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Beyond TGFβ: roles of other TGFβ superfamily members in cancer

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Abstract

Much of the focus on the transforming growth factor-β (TGFβ) superfamily in cancer has revolved around the TGFβ ligands themselves. However, it is now becoming apparent that deregulated signalling by many of the other superfamily members also has crucial roles in both the development of tumours and metastasis. Furthermore, these signalling pathways are emerging as plausible therapeutic targets. Their roles in tumorigenesis frequently reflect their function in embryonic development or in adult tissue homeostasis, and their influence extends beyond the tumours themselves, to the tumour microenvironment and more widely to complications of cancer such as cachexia and bone loss.

The transforming growth factor-β (TGFβ) superfamily comprises the TGFβs, activins, NODAL, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and anti-Mullerian hormone (AMH). Over the past three decades much emphasis has been given to the role of the TGFβs themselves in cancer. In this Review, we focus on the other family members, in particular, the BMPs, activins, NODAL and GDFs (collectively referred to as BANGs). These ligands are well known for their functions in early vertebrate development and in adult tissue maintenance[1–3], but it is now increasingly apparent that they also have crucial roles in both tumour development and dissemination. A recent surge of papers has revealed that many of their roles in cancer represent a redeployment of their roles in early development, or a perturbation of their roles in tissue homeostasis. In cancer, the role of some of the BANGs is to regulate the balance between the self-renewal and the differentiation of cancer stem cells (CSCs), and, in the case of NODAL, also to increase the plasticity of tumour cells. Moreover, the interplay between the BMPs and their antagonists, which is fundamental for the patterning of early embryos, determines the aggressiveness of primary tumours and the ability of disseminated tumour cells to exit from dormancy and establish metastases. After an introduction to the TGFβ superfamily and an outline of their mechanism of signalling, we briefly discuss the regulation of the BANGs and their

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functional roles in early vertebrate development. We then focus on recent work that demonstrates how tumour cells hijack the normally well-controlled functions of these ligands to enable the cells to grow at primary sites, and to disseminate and survive at distant sites. In the final section we discuss the therapeutic opportunities that arise from this emerging knowledge.

Signalling by TGFβ superfamily members

There are more than 30 TGFβ superfamily ligands in the human genome, which can be divided into a number of subfamilies on the basis of sequence similarity and function (TABLE 1). They are found in all metazoans and arose with multicellularity, with the activin and BMP families being the most ancient[4,5]. In all cases, the ligands are synthesized as precursors, with a large prodomain and a carboxy-terminal mature domain, and the mature ligands are cleaved from the precursor by proprotein convertases[6,7]. The ligands form dimers, which can be homomeric or heteromeric and are held together by disulphide bonds.

The mechanism of signalling for all the ligands is fundamentally the same (FIG. 1a). Each ligand requires two types of serine/threonine kinase receptors to signal, a type I and a type II (see TABLE 1 for the known ligand–receptor interactions)[8]. For some ligands, additional co-receptors are required for optimal ligand binding to the type I–type II receptor complex (TABLE 1). In the activated receptor complex the constitutively active type II receptor phosphorylates the type I receptor on several serines and threonines in a highly conserved glycine- and serine-rich domain, close to the membrane-spanning region. This phosphorylation activates the type I receptor kinase and provides a binding site for the downstream substrates, the receptor-regulated SMADs (R-SMADs)[9]. Although the SMADs are not the only molecules that can transduce TGFβ superfamily signals to the nucleus[10], they are by far the best understood, particularly for the BANGs, and thus we focus on this signalling pathway. The traditional view of TGFβ superfamily signalling is that BMPs and GDFs signal through SMAD1, SMAD5 and SMAD8, and that TGFβs, activins and NODAL signal through SMAD2 and SMAD3 (FIG. 1a). However, this view has been revised following the finding that TGFβ induces phosphorylation of SMAD1 and SMAD5 in many cell types in addition to SMAD2 and SMAD3 (REFS 11–14).

Receptor-mediated phosphorylation allows the R-SMADs to form heteromeric complexes with another member of the SMAD family, SMAD4 (REF. 9). SMAD4 thus occupies a central position in the signalling pathways downstream of all of the ligands, being required for many, although not all, responses[15,16]. The R-SMADs also form homomeric complexes, and complexes with other activated R-SMADs[12,17]. The activated SMAD complexes accumulate in the nucleus, where they directly regulate transcription, both positively and negatively. The amino-terminal domains of SMAD4, and of all the R-SMADs except SMAD2, bind DNA directly, but have a fairly low affinity and low specificity. SMAD3 and SMAD4 recognize the sequence AGAC or its reverse complement, and SMAD1, SMAD5 and SMAD8 seem to preferentially bind GC-rich elements with the sequence GRCGNC. The SMAD complexes bind repeats of these sequences or bind in conjunction with other transcription factors[18]. The first identified SMAD-interacting transcription factor was FOXH1 (originally called FAST1), which recruits SMAD2–SMAD4 complexes to DNA[19].

SMAD complexes recruit further chromatin remodelling factors to regulate transcription[18]. In the nucleus, SMAD phosphatases dephosphorylate the R-SMADs, allowing their export to the cytoplasm. Although several candidates have been proposed, the identity of these phosphatases remains controversial[20].

The SMAD pathways were originally thought to be unidirectional and linear, but are actually dynamic networks, as the SMADs shuttle between the cytoplasm and nucleus, in both the absence and the presence of signal (FIG. 1a). In the presence of signal this allows the SMADs to continuously monitor levels of activated receptor, meaning that the level of activated SMADs in the nucleus continuously reflects the levels of activated receptors in the cytoplasm[21]. TGFβ superfamily ligands frequently function as morphogens, with different doses of ligand eliciting different responses (see below). The nucleocytoplasmic shuttling behaviour of the SMADs, as well as the lack of amplification steps, makes these pathways highly suitable for interpreting morphogen gradients[21].

The TGFβ superfamily–SMAD pathways are subject to numerous levels of regulation[21,22]. One of the most important levels of regulation for the BANGs, which is pertinent to this Review, is the interaction of the ligands with extracellular antagonists that prevent, directly or indirectly, their binding to receptors (FIG. 1b). The output of the signalling pathways thus crucially depends not only on ligand levels, but also on the levels and activities of their antagonists. Ligand levels alone are thus rarely good predictors of signalling activity. In addition, TGFβ superfamily pathways are modulated by other signalling pathways. This modulation occurs at a number of different levels. For example, the R-SMADs themselves are phosphorylated by MAPKs, glycogen synthase kinase 3β (GSK3β) and cyclin-dependent kinases (CDKs) at a number of sites in their middle linker region. Linker phosphorylation is thought to affect the stability of the SMADs, and hence the levels of activated nuclear SMADs. As a result, the activity of the SMADs is regulated by growth factors that signal through the MAPK pathways, by stimuli that regulate GSK3β activity and by the cell cycle[23]. TGFβ superfamily signalling responses are also modulated by other signalling pathways at the level of promoters or enhancers of target genes; a recent example being the requirement of WNT and activin signalling for the activation of the mesodermal gene mix paired-like homeobox (*MIXL1*) in human embryonic stem cells (HESCs)[24] (see below).

Furthermore, the TGFβ superfamily pathways themselves are known to antagonize each other, and several mechanisms have been uncovered. This can occur at the level of the ligands themselves — for example, GDF3 directly inhibits BMP signalling[25] — but also at the level of the SMAD complexes. Antagonism of BMP signalling by TGFβ involves inhibitory complexes formed between phosphorylated SMAD3 and SMAD1 or SMAD5 in response to combined TGFβ and BMP signalling[17]. In other situations, limiting amounts of SMAD4 may account for the antagonism observed between activin or NODAL and BMP or GDF signalling in early *Xenopus laevis* embryos[26]. Finally, the inhibitory SMADs, SMAD6 and SMAD7, which are upregulated in response to most TGFβ superfamily members, inhibit TGFβ superfamily signalling by recruiting E3 ubiquitin ligases to the activated type I receptors to induce their degradation[27]. This mechanism may explain the antagonism between NODAL and BMP signalling in mouse embryonic stem cells[28].

Roles of BANGs in development

Studies predominantly in mice, fish and frogs have revealed that the BANGs have crucial roles in early vertebrate development. We discuss them briefly below to set the scene for a discussion of how these activities are redeployed and perturbed during tumorigenesis in adult animals.

The earliest role of NODAL in the mouse embryo is at the blastocyst stage, where it is responsible for maintaining the determinants of pluripotency, such as *Oct4* (also known as *Pou5f1*) and *Nanog*[29]. After implantation, a gradient of NODAL signalling defines the proximal–distal axis, which in turn establishes the anterior–posterior axis of the embryo[30]. Graded NODAL signalling, in conjunction with WNT and BMP signalling, is subsequently essential for mesoderm and endoderm formation and patterning. At later stages, NODAL is required for left–right axis formation and patterning. In all these contexts, spatial NODAL activity is shaped and regulated by the activity of the antagonists LEFTY and cerberus-related proteins[30,31]. During these developmental processes, NODAL signalling is important not only for specifying cell fates, but also for governing cell-sorting behaviour, and inducing epithelial-to-mesenchymal transition (EMT)[30]. Roles for NODAL signalling in pluripotency and differentiation are also evident in HESCs, which express NODAL, GDF1 and GDF3 and the obligate receptors, including the co-receptor CRIPTO (also known as TDGF1)[32]. Low levels of NODAL are required for self-renewal, and higher levels promote differentiation to mesendoderm *in vitro*[33,34], illustrating an important role for signal strength as a determinant of outcome. The mechanism underlying this has recently been illuminated by the finding that high levels of PI3K activity, which are induced by ligands such as heregulin and insulin-like growth factor 1 (IGF1), are required for activin to induce pluripotency in HESCs, whereas low levels of PI3K are required for activin to induce mesendoderm differentiation[24]. This probably also applies to NODAL as it shares the same receptors and downstream signalling pathway. Crucial to this mechanism is the ability of PI3K to regulate ERK and WNT signalling. High levels of PI3K suppress these pathways, and, in the absence of PI3K activity, WNT and ERK signalling are activated and activin- or NODAL-induced phosphorylated SMAD2 or SMAD3 cooperates with β-catenin-TCF transcription factor complexes to specifically induce mesendodermal genes, as well as genes required for EMT.

The BMPs also act in gradients in early embryos to establish the embryonic axes and pattern the tissues across them. Similar to NODAL, this spatial activity is moulded by the activity of secreted ligand antagonists[22]. A good example of this is the ventral-dorsal gradient of BMP activity that is formed before gastrulation in fish and frog embryos, which is dependent on the production of BMP antagonists such as chordin and noggin on the dorsal side[22]. This gradient is required to specify and pattern the mesoderm and ectoderm. At later stages of development, BMPs and the related GDF ligands are required for the formation of many different organs, such as for the regulation of limb, tooth, kidney, skin, muscle, vascular, haematopoietic and neuronal development, in conjunction with other signalling pathways such as WNT, receptor tyrosine kinases, Hedgehog and Notch[35,36]. Consistent with these developmental roles, BMPs and GDFs are essential in the adult for tissue homeostasis, regulating somatic stem cells and controlling differentiation, often in conjunction with the

same signalling pathways with which they interact in development[2] (discussed below). By contrast, NODAL is predominantly expressed in the adult in pathological contexts[37].

Roles of BANGs in the tumour parenchyma

Many tumours are phenotypically heterogeneous with only a subpopulation of cells, the CSCs, capable of initiating and sustaining tumorigenesis[38]. CSCs may originate from the adult somatic stem cell through the perturbation of normal stem cell self-renewal processes, or from more differentiated cells by the reacquisition of stem cell-like characteristics. During tumorigenesis, the normal hierarchical organization of the tissue breaks down owing to the failure of homeostatic processes and varying degrees of differentiation blockade. Furthermore, embryonic programmes specifying stem cell expansion, cellular migration and phenotypic plasticity are often inappropriately reactivated. The central role of BANGs in regulating these processes in embryogenesis and adult homeostasis makes BANG signalling a frequent target for disruption in cancer. The literature suggests particularly important roles for BMPs and NODAL in modulating the tumour parenchyma, whereas the most well-characterized effects of the activins and GDFs target the tumour microenvironment and host organs (discussed below).

Aberrant BMP signalling disrupts stem cell self-renewal and differentiation.

In adult somatic tissues, BMPs have roles at two major levels in the cellular differentiation hierarchy (FIG. 2a). At the apex of this hierarchy, BMPs limit self-renewal of the somatic stem cells, frequently by opposing important regulators of stemness, such as the WNT pathway. Further down the hierarchy, they can specify cell fate and promote differentiation in proliferative progenitor cells. This organizational structure is well-characterized in the intestine (BOX 1), skin and brain[1,2,39–42]. The BMPs are often produced by specialized mesenchyme, and their activity in the stem cell niche is regulated by transient or highly localized expression of antagonists to permit controlled stem cell self-renewal for tissue maintenance and repair. This delicately balanced process can be disrupted in a number of ways in tumorigenesis (FIG. 2b).

In the colon, two familial polyposis syndromes have been genetically linked to aberrant BMP signalling and altered stem cell dynamics. Juvenile polyposis syndrome (JPS) is an inherited condition in which patients develop hamartomatous polyps in the intestine, which are associated with an increased risk of adenocarcinoma. Germline mutations in the type I receptor activin receptor-like kinase 3 (*ALK3*; also known as *BMPR1A*) are seen in 20–25% of JPS cases, with a further 15–20% of cases having mutations in *SMAD4* (REF 43). In the clinically-related hereditary mixed polyposis syndrome (HMPS), the causative mutation is a duplication of an upstream region of the *GREM1* locus that drives ectopic overexpression of the BMP antagonist gremlin 1 throughout the intestinal epithelium and thus disrupts the tightly controlled ligand–antagonist balance[44]. Causality was confirmed in mouse models, in which the overexpression of another BMP antagonist, noggin, or the conditional inactivation of *ALK3*, drove the development of hamartomatous polyps in the intestine[41,45]. Polyposis was associated with stem cell expansion and crypt fission, reflecting a crucial homeostatic role for BMPs in limiting intestinal stem cell self-renewal. In a recurrent theme of BMP–

WNT antagonism[46], the underlying molecular mechanism involves BMP blockade of WNT signalling[41].

In contrast to these familial syndromes, the evidence for BMP involvement in sporadic colorectal cancer (CRC) points to a role for the loss of BMP signalling in the late adenoma-to-carcinoma transition, rather than as an initiating event[1] (BOX 1). Use of nuclear phosphorylated SMAD1, SMAD5 or SMAD8 as a marker showed that BMP signalling is inactivated in ~70% of CRCs at the late adenoma stage or beyond, suggesting that BMPs pose a substantial barrier to tumour progression at this stage[1]. Somatic mutations in *SMAD4* or *BMPR2*, or epigenetic silencing of *BMP2*, contribute to breaching this barrier[1]. As BMPs induce intestinal cell maturation[47] and can oppose inducers of EMT[48], loss of BMP signalling in adenomas may permit the development of a migratory, invasive cell state through impaired lineage-specific differentiation and failure to maintain the epithelial phenotype.

The ability of BMPs to maintain a hierarchically organized tissue architecture also breaks down in brain cancer. In glioblastoma, this process can occur by reversion from an adult to an early embryonic pattern of BMP signalling. Early in development, BMP signalling through ALK3 promotes proliferative expansion of neural stem cells[49]. However, in the later maturation phase, upregulation of ALK6 (also known as BMPR1B) qualitatively alters cellular responses so that BMP signalling blocks the proliferation of neural precursors and instead drives their differentiation[49]. Similar to their role in late embryogenesis, BMPs are also active in the adult brain stem cell niche in the subventricular zone where they limit stem cell self-renewal and promote an astroglial fate[50]. This control mechanism is retained in some glioblastomas[50], but CSCs from nearly 20% of human glioblastomas are rendered unresponsive to the anti-proliferative and differentiation-inducing effects of BMPs by epigenetic silencing of the *ALK6* promoter[51]. In such cases, treatment with BMPs actually expanded the CSC population through the activation of ALK3, suggesting that the glioblastoma cells had reacquired a BMP response pattern that was characteristic of early embryogenesis.

In the skin, endogenous BMP control mechanisms may be overridden by counteracting signals from the tumour-educated stroma. Gremlin 1 is highly expressed in stromal cells of basal cell carcinomas, but not in stromal cells of normal skin, and creates a permissive niche for CSC self-renewal by opposing BMP signalling[52]. Stromal expression of gremlin 1 was also seen in many breast, lung, colon, pancreatic and oesophageal tumours, suggesting broader relevance[52]. However, it is clear that normal tissue homeostasis is maintained by a very delicate balance of BANG ligands, antagonists and receptor subtypes, and that perturbation of this balance may have different outcomes depending on the situation. Thus, although the BMPs and related BANGs may inhibit CSC self-renewal in intestinal, brain, skin, liver and breast cancers[41,51–55], overproduction of BMP2 and BMP4 in response to a naturally occurring oncogenic fusion gene, *CBFA2T3–GLIS2*, drives the expansion of haematopoietic progenitors in acute megakaryoblastic leukaemia[56], and BMP2, BMP4 and BMP6 made by cancer-associated mesenchymal stem cells may cause the expansion of ovarian CSCs[57]. Qualitative aspects (for example, the identity of the type I receptor) and quantitative aspects of BMP signalling, as well as the molecular context for BMP signal

interpretation, can have major effects on the biological outcome and may contribute to these differences.

Reactivated NODAL signalling promotes phenotypic plasticity and stemness in advanced cancers.

NODAL is not normally expressed in adult tissues, with the exception of organs that undergo widespread remodelling, such as the placenta, endometrium and lactating mammary gland[37,58]. However, aggressive tumour cells, which share characteristics with embryonic progenitors in terms of self-renewal and plasticity, have been shown to both secrete and respond to NODAL. This phenomenon was first shown in melanoma cells using blastula stage zebrafish embryos as a biosensor for NODAL[59]. The amount of NODAL secreted by different melanoma cell lines correlated with tumour aggressiveness. These findings have subsequently been extended to prostate, breast and testicular tumours[60–62]. In embryonic development, NODAL requires the co-receptor CRIPTO to signal and, indeed, CRIPTO is also widely overexpressed in tumour cells from many different origins. In breast cancer, CRIPTO expression levels correlate with poor prognosis[63], and in testicular tumours the amount of NODAL and CRIPTO produced was proportional to invasiveness and number of malignant cells[62]. The finding that CRIPTO is co-expressed with NODAL in melanoma cells, pancreatic tumour cells and breast carcinoma cells[64,65], suggests that in tumour cells NODAL signals via its canonical pathway. Further parallels exist between NODAL secretion in development and in cancer as the same signalling pathway (Notch) that is required for NODAL production during the establishment of left–right asymmetry in vertebrate embryos also facilitates NODAL expression in melanoma[66]. In contrast to the situation during development, however, in which NODAL signalling is normally tempered by ligand antagonists such as LEFTY and cerberus-related proteins, NODAL signalling in breast carcinoma or melanoma cells seems to be unopposed, as *LEFTY* is not expressed in these cells[65] (FIG. 3). In melanoma this is explained by methylation of the *LEFTY* gene, which renders it transcriptionally inactive[67].

The major tumour-promoting role of NODAL signalling in melanoma seems to be to drive cells to a less differentiated, more plastic phenotype[59] (FIG. 3). Many aggressive melanomas simultaneously express markers of multiple lineages (mesenchymal, epithelial and endothelial), a feature that is dependent on NODAL signalling and favours functional adaptation of the tumour to hostile growth conditions. In a particularly striking example, NODAL promotes the formation of *de novo* vascular networks by melanoma cells, a process that is termed vascular mimicry, which *in vivo* may contribute to the perfusion of rapidly growing tumours[66,68]. Inhibition of NODAL signalling in melanoma cells reversed this phenotype and inhibited their ability to undergo anchorage-independent growth, to invade extracellular matrix and to form tumours in mice[59]. In breast cancer, NODAL also potentiates tumorigenesis by promoting tumour vascularization through facilitating endothelial cell migration and tube formation[69]. This is not a direct effect of NODAL on the endothelial cells, but is rather due to NODAL-induced upregulation of pro-angiogenic factors such as platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) in the tumour cells[69].

A prominent role of NODAL signalling in the developing mouse embryo and in HESCs is to drive pluripotency and self-renewal (FIG. 3), and a similar role for NODAL has recently been demonstrated in pancreatic cancer, where NODAL is required to drive CSC self-renewal[64]. Pharmacological inhibition of NODAL signalling in the CSCs abolished their self-renewal capacity, and, in combination with a chemotherapeutic agent, gemcitabine, abolished tumorigenicity in a mouse model of pancreatic cancer. The source of NODAL in pancreatic tumours *in vivo* is not only the tumour cells themselves, but also the pancreatic stellate cells that form a niche for the CSCs, promoting their self-renewal and invasiveness[70]. These activities of NODAL and CRIPTO in cancer cells, coupled with the absence of NODAL and only low levels of CRIPTO in normal adult tissue, make the NODAL signalling pathway a very attractive target for tumour therapy (discussed below).

BMPs pose natural barriers to tumour progression and metastasis.

The development of metastases requires the expression of new phenotypes in the tumour cell to facilitate escape from the primary site and to permit adaptation to hostile environments. Recent work suggests that many tumour cells of epithelial origin must acquire sufficient phenotypic plasticity so that they can cycle between a proliferative epithelial state and a motile, invasive but non-proliferative mesenchymal state[71–73]. The plasticity that is necessary for EMT can also enhance stem cell-like properties[74]. BMPs can oppose inducers of EMT, including TGFβ, in many cell types[48,53,75], and may thus present a natural barrier against progression to an invasive state at the primary site (FIG. 2b). Consistent with this idea, BMP signalling in sporadic CRC is frequently inactivated at the late adenoma-to-carcinoma transition when invasion occurs[1], and *SMAD4* loss promotes invasion and metastasis in prostate cancer[76]. However, BMPs can stimulate invasion in some *in vitro* models[77].

Common sites for metastatic dissemination, such as lung and bone, are characterized by particularly high levels of BMP expression[78,79], which can affect the newly arrived tumour cells in a variety of ways (FIG. 2b). As BMPs can induce mesenchymal-to-epithelial transition (MET)[80], it is possible that BMPs may promote reversion to the epithelial state that is a precondition for CSC self-renewal in carcinomas that do not have a constitutive mesenchymal phenotype[73]. However, the high BMP levels then pose an important protective barrier to further steps in the metastatic colonization process. A screen for genes that promote the post-dissemination phase of breast cancer metastasis to the lung identified the BMP antagonist COCO (also known as DAND5 and cerberus 2) as a key player[78]. In the absence of COCO, solitary tumour cells in the lung showed active BMP signalling and failed to establish clinically meaningful metastases, suggesting that locally high BMP levels enforce a state of dormancy. Overexpression of COCO allowed a few dormant tumour cells to overcome this BMP barrier, and to establish metastatic outgrowths. Mechanistically, COCO selectively induced a self-renewing stem cell-like phenotype by reversing the BMP-induced repression of core stem cell transcription factors[78].

As in the lung, BMPs in the bone can also prevent the colonization of disseminated tumour cells. BMP7 secreted by bone marrow stromal cells maintained the dormancy of prostate cancer cells in bone through the induction of a reversible senescent state in prostate CSCs[79].

Although the mechanism for escape from BMP7 was not identified, bone-seeking metastatic breast cancer cells have been shown to overexpress the BMP antagonist noggin, which enhances their tumour-initiating and metastatic activity[81]. Interestingly, BMP7 treatment specifically inhibited the proliferation of prostate cancer cells in the bone, but not in the prostate itself, suggesting that local microenvironmental factors may generate divergent responses at the primary and metastatic sites[75]. BMP7 also reduced the size of the CSC population and suppressed bone metastasis in a breast cancer model[53]. However, other studies have shown a stimulatory effect of BMP2 on breast cancer metastasis to the bone[82], and BMP4 was implicated in a vicious cycle of prostate cancer and bone stromal cell interaction, leading to enhanced osteoblastic metastases[83]. Whether these discrepancies are due to differences in BMP ligands, influences of the microenvironment or genetic wiring patterns of the tumours remains to be clarified.

BANGs sculpt the tumour microenvironment

BANGs not only affect the tumour parenchyma, but they can also fuel an unhealthy dialogue between tumour cells and host cells that fosters the generation of a protumorigenic microenvironment. Tumour cells can interact with mesenchymal cells in the stroma to generate detrimental BMP and antagonist expression patterns[52,57,83], and effects of BANGs on the vascular and immune components in the tumour bed are also emerging. The type I receptor ALK1 has a key role in regulating angiogenesis and vasculogenesis in early embryogenesis, mediating a complex interplay of signals from BMP9, BMP10 and the TGFβs[84]. ALK1 expression is low in adult vasculature, but it increases in neo-angiogenic vessels in wounds and cancer, and may represent an Achilles heel in the angiogenic process[84]. In preclinical models, ALK1-based ligand traps or antibodies significantly decreased the growth and angiogenesis of tumours[85,86], confirming the involvement of ALK1 signalling. NODAL can also increase the tumour blood supply, both through pro-angiogenic effects on endothelial cells[69] and through the promotion of vascular mimicry[87] (FIG. 3).

Aberrantly activated immune cells are a prominent feature of the tumour ecosystem, resulting from subversive effects of the tumour on the immune response. TGFβs are well known as potent immunosuppressive factors, but activins are also emerging as players[88]. Like TGFβ, activin A can drive macrophage differentiation towards a tumour-promoting M2 phenotype, as well as skewing T cell differentiation towards T helper 2 (T_H2) or regulatory T cell (T_{Reg}) fates and thereby suppressing antitumour immune responses[88]. Activin A is overexpressed in human skin cancer, as well as other tumours, and transgenic expression of activin A in mouse skin enhanced tumorigenesis through effects specifically on the local immune response, by increasing the number of T_{Reg} cells and suppressing cytotoxic γδT cells[89]. Similarly, the divergent BANG GDF15 is overexpressed by many tumours and contributes to immune escape in gliomas by suppressing the cytotoxicity of T cells and natural killer cells[90]. Thus, aberrant expression of BANGs that signal through SMAD2 and SMAD3 can powerfully modulate the immune response to promote tumour progression.

Roles for BANGs in complications of cancer

Many tumours show increased expression of BANGs[91–94], and chemotherapy can also increase BANG production[95,96]. It is becoming increasingly clear that this aberrant expression of BANGs can have clinical consequences beyond the tumour and its immediate microenvironment. BANGs are important regulators of normal homeostasis in muscle, bone and the haematopoietic system, and tumour- or treatment-induced increases in BANGs of the activin or GDF subfamilies can adversely affect all these tissues. *In vitro* experiments had generated conflicting views of the possible roles of individual BANGs, particularly in osteogenesis and erythropoiesis[95]. However, use of ligand traps to probe the integrated effects of endogenous BANGs *in vivo* has clarified BANG involvement in these tissues in preclinical models, and has generated leads for therapeutic development (see below).

Cachexia is a wasting syndrome that affects the majority of patients with advanced cancers, and may account for up to one-third of cancer-related deaths[94]. Furthermore, >75% of cancer patients develop anaemia either as a direct result of their cancer, or in response to therapy[95]. A role for activin in cachexia was first suggested by the phenotype of mice lacking the inhibin-α chain (*Inha*-knockout mice), which had increased circulating activin levels and which developed gonadal tumours and cachexia[97]. Subsequently, myostatin (also known as GDF8; originally named for its ability to regulate muscle mass) and GDF15 were also implicated in this process[98,99]. In an exciting recent advance, an activin receptor IIB (ACTRIIB)-based ligand trap that binds activin and myostatin, not only prevented on-going anorexia and muscular wasting in multiple preclinical models of cancer cachexia, but also fully reversed existing skeletal and heart muscle atrophy, leading to significantly prolonged survival[94]. Similarly, in the context of cancer anaemia, an ACTRIIA-based ligand trap reduced the anaemia that was induced by treatment with the chemotherapeutic paclitaxel in preclinical models[95], confirming an important role for an endogenous ACTRIIA ligand, probably activin A, in suppressing erythropoiesis *in vivo.*

Bone loss occurs with particularly high incidence in myeloma, lung, breast and prostate cancers, and causes severe pain and also increases the risk of death[100]. Circulating activin levels are increased in patients with breast and prostate cancer who have bone metastases, as well as in patients with advanced multiple myeloma[95]. ACTRIIA and ALK3 ligand traps increase bone formation and quality in normal mice, suggesting that endogenous BANG ligands of both the activin family and the BMP2 and BMP4 family negatively regulate bone formation by altering the balance of osteoblast and osteoclast activity[101,102]. Extending these observations to the cancer setting, an ACTRIIA ligand trap significantly reduced the osteolytic effect of multiple myeloma in a xenograft model[93]. Antagonism of activins released by tumour-conditioned bone marrow cells also blocks direct tumour-promoting effects[93]; so, BANG antagonism in this context can be of dual benefit to the patient.

Therapeutic implications and advances

From the discussions above, the reductionist view is that BANGs that signal through SMAD2 and SMAD3 (activins, NODAL and myostatin) are frequently overexpressed and have deleterious effects in cancer development, whereas BANG signalling through SMAD1,

SMAD5 and SMAD8 (by BMPs) is frequently inactivated, with consequent loss of tumour suppression. Strategies are under development to reverse both types of aberration (FIG. 4). The promiscuous interrelationship between the BANGs and their receptors creates both opportunities and problems for therapeutic targeting. The highest degree of specificity is achieved by targeting a given ligand with specific antibodies, whereas targeting the receptor kinases with small-molecule antagonists has the broadest effect owing to the high structural relatedness of the ALK kinases[103]. Building on promising preclinical results, there are currently six BANG-targeted therapeutic agents in early phase clinical trials in cancer patients (TABLE 2).

Taking aim at the tumour cell itself, the NODAL-CRIPTO pathway makes a particularly attractive target. NODAL is absent in most normal adult tissues and CRIPTO is only expressed at low levels, but both are frequently reactivated in tumours, creating a viable therapeutic window[104]. BIIB015 (Biogen-Idec), a CRIPTO-specific monoclonal antibody conjugated to a maytansine toxin, was well-tolerated in a Phase I trial, but further development was discontinued owing to company reprioritization. The kinase inhibitor LY2157299 (Eli Lilly) was originally developed and taken into the clinic as an inhibitor of TGFβ signalling[105]. However, LY2157299 also inhibits the activity of ALK4 and ALK7 in addition to the TGFβ-specific ALK5. Thus, some of the observed efficacy may be attributable to inhibition of activin or NODAL signalling. Indeed, given the many tumour-promoting effects of activins and NODAL, the combined inhibition of TGFβ, activin and NODAL pathways has appeal as a therapeutic strategy, providing the side effects are tolerable. Early clinical results are encouraging, as the drug is well-tolerated with indications of efficacy in malignant glioma[105]. Targeting the tumour stroma, an ALK1-specific antibody (PF-03446962; Pfizer) and an ALK1-based ligand trap (ACE-041 (Dalantercept; Acceleron Pharma)) are now in early phase clinical trials as anti-angiogenics in a variety of human solid tumours[95]. ALK1 was implicated as an escape mechanism in acquired resistance to anti-VEGF therapeutics, so combined antagonism of ALK1 and VEGF may ultimately be desirable[86]. Finally, on the basis of compelling preclinical data showing that activins and myostatin have deleterious effects on host tissues, an ACTRIIA-based ligand trap (ACE-011 (Sotatercept; Celgene Corp/Acceleron Pharma)) and a myostatin-specific antibody (LY2495655; Eli Lilly) are in clinical trials to treat cancer-induced cachexia, anaemia and bone loss[95]. Early results from Phase I trials show that both agents are well-tolerated, with evidence for improvement in haemoglobin levels and markers of bone formation–destruction balance (ACE-011), and increases in muscle volume and function (LY2495655).

All the above strategies are based on antagonizing the deleterious effects of BANGs in cancer. However, in contrast to NODAL and activins, many of the actions of BMPs in the carcinogenic process are anti-tumorigenic, and therapeutic strategies are being sought that may restore or enhance these effects. Preclinical data suggest that treatment with BMPs, or strategies to boost the activity of endogenous BMPs, may be effective alone or as adjuncts to chemotherapy. CSCs are intrinsically resistant to therapy[106], but treatment of xenografted brain or colon tumours with BMP2 or BMP4 increased tumour cell differentiation, thereby restoring sensitivity to chemotherapeutics[50,107]. Moreover, reactivation of *ALK6* by knockdown of enhancer of zeste homolog 2 (EZH2) restored tumour suppressive effects of endogenous BMPs in glioblastoma cells[51], suggesting potential for drugs targeting histone

methylation. However, context is crucial, as high BMP4 expression was associated with the induction of EMT and resistance to cisplatin in gastric cancer[108]. Thus, depending on the tumour type, the specific BANG and the molecular context, it may be desirable to either enhance or block BANG activity to achieve maximum therapeutic benefit. Success in this area will depend on improved understanding of the underlying biology and the identification of good predictive biomarkers.

Conclusions

Having been overshadowed for many years by the focus on the TGFβs in human cancer, the BANGs are now emerging as key players in the tumorigenic process and as viable therapeutic targets. Deregulated BANG signalling can influence all stages of tumorigenesis from initiation, to seeding and growth of metastases. Moreover, because of their essential roles in maintaining normal tissue homeostasis, the consequences of perturbing BANG function are not restricted to the tumour cells themselves and the tumour microenvironment, but have more general effects in the body, as demonstrated by the effects of misregulated BANGs in cachexia, a common complication of cancer. General principles concerning the regulation and *in vivo* activity of many of the BANGs have emerged from studies in developmental systems. The discovery that the roles of the BANGs in cancer frequently represent the redeployment of their functions in embryonic development suggests that further insights may come via this route. Similarly, it is clear that BANG signals are propagated and interpreted in the context of a complex interactive network of signalling pathways, and network interactions first identified in embryogenesis are also frequently found to be at work in tumorigenesis.

Many questions are still outstanding. Our knowledge of the role of NODAL signalling in cancer is only rudimentary, and much more remains to be learnt regarding how it becomes expressed in tumours and exactly how it contributes to cancer progression. The BMP antagonists are also emerging as key players in tumorigenesis. Their crucial role in ‘reining in’ BMP activity in the context of normal tissue homeostasis is highlighted by the demonstration that increased expression of *GREM1* underlies the hereditary syndrome HMPS. Clearly, as in embryogenesis, fairly small changes in flux through BANG signalling pathways can have a major effect on biological outcome. Our understanding of how these antagonists are normally so tightly regulated and what determines their specificity for different BANGs is far from complete. Moreover, some of the genetic lesions in pathway components responsible for certain tumours, such as the epigenetic silencing of *ALK6* in glioblastoma, reveal our relative ignorance about how the BANGs signal. From *in vitro* analysis, the type I receptors ALK3 and ALK6 seem redundant, but signalling through these different receptors evidently has completely different consequences in the context of glioblastoma.

The work on BANGs is now at an exciting stage as we start to understand how these ligands contribute to cancer and await the results of clinical trials to discover whether their activity can be manipulated in patients for therapeutic benefit. Much progress is expected in the next few years.

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Glossary

Mesoderm
The middle germ layer of the developing embryo. Gives rise to the musculoskeletal, vascular and urinogenital systems, and to connective tissue (including that of the dermis).

Endoderm
The innermost of the three germ layers of the developing embryo. It differentiates to form the linings of two tubes in the body: the digestive tube, which extends the entire length of the body and the respiratory tube. Buds from the digestive tube form the liver, gall bladder and pancreas.

Cell-sorting behaviour
The process by which a heterogeneous population of cells with different attractive and repellent properties migrate and sort themselves into homogeneous populations.

Epithelial-to-mesenchymal transition (EMT)
Conversion from an epithelial to a mesenchymal phenotype, which is a normal process in embryonic development. In carcinomas, this transformation results in altered cell morphology, the expression of mesenchymal proteins and increased invasiveness.

Mesendoderm
The term given to an embryonic tissue layer that can differentiate into mesoderm and endoderm.

Ectoderm
The outermost of the three germ layers of the developing embryo. It differentiates to form the nervous system, tooth enamel, the epidermis, hair, nails and the lining of mouth, anus, nostrils and sweat glands.

Hamartomatous polyps
Intestinal polyps in patients with juvenile polyposis syndrome. They are characterized by increased crypt formation and cell proliferation but otherwise normal epithelial cell maturation, and are associated with an abnormally expanded mesenchymal component with a pronounced inflammatory infiltrate. Unlike intestinal adenomas, they do not show epithelial dysplasia.

Mesenchymal-to epithelial transition (MET)
The conversion of non-polarized and motile mesenchymal cells into polarized epithelial cells.

Ligand traps

Chimeric proteins that typically contain the ligand-binding domain of a receptor coupled to the Fc domain of an immunoglobulin. This generates an antibody-like ligand antagonist with the ligand specificity and high affinity of the parent receptor, coupled with the *in vivo* stability and distribution characteristics of the parent immunoglobulin.

Osteoblast

A cell responsible for bone formation.

Osteoclast

A cell that breaks down mineralized bone and that is responsible for bone resorption.

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At a glance

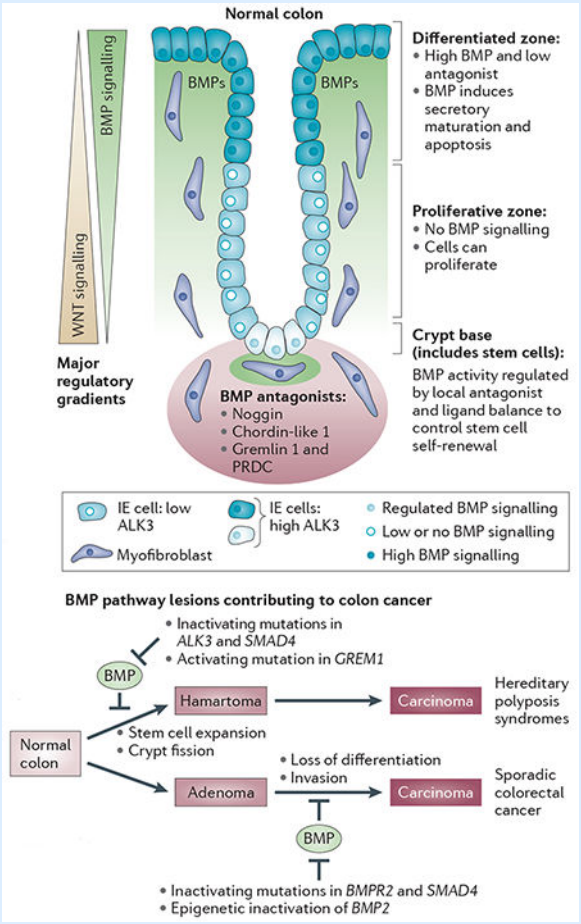
- The bone morphogenetic proteins (BMPs), activins, NODAL, and growth and differentiation factors (GDFs) (collectively referred to as BANGs) have essential roles in early embryonic development and in regulating tissue homeostasis in adults, frequently acting in gradients shaped by the activity of ligand antagonists. Their roles in human cancer frequently constitute a redeployment of their activities in embryonic development or a perturbation of their roles in tissue homeostasis.
- Abberant BMP signalling disrupts stem cell self-renewal and differentiation, and can contribute to tumour formation. This may occur, for example, at the level of genetic or epigenetic changes resulting in the overexpression of BMP antagonists, or the loss of BMP receptors, ligands or SMAD4.
- NODAL is not expressed in most normal adult tissues, but is expressed, along with the co-receptor CRIPTO, in many different tumours. It promotes phenotypic plasticity, which is important for tumour progression, and can positively regulate cancer stem cell self-renewal.
- High BMP signalling provides a natural barrier to tumour progression and metastasis. In the primary tumour, BMP signalling inhibits epithelial-to-mesenchymal transition (EMT), which can prevent tumour invasion. At metastatic sites, high BMP signalling prevents tumour cell colonization by enforcing a dormant state. This can be reversed by tumour-expressed BMP antagonists.
- BANGs sculpt the tumour microenvironment by promoting angiogenesis and suppressing immune responses.
- Increased expression of BANGs in tumours and as a result of chemotherapy can contribute to severe complications of cancer such as cachexia, anaemia and bone loss.
- Strategies are under development to target BANG signalling for therapeutic ends. These include inhibiting NODAL–CRIPTO signalling in the tumour cells, reducing tumour angiogenesis by inhibiting activin receptor-like kinase 1 (ALK1), and inhibiting activin receptors and myostatin to treat cachexia, anaemia and bone loss. Therapies that aim to increase BMP activity are also being developed.

Box 1 |

BMP signalling in the colonic mucosa

Homeostasis in the colon is regulated by opposing gradients of bone morphogenetic protein (BMP) and WNT pathway activation (see the figure; top part). As in embryogenesis, the BMP signalling gradient and localized BMP signalling domains in the colon are partly established by the balance between BMP ligands and their antagonists. BMP4 and other BMPs are expressed in mesenchymal cells of the intravillus and intercrypt regions, as well as in mesenchymal cells adjacent to the intestinal stem cell. BMP signalling is active in the intestinal stem cells in the crypt base and in the differentiating cells of the villus. There is no BMP signalling in the cells of the proliferative zone, which have very low expression of activin receptor-like kinase 3 (ALK3; also known as BMPR1A). Several BMP antagonists are expressed in subepithelial myofibroblasts at the crypt base, where they contribute to the stem cell niche and override BMP signalling in a regulated manner to permit WNT-driven stem cell self-renewal. BMP signalling in the differentiating zone of the villus is required for proper maturation of the secretory cells and apoptosis of mature colonic cells. There are also important interactions with the Notch and PI3K pathways that are not discussed here. Various lesions in the BMP pathway can contribute to colon cancer (see the figure; lower part). Germline mutations in BMP pathway components serve as initiating lesions that lead to increased stem cell expansion in hereditary polyposis syndromes that progress to carcinoma via a hamartomatous route. In sporadic colorectal cancer, the BMP pathway is typically compromised later in the disease process by somatic mutations or epigenetic silencing events, and the transition from *in situ* adenoma to invasive carcinoma is promoted.

BMPR, BMP receptor; *GREM1*, gremlin 1; IE cell, intestinal epithelial cell. Figure is modified, with permission, from REF. 42 © (2007) Proc. Natl Acad. Sci. USA.



DATABASES

CtinicalTrials.gov: http://www.clinicaltrials.gov NCT00557856 | NCT00674947 | NCT00747123 | NCT00996957 | NCT01190644 | NCT01220271 | NCT01246986 | NCT01373164 | NCT01486368 | NCT01505530 | NCT01524224 | NCT01562405 | NCT01582269 | NCT01642082 | NCT01682187 | NCT01712308 | NCT01720173

FURTHER INFORMATION

Lalage M. Wakefield's homepage: http://ccr.cancer.gov/staff/staff.asp?profileid=13665

Caroline S. Hill's homepage: http://www.london-research-institute.org.uk/research/caroline-hill

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

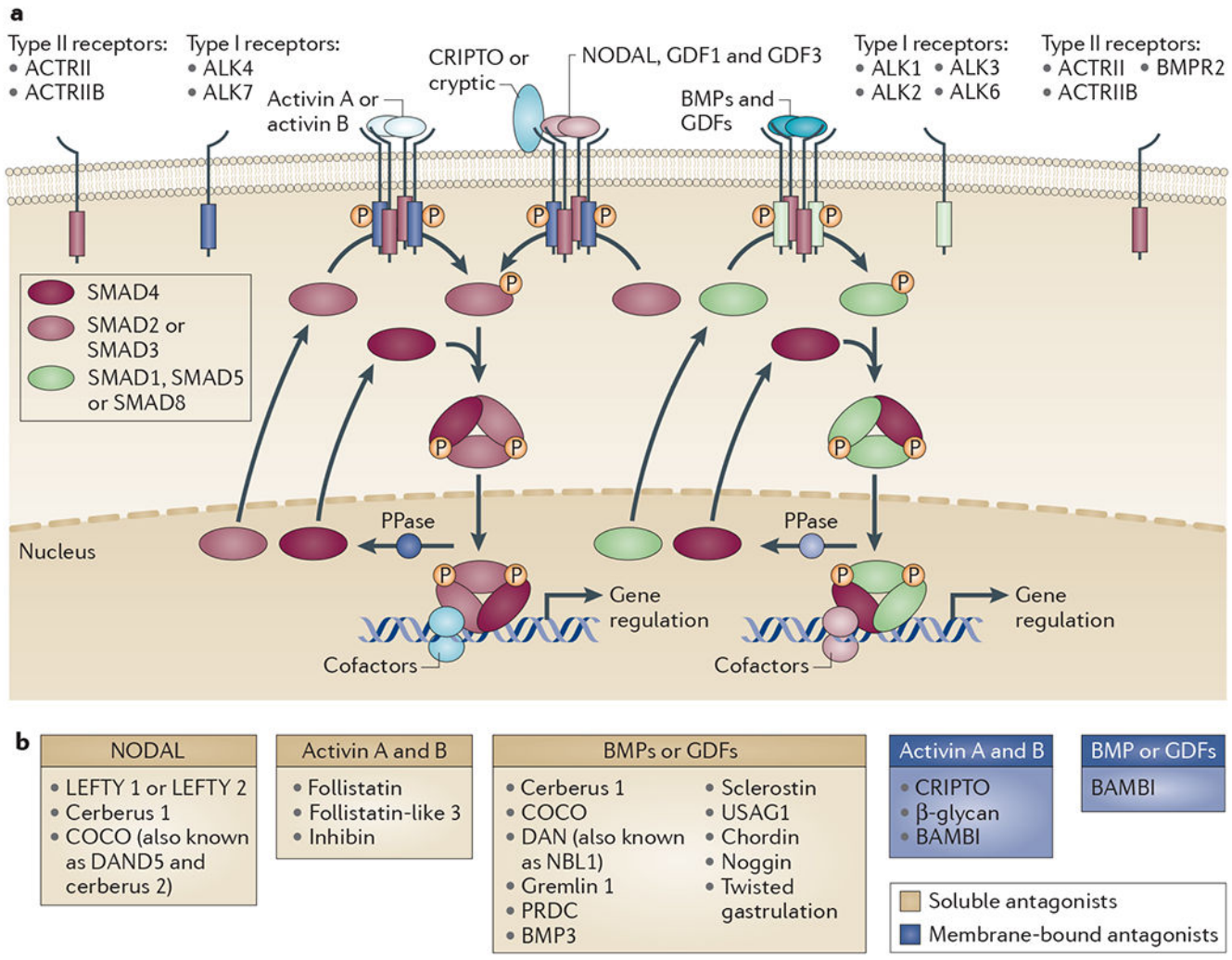


Figure 1 |. Signalling downstream of TGFβ superfamily ligands.

a | The core signalling pathway through the SMADs is shown for the bone morphogenetic proteins (BMPs), activins, NODAL, and growth and differentiation factors (GDFs) (BANGs). The pathways downstream of the transforming growth factor-βs (TGFβs) are not shown. Specific ligands bring together different combinations of type I and type II receptors, as indicated. In humans there are a total of seven type I receptors, known as activin receptor-like kinases (ALK1–7), and five type II receptors, with individual ligands binding different combinations (TABLE 1). Six type I receptors and three type II receptors mediate BANG signalling. The receptors all have a cysteine-rich extracellular domain, a single-pass transmembrane domain and an intracellular kinase domain. NODAL, GDF1 and GDF3 signalling also requires the co-receptors CRIPTO or cryptic, which are members of the EGF–CFC family (named after their epidermal growth factor (EGF)-like motif, and a novel cysteine-rich domain with the founding members CRIPTO, FRL1 and cryptic)[21,32]. The type I receptors dictate which receptor-regulated SMADs (R-SMADs) are phosphorylated (P) in response to which ligand. ALK1, ALK2, ALK3 and ALK6 phosphorylate SMAD1, SMAD5 and SMAD8, whereas ALK4, ALK5 and ALK7 phosphorylate SMAD2 and SMAD3 (REF.

9).The receptor-mediated phosphorylation of the R-SMADs occurs at their extreme carboxyl termini on two serines in an S-M-S or S-V-S motif. R-SMAD phosphorylation promotes complex formation with SMAD4 and subseguent accumulation in the nucleus. **b** | TGFβ superfamily ligand antagonists CRIPTO and β-glycan, which are co-receptors for NODAL, GDF1 and GDF3, and TGFβ, respectively (TABLE 1), act as inhibitors of activin signalling[109]. BAMBI, BMP and activin membrane-bound inhibitor; PPase, phosphatase; PRDC, protein related to DAN and cerberus (also known as gremlin 2); USAG1, uterine sensitization-associated gene 1 protein (also known as SOSTDC1).

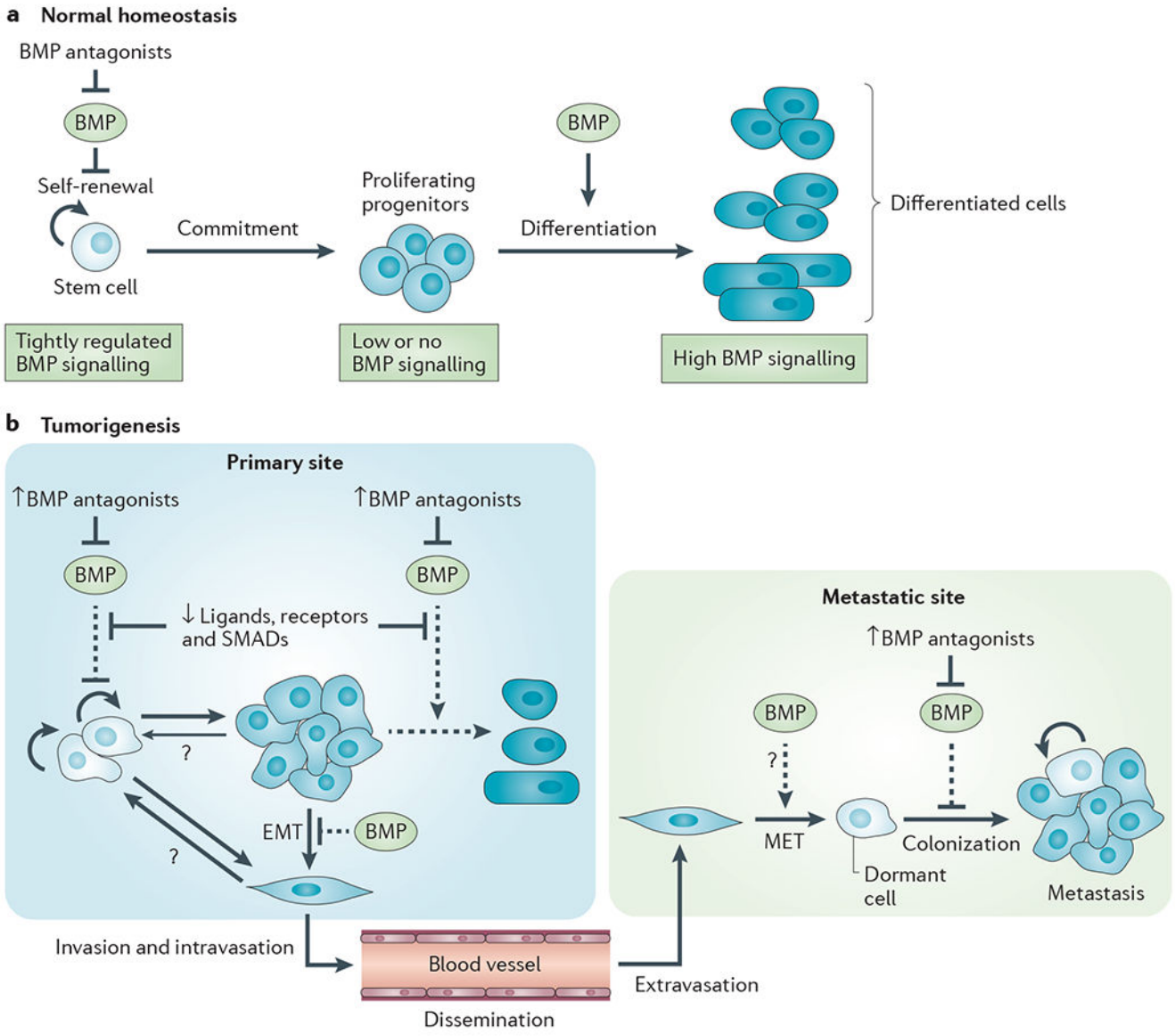


Figure 2 |. Roles for BMPs in normal tissue homeostasis and tumorigenesis.

a | Simplified schematic showing roles of bone morphogenetic proteins (BMPs) in normal adult homeostasis in organs such as the intestine, brain and skin. **b** | Schematic for aberrant BMP signalling in epithelial tumorigenesis. At the primary tumour site, impaired BMP signalling interacts with other oncogenic lesions to promote tumorigenesis. BMP antagonists are frequently overexpressed either by the tumour cells or by the tumour-educated stroma. The BMP ligands, receptors and downstream signalling components can be disabled through genetic or epigenetic targeting, or by aberrant regulation of expression in the dysfunctional tumour environment. As a result of compromised BMP signalling, stem cell self-renewal pathways are hyperactivated, and cellular maturation and differentiation are blocked or incomplete. Furthermore, some tumour cells may respond to oncogenic cues by undergoing an epithelial-to-mesenchymal transition (EMT), leading to increased cell motility, invasiveness and an increased probability of acquiring stem cell-like characteristics. When

the activity of the BMP pathway is compromised, one of the natural barriers to EMT is eliminated. Tumour cells can then leave the primary tumour site and disseminate through the circulation to distant organs. Some commonly colonized sites such as the bone and lung express naturally high levels of endogenous BMPs. The local BMPs may promote a mesenchymal-to-epithelial transition (MET) in the newly arrived tumour cell. However, they also maintain the disseminated tumour cells in a dormant state, a barrier to successful metastasis that can be overcome in tumour cells expressing high levels of BMP antagonists. Dashed arrows represent normal functions of BMPs that are compromised in tumorigenesis.

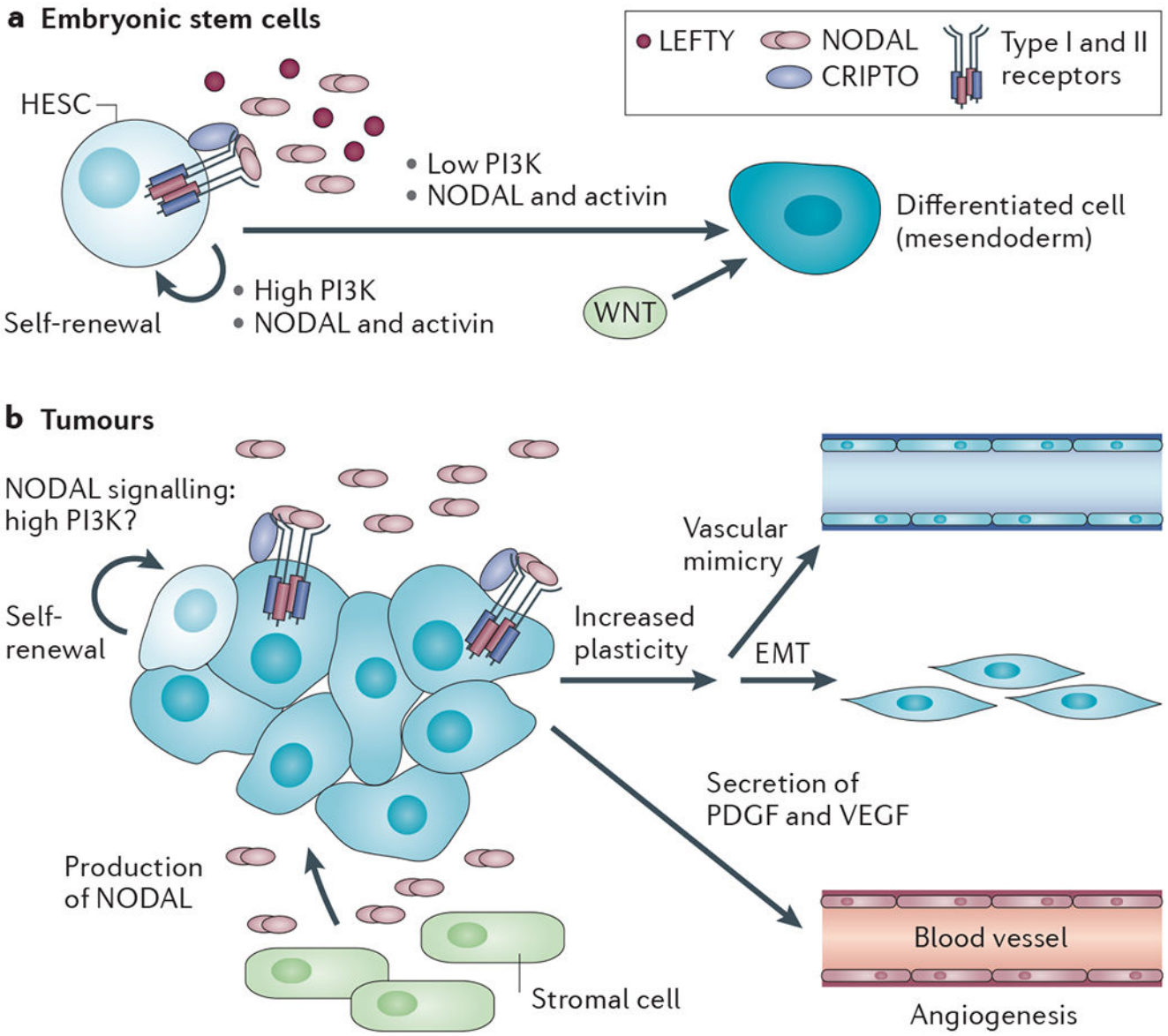


Figure 3 |. Role of NODAL signalling in HESCs and in cancer.

a | Human embryonic stem cells (HESCs) express NODAL (and the related ligands growth and differentiation factor 1 (GDF1) and GDF3), in addition to the relevant type I and type II receptors and CRIPTO, and are thus competent to signal. They also express antagonists such as LEFTY Activin and NODAL signalling induces self-renewal in HESCs in cooperation with high PI3K signalling, but induces differentiation to mesendoderm when PI3K signalling is low or absent, when it cooperates with WNT signalling. **b** | In many different types of cancer, NODAL is produced both by tumour cells and by stromal cells, such as pancreatic stellate cells. Tumour cells also express CRIPTO, but not LEFTY. NODAL signalling is important for self-renewal of cancer stem cells (CSCs) and this may be influenced by high PI3K signalling in tumours as a result of, for example, high epidermal growth factor (EGF) signalling, mutations in *PTEN* or mutations in *PIK3CA* (which encodes

the PI3K p110α subunit) itself[110]. NODAL promotes plasticity of tumour cells — for example, inducing epithelial-to-mesenchymal transit ion (EMT) — or development of a vascular network in the case of aggressive melanoma. NODAL also promotes the secretion of the pro-angiogenic factors platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF), which act on endothelial cells to promote angiogenesis.

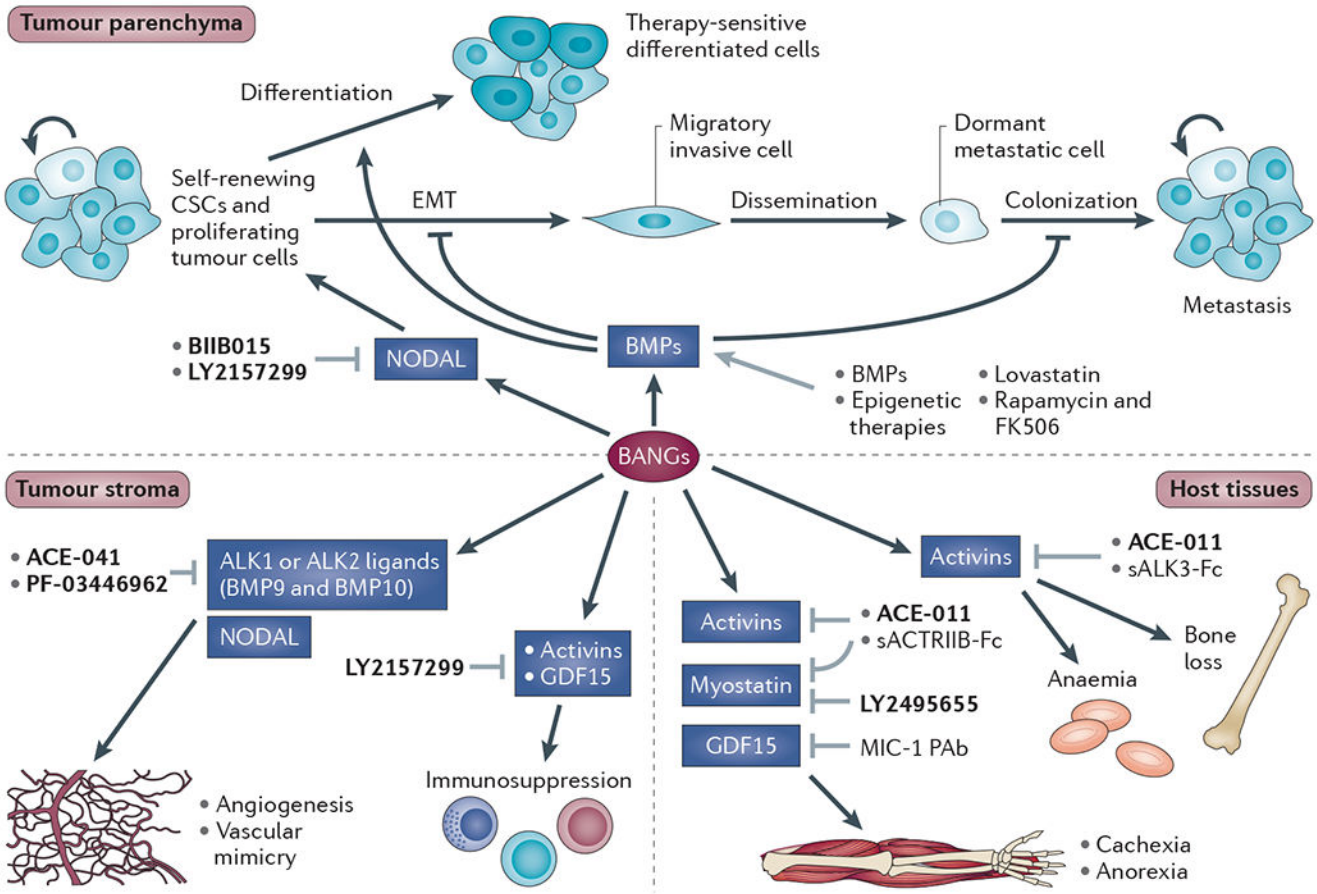


Figure 4 |. Therapeutic approaches to targeting the BANGs in cancer.

Dysregulation of bone morphogenetic proteins (BMPs), activins, NODAL, and growth and differentiation factors (GDFs) (BANGs) can have deleterious effects on the tumour parenchyma, the tumour stroma and on host tissues that are not directly involved in the tumorigenic process. Broadly, most therapeutic strategies to date have been aimed at either enhancing BMP activity or antagonizing BANGs of the activin and NODAL superfamily. For more details of therapeutic agents under clinical development (shown in bold) see TABLE 2. The other therapeutic agents shown are still at the preclinical development stage. Of these, the BMP ligands (some of which are already US Food and Drug Administration (FDA)-approved for fracture healing and lumbar fusion) have been used to induce cancer stem cell (CSC) differentiation and to restore response to chemotherapeutics[50,107]. Genetic knockdown of enhancer of zeste homolog 2 (EZH2), a component of the Polycomb repressor complex that is highly expressed in many tumours, restored tumour suppressive BMP responses in glioma cells, suggesting promise for epigenetic therapies that can reverse gene silencing[51]. Interestingly, some drugs developed in other contexts can activate BMP signalling. Lovastatin, a cholesterol-lowering agent, restored response to 5-fluorouracil by reactivating epigenetically silenced BMP2 in colorectal and gastric cancers[111], and the immunosuppressive agents FK506 and rapamycin can also activate BMP signalling[112,113]. Soluble activin receptor-like kinase 3-Fc (sALK3-Fc) and soluble activin receptor IIB-Fc (sACTRIIB-Fc) are ligand traps that have shown therapeutic promise as BANG antagonists

in preclinical studies but that have not been taken into the clinic[94,101]. The grey arrows indicate interventions that may affect BANG signalling in cancer and cancer-associated processes. MIC-1 PAb, polyclonal antibody to GDF15.

Table 1 |

Ligand-receptor usage in TGFβ superfamily signalling*

Ligand	Type I receptor	Type II receptor	Co-receptors
Inhibin-α	No type I receptor	ACTRII	ND
Activin-βA	ALK4	ACTRII and ACTRIIB	ND
Activin-βB	ALK4 and ALK7	ACTRII and ACTRIIB	ND
Activin-βE	Unknown receptor	Unknown receptor	ND
Activin-βC	Unknown receptor	Unknown receptor	ND
GDF1	ALK4 and ALK7	ACTRII and ACTRIIB	CRIPTO and cryptic
GDF3	ALK4 and ALK7	ACTRII and ACTRIIB	CRIPTO and cryptic
NODAL	ALK4 and ALK7	ACTRII and ACTRIIB	CRIPTO and cryptic
BMP3	No type I receptor	ACTRIIB	ND
BMP3B (also known as GDF10)	ALK4	ACTRII	ND
GDF11	ALK4 and ALK5	ACTRII and ACTRIIB	ND
Myostatin (also known as GDF8)	ALK4and ALK5	ACTRIIB	ND
GDF9	ALK4	BMPR2	ND
TGFβ1	ALK1≠ and ALK5	TGFBR2	β-glycan and endoglin
TGFβ2	ALK1 and ALK5	TGFBR2	β-glycan and endoglin
TGFβ3	ALK1 and ALK5	TGFBR2	β-glycan and endoglin
GDF15	Unknown	TGFBR2	ND
BMP9	ALK1	ACTRII and BMPR2	ND
BMP10	ALK1	ACTRII and BMPR2	ND
BMP2	ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
BMP4	ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
GDF5 (also known as BMP14)	ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
GDF6	ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
GDF7	ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
BMP5	ALK2, ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
BMP6	ALK2, ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND

Ligand	Type I receptor	Type II receptor	Co-receptors
BMP7	ALK2, ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
BMP8	ALK2, ALK3 and ALK6	ACTRII, ACTRIIB and BMPR2	ND
BMP15	ALK6	BMPR2	ND
AMH	ALK2 and ALK3	AMHR2	ND

ACTR, activin receptor; ALK, activin receptor-like kinase; AMH, anti-Müllerian hormone; AMHR, AMH receptor; BMP, bone morphogenetic protein; BMPR, BMP receptor; GDF, growth and differentiation factor; ND, not determined; TGFβ, transforming growth factor-β; TGFBR, TGFβ receptor.

*The ligands are arranged according to receptor usage. Where multiple type I, type II and co-receptors are listed for a given ligand, multiple permutations are possible.

≠ALK1 is endothelial specific.

Table 2 |

BANG-targeted therapeutics under clinical development for cancer treatment

Agent (Company)	Molecular target	Agent type	Biology targeted	Stage of development	Indication or patient population	Clinical trial identifier and status
BIIB015 (Biogen Idec)	CRIPTO	Antibody with 'toxic payload': humanized CRIPTO-specific monoclonal (IgG1) conjugated to maytansinoid derivative DM4	Tumour targeting	Phase I	Relapsed or refractory solid tumours	NCT00674947 (completed; further development discontinued)
LY2157299 (Eli Lilly)	ALK4, ALK5 and ALK7	Small-molecule kinase inhibitor	Tumour targeting	Phase I	Advanced or metastatic cancer	NCT01682187 (complete)
			Tumour targeting	Phase IB/IIA	Malignant glioma in combination with radiochemotherapy	NCT01220271 (recruiting)
			Tumour targeting	Phase IB/IIA	Metastatic cancer and advanced pancreatic cancer	NCT01373164 (recruiting)
			Tumour targeting	Phase II	Hepatocellular carcinoma	NCT01246986 (recruiting)
			Tumour targeting	Phase II	Recurrent glioblastoma	NCT01582269 (recruiting)
PF-03446962 (Pfizer)	ALK1	Fully human ALK1-specific monoclonal antibody (IgG2)	Angiogenesis	Phase I	Advanced solid tumours	NCT00557856 (recruiting)
			Angiogenesis	Phase II	Malignant pleural mesothelioma and previous cytotoxic chemotherapy	NCT01486368 (recruiting)
ACE-041 (Dalantercept; Acceleron Pharma)	ALK1 ligands (BMP9 and BMP10)	Ligand trap: human ALK1-Fc fusion (IgG1)	Angiogenesis	Phase I	Advanced solid tumours or refractory multiple myeloma	NCT00996957 (ongoing but not recruiting)
			Angiogenesis	Phase II	Recurrent or persistent endometrial cancer	NCT01642082 (recruiting)
			Angiogenesis	Phase II	Ovarian, fallopian tube or primary peritoneal cancer	NCT01720173 (planned)
ACE-011 (Sotatercept; Celgene Corp/Acceleron Pharma)	ACTRIIA ligands (activins, BMP10, myostatin and GDF11)	Ligand trap: human ACTRIIA-Fc (IgG1)	Bone loss	Phase IIA	Patients with multiple myeloma who have osteolytic lesions receiving concomitant MPT	NCT00747123 (completed)
			Tumour targeting, anaemia and bone loss	Phase I	Relapsed or refractory multiple myeloma treated with dexamethasone and lenalidomide	NCT01562405 (temporarily suspended pending amendment)
			Anaemia	Phase II	Anaemia in solid tumours	NCT01190644 (recruiting)
			Myelofibrosis and anaemia	Phase II	Myelofibrosis and anaemia in myeloproliferative neoplasms	NCT01712308 (planned)

Agent (Company)	Molecular target	Agent type	Biology targeted	Stage of development	Indication or patient population	Clinical trial identifier and status
LY2495655 (Eli Lilly)	Myostatin	Fully humanized myostatin-specific antibody	Cancer-related cachexia	Phase I	Advanced cancer	NCT01524224 (ongoing but not recruiting)
			Cancer-related cachexia	Phase II	Patients with locally advanced or inoperable metastatic pancreatic cancer receiving chemotherapy (gemcitabine)	NCT01505530 (recruiting)

ACTRIIA, activin receptor IIA; ALK, activin receptor-like kinase; BANG, BMPs, activins, NODAL, and GDFs; BMP, bone morphogenetic protein; GDF, growth and differentiation factor; igG, immunoglobulin G; MPT, melphalan, prednisolone and thalidomide.