



Mini-review

Bone morphogenetic proteins in tumour associated angiogenesis and implication in cancer therapies

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ABSTRACT

Bone morphogenetic protein (BMP) belongs to transforming growth factor- β superfamily. To date, more than 20 BMPs have been identified in humans. BMPs play a critical role in embryonic and postnatal development, and also in maintaining homeostasis in different organs and tissues by regulating cell differentiation, proliferation, survival and motility. They play important roles in the development and progression of certain malignancies, including prostate cancer, breast cancer, lung cancer, etc. Recently, more evidence shows that BMPs are also involved in tumour associated angiogenesis. For example BMP can either directly regulate the functions of vascular endothelial cells or indirectly influence the angiogenesis via regulation of angiogenic factors, such as vascular endothelial growth factor (VEGF). Such crosstalk can also be reflected in the interaction with other angiogenic factors, like hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF). All these factors are involved in the orchestration of the angiogenic process during tumour development and progression. Review of the relevant studies will provide a comprehensive prospective on current understanding and shed light on the corresponding therapeutic opportunity.

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Introduction

Blood vessels form a complex network that transports blood, nutrients, oxygen and metabolic waste through our bodies, from the respiratory and digestive systems to the kidneys and lungs – for discharge and exchange. These blood vessels also transfer messages from one organ/tissue to other distant parts of the body, via hormones, proteins and other messengers. Formation of new blood vessels (vasculogenesis/angiogenesis) is an essential process for embryonic and postnatal development and also tissue repair (wound healing and endometrial angiogenesis during the menstrual cycle in women). Aberrant angiogenesis has been implicated in different disorders. Insufficient new vasculature may lead to defect in development and more suffering from certain disorders such as coronary heart disease, stroke and chronic wound [1–3]. Uncontrolled and excessive angiogenesis can result in haemangioma and underlie certain pathological changes in patients with certain chronic diseases such as diabetes [4–6]. Angiogenesis also contributes to tumour growth and provides a pathway for the dissemination of malignant tumour cells [7,8]. This has been one of the most heavily invested research areas for different disorders, in particular for treat-

ment of various solid tumours to stop tumour growth and prevent the haematogenous dissemination of cancer cells [9].

Angiogenesis is a complex process consisting of multiple steps which require a variety of growth factors and cytokines to initiate and coordinate in a spatiotemporal setting. Hypoxia is the primary factor for triggering angiogenesis via hypoxia inducible factors (HIFs), which subsequently upregulate pro-angiogenic factors. Pro-angiogenic factors such as VEGF, FGF, TNF- α , and certain interleukins can be produced by cancer cells, stromal cells, inflammatory cells and endothelial cells. These factors promote angiogenesis either by endothelial cell sprouting from pre-existing vessels [10] or by recruitment of endothelial progenitor cells (EPCs) derived from circulating bone marrow cells [11]. The formation of new blood vessels involves a series of coordinated biological processes such as cell proliferation, guided migration, differentiation and cell-cell communication [12]. There are three ways of forming new capillaries: sprouting growth, longitudinal division and intussusceptive growth [13,14].

Sprouting angiogenesis, which is the most commonly studied mechanism, starts with a tip cell that moves along a gradient of pro-angiogenic factors and degradation of basement membrane, followed by the growth of a trunk with proliferating endothelial stalk cells. These endothelial cells will form a lumen, recruit pericyte, fibroblast and smooth muscle cells (for bigger vessels) to surround the nascent vessel, and so the basement membrane will be reformed.

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The tip cells are essential at the start of sprouting by degrading basement membrane, migrating through extracellular matrix (ECM) [10]. The elongation of the new vessel relies on the proliferation of stalk cells which immediately follow the tip cells. Eventually, when tip cells encounter the tip of other sprouts, the migration will be inhibited and new cell–cell adhesion will be established at the joining point. The formation of a lumen consists of: the recruitment of pericytes and fibroblasts to new vessels, the deposition of ECM proteins and the terminal differentiation of ECs leading to vessel maturation and quiescence [12].

Angiogenesis is an important event during the development and progression of both primary and secondary tumours [15,16]. Tumour associated angiogenesis is pivotal for a solid tumour to grow beyond a size (2–3 mm) that is restricted by interspatial diffusion of nutrients and it also provides a fast and easier route for cancer cells to spread to other parts of the body. Such angiogenesis is affected by multiple factors and various cells in the tumour [9]. Bone morphogenetic proteins (BMPs) belong to the transforming growth factor- β (TGF- β) superfamily. They are involved in angiogenesis by either directly acting on endothelial cells or mediating interactions among different cell types in the tumour microenvironment [17,18]. In the current review, we are trying to focus on these two areas to highlight the importance they have in tumour associated angiogenesis and therapeutic potential.

BMP and BMP signalling

To date, more than 20 BMPs have been identified. These BMPs play vital roles in intramembraneous/endochondral bone formation and formation of cartilage. BMPs are also essential for certain developmental processes and homeostasis of various tissues and organs such as teeth, kidneys, prostate, breasts, skin, hair, muscle, heart and neuron [18–25].

BMP precursor proteins have an amino-terminal pro-region and a carboxy-terminal ligand containing seven conserved cysteines [26–28]. Six cysteines at the C-terminal tail construct a cysteine knot, whilst the seventh cysteine contributes to the dimerisation of ligands [29]. BMP precursor proteins can be proteolytically activated at the sequence of R-X-K/R-R or R-X-X-R by proprotein convertases (PCs), such as furin, proprotein convertase subtilisin/kexin type 6 (PCSK6) and proprotein convertase subtilisin/kexin type 5 (PCSK5) [30–33]. The pro-region of the precursor BMP protein stabilises the processed ligands [31]. Following the cleavage, BMP ligands can form homodimer and heterodimer molecules. Certain heterodimers such as BMP-4/-7, BMP-2/-6, BMP-2/-7 and BMP7-/GDF-7 tend to be more effective than their homodimers [29,34–36].

BMP signal transduction

BMP signals are mediated by type I and type II serine/threonine kinase receptors. Six of the type I receptors and three of the type II receptors have been shown to mediate BMP signalling [24]. BMPRI1A, BMPRI1B and BMPRI2 are specific to the BMPs, whilst ACVRL1, ACVR1, ACVR1B, ACVR2B, and ACVR2A are the receptors shared with activin; TGFBR1 (ALK5) is known as the type I receptor for TGF- β 1, 2 and 3. Both type I and type II receptors consist of a N-terminal extracellular ligand binding domain, a single transmembrane region, and a C-terminal serine/threonine kinase domain [37]. BMP ligands exhibit higher affinity with type I receptor. Upon binding with BMP ligands, a ligand–receptor complex consisting of a homodimer/heterodimer of BMP ligands, type-I and type-II receptors are formed rapidly. The type-II receptors transphosphorylate the GS domain of the type-I receptors leading to an activation of downstream cascades [38].

Smad proteins are important intracellular molecules mediating signals from the BMP receptors. The Smad family comprises

receptor regulated Smads (R-Smads, Smad-1, -2, -3, -5 and -8), common mediator Smads (Co-Smad, Smad-4), and inhibitory Smads (I-Smads, Smad-6 and -7) [39,40]. R-Smads 1, 5 and 8 are substrates of the type I receptors (ALK-1, ALK-2, ALK-3 and ALK-6), whilst R-Smads 2 and 3 are substrates of the type I receptors (ALK-4, ALK-5 and ALK-7) [40–43]. Smad2 and 3 in complex with Smad4 bind specifically via their MH1 domains to Smad binding elements (SBE), i.e. AGAC/GTCT at promoter of target genes [44]. Smad1 binds with low affinity to SBEs and preferentially binds to a GC-rich sequence, such as GCCGNCGC [45].

Binding of BMP ligand to preformed hetero-oligomeric complexes (PFC) leads to activation of the Smad dependent pathway [39,46]. The pathway-restricted Smads (R-Smads, Smads1, 2, 3, 5 or 8) are recruited and translocated into nuclei with assistance of Smad-4 and regulates the transcription of target genes; this is known as the Smad dependent pathway. Upon activation, the Smad-complex will translocate into the nucleus, R-Smads will interact with transcriptional coactivators and repressors to regulate expression of target genes, including ID1, Smad-6 and -7.

BMPs have a higher affinity with the type-I receptors. Thus, BMP ligand can also bind to ALK3 or ALK6, and then recruits BMPRII into a hetero-oligomeric complex (BMP-induced signalling complexes, BISC); this leads to the activation of the Smad independent pathway [39,46]. X-linked inhibitor of the apoptosis protein (XIAP) functions as an adaptor protein bridging between the type I receptor and TGF- β activated binding protein (TAB1/2/3), which is an activator of the MAPKKK TGF- β activated tyrosine kinase 1 (TAK1) [47–49]. TAK1 can subsequently activate mitogen-activated protein kinase (MAPK) pathways [46,50,51]. TAK1 can also activate Jun N-terminal kinases (JNKs), NF- κ B (NF- κ B), and Nemo-like kinase (NLK) [52–54].

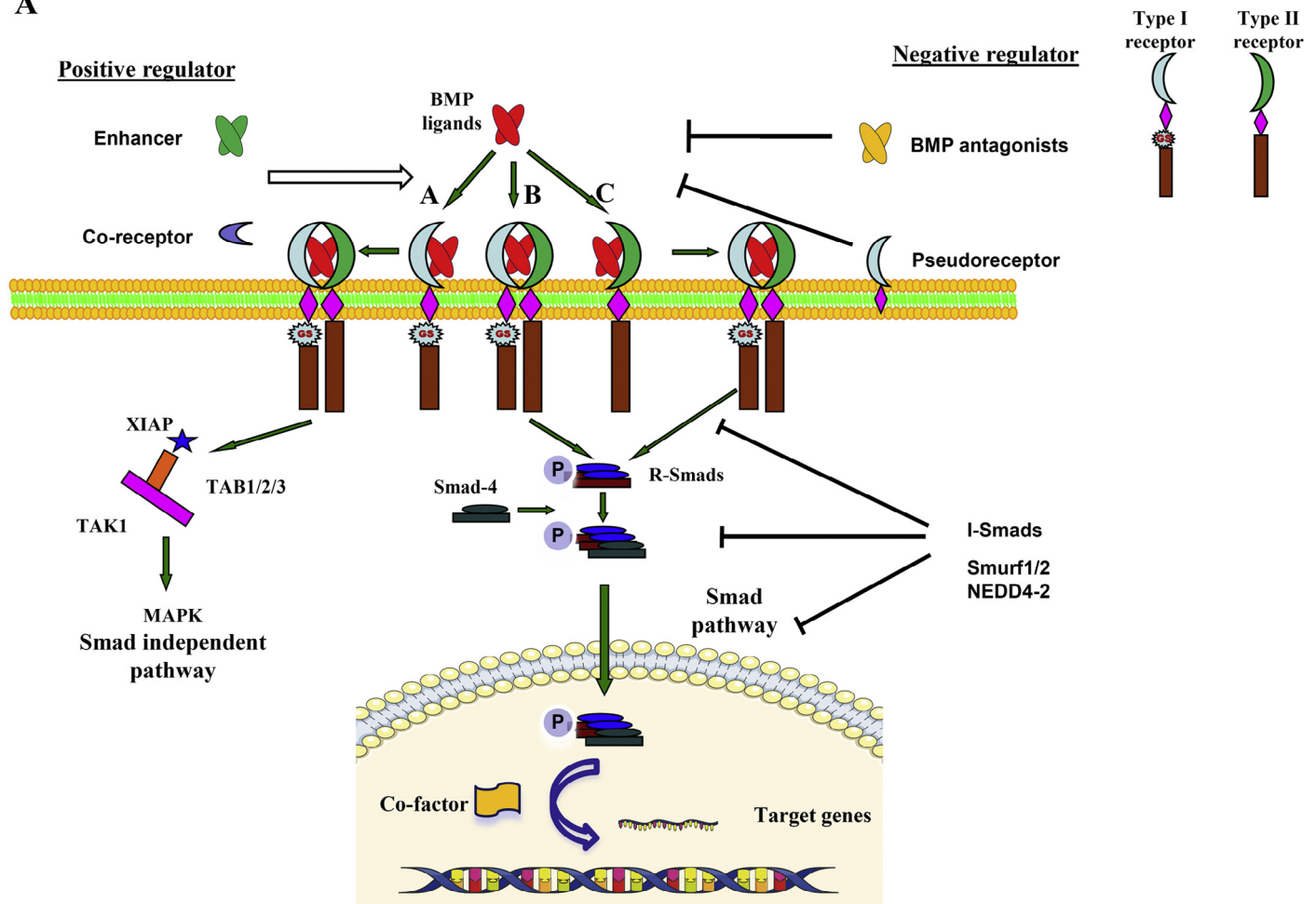
Regulation of BMP signalling

BMP signalling can be regulated extracellularly during the process of ligand binding to the receptors, or intracellularly during signal relay and regulation of their target genes (Fig. 1). The most important molecules that influence BMP signalling extracellularly are BMP antagonists including Noggin, Follistatin, Gremlin, Chordin, Chordin-like, etc. The antagonists can competitively bind to BMP receptors. Expression of BMP antagonists can be induced by BMP ligands. For example, noggin expression in osteoblasts can be induced by BMP2, 4 and 6 [55]. This feedback regulation of BMP antagonists has also been observed in prostate cancer cells [56]. Such feedback regulation helps cells to justify their response to BMP ligands which also operate through regulation of the pseudoreceptor and I-Smads [57,58].

Along with the BMP antagonists, co-receptors and pseudoreceptors can also coordinate binding of BMP ligands to the receptors. BMP and activin membrane bound inhibitor (BAMBI) is a pseudoreceptor for serine-threonine kinase receptor which lacks the intracellular serine/threonine kinase domain. Binding of BAMBI to the ligands can inhibit subsequent signalling [59]. In contrast to the negative regulators, co-receptors for BMP ligands can enhance their signalling. Repulsive guidance molecules (RGMs, A, B and C) can enhance signalling of BMP2 and BMP4 as co-receptors [60–62].

I-Smads suppress TGF- β signalling by either preventing R-Smad activation or competitively binding with Smad4 to block the formation of Smad hetero-complex [63,64]. Smad7 can target both TGF- β /activin and BMP signalling, whilst Smad6 is more specific for BMP signalling [63,65]. Such inhibitory effects of I-Smads can be enhanced when cells are exposed to BMP ligands due to feedback regulations [66,67]. The action of I-Smads can be fine-tuned by an association with the SH3 domain of STAM (AMSH) which can directly bind to Smad6 and thus interferes with the interactions between Smad6 and the activated BMP type I receptors [68]. To reg-

A



B

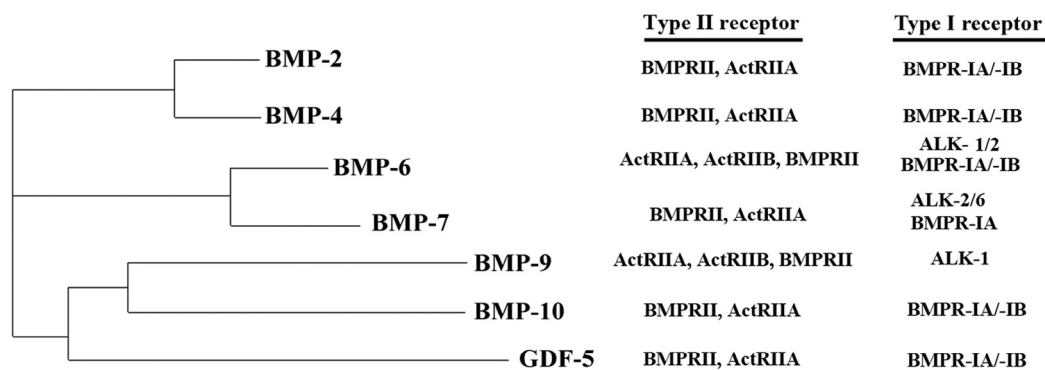


Fig. 1. BMP signal transduction. (A) BMP signalling pathways. BMP ligands bind to type I and type II simultaneously which leads to activation of the Smad-dependent pathway. Activation, transport and regulation of target genes by R-Smads require Co-Smad. If BMP ligands bind to the type I receptors first and then recruit the type II receptors which activates TAB1/2/3 through XIAP, this would lead to the activation of Smad independent MAPK pathway. Regulation of BMP signaling can occur extracellularly during the process of ligand binding to receptor, and intracellularly during the signal transduction. (B) BMPs and their specific receptors. Key downstream signalling molecules of BMP/GDF through the use of a dendrogram tree. Phylogenetic analysis was performed using the Clustal Omega (<http://www.ebi.ac.uk/Tools/msa/clustalo/>), and the dendrogram tree was plotted using the Treeview (Version 1.6.6, <http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>).

ulate gene transcription, Smad-1, -5 and -8 need assistance from other transcription factors due to their low affinity in binding with SBEs. These helpers include Forkhead box HI (FOXHI) [69], P53 [70], Runx transcription factors [71], Smad interacting protein-1 (SIP-1) [72] and ATF-2 [73]. Transcriptional co-activators and repressors are also involved in the regulation of Smad signalling. P300 and CREB-binding protein (CBP) can enhance transcriptional activities of Smads

[74]. Sloan-Kettering retrovirus (Ski) can inhibit BMP signalling via interaction with Smad-1, -3, -5 and -4 [75–77]. The transcriptional activities can also be balanced between BMPs and other TGFβ family members. For example, Ski and SnoN degradation can be triggered by TGFβ signalling which may thus enhance Smads-induced transcription [78]. In addition, Smads in the intracellular pool are also regulated by HECT type E3 ligases known as Smad Ubiquitination

Regulatory Factors (Smurf) 1 and 2. Smurf 1 can either directly interact with Smad 1/5 to facilitate their degradation [79] or indirectly interact with the BMP type I receptor through I-Smad 6 and 7 leading to a degradation of the receptors [80]. Smurfs also participate in the translocation of I-Smads from the nucleus into the cytoplasm and enhance I-Smad interaction with the type-I receptors [81]. NEDD4-2 (neural precursor cell expressed, developmentally down-regulated 4-2) which is structurally similar to Smurfs can directly bind with Smad-2, -3 and -7 leading to degradation of these proteins [82,83].

Aberrant BMP signalling in cancer

Since BMPs play profound roles in the bone formation and regulation of cell differentiation, their involvement in tumorigenesis and cancer progression has been a hot spot for cancer research. Aberration in BMP expression and their signalling has been observed in a variety of solid tumours and disease specific bone metastasis [18].

Dysregulated BMP and BMP signalling at the primary tumour

Breast cancer and prostate cancer have been intensively studied for the involvement of BMPs in their disease progression due to the unique feature of their metastasis to bone. They are the leading cancers seen in women and men respectively in developed countries. BMP-6 overexpression is associated with higher grade, primary tumours and advanced prostate cancer with metastasis [84–87]. BMP-6 may also play a role in the progression of androgen independent prostate cancer [85,87]. Downregulated expression of BMP-2, BMP-4, BMP-7, BMP-9, BMP-10 and GDF15 has been observed in prostate cancer during its development and progression [88–94]. Similarly, reduced expression of BMPs, including BMP-2, BMP-4, BMP-6, BMP-7, BMP-12, BMP15 and GDF9a, has been evident in breast cancer and is associated with poor prognosis of the patients [95–97]. Although the expression of specific BMPs, such as BMP-2, 4, 6 and 7, in breast cancer remains controversial [95,98–101], the aberrant BMP expression plays a role in the development and progression of both prostate and breast cancers.

The expression of BMPRIA, BMPRII, and BMPRII in human prostate cancer tissues is frequently reduced – or absent – from high-grade (poorly differentiated) prostate tumours which plays an important role in the disease progression [92,102–104]. Elevated expression of BMPRII was associated with high tumour grade, high tumour proliferation, cytogenetic instability, and a poor prognosis in oestrogen receptor-positive carcinomas [105]. Decreased levels of BMPRII have also been observed in breast cancer, which are associated with poorer prognosis [106].

Perturbed Smad expressions and signalling have also been indicated in the primary tumours. Loss of Smad 4 is related to the progression of prostate cancer towards a more aggressive phenotype suggesting that it is a tumour suppressor [90]. Activation of the Smad pathway of BMPs (Smad1/5/8) and TGF- β (Smad2) has also been reported in breast cancer cells in both primary tumours and bone metastases [107].

BMP in bone metastasis

Primary prostate tumours and metastatic prostate tumours exhibit differential expression profiles of BMP and BMP signalling molecules. The differential expression has an intimate relationship with the disease progression, in particular the bone metastases. BMP6 is highly expressed by both primary prostate tumours and metastatic bone lesions. In contrast, BMP7 and GDF15 are suppressed in prostate cancer cells at primary tumours and are upregulated in the metastatic cancer cells at bone lesions [89,108].

Decreased expression of BMP7 in primary tumours is associated with bone metastases. BMP7 is able to inhibit the growth of breast cancer tumours at primary sites and in bone *in vivo* [109]. BMP-2 can promote metastasis of breast cancer cells to bone [110]. On the other hand, lack of BMP antagonists in breast cancer may contribute to the osteoblastic lesions of breast cancer [111]. 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. However, targeting Noggin was able to reduce osteoblastic activity, but had little influence on bone resorption and tumour growth [112]. Meanwhile, increased expression of BMP receptors and activation of BMP signalling have also been implicated in breast cancer and the corresponding bone metastasis from the tumour. Activation of the Smad pathway of BMPs (Smad1/5/8) and TGF- β (Smad2) has been revealed in the nuclei of breast cancer cells at both primary tumours and bone metastasis [107]. These studies collectively suggest that BMPs are involved in bone metastasis.

Since metastatic cancer cells spread to the bone, interactions between the tumour cells and its residing microenvironment constantly affect the subsequent development of a secondary tumour. Such a crosstalk coordinates functions of cancer cells and host cells. BMPs released from tumour cells influence remodelling of the bone by regulating osteoblastic and osteoclastic activity. Such mutual influence is also reflected by the BMPs that are enriched in the bone environment. In addition to the promotion of the motility and invasion of cancer cells, they are also able to induce the expression of other growth factors, which then enhances the vicious circle of bone–tumour–bone interactions. For example, BMP2 induced osteoprotegerin (OPG) expression in PC-3 cells inhibits osteoclastogenesis which may contribute to the osteoblastic lesions [113]. BMP-7 induces VEGF expression in prostate cancer cells, which leads to an enhanced pro-osteoblastic activity in the tumour [114].

Taken together, aberrant expression of BMPs and BMP signalling molecules has been implicated in a variety of solid tumours and disease specific bone metastasis. Most BMPs elicit inhibitory effects on proliferation of cancer cells through their receptor signalling. Certain BMPs, including BMP-2 and BMP-7, can promote epithelial mesenchymal transition (EMT) and enhance cell invasion and motility, leading to a more aggressive phenotype and contribute to the subsequent dissemination of cancer cells [115,116]. On the other hand, contrasting evidences also showed that BMPs, for example, BMP-6 and BMP-7, were able to reverse EMT and inhibit aggressive traits of cancer cells [56,94,109,117]. The phenotypic profile of BMPs, BMP receptors and intracellular signalling molecules can be modified by sexual hormones and growth factors in order to coordinate biological behaviours of cancer cells during the disease progression. The altered expression of certain BMPs may assist cancer cells to metastasise to bone. In addition to their direct effect on cancer cells, BMPs can also regulate angiogenesis thus indirectly contributing to tumour growth and metastasis (Fig. 2).

BMP and angiogenesis

Angiogenesis is critical for the development and progression of both primary and secondary tumours. It has been demonstrated that BMPs, including BMP-2, 4, 6, 7 and GDF5, are capable of inducing angiogenesis [118–121]. BMP receptors play an important role in mediating the angiogenesis induced by BMPs. For example, BMPRII and BMPRI are upregulated in endothelial cells during tubule formation [122], whilst activation of ALK1/Smad-1/5 pathway induces angiogenesis, whereas ALK5/Smad-2/3 pathway signalling is involved in the angiogenesis at a later stage [123]. In addition to BMP ligands and their receptors, BMP antagonists are also involved in angiogenesis. Gremlin for example shows higher expression in pituitary tumours and is associated with angiogenesis [124]. However, Gremlin may directly target vascular endothelial cells to

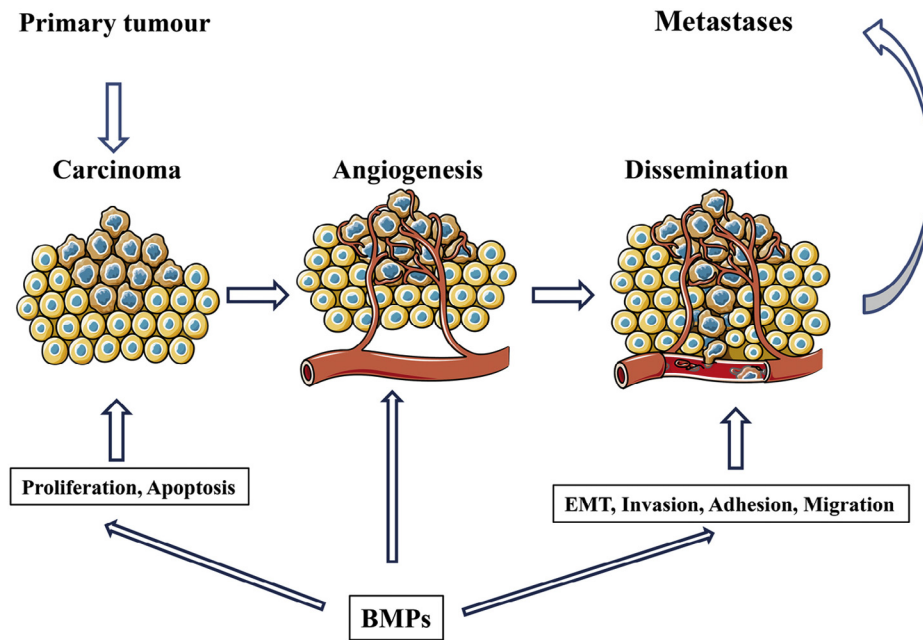


Fig. 2. BMP in cancer and cancer metastasis. Dysregulated BMP signalling assist cancer cells dynamically during the development and disease progression, in particular during angiogenesis.

promote angiogenesis rather than interacting with BMP ligands [125]. Tumour associated angiogenesis is orchestrated by interactions among cancer cells with the local microenvironment at both primary tumour and secondary tumour. In the following text, the way that BMPs directly act on the vascular endothelial cells and also what roles they play in the orchestrated angiogenesis in tumours will be highlighted.

Direct effect of BMP on vascular endothelial cells

The direct effect on endothelial cells by certain BMPs, such as BMP-2, -4 and -9, has been recently reviewed, specifically for their implication in endothelial dysfunction and corresponding vascular diseases other than tumours [126]. In addition to their potent effect on bone formation, BMP-2, 4, 6, 7 and GDF-5 can also promote angiogenesis [118–121]. For example, GDF-5 exhibited pro-angiogenic effect in a chorio-allantoic membrane (CAM) assay, but not for BMP-2 [118]. However, BMP-2 can promote angiogenesis in an *in vivo* lung cancer tumour model [127]. BMP-2 promotes migration and tubule formation of human umbilical vein endothelial cells with little effect on proliferation and apoptosis [128]. BMP-2 can also suppress expression of apelin in vascular endothelial cells which may have profound implication during angiogenesis [129]. Disable homolog 2 (Dab2) has been revealed as an important factor in BMP-2 induced angiogenesis. It provides a molecular basis for understanding the context-dependent proangiogenic function of BMP signalling [130]. During caudal vein plexus (CVP) formation in zebrafish, BMP induces the extension of endothelial filopodia and their migration via Arhgef9b-mediated activation of Cdc42. Active Cdc42 binds to and stimulates Formin-like 3 (an actin-regulatory protein of the formin family) which, in turn, promotes angiogenic sprouting of the CVP [131]. BMP-2 can also promote proliferation of aortic endothelial cells [127] and stimulate migration of vascular smooth muscle cells [132]. A similar effect on smooth muscle cell migration has also been observed for BMP-4 and BMP-7 [133,134]. It suggests that BMPs can regulate angiogenesis by coordinating different cells.

Being different from other BMPs, BMP-9/-10 exhibit an inhibitory effect on angiogenesis by suppressing proliferation and migration of endothelial cells [135]. BMP-9/ALK1-induced CV2 and matrix Gla protein (MGP) can inhibit the angiogenic effect of BMP-4 by disrupting complex formation between BMP-4 and BMP-9, and their receptors ALK2 and ALK1 [136]. Furthermore, BMP-9 can inhibit lymphangiogenesis via ALK-1 during development and cancer progression [137].

Endothelial cells cultured on type-I collagen can spontaneously form tubular structures which exhibit increased expression of BMPR-IB and BMPR-II. The CAM assay also showed the important role played by these BMP receptors in angiogenesis [122]. Downstream of BMP receptors, Smads play a vital role in mediating signal transduction for BMPs. Smads are the transcriptional regulators of BMP target genes, in particular the VEGF. Smad3 expression in rat proximal tubular cells resulted in an induction of endothelial cell proliferation with an up-regulation of VEGF-A, whilst Smad2 induced expression of thrombospondin-1 – this suggests that these two Smads have opposing roles during angiogenesis [138]. However, Smad-3 in a gastric cancer cell line (SNU484) gastric cancer cells results in a down-regulation of VEGF expression. Smad-3 also suppresses *in vivo* growth of these cancer cells with impaired angiogenesis [139]. Overexpression of Smad-4 pancreatic cancer cells suppresses VEGF expression and up-regulates thrombospondin-1 expression leading to reduced new vasculature [140].

BMP antagonists play important roles in justification of cell response to BMP ligands. Noggin can attenuate the pro-angiogenic effect of BMP-7 [114]. Drm/gremlin has been identified as a pro-angiogenic factor expressed by endothelium through promoting migration and invasion. Interestingly, BMP4 does not affect the pro-angiogenic effect of Drm/gremlin, instead, the two work together to promote angiogenesis *in vitro* and *in vivo*. It suggests that Drm/gremlin can interact directly with endothelial cells to modulate angiogenesis in a relatively independent manner [125]. Higher expression of Gremlin has also been revealed in pituitary tumours which is associated with angiogenesis [124]. In a study of lung cancer, BMP-9 and Follistatin were used as angiogenic biomarkers for as-

sisting differential diagnosis of malignant tumours from benign tumours. BMP-9 is elevated in malignant tumours whilst Follistatin is decreased [141]. BMP-binding endothelial cell precursor-derived regulator (BMPER) can regulate migration of endothelial cells in a dose-dependent manner, i.e. a promoting effect at lower concentration and an inhibitory effect at higher concentration [142]. Tsg and BMPER interfere with each other to enhance their angiogenic effect [143]. BMPER-coordinated BMP-4 signalling relies on LDL receptor-related protein 1 (LRP1), mediated endocytosis, and subsequent Smad activation [144].

BMP-mediated angiogenic effect in the crosstalk between tumour cell and local microenvironment

The process of angiogenesis is regulated by altering the balance of pro-angiogenic and anti-angiogenic factors. One of the most well-known pro-angiogenic factors is VEGF, which is frequently over-expressed in cancers and can be regulated by many signalling pathways, including TGF- β and BMP [145]. BMP-9 for example, when signalling via ALK-1 and BMPR-II in endothelial cells, activates Smad1/5 downstream signalling and has been shown to inhibit the proliferation of endothelial cells, as well as block VEGF mediated angiogenesis [146]. Secretion of BMP and angiogenic factors comes from different cell types in a tissue. An *in vitro* 3D cell model comprising adipose-derived stromal cells and endothelial cells revealed such differential resources for these soluble factors [147].

The experimental evidence suggests that BMPs can promote angiogenesis indirectly through up-regulation of the expression of VEGF in both cancer cells and osteoblasts. Dai et al. have demonstrated that it is possible for BMP-7 to promote osteosclerosis through VEGF in the skeletal metastases from prostate cancer [114]. BMP-7 can also upregulate VEGFR in vascular endothelial cells [148]. BMP-2 up-regulates VEGFR through p38 pathway human adipose derived stromal cells [149]. Bone induction by rhBMP-2 can be blocked by the anti-angiogenic agent (TNP-470) [119]. These pieces of evidence suggest that angiogenesis is an integrated part of BMP induced bone formation.

BMP-induced angiogenesis can also be synergised by basic fibroblast growth factor (bFGF) and TGF- β 1 [150]. This synergistic effect on angiogenesis has also been observed for BMP-2 and VEGF in electron beam melting-fabricated porous titanium implant delivery of these factors for orthopaedic application [151]. In human bone marrow stromal cells (hBMSCs), BMP-5 and -6 can upregulate SDF-1, BMP-2 induces b-FGF, whilst VEGF can be up-regulated by BMP-2 and -4 [152]. In contrast to TGF- β 1 and most BMPs, BMP9 has been shown to inhibit the proliferation of endothelial cells, as well as block VEGF mediated angiogenesis [146].

Cross talk between Delta-like ligand 4 (DLL4)-notch and BMP signalling plays a critical role in the tip-stalk formation during angiogenesis [153]. BMP-9 and BMP-10 can upregulate the expression of genes involved in Notch signalling (Jagged 1, DLL4, Hey1, Hey2 and Hes1) in vascular endothelial cells thus coordinating postnatal vascular remodelling [154]. ALK1 mediated BMP signalling can regulate Hey1 and Hey2, but not other Notch target genes (Hes1, Hes5, ephrinB2 and Dll4) [155]. VEGF and Dll4/Notch signalling operates through a negative feedback loop that regulates endothelial tip and stalk cells to ensure adequate vessel branching and function. Neuropilin-1 (Nrp1) suppresses the stalk cell phenotype by limiting Smad2/3 activation through ALK1 and ALK5. Notch downregulates Nrp1 leading to a relief of ALK1 and ALK5 mediated Smad signalling [156]. Osterix (Osx) is a BMP-2 inducible osteoblast specific transcription factor which is essential for osteoblast differentiation and bone formation. Proline-rich regions of Osx are important for its binding to the VEGF promoter and consequent up-regulation of VEGF. Osx and HIF-1alpha can cooperatively regulate VEGF expression [157]. CXXC5 containing CXXC-type zinc-

finger is a transcription factor of Flk-1. It can mediate BMP signalling for differentiation and migration of vascular endothelial cells during angiogenesis and vascular development [158].

Hepatocyte growth factor (HGF) and its receptor, cMet signalling, plays a vital role in tumour angiogenesis and has been a target for the research of anti-angiogenesis [159–161]. HGF can upregulate the expression of BMP-7 and BMP receptors in prostate cancer cells [162,163]. Our recent study also showed that HGF was able to upregulate BMP coreceptor (RGMb) in vascular endothelial cells which mediated HGF and BMP-7 induced angiogenesis [164]. It suggests that HGF/BMP-7 may work together to enhance angiogenesis in tumours. Smad-2, -3 and -4 can also mediate differential regulation on HGF transcription. Smad-2 binds with higher affinity with co-repressors of HGF promoter, whilst Smad-3/Smad-4 are more likely to work together with a co-activator. Therefore, targeting different Smads could elicit contrasting effects on HGF promoted angiogenesis [165].

BMP Smad signalling can also upregulate the expression of platelet-derived growth factor alpha (PDGFA) during glanulosa cell tumourigenesis [166]. BMP-2 and fibrin-binding platelet-derived growth factor-BB (PDGF-BB) can promote the formation of bone and new vasculature [167]. BMP-4 stimulated fibroblasts can release more inflammatory factors and MMPs [168]. BMP-4 suppress TNF induced expression of MMP-9 through the Smad dependent pathway [169].

A microenvironment can foster interactions between cancer cells and other types of cell to promote angiogenesis. TGF- β plays an important role in the crosstalk within the tumour microenvironment including interactions among stromal cells, endothelial cells and tumour cells. Stromal cell-released TGF- β induces both ligand-dependent and ligand-independent activation of androgen receptor (AR) in prostate cancer cells through an up-regulation of BMP-6 and IL-6 [170]. Extracellular matrix is also involved in orchestrated angiogenesis. 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 [122]. Enamel matrix derivative (EMD) can promote angiogenesis and osteogenesis. Low molecular weight protein pools (7–17 kDa) of EMD can induce BMP signalling and increase osterix and VEGF-A expression [171]. VEGF and FGF-2 are more helpful in the initial activation phase during the angiogenesis process, whilst BMP-2 can synergistically work together with these two angiogenic factors in a more effective way by delivering at the maturation stage [172]. BMP-2 and fibrin-binding platelet-derived growth factor-BB (PDGF-BB) can promote the formation of bone and new vasculature [167]. Pregnancy-associated plasma protein-A2 (PAPP-A2, pappalysin-2) is a large metalloproteinase, known to be required for normal postnatal growth and bone development in mice. Papp-a2 regulates BMP signalling via a proteolysis of insulin-like growth factor binding protein-3 (IGFBP-3) and also Notch signalling by an independent mechanism [173].

Bone marrow stem cells (BMSCs) implanted with porous silk scaffolds (silk fibroin) can differentiate into endothelial cells and osteoblasts in the presence of VEGF and BMP-2 [174]. Localised release of VEGF and BMP-2 can promote bone regeneration, in part by facilitating the mobilisation of bone marrow stem cells and the subsequent differentiation into endothelial and osteogenic lineages respectively [175]. BMP-6 can induce IL-1alpha in macrophages through a cross-talk between NF-kappaB and Smad1 signalling leading to an action on endothelial cells [176]. In BMP-2 induced bone formation, α SMA⁺ and Tie2⁺ progenitor lineages make distinct cellular contributions to bone formation, angiogenesis, and resorption/remodelling. Co-staining of tartrate-resistant acid phosphatase activity (TRAP, an osteoclast marker) and CD31 (vascular endothelia marker) was seen in the Tie2-lineage endothelial progenitor cells, whilst the α SMA-lineage cells contribute to osteoblastic

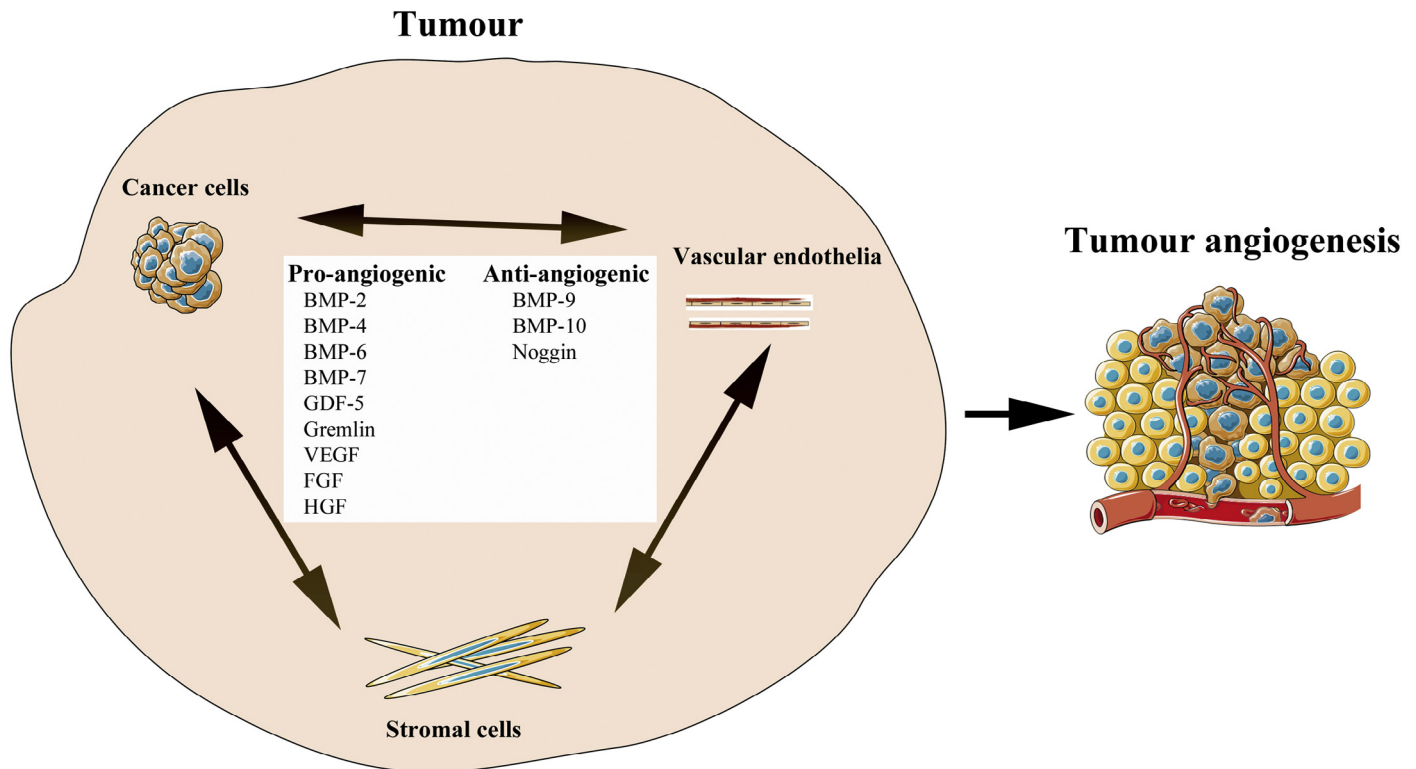


Fig. 3. BMP in orchestrated tumour angiogenesis. Multiple cell types exist in a tumour, a vicious tri-angle can be triggered by the cancer cells. BMPs play a vital role in these types of crosstalk and resulted in a promoted angiogenesis which can facilitate tumour growth and dissemination.

lineage [141]. FGF-2 can up-regulate BMP-2 to promote osteoblastic differentiation and bone formation in periodontal regeneration [177]. These pieces of evidence have opened up an avenue leading to a better understanding of complicated interactions among different cell types in bones during bone formation and angiogenesis, which is vital for tumour associated angiogenesis and bone metastasis (Fig. 3).

Therapeutic potential by targeting BMPs and their signalling

The variety of effects exhibited by some BMPs offer us with different choices of approach. We would then use these molecules for tumour associated angiogenesis. For example, BMP-2, -4, -6, -7 and GDF-5 are pro-angiogenic factors, whilst BMP-9 and -10 are anti-angiogenic factors. The natural BMP antagonist, Noggin, can be used to target pro-angiogenic BMPs. In contrast, recombinant BMP-9 and -10 can be used to suppress angiogenesis. BMP-9 and BMP-10 have both also shown inhibitory effects on growth and motility of certain cancer cells [92,93,178]. On the other hand, attenuation of BMP-9 induced ALK1 signalling with neutralising antibody was able to inhibit endothelial cell sprouting [179]. It suggests that ALK1 can be targeted for prevention of tumour associated angiogenesis. PF-03446962 (Pfizer) and Dalantercept (also known as ACE-041, Acceleron Pharma) targeting ALK1 have been shown as potent inhibitors for blocking development of blood vessels. PF-03446962 is a monoclonal antibody against ALK1 which exhibits a dose-dependent anti-angiogenic activity [180]. Dalantercept is a soluble chimeric protein (ALK1-Fc) which displays high affinity binding with BMP-9 and BMP-10 and therefore suppresses tumour growth by inhibiting angiogenesis [181]. Both are currently being tested as anti-angiogenic agent alone or in conjugation with other anti-angiogenic or anti-cancer agents in different clinical trials to treat advanced malignancies and treat/prevent metastatic tumours (Table 1). A recent study showed that targeting ALK1 using a small molecule inhibi-

tor (K02288) was able to inhibit angiogenesis through suppressing signalling of both Smad and Notch pathways [182]. However, anti-angiogenic potential of the small inhibitor is yet to be examined in clinical trials. BMP signalling is required for remodelling of plexus into structured blood vessels during zebrafish fin regeneration. Inhibition of BMP signalling using DMH1 blocks maturation of the new vasculature [183]. Endoglin also known as CD105, mediates a transition of endothelial cells from quiescent to active status during angiogenesis through preferential phosphorylation of Smad 1/5/8 (endoglin is also a coreceptor for BMP-9). A monoclonal antibody against Endoglin (TRC105) has exhibited anti-angiogenic potential and may achieve greater effect in a combination with bevacizumab. TRC105 is currently being tested in phase 1b and phase 2 clinical trials [184] (Table 1).

The expression of BMP-7 for example is decreased or absent from primary tumours which may be related to the shift in phenotype from androgen-dependent to androgen-independent activity within the cancer cells [91]. Loss of endogenous BMP-7 may encourage the prostate cancer cells to be more aggressive [56]. However, BMP-7 can be re-expressed in metastatic cancer cells in bone [108]. This needs to be considered when we select BMP for targeting cancer cells and angiogenesis.

The VEGF pathway is critical for tumour angiogenesis. However, anti-angiogenic therapy targeting VEGF can be limited by intrinsic and acquired resistance. Understanding of BMP for its implication in tumour associated angiogenesis may provide more targets. We wish to fill the therapeutic gap of solely targeting the VEGF pathway. Alternative targets can be selected from BMP signalling as aforementioned. Furthermore, HGF is a key cytokine in promoting cancer metastasis and angiogenesis. Our recent studies have demonstrated that HGF can up-regulate the expression of BMP-7 and BMP receptors in prostate cancer cells, and RGMB in vascular endothelial cells [162–164]. Targeting HGF and BMP signalling may prevent tumour associated angiogenesis. Collectively, these will provide a

Table 1
Relevant clinical trials.

Target	Specific agent and the anti-angiogenesis effect	Agent(s) used in the trial	Tumour type	Clinical trial No./Phase
CD105 (Endoglin)	TRC105 is a novel, clinical stage antibody to endoglin, which is a protein that is overexpressed on endothelial cells and is essential for angiogenesis, the process of new blood vessel formation. Anti-angiogenesis; treat primary tumours; treat/prevent advanced and metastatic tumours	TRC105 + Avastin® (bevacizumab)	Kidney cancer	NCT01727089**/Phase 2B randomised
		TRC105	Prostate cancer	NCT01090765***/Phase 1 & 2
		TRC105	Urothelial carcinoma	NCT01328574***/Phase 2A
		TRC105 + Nexavar® (sorafenib)	Liver cancer	NCT01306058*/Phase 1B/2A
		TRC105	Liver cancer	NCT01375569**/Phase 2A
		TRC105 + Avastin® (bevacizumab)	Glioblastoma	NCT01648348*/Phase 1B/2B randomised
		TRC105 + Avastin® (bevacizumab)	Glioblastoma	NCT01564914**/Phase 2A
		TRC105 + Avastin® (bevacizumab)	Choriocarcinoma	NCT02396511*/Phase 2
		TRC105	Ovarian cancer	NCT01381861***/Phase 2A
		TRC105 + Xeloda® (capecitabine)	Metastatic breast tumours	NCT01326481**/Phase 1B
		TRC105 + Inlyta® (axitinib)	Advanced renal cell cancer	NCT01806064*/Phase 1B/2B randomised
		TRC105 + Votrient® (Pazopanib)	Advanced soft tissue sarcoma	NCT01975519*/Phase 1B/2A
		TRC105 + Avastin® (bevacizumab)	Advanced solid tumours	NCT01332721***/Phase 1B
ALK1	Dalantercept is an investigational protein therapeutic that inhibits angiogenesis by preventing BMP9 and BMP10 from interacting with ALK1. Treat primary advanced tumours, recurrent tumours and metastatic tumours; Prolongs progression free survival PF-03446962 is a novel monoclonal antibody against ALK1 which shows dose-dependent anti-angiogenic activity.	Dalantercept (Recombinant ALK-1 Inhibitor Receptor-Fusion Protein, also known as ACE-041)	Advanced or metastatic solid tumours	NCT00582985***/Phase 1
		Dalantercept + axitinib	Ovarian cancer and primary peritoneal carcinoma	NCT01720173**/Phase 2
		Dalantercept + sorafenib	Advanced renal cell carcinoma	NCT01727336*/Phase 2
		ACE-041	Advanced adult hepatocellular carcinoma	NCT02024087*/Phase 1 and 2
		Dalantercept	Advanced solid tumours multiple myeloma	NCT00996957***/Phase 1
		Dalantercept	Recurrent or persistent endometrial cancer	NCT01642082**/Phase 2
		Dalantercept	Squamous cell carcinoma of the head and neck	NCT01458392**/Phase 2
		PF-03446962 + Regorafenib	Colorectal cancer	NCT02116894*/Phase 1
		PF-03446962	Transitional cell carcinoma of bladder	NCT01620970*/Phase 2
		PF-03446962	Hepatocellular carcinoma	NCT01911273 Terminated
PF-03446962	Malignant pleural mesothelioma	NCT01486368 ***/Phase 2		
PF-03446962	Neoplasms	NCT01337050***/Phase 2		
PF-03446962	Advanced solid tumours	NCT00557856***/Phase 2		

Note: *Recruiting or enrolling; **Ongoing; ***Completed. More information of the trials can be found at <https://clinicaltrials.gov/ct2/home>.

comprehensive understanding of how angiogenesis is instigated in the tumour and what should be considered for personalised disease management.

A recent review has summarised the mechanism leading to increased endothelial cell growth and angiogenesis with a focus on miR-26 [185]. MiR-26a expression is decreased by pro-angiogenic stimuli such as VEGF, bFGF, and TNF- α in endothelial cells (ECs). MiR-26a inhibits SMAD1 by binding to its 3'-UTR, leading to a down-regulation of ID1 and an increased expression of p21WAF1/CIP1 and p27. In addition, MiR-885-3p can interact with BMPR1A and consequently results in an inhibition of Smad 1/5/8 signalling and downregulation of ID-1 leading to impaired angiogenesis [186]. It suggests that these microRNAs have a certain anti-angiogenic potential yet to be fully examined.

Above all, BMPs and their signalling pathways play critical roles in the development, progression, and metastasis of various cancers. Together with their involvement in angiogenesis, promising targets should be fully investigated for their therapeutic potential.

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Conflict of interest

None.

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