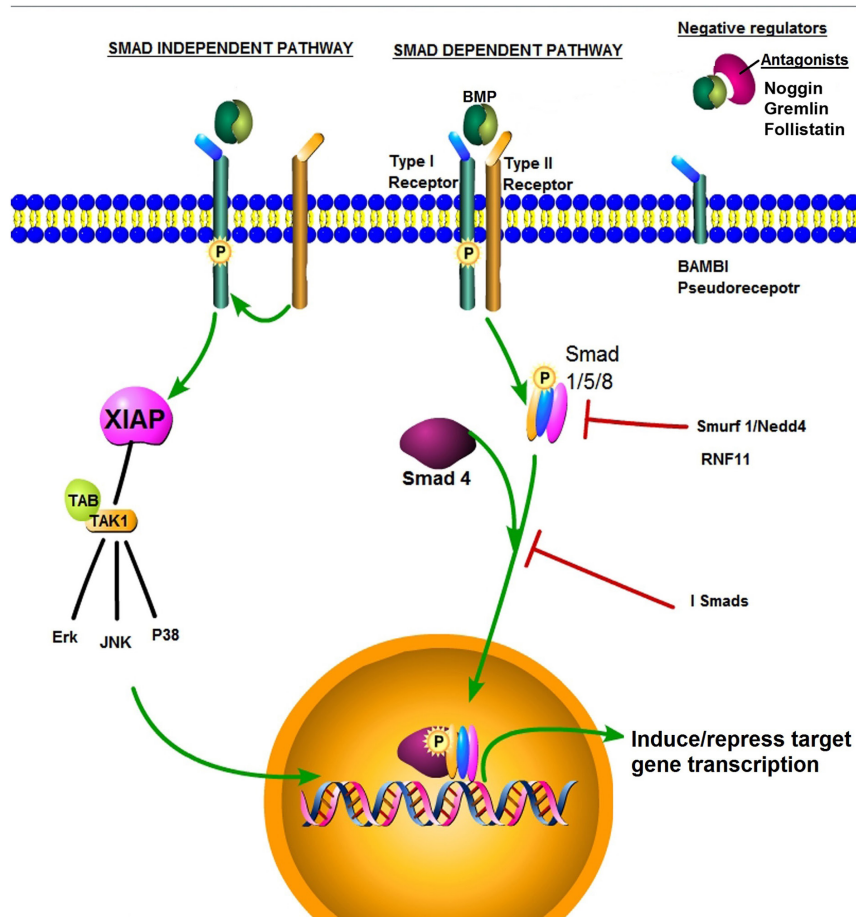

Figure 1
BMP signalling utilises canonical Smad dependant pathways and non-canonical Smad-independent pathways. According to combination and sequence of receptor complex formation or recruitment if the BMP ligand (two tone sphere) binds to a preformed heteromeric receptor complex this results in Smad dependant signalling. The Smad 1/5/8 complex binds to Smad 4 and translocates to the nucleus whereby they interact with transcription factors, or directly binding to DNA to regulate gene transcription. If the BMP binds to a type I receptor and recruits the type 2 receptor to the complex, Smad-independent signalling results. There are multiple levels of regulation, including inhibitory Smads 6 and 7 (I-Smads), pseudoreceptors lacking the serine/threonine kinase domain (BAMBI), ubiquitination and degradation (Smurf/Nedd4/RNF11) and target gene transcription of negative regulators (such as the BMP antagonists) functioning as negative feedback. There are myriad other BMP target genes in breast cancer such as ID1, Snail, Zeb1, p21, PTEN, MMPs and ER that affect cancer cell functions. The figure was made with pathway builder tools from www.proteinlounge.com. A full colour version of the figure is available at http://dx.doi.org/10.1530/ERC-17-0139

prevent complex formation between R-Smads and Smad-4, thus affecting transcriptional regulation in the nucleus, which was well reviewed previously (Miyazono 2008, Bragdon et al. 2011). They bind to activated type I receptors blocking activity and promoting the degradation of receptors through ubiquitination pathways (Nohe et al. 2004).

Smad ubiquitination regulatory factor 1 (Smurf1) is the ligase of Smad 1/5/8, amongst other substrates. In turn, Smurf2 induces degradation of Smurf 1. Downregulation of Smurf1 in breast cancer MDA-MB-231 cells impaired migration (Xie et al. 2013) whereas Smurf 2 knockdown in these cells resulted in enhanced migration in vitro and metastasis in vivo (Jin et al. 2009). The ubiquitin ligase ring finger protein 11(RNF11) interacts with Smurf1 & 2, Smad 4 and other ubiquitin ligases to modulate BMP signalling pathways and both mRNA and protein have been found at high levels in breast tumours (Azmi & Seth 2005).

Further regulation is provided by secreted extracellular BMP antagonists (Walsh et al. 2010). BMP antagonists can block the binding of BMPs to their receptors by directly binding to the BMP ligands. These antagonists are often BMP transcription target genes, forming an important regulatory feedback loop for normal tissue development (Alarmo & Kallioniemi 2010). In developing breast epithelium, the interplay between BMPs 2 and 4 and antagonist Noggin is essential for normal ductal elongation and myoepithelial compartmentalisation (Cowin & Wysolmerski 2010, Forsman et al. 2013). Noggin, Chordin and Gremlin appear to be upregulated (but not mutated) in breast cancer, but their potential role in breast tumourigenesis has not been well studied and in other cancers they are both pro and anti-tumourigenic (Walsh et al. 2010, Owens et al. 2015). Finally, Betaglycan (TGF-β receptor III) binds BMPs 2, 4, 7 and GDF5, helping to mediate BMP signalling. Its expression in breast cancer models suppresses BMP-induced invasion and migration through ligand sequestration when in soluble form (Gatza et al. 2014).

BMP signalling is heterogeneous and complex, with multiple regulatory influences that are currently implicated in breast cancer, but not yet fully elucidated.

BMPs in breast tumourigenesis

Influence of BMPs in cell cycle and proliferation

BMPs are able to regulate the growth of breast cancer cell lines (Table 1). BMP-2, BMP4, BMP-6, BMP-9, BMP-10, BMP-15 and GDF9a impede the proliferation of breast cancer cells (Hanavadi et al. 2007, Du et al. 2008, Alarmo & Kallioniemi 2010, Ye et al. 2010, Ren et al. 2014a). Inhibition of BMP signalling dramatically downregulates protein levels of mitotic checkpoint components BUB3, Hec1, TTK and MAD2, leading to cell division

Table 1 BMP in breast cancer.

	Expression in breast cancer		Function in breast cancer cells				Effect in vivo	
	Primuary tumour	Bone metastases	Proliferation	Apoptosis	Motility	EMT	Primary	Bone
BMP-2	↓/↑		↓	↓/↑	↑	↑	↑	↑
BMP-4	↑		↓/↑		↓/↑	↑	↓/↑	
BMP-5	↑							
BMP-6	↓/↑		↓	↓	↓	↓		
BMP-7	↓/↑	↓/↑	↓/↑		↑	↓	↓	↓
GDF9A	↓		↓		↓			
BMP-9			↓	↑	↑		↓	↓
BMP-10	↓		↓		↓			
BMP-15	↓		↓		↓			
BMPR-IA		↑	↑		↑	↓/↑	↑	↑
BMPR-IB	↓/↑		↓		↑			
BMPR-II	↑		↑		↓			
NOGGIN		↑						↓

Expression of BMPs in breast cancer shown in the table are based on literature: BMP-2 (Soda et al. 1998, Clement et al. 2000, Ghosh-Choudhury et al. 2000a,b, Pouliot & Labrie 2002, Reinholz et al. 2002, Clement et al. 2005, Raida et al. 2005a, Katsuno et al. 2008); BMP-4 (Alarmo et al. 2007, 2013, Ketolainen et al. 2010, Guo et al. 2012, Ampuja et al. 2013, 2016, Owens et al. 2013, Cao et al. 2014); BMP-5 (Bobinac et al. 2005, Davies et al. 2008); BMP-6 (Clement et al. 1999, Yang et al. 2007, 2009, Du et al. 2008, 2009, Lian et al. 2013, Hu et al. 2016); BMP-7 (Schwalbe et al. 2003, Alarmo et al. 2006, 2007, 2008, 2009, Buijs et al. 2007, Sakai et al. 2012); GDF9A/BMP-15 (Hanavadi et al. 2007); BMP-9 (Wang et al. 2011, Ren et al. 2014a,b); BMP-10 (Ye et al. 2010); BMPR-IA (Katsuno et al. 2008, Pickup et al. 2015a); BMPR-IB (Helms et al. 2005, Bokobza et al. 2009, Allison et al. 2016); BMPR-II (Pouliot et al. 2003, Owens et al. 2012); Noggin (Tarragona et al. 2012). Up arrows indicate upregulated (expression) or promote (function and effect), whilst the down arrows indicate downregulated (expression) or inhibit (function and effect).

and tumourigenesis, whereas an upregulation of BMP signalling has the converse effect in breast cancer cells (Yan et al. 2012).

Studies have focused on the effects of BMP-2, which has a direct anti-proliferative effect on tumour cells at a very high concentration in vitro (Soda et al. 1998). A kinase inactive type II TGF-β receptor (dnTbetaRII) eliminated the anti-proliferative effect of BMP-2 in breast cancer cells by preventing the phosphorylation of Smad-1 (Dumont & Arteaga 2003). Interestingly, Waite and coworkers demonstrated that BMP-2 increases PTEN expression in MCF-7 cells with resultant decreased proliferation. PTEN is a tumour suppressor affecting proliferation by modulating the PI3K/Akt pathway. Mutations in PTEN are associated with Cowden's disease, in which there is a markedly increased risk of breast carcinoma. In the presence of BMP-2, association of PTEN with ubiquitin conjugating proteins was reduced, indicating BMP-2 may decrease PTEN degradation, thus increasing the pool of available PTEN and resulting in inhibition of cellular proliferation (Waite & Eng 2003). The presence and composition of BMP receptor complexes can also influence the effect of BMPs on breast tumourigenesis, as although BMPR-IB mediates an inhibition of breast cancer proliferation, BMPR-IA (ALK-3) contributes to progression of breast cancer at primary and secondary sites (Katsuno et al. 2008). BMPR-II promotes BMP-induced proliferation in breast cancer cells (Bokobza et al. 2009) and over-expression of a dominant negative BMPR-II in T-47D breast cancer cells led to an arrest of cancer cells at the G1 phase of the cell cycle (Pouliot et al. 2003).

With regard to cell cycle, it appears that many BMPs have direct and indirect anti-proliferative effect in breast cancer, but this may be subject to aberrations in the balance or function of BMP receptors.

Influence of BMPs on apoptosis

BMP-2 under routine culture conditions, shows pro-apoptotic effect in MCF-7 breast cancer cells, in which the expression and function of apoptosis related genes, particularly protein kinase R (PKR), and subsequent activation of its substrate eIF2α are regulated by BMP signalling (Steinert et al. 2008). In MDA-MB 231 cancer cells, overexpression of Neogenin (a co-receptor for BMPs) significantly increased apoptosis whilst inhibiting BMP-2 induced phosphorylation of Smad1/5/8. This interesting new player appears to modulate BMP/Smad signalling, resulting in the observed effect on apoptosis

(Zhang et al. 2015). The exact mechanism is not yet known, but Neogenin forms a complex with repulsive guidance molecules (RGM) (Bell et al. 2013), such as RGMB, which are also co-receptors for BMP signalling. In MDA-MB-231 cells, knockdown of RGMB promoted survival, reduced Caspase 3 expression and promoted growth and migration via regulation of Smad dependant and independent BMP signalling (Li et al. 2012).

However, under different experimental conditions, without supplement of serum, BMP-2 increases the resistance of MCF-7 breast cancer cells to hypoxia-induced apoptosis, via the activation of both the MAPK pathway and ID-1, and suppression of Caspase-3 (Clement et al. 2000, Raida et al. 2005a). The other example is BMP-6, which inhibits proliferation through an upregulation of miRNA-192 and resultant repression of cell cycle progression in MDA-MB-231 cells (Du et al. 2008). However, under deprivation of serum, BMP-6 protects MDA-MB-231 cancer cells from stress-induced apoptosis through upregulation of survivin, via the Smad dependent pathway, and activation of p38 via the Smad-independent pathway, with both contributing to the anti-apoptotic effect of BMP-6 (Du et al. 2008).

It appears with regard to apoptosis in breast cancer that BMPs may have a dual role dependent on cellular conditions, being pro-apoptotic unless under conditions of cellular stress. However, the evidence from in vitro study warrants further exploration for their possible role in therapeutic resistance.

Expression of BMPs and clinical correlations

In clinical breast cancer samples, decreased mRNA expression of BMP-2, BMP-7, BMP-10 and GDF-9a (an analogue of BMP-15/GDF-9b) were seen and associated with poor clinical outcomes (Reinholz et al. 2002, Buijs et al. 2007, Hanavadi et al. 2007, Davies et al. 2008, Ye et al. 2010). In contrast, BMP-2, BMP-4, BMP-5 and BMP-7 expression has been reported as elevated in breast tumours and the latter two associated with poor prognosis (Bobinac et al. 2005, Raida et al. 2005a, Alarmo et al. 2006, 2007, Davies et al. 2008). The key may be that BMPs have bidirectional actions in breast cancer, such as BMP-4, which not only suppresses breast cancer cell growth, but also promotes invasion and migration. Immunohistochemistry studies associated BMP-4 expression with low proliferation tumours, but also increased recurrence (Alarmo et al. 2013). Interestingly, this may be supported by the finding of increased transcript levels of BMP-4 and its receptor

BMPRII in the peripheral blood of breast cancer patients in advanced disease (Gul *et al.* 2015). Another potentially bidirectional BMP, both increased and decreased BMP-7 expression in primary breast tumours has been correlated with disease-specific bone metastases (Buijs *et al.* 2007, Alarmo *et al.* 2008).

The difference in findings from clinical samples may reflect the heterogeneity of breast cancer and the crosstalk of BMP signalling with a variety of other signalling pathways critical in breast tumourigenesis.

BMPs and clinical subtypes of breast cancer

It has become clear that breast cancers are heterogeneous, with distinct subtypes based on molecular profile (Cancer Genome Atlas Network 2012), and clinically, treatments are increasingly directed toward molecular markers such as the hormone receptors. The development of hormonal therapies confirmed a distinction in behaviour between oestrogen receptor (ER)-positive and ER-negative breast cancers. Tamoxifen was initially used as a treatment for all breast cancers, but it later became apparent that only those tumours expressing hormone receptors benefit. The introduction of trastuzumab (Herceptin) has also introduced tumour profiling of HER2 expression as a standard in clinical care. More recently, gene expression profiling has made its way into the clinic to predict those with early breast cancer at risk of relapse that would benefit from chemotherapy. Receptor status appears to influence the effect of BMP signalling as seen in clinical studies and several *in vitro* studies, which could make BMP/BMPR status another important profiling marker.

BMPs and oestrogen receptor signalling

Oestrogen regulates the expression of BMPR-IA, BMPR-IB, ActRIIA and ActRIIB, but has no effect on the expression of ActR1 and BMPR-II (Takahashi *et al.* 2008). Elevated expression of BMPR-IB was associated with high tumour grade, high tumour proliferation, cytogenetic instability and a poor prognosis in ER positive carcinomas (Helms *et al.* 2005). A decreased level of BMPR-IB associated with poor prognosis in a majority of ER-negative tumours (Bokobza *et al.* 2009).

The expression of BMP-7 highly correlates with the expression level of ER, although BMP-7 expression reduces in response to oestrogen (Schwalbe *et al.* 2003, Alarmo & Kallioniemi 2010). BMP-2 expression is significantly higher in the ER-negative tumours

(Julien *et al.* 2011). Silencing of ERα results in resistance to effects of oestradiol increased BMP-2 expression, and genetic changes associated with epithelial–mesenchymal transition (EMT) (Al Saleh *et al.* 2011). BMP-6 mRNA has been declared both increased and reduced in comparison with non-tumour margins (Clement *et al.* 1999). Studies show over-expression of BMP-6 particularly in ER positive cell lines and tumour samples (Ong *et al.* 2004, Zhang *et al.* 2007), however, BMP-6 inhibits oestrogen-induced mitosis of ER positive breast cancer cells (Takahashi *et al.* 2008) and targeting BMP-6 in MCF-7 cells using shRNA knockdown promoted cell proliferation (Lian *et al.* 2013).

The mechanism of these interactions is being explored. BMP-6 appears to be activated in a dose-dependant manner by oestrogen through interaction of ER with sites on the BMP-6 promoter region (Zhang *et al.* 2005). In addition, BMP-6 promoter methylation status correlates with ER status in breast cancer. Methylation of the BMP-6 gene promoter has been detected in ER-negative MDA-MB-231 cells; whereas in ER positive MCF-7 and T47D, the BMP-6 gene promoter remains demethylated. In 33 breast tumour specimens, hypermethylation of BMP-6 was observed in all ER-negative cases whereas lower methylation frequency was observed in ER positive cases (Zhang *et al.* 2007).

Oestrogen interferes with the biological function of BMP-2 by inhibiting the activation of Smad, as a result of biochemical interaction between Smad and ERα (Yamamoto *et al.* 2002). Smad-4 can associate with cytoplasmic ERα, preventing the transcriptional regulation mediated by ERα (Wu *et al.* 2003). In MDA-MB-231 cells BMP-2 treatment induced the expression of a splicing variant of ER (ERα-36) in a dose-dependent manner, and growth of MDA-MB-231 cells could be stimulated by oestradiol, even though they were insensitive to it before BMP-2 induction. When the BMP-2 signalling pathway was silenced by si-BMPRIA and si-BMPRIB, the ERα-36 induction was eradicated (Wang *et al.* 2012).

In a bidirectional manner, BMPs as well as being affected by ER signalling can in turn have an effect on ER signalling. BMP-2 inhibits oestradiol-induced proliferation of breast cancer cells, via upregulation of cyclin kinase inhibitor p21, which in turn inhibits the oestradiol-induced cyclin D1-associated kinase activity (Ghosh-Choudhury *et al.* 2000a). The Smad dependent signalling is indispensable for BMP-2 induced p21 expression and the consequent inhibitory effect on cell proliferation (Pouliot & Labrie 2002).

It is evident that the ER status has a bearing on the cells' response to BMPs and vice versa, at both nuclear and cytoplasmic level, involving signalling cross talk and transcriptional regulation. Once again it is likely that the resultant influence on breast tumours and their response to hormonal therapies depend on the balance of these interactions between the signalling pathways.

BMPs and other signalling crosstalk

Co-regulation of the growth of breast cancer cells can occur between the BMP and other cell signalling pathways. This includes other members of the TGF-β super family (Katsuno *et al.* 2008), epidermal growth factor (EGF) (Schwalbe *et al.* 2003), hepatocyte growth factor (HGF) and HGF receptors (Imai *et al.* 2005, Ye *et al.* 2007*a*, 2008) and Wnt signalling (Guo & Wang 2009).

The upregulation of p21 by BMP-2 prevents EGF-induced proliferation of MDA-MB-231 breast cancer cells (Ghosh-Choudhury *et al.* 2000*b*). This may reflect why MDA-MB-231, an ER-negative tumour cell line, responds to recombinant human BMP-2 with a more significantly reduced proliferation, in comparison with the ER positive MCF-7 cells (Arnold *et al.* 1999).

BMP-4 is considered as an inhibitor of breast cancer cell growth, but can also have a synergistic effect on proliferation of breast cancer cells induced by fibroblast growth factor (FGF), EGF and HGF (Montesano *et al.* 2008). EGF treatment of breast cancer cells *in vitro* upregulated BMP-4 signalling via the Smad pathway, leading to suppression of matrix metalloprotease (MMP) 9. This suppression was attenuated with an addition of BMP-4 antagonist Gremlin or Smad 6 (Laulan & St-Pierre 2015). In addition, BMP-6 in breast cancer cells can be upregulated by EGF and other EGFR ligands such as transforming growth factor-α, amphiregulin and betacellulin (Clement *et al.* 1999). Conversely, EGF, FGF and HGF activated MAPK/ERK results in a phosphorylation of the linking region of Smad1/5/8 leading to a reduced nuclear translocation and a suppression of BMP target genes (Kretzschmar *et al.* 1997, Guo & Wang 2009). BMPs exert reciprocal effects, suppressing EGF-induced gene transcription through MAPK/ERK-1 signalling (Ghosh Choudhury *et al.* 1999). BMP-9 inhibits the proliferation and metastasis of SK-BR-3 breast cancer cells via decreasing HER2 expression and inactivating ERK1/2 and PI3K/AKT signalling pathways (Ren *et al.* 2014*a*).

SOSTDC1, a secreted regulator of both BMP and Wnt signalling pathways, is under expressed in breast cancer and can differentially affect signalling induced by Wnt3a, BMP-2 and BMP-7. In breast cancer cells, SOSTDC1 modestly increases Wnt3a signalling, decreases BMP-7 signalling, whilst eliciting little effect on BMP-2-induced signalling (Clausen *et al.* 2011).

This highlights the important influence of other signalling pathways and the canonical or noncanonical BMP signalling pathways, and may be one of the reasons for the varied and sometimes contradictory study outcomes regarding BMPs in breast cancer.

BMPs and the androgen receptor

More recently androgen receptor status has become a focus of research, particularly in relation to treatment resistance. Reported as either tumour suppressor or promoter, its expression has been linked to both good and poor prognosis (Feng *et al.* 2017). In ER positive tumours that respond to neoadjuvant endocrine therapy, AR mRNA and protein expression decreases, whereas in tumours those fail to respond, AR mRNA does not decrease. AR over-expression increases tamoxifen resistance in breast cancer models *in vitro* and *in vivo*. In a clinical cohort, a high AR:ER ratio was shown as an independent risk for failure of tamoxifen treatment and poor survival (Cochrane *et al.* 2014).

Upon an ERK-mediated phosphorylation, BMP-activated Smad1 can bind to AR leading to an inhibition of AR-induced transcription and its corresponding effect on cellular functions of prostate cells (Guo & Wang 2009). It is not yet known whether similar interactions between BMP signalling and AR are found in breast cancers, and this would be a novel area of exploration and possible targeted therapy for endocrine treatment resistant breast cancers.

BMPs and progression of breast cancer

BMPs in epithelial–mesenchymal transition (EMT)

EMT is an important event during the development and progression of cancer, causing disruption of epithelial homeostasis that may lead to carcinogenesis; it can also transform the indolent tumour cells into a more aggressive colony, leading to metastasis (Larue & Bellacosa 2005, Lamouille *et al.* 2014). The early steps of metastasis, such as invasion and extravasation are facilitated by cells acquiring mesenchymal traits, however, the ability to colonise distant tissues and form macroscopic metastases may be facilitated more by epithelial properties, and thus

the breast cancer cell at any one time may be triggered towards either EMT or MET by differential BMP signalling.

EMT regulated by BMPs has been implicated in foetal and postnatal development of different organs and tissues, including mammary gland development, where BMP-2 and 4 have essential roles in both epithelial and mesenchymal differentiation (Nakajima *et al*. 2000, Romano & Runyan 2000, Hens & Wysolmerski 2005).

In vitro, BMP-4 subverts the ability of mammary epithelial cells to form polarized lumen-containing structures, and also endows them with invasive properties, demonstrating a direct effect promoting a mesenchymal phenotype (Montesano 2007). TGF-β and BMP-2 signalling in murine mammary cancer cell lines results in transcription of genes that suppress the epithelial phenotype. miR-200 counteracts this by targeting the BMP-2 downstream transcription factors responsible for epithelial gene repression, such as Crtap, Fhod1, Smad2, Map3k1, Tob1, Ywhag/14-3-3γ, Ywhab/14-3-3β, Smad5, Zfp36, Xbp1, Mapk12 and Snail (Perdigao-Henriques *et al*. 2016). BMP-2 appears to promote motility and invasiveness of MCF-7 and MDA-MB-231 cells, both *in vitro* and *in vivo* (Clement *et al*. 2005, Katsuno *et al*. 2008). BMP-2 upregulation of target gene ID-1 (which activates pathways involved in tumour progression) may contribute to this effect (Gautschi *et al*. 2008) (Fig. 2).

BMP receptors are also important in EMT, with application of a type I BMPR inhibitor to mice reducing key EMT-related genes such as Snail, Twist, Zeb1 and Zeb2

(Balboni *et al*. 2013, Owens *et al*. 2015). In humans, high BMPRIA expression correlates with poor survival (Pickup *et al*. 2015a). Knockdown of BMPRIA *in vivo* delayed tumour onset, and also subsequent growth of tumours and improved survival, despite conversely seeming to induce EMT-like tumour transitions, such as increased Vimentin (Pickup *et al*. 2015a).

Not all BMPs induce EMT, and some appear to promote MET, reducing the aggressive properties of tumour cells. In murine mammary epithelial cells (NMuMG), BMP-7 was not able to induce EMT whereas TGF-β1 could (Piek *et al*. 1999). BMP-7 is able to increase cytokeratin expression, and decrease vimentin in breast cancer cells *in vitro* and *in vivo*, leading to an epithelial-like phenotype (Buijs *et al*. 2007). This effect is also seen with BMP 6, which restores E-cadherin-mediated cell-to-cell adhesion and prevents breast cancer metastasis through the downregulation of miR-21 and δEF1 (ZEB1, whose expression associates with invasive breast cancer phenotype) (Yang *et al*. 2007, Du *et al*. 2009, de Boeck *et al*. 2016).

BMPs effect on tumour microenvironment, migration and invasion

Tumour microenvironment and the interaction between tumour cells and surrounding support cells are important for the progression and invasion of tumours (Fig. 2). Stimulation of fibroblasts by BMP signalling can promote breast tumour cell invasion and increased inflammatory
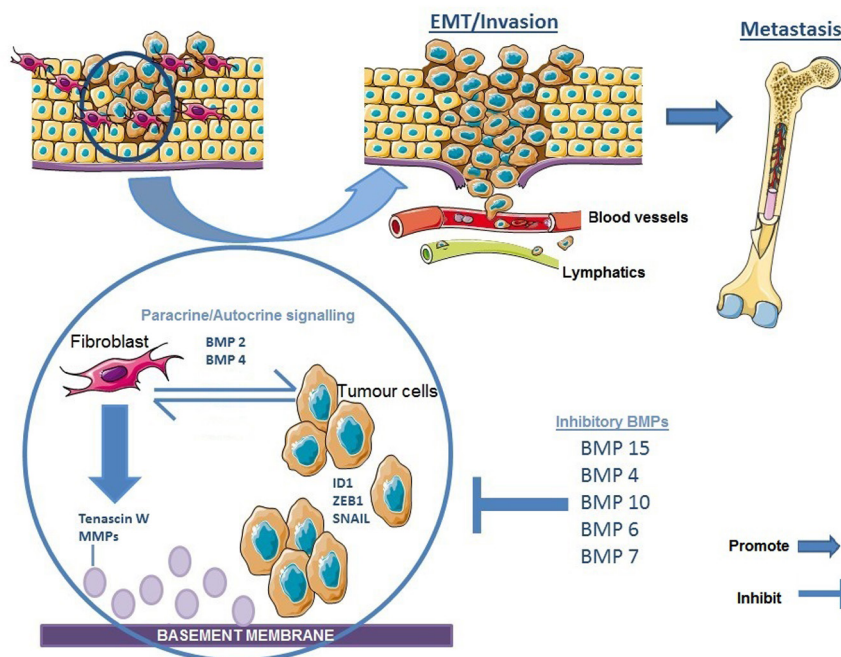

Figure 2
BMPs mediate crosstalk between breast cancer cells and microenvironment during disease progression. BMP signalling by tumour-associated stromal cells (or cancer associated fibroblasts) alter the expression of EMT-related genes in breast cancer cells such as ZEB1, with BMP-2 and 4 in particular, promoting the mesenchymal phenotype. Likewise, breast cancer cells secreting BMP-2 and 4 can stimulate fibroblasts to produce chemokines and enzymes (Tenascin W, MMPs) that promote invasion and motility. Breast cancer cells are then able to disseminate to bone via lymphovascular invasion. However, there are BMPs that reduce invasion (BMP-6, BMP-10, BMP-15) and others who have a dual or bidirectional role dependant on cellular context, such as BMP-4. The figure was created using Servier Medical Art tools http://servier.com. A full colour version of the figure is available at http://dx.doi.org/10.1530/ERC-17-0139

cytokine production (Owens et al. 2012). Loss of BMPRII in murine fibroblasts promoted tumour metastasis and sustained inflammatory cell infiltration (Pickup et al. 2015b). This suggests BMPRII can have both direct suppressive effects on tumour cells, but also indirectly via regulation of inflammation in the tumour-associated stroma (Owens et al. 2012, Pickup et al. 2015b).

In triple negative MDA-MB-468 cells, upregulated BMPRIB showed increased migratory capacity in response to BMP-2, which was abrogated by the BMPR antagonist dorsomorphin (Allison et al. 2016). An analogue of dorsomorphin (DMH1), much more highly selective for type 1 BMPR, can attenuate the pro-tumour microenvironment by altering the expression of certain genes (such as ID-1 and matrix metalloproteases-MMPs) in fibroblasts, lymphatic vessels and macrophages in a mouse model (Owens et al. 2015).

BMP-2 may contribute to the invasiveness of tumour cells via induction of the extracellular matrix glycoprotein Tenascin-W in the tumour-surrounding stroma. Smad-independent signalling through p38 and JNK pathways is involved in BMP-2 induction of Tenascin-W and overexpression of Tenascin-W in the stroma of breast cancer promotes invasion and migration of cancer cells through an interaction with α8 integrin (Scherberich et al. 2005).

Treatment with BMP-4 increased invasion and migration in both breast cancer cell lines and a mouse model (Ketolainen et al. 2010, Guo et al. 2012, Ampuja et al. 2013, 2016). CCN6 is an extracellular matrix associated protein that has been shown in vitro and in vivo to directly antagonise this BMP-4 mediated invasiveness and metastases (Pal et al. 2012). A similar effect to BMP-2 on stromal cells appears to be true with BMP-4 treatment in mammary stromal fibroblasts. Fibroblasts stimulated with BMP-4 enhanced MCF-7 cell invasion, and these effects were inhibited by DMH1. BMP-4 increased MMP-3 and IL-6 in conditioned medium from treated mammary fibroblasts, suggesting BMP-4 can influence the tumour microenvironment to promote breast cancer invasion (Owens et al. 2013).

Interestingly, BMP-4 inhibits aggressiveness in different breast cancer cell lines under different experimental conditions. Overexpression of N-myc downstream-regulated gene 2 (NDRG2) in MDA-MB-231 cells induced BMP-4 and inhibited expression of MMP-1, -3 and -9 compared to control. When BMP-4 was neutralised with anti-BMP-4 antibody, MMP-9 expression recovered and migratory capacity of the cells increased.

Application of rhBMP-4 to wild type MDA-MB-231 cells suppressed MMP-9 expression and activity, reducing migration and invasion (Shon et al. 2009). Additionally, in a mouse model, BMP-4 suppressed metastasis, seemingly by regulating anti-tumor immune responses (Cao et al. 2014).

Forced expression of GDF-9a/BMP-15 in breast cancer cells also reduced invasiveness in vitro, as does BMP-10 (Hanavadi et al. 2007, Ye et al. 2010). Hu and coworkers showed that BMP-6 markedly downregulated matrix metalloproteinase-1 (MMP-1) expression at both the mRNA and protein levels in MDA-MB231 cells, inhibiting invasion, and this effect was significantly attenuated by overexpression of MMP-1. BMP-6 also increased adhesion and cell-cell contacts in these cells (de Boeck et al. 2016, Hu et al. 2016).

The above experimental evidence suggests that BMPs can differentially influence tumour invasion by regulating the balance of MMPs, extracellular matrix components, cytokines and immune or inflammatory cells in the tumour microenvironment.

BMPs and angiogenesis in breast cancer

Tumour angiogenesis has been shown to be important in breast cancer progression and metastasis (Ribatti et al. 2016) and current knowledge regarding the role of BMPs and angiogenesis has been well reviewed here (Ye & Jiang 2016) (Fig. 3). In general, experimental evidence suggests that BMPs promote angiogenesis indirectly through upregulation of the expression of vascular endothelial growth factor (VEGF) (Yeh & Lee 1999, Deckers et al. 2002, Dai et al. 2004).

Regarding breast cancer specifically, one study has reported that BMP-2 promotes breast tumour related angiogenesis through stimulating p38 MAPK pathway and ID-1 expression (Raida et al. 2005b). Conversely, Chi and coworkers demonstrated overexpression of BMP antagonist Coco (DAND5) in breast cancer cells promoted micro-vascular formation in vitro and in mouse xenograft tumours, although the mechanism has not been clarified. They also found Coco positivity in breast cancer patient serum correlated with relapse and poor survival, although this could be due to its influence on other aspects of tumour progression (Chi et al. 2016). Current ongoing clinical trials are examining the effect of BMP-9 and 10 blocking agents as anti-angiogenic treatments for solid tumours, which is further addressed below. The limited literature regarding BMPs in breast tumour angiogenesis
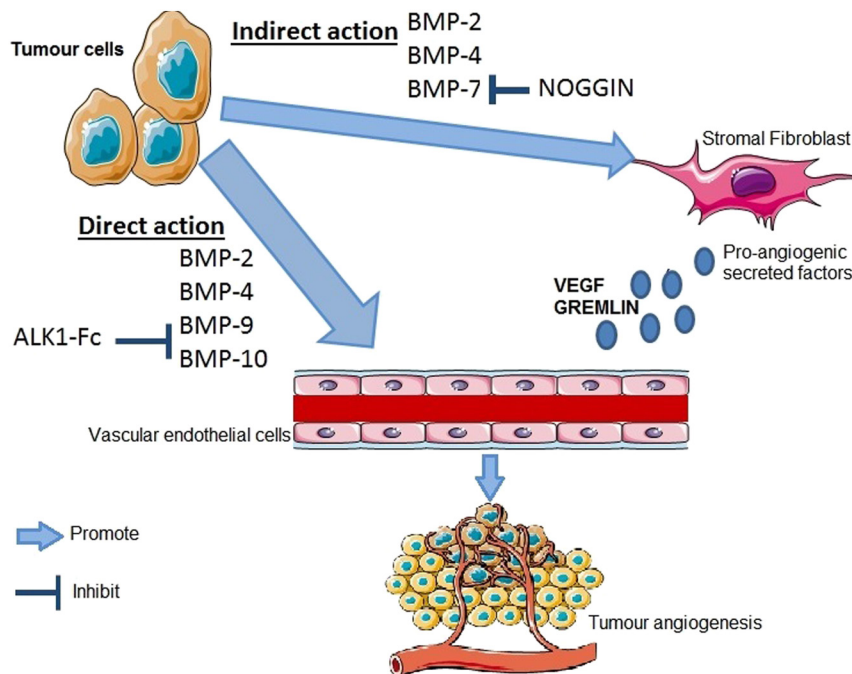
Figure 3

It has been demonstrated that BMP-2, -4, -6, -7, and GDF5, are capable of directly inducing angiogenesis in vascular endothelial cells, whilst effect of BMP 9/10 seems more context dependent. BMP-9 has biphasic effects on endothelial cells. High doses can inhibit endothelial proliferation and migration, whereas low doses can have stimulatory effects. Indirectly, BMPs can act on stromal cells to promote the secretion of pro- angiogenic factors such as VEGF. This can be attenuated by BMP antagonism such as Noggin attenuating the pro-angiogenic effect of BMP 7. Another BMP antagonist, gremlin has a BMP independent function promoting angiogenesis by interaction with VEGF receptors. The figure was created using Servier Medical Art tools http://servier.com. A full colour version of the figure is available at http://dx.doi.org/10.1530/ERC-17-0139

makes this a rich area for study, particularly considering the potential therapeutic applications.

BMPs and dissemination of breast cancer to bone

BMPs and the bone environment

A now traditional view of cancer metastasis is Paget's 'seed and soil' hypothesis, namely that the cancer cell will deposit and grow only if the environment is favourable towards that cancer cell, i.e., both the seed and the soil have to be mutually compatible. Bone metastasis involves cancer cell dissemination from the primary tumour, extravasation into the blood stream and occupation of the bone marrow space. As vital regulators of bone formation, BMPs have been of great interest in this field with several studies examining their role in bone metastases. In normal bone physiology and turnover, BMP signalling is essential for differentiation of mesenchymal stem cells (MSCs) and maturation into chondroblasts and osteoblasts, resulting in bone formation. BMP-2, BMP-4, BMP-6, BMP-7, BMP-9, BMP-12 and BMP-13 induce MSCs differentiation, but not all are osteoinductive in nature (Alarmo & Kallioniemi 2010, Carreira et al. 2014). BMPs also induce osteoblasts to produce certain factors influential to osteoclast maturation and function. Thus, BMPs are an integral part of the bone environment (Alarmo & Kallioniemi 2010, Rahman et al. 2015).

Aberration in BMPs and bone metastasis

In breast cancer, BMP-induced transcriptional pathways are active in bone metastatic lesions *in vivo* and dominant negative BMP receptors reduced bone metastases *in vivo* (Katsuno et al. 2008). Decreased expression of BMP-7 in primary tumours correlates with bone metastases and BMP-7 is able to inhibit the growth of breast cancer tumours in bone *in vivo* (Buijs et al. 2007). Conversely, other studies have shown BMP-7 overexpression in primary tumours associated with bone metastases (Alarmo & Kallioniemi 2010). In murine 4T1E/M3 mammary cells, which are highly metastatic to bone, expression of BMP-7, BMPR and phosphorylated Smad1/5/8 is upregulated. These highly invasive features are attenuated when BMP-7 is inhibited (Sakai et al. 2012). BMP-9 inhibits the growth of breast cancer cells *in vitro* and *in vivo*, and also suppresses the growth of tumour cells in bone (Wang et al. 2011, Ren et al. 2014b). Downregulation of connective tissue growth factor (CTGF) by BMP-9 is involved in the inhibition of tumour growth in bone (Ren et al. 2014b).

Breast cancer cells themselves can acquire an osteoblast-like phenotype, by ectopically expressing bone matrix proteins such as bone sialoprotein (BSP), osteopontin (OPN), osteoprotegerin (OPG) and osteoblast-specific cadherins (Ibrahim et al. 2000, Kapoor et al. 2008, Tan et al. 2016). Tan and coworkers (Tan et al. 2016) showed that breast cancer cells with induced EMT exhibited an elevated level of bone-related genes (BRGs)

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and osteoblast-like features in an exposure to BMP-2. Breast cancer cells expressing these BRGs preferentially metastasise and survive in bone. It also interestingly made cells more resistant to chemotherapy. These effects were reversed with Noggin application, or knockdown of runt-related transcription factor 2 (RUNX2), which regulates bone remodelling, and osteogenic differentiation (Alarmo & Kallioniemi 2010, Carreira et al. 2014, Tan et al. 2016). This osteomimicry induced by BMPs may be one of the reasons breast cancer cells home to bone tissue and survive in the bone microenvironment (Rucci & Teti 2010). In other words, BMP signalling in breast tumours could create a subset of bone-specific metastatic cells: the right kind of 'seed' for a specific soil.

Regulators of BMP signalling in bone metastases

BMPs and their antagonists can also influence the bone microenvironment, for example, orthotropic implant of silk scaffolds carrying BMP-2 showed increased metastatic spread of breast cancer cells to bone in vivo (Moreau et al. 2007). Conditioned medium (CM) from HT-39 breast cancer cells promoted osteoblastic behaviour in osteoprogenitor cells. This effect was blocked by addition of Noggin (Bunyaratavej et al. 2000). High expression levels of Noggin are associated with bone metastases in both cell line/murine models and clinical samples of breast cancer bone metastases (Tarragona et al. 2012). Upregulation of Noggin and Follistatin by ZEB1 in breast cancer cells induced differentiation of osteoclasts in vitro, suggesting an osteolytic influence in the bone microenvironment (Mock et al. 2015), however the role BMP antagonists play in coordinating the osteoblastic and osteolytic activities in bone metastatic lesions are far from being clear.

Regulation of BMP signalling by oestrogen and ER may also contribute to osteoblast differentiation and thus may influence the bony metastatic niche. The selective estrogen receptor modulator raloxifene increased the activity of the BMP-4 promoter in U-2 OS osteoblast-like cells. ER-α is thought to be indispensable for this effect on the BMP-4 promoter and may be part of the mechanism of this agents in reducing both osteoporosis and breast cancer risk (van den Wijngaard et al. 2000).

Oestradiol enhances BMP-4-induced expression of osteoblastic markers (Runx2, osterix, osteocalcin) in osteoprogenitor cells. In contrast, the expression of ER-α and endogenous BMP-4 was suppressed by BMP-4 treatment regardless of the presence of oestrogen, implying the presence of a negative feedback loop for osteoblast differentiation (Matsumoto et al. 2013).

In osteoblasts, BMP-6 reporter activity increased with anti-oestrogen treatment, and decreased with oestradiol treatment, providing evidence that ER regulates BMP-6 differentially in breast and bone, and ERα-dependent pathways (such as BMPs) may influence skeletal secondary formation in breast cancer, which is consistent with the previous observation that patients with ER positive breast tumours are more likely to develop skeletal metastases (Ong et al. 2004).

In summary, BMPs and their regulators, such as BMP antagonists or ER signalling, can also result in a bone environment receptive and supportive of metastases, and influential in the balance between osteogenesis and osteolysis, i.e., they affect the bone 'soil' conditions.

BMPs and breast cancer relapse

As a consequence of improvements in breast cancer treatments, nearly 80% of women survive at least 10 years after their diagnosis (Cancer Research UK 2017). For women with triple negative disease there is high risk of early recurrence, reflecting the aggressive nature of this subtype, and the lack of targeted treatments. For receptor-positive tumours, the risk of recurrence is lower, but continues potentially for decades after diagnosis (Yamashita et al. 2016).

Relapse of disease is often attributed to cancer stem cells, cells with tumour initiating capacity and the ability to evade the effects of chemotherapy by remaining in an alive but quiescent or dormant state, only to clinically manifest at a later point, causing symptoms and death (Oskarsson et al. 2014).

BMPs and breast cancer stem cells

Mammary tissue inevitably contains stem and progenitor cells, undergoing cycles of quiescence and proliferation throughout mammary development, maturation and involution (Woodward et al. 2005). Stem cells share many of the characteristics of cancer cells, including the ability to proliferate through a process of self-renewal and a loss of contact inhibition and BMPs seem to play a role in stem cell and progenitor determination.

BMP-2 enhanced production of luminal progenitors in MCF10A mammary cells, whereas BMP-4 prevented differentiation. BMP-4 redirected these cells towards an immature progenitor phenotype, suggesting a balance between BMP-2 and BMP-4 defines mammary cell fate (Clement et al. 2017).

In studies with human embryonic stem (hES) cells, BMPs promote differentiation, dependent on the feeder cells on which the hES are grown and in the context of other signalling pathways. For example, in the presence of FGF signalling BMP induces hES cells to differentiate into the trophoblast lineage. In the presence of FGF and BMP antagonist Noggin, hES cells can be maintained in the pluripotent state. This implicates the balance of BMP and antagonists in the switch between states of self-renewal and differentiation (Varga & Wrana 2005).

In breast cancers, the influence of BMP signalling on stem cell populations is not yet clear, and varies dependant on experimental conditions. A BMP2/7 heterodimer strongly reduced the size of a breast cancer stem cell population *in vitro,* and *in vivo* was able to inhibit formation of bone metastases (Buijs *et al.* 2012). Conversely, in separate studies, a BMPR inhibitor reduced stem cell populations and clonogenic capacity in established mammary epithelial cell lines and primary murine tumor cells (Balboni *et al.* 2013).

Autocrine BMP-4 signalling maintained the stem cell phenotype of an A17 invasive mesenchymal cell line, whereas BMP-4 inhibition by dorsomorphin resulted in epithelial-like traits, by downregulating Snail and Slug transcription factors, resulting in loss of stem-features and self-renewal ability (Garulli *et al.* 2014). It may be that differential BMPs and receptor profiles in autocrine and paracrine signalling result in the variety of effect on breast stem cell populations.

BMPs and quiescence

As well as influencing stem cells, some studies suggest BMPs could induce stem cell quiescence, which would have important implications for disease relapse. When expression of tumour suppressor ΔNp63α was induced in MCF-7 cells, the BMP target gene ID-1 was upregulated and proliferation significantly reduced. There was an increase in proportion of progenitor like cells, and cells in reversible G0 cell phase. The authors suggest BMP signalling induced quiescence in MCF7 cells, mediated by ΔNp63α (Amin *et al.* 2016).

Gao and coworkers demonstrated that paracrine BMP signalling suppresses cancer stem cell traits, and that BMP antagonist Coco reactivates dormant metastatic breast cancer cells in the lungs. Coco induced a self-renewing stem cell-like phenotype in the metastatic cells by blocking the BMP-induced repression of core stem cell transcription factors (Gao *et al.* 2012).

Therapies usually target proliferating cells, thus quiescence in disseminated breast cancer cells can result in evasion of treatment and disease relapse, potentially many years later (Zhang *et al.* 2013). BMP signalling influences both self-renewal of cells and the switch between active proliferation and quiescence. This is a key area for further development in treating, predicting and preventing relapse, which remains a significant clinical challenge in breast cancer.

## Implications in breast cancer therapeutics

BMPs are instrumental in the differentiation of bone marrow mesenchymal stem cells into bone producing osteoblasts in normal bone turnover. BMPs may swing the balance between osteolysis and osteogenesis, as they promote osteoblast differentiation, which directly promotes osteogenesis and is thus the reason recombinant BMP-2 is utilised in orthopaedic surgery. But as BMPs promote the production of receptor activator of nuclear factor kappa-B ligand (RANKL) by osteoblast precursors, this indirectly promotes osteoclastogenesis and bone resorption. In addition, BMP and Wnt pathways are major regulators of normal osteogenesis.

Targeting Wnt/BMP signalling

The Wnt inhibitors sclerostin and dickkopf 1 (DKK1) act physiologically as downstream molecules of BMP signalling to inhibit canonical Wnt signalling and therefore negatively regulate bone mass. Tumour production of DKK1 and sclerostin is thought to contribute to osteolytic bone lesions (Lipton *et al.* 2009, Chen *et al.* 2012). A DKK1-neutralizing antibody is in clinical trials for multiple myeloma, and sclerostin-neutralizing antibodies have been developed for osteoporosis. Bortezomib is a proteasome inhibitor, which inhibits osteoclast formation and bone resorption while enhancing osteoblastic differentiation and mineralisation *in vitro*. The detailed mechanism is unclear but may result from decreased DKK1. The fact that BMP signalling acts upstream makes BMP antagonism and interaction with Wnt signalling a future area of exploration for bone metastases therapeutics (Lipton *et al.* 2009, Suvannasankha & Chirgwin 2014) (Fig. 4).

Targeting osteoclast activity

In breast cancer bone metastasis, parathyroid hormone-related peptide (PTHRP) released from tumour cells
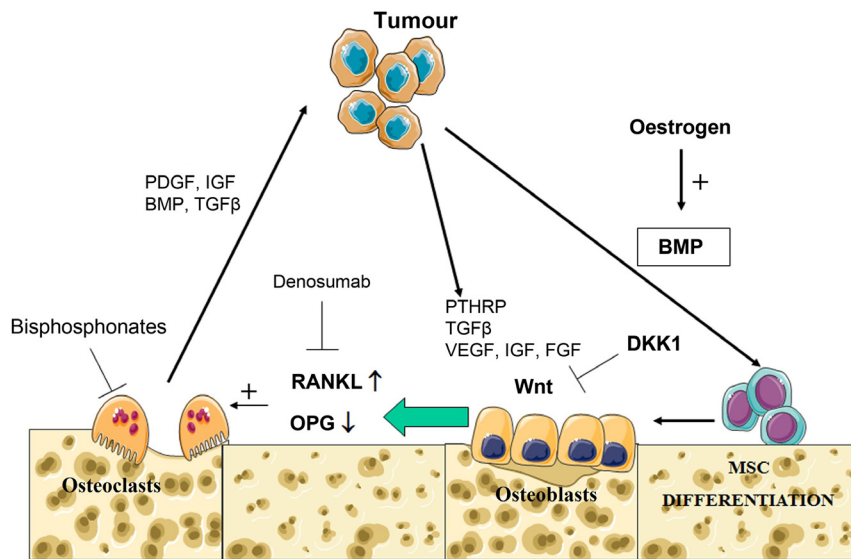
Figure 4

BMPs are a vital component of normal bone turnover, stimulating the differentiation of mesenchymal stem cells (MSC) into osteoblasts, which is promoted by oestrogen. Osteoblasts secrete RANKL and OPG. If the balance of RANKL is higher than its inhibitor OPG, RANKL binds to osteoclast precursors and results in their maturation and resultant osteolysis. In the vicious cycle of bone metastases, tumour cells secrete many factors which encourage osteoblast production of RANKL and downregulation of OPG, resulting in net osteolysis, which further releases factors that support the survival and proliferation of the tumour cells. Wnt signalling encourages osteoblast maturation and OPG secretion. DKK1, downstream of BMP signalling, inhibits Wnt signals, and thus tips the balance towards osteolysis and reduced bone mass, making DKK1 a therapeutic target in osteoporosis and osteolytic bone metastases. In the current management of osteolytic bone lesions, denosumab is a RANKL inhibitor, reducing osteoclast maturation and function, whereas bisphosphonates penetrate the bone environment, binding to calcium and are then taken up by osteoclasts resulting in osteoclast apoptosis. The figure was created using Servier Medical Art tools http://servier.com. A full colour version of the figure is available at http://dx.doi.org/10.1530/ERC-17-0139

up-regulates the expression of RANK-L in preosteoblasts, whilst repressing the expression of osteoprotegerin (OPG, which normally acts to inhibit RANK-L function), leading to a stimulation of osteoclasts and consequent bone resorption. Osteoclastic activity in turn increases the production of factors that increase PTHRP production, including TGFβ, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and BMPs, and this supports the survival of the tumour (Suvannasankha & Chirgwin 2014, Yardley 2016). The only current treatments for skeletal events in breast cancer are focused on inhibiting osteoclast function, thus reducing bone resorption. Bisphosphonates bind to bone mineral and are then taken up by osteoclasts, resulting in apoptosis. Denosumab is a monoclonal antibody against RANKL, reducing osteoclast differentiation (Steger & Bartsch 2011) (Fig. 4). The use of bisphosphonates and denosumab is currently a palliative measure, because they have not been consistently shown to improve survival or prevent bone metastasis. Moreover, breast cancer patients who develop pathological fracture have a significant 32% increased risk of death relative to patients without a fracture (Lipton et al. 2009). Thus, there is a need for developing agents that act to prevent bone metastasis, and BMPs are under explored in this capacity.

Targeting phosphoinositide-3-kinase-Akt-mTOR pathway

Another area of therapeutic interest more recently is the phosphoinositide-3-kinase (PI3K)-Akt-mTOR pathway: a key mediator of cellular proliferation, apoptosis, migration and angiogenesis, which is commonly activated in breast cancer, conferring resistance to hormonal therapy and trastuzumab. In lung cancer cells, BMP-2 regulates cellular transformation by activating the PI3K/mTOR pathway, which was completely inhibited by the mTOR inhibitor rapamycin. In breast cancer models, BMP-2 has also been shown to induce the proto-oncogene PI3K in osteoblasts to regulate differentiation. mTOR blockade suppresses RANKL and increases OPG secretion by the bone marrow stroma. mTOR inhibitors are part of ongoing trials regarding hormone receptor-positive treatment resistant tumours, although the apparent involvement of PI3K/mTOR in bone makes it of interest for bone metastasis (Royce & Osman 2015, Zhang et al. 2016).

BMP specific inhibitors

The BMP small molecule inhibitors dorsomorphin and LDN 193189 reverse stem-like features in breast cancer cells and reduce invasiveness, and have been used in several breast cancer studies to abrogate BMP signalling.

However, as yet, have not been advanced towards further development for clinical testing in malignancy with propensity to metastasise to bone. One agent that directly affects BMP signalling and is in clinical trials for solid tumours is dalantercept. A soluble chimeric ALK 1 receptor-like protein (ALK1-Fc), which displays high affinity binding with BMP-9 and BMP-10, preventing their interaction with the type 1 receptor ALK1. This results in inhibition of angiogenesis and suppresses tumour growth (Hawinkels et al. 2016) (Fig. 3). Initial studies show ALK1-Fc decreased metastasis formation in a breast cancer model (Cunha & Pietras 2011). In mice, treatment with ALK1-Fc did not result in decreased tumour size, but seemed to remodel tumour vasculature, with increased perfusion and reduced hypoxia. A temporary improvement of tumour perfusion could result in a better delivery and efficacy of chemotherapy, and indeed, pretreatment with ALK1-Fc made tumours more sensitive to cisplatin, repressing disease progression (Hawinkels et al. 2016). BMPs (particularly 9 and 10) may thus have an important role in primary tumour and bone metastases vascular remodelling, and not only angiogenesis itself. Targeting ALK1 and its ligands are the focus of ongoing clinical trials for anti-angiogenic therapies in breast cancer and other solid tumours, the results of which are awaited with interest.

Conclusions

Considering the prospect of relapsed disease and treatment resistance in breast cancer patients, and the significant burden of skeletal metastases in particular, we currently have only palliative measures for skeletal related events, and no bone-specific predictors, biomarkers or preventative therapies for bone metastases.

Aberrant expression of BMPs and BMP signalling has been implicated in breast cancer and disease-specific bone metastasis. BMPs are, as the evidence suggests, part of a highly complex, contextual and contrary signalling pathway, where balance is key to effect. The more recent studies have demonstrated BMP signalling activity in both breast primary tumours and bone metastases, contributing to EMT, angiogenesis, invasion, stemness and quiescence, bone-related phenotypes, osteogenesis and osteolysis.

These findings collectively indicate a promising therapeutic value for BMPs and their antagonists in the management of bone metastases by influencing the propensity to disseminate to and survive in the bone microenvironment. In altering the balance of bone turnover to reduce osteolysis and the morbidity associated with it, they may also be a useful adjunct to the RANKL inhibitors currently used to palliate osteolytic lesions. The current clinical trials targeting ALK-1 BMP receptors to influence tumour angiogenesis and effectiveness of chemotherapies clearly show that the BMP pathway contains a wealth of potential.

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