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Invited Mini Review

BMPs and their clinical potentials

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Bone morphogenetic protein (BMP) signaling in diseases is the subject of an overwhelming array of studies. BMPs are excellent targets for treatment of various clinical disorders. Several BMPs have already been shown to be clinically beneficial in the treatment of a variety of conditions, including BMP-2 and BMP-7 that have been approved for clinical application in nonunion bone fractures and spinal fusions. With the use of BMPs increasingly accepted in spinal fusion surgeries, other therapeutic approaches targeting BMP signaling are emerging beyond applications to skeletal disorders. These approaches can further utilize next-generation therapeutic tools such as engineered BMPs and ex *vivo*-conditioned cell therapies. In this review, we focused to provide insights into such clinical potentials of BMPs in metabolic and vascular diseases, and in cancer. [BMB reports 2011; 44(10): 619-634]

INTRODUCTION

Bone Morphogenetic Proteins (BMPs) represent the largest subset within the Transforming Growth Factor (TGF)-β superfamily. They are now known to be involved in such a wide variety of processes that several investigators even suggested to change their name from 'Bone' to 'Body' Morphogenetic Proteins (1, 2). Although they share some fundamental similarities with other members of the TGF-β superfamily, the pleomorphic functions of BMPs led to their signaling functions being regulated at such complex levels that far exceed those imposed on the other members of the TGF-β superfamily (Fig. 1). One hallmark feature of BMPs is that there is a high degree of promiscuity in the interaction of ligands with their receptors and regulators that are also shared with other members of the TGF-β superfamily (Table 1, 2). Hence, the final outcome of BMP signal transduction is highly dependent on spatiotemporal circumstances in addition to the endocrine properties of those that are secreted into circulation. This requires careful examinations of potentially-overlapping signaling pathways to understand molecular mechanisms of their *in vivo* biological activity, thus to evaluate their potential clinical benefits. Nonetheless, the importance of BMP signals in pathophysiology cannot be overstated because of the multitude of dysregulated BMP signaling in numerous pathological processes. Clinically, BMP family members have been associated with a number of pathologies, including obesity, diabetes, various vascular diseases as well as cancer and its related comorbidities. This review will discuss selected clinical targets of the BMP signaling whose therapeutic potential have been substantiated through both *in vitro* and *in vivo* studies.

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METABOLIC DISEASES

Obesity

The last decade has witnessed an ever-growing surge in the obesity pandemic, making obesity one of the most serious predicaments among the metabolic diseases. Rates of obesity in developed countries such as the United States exceed 33% of the population primarily caused by changes in behavior and lifestyle. Moreover, the trend toward global obesity creates a substantial increase in incidences of various metabolic syndromes, such as type 2 diabetes mellitus, liver steatosis and cirrhosis, hypertension, coronary heart disease, as well as neurodegenerative Alzheimer's disease and even some cancers (3-7). Treatment of obesity-related morbidities has imposed a huge economic burden on societies, with direct annual cost estimates for medical spending due to obesity in the United States to date being up to $147 billion for adults and $14.3 billion for children (8). Current treatment of human obesity is limited to behavioral adjustments, control of satiety, induced mal-absorption in the intestine and a variety of surgical procedures with limited efficacy, undesirable side effects, and unknown long-term consequences (9). For instance, almost all current anti-obesity drugs target the brain as appetite suppressants with a variety of adverse side effects (10, 11). Recently, BMP signaling has emerged as a promising new approach to the problem, targeting obesity from the roots. The effects of BMP signaling on adipogenesis suggest treatments that target the adipocyte-differentiation process itself and metabolic activities that favor energy expenditure.

Adipose tissue is a central player in systemic energy metabolism that exists in two forms with both shared and unique char-

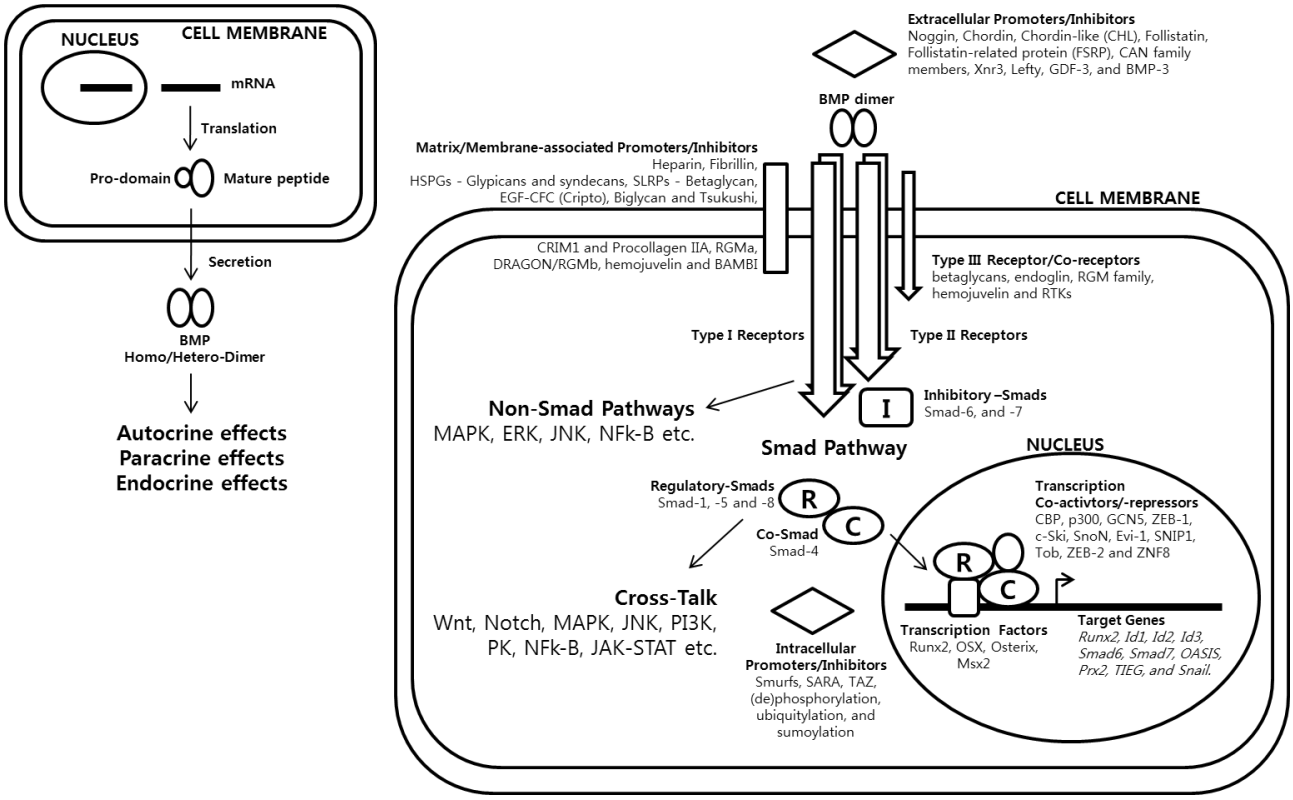

Fig. 1. BMP synthesis to signaling.

Table 1. Human BMPs

Ligand	Alternate names	Gene locus	Known receptors		
			Type I receptors	Type II receptors	Type III receptors
BMP-1	BMP-1 is a metalloproteinase, not a formal member of the TGF-β superfamily.				
BMP-2	BMP-2A, XBMP2, xBMP-2, MGC114605	20p12	ALK-2, ALK-3, ALK-6	BMPR-II; ActR-IIA, ActR-IIB	TGF-βR-III; Endoglin
BMP-3	Osteogenin, BMP-3A	14p21.21	ALK-4	ActR-IIA, ActR-IIB	
BMP-4	BMP-2B, BMP2B1, ZYME, OFC11, MCOPS6	14q22-q23	ALK-2, ALK-3, ALK-5, ALK-6	BMPR-II; ActR-IIA	TGF-βR-III
BMP-5	MGC34244	6p12.1	ALK-3		
BMP-6	Vgr1, DVR-6	6p24-p23	ALK-2, ALK-3, ALK-6	BMPR-II; ActR-IIA, ActR-IIB	
BMP-7	OP-1	20q13	ALK-2, ALK-3, ALK-6	BMPR-II; ActR-IIA, ActR-IIB	
BMP-8A	OP-2, FLJ14351, FLJ45264	1p34.3	ALK-2; ALK-3; ALK-4; ALK-7	BMPR-II; AMHR-II;	
BMP-8B	OP-3, PC-8, MGC131757	1p35-p32	ALK-3; ALK-6	BMPR-II; AMHR-II	
BMP-9	GDF-2	10q11.22	ALK-1, ALK-2	BMPR-II; ActR-IIA, ActR-IIB	Endoglin
BMP-10	MGC126783	2p13.3	ALK-1, ALK-3, ALK-6	BMPR-II; ActR-IIA, ActR-IIB	
BMP-11	GDF-11	12q13.2	ALK-3, ALK-4, ALK-5, ALK-7	ActR-IIA, ActR-IIB	
BMP-12	GDF-7, CDMP-3	2p24.1	ALK-3, ALK-6	BMPR-II;ActR-IIA, ActR-IIB	
BMP-13	GDF-6, CDMP-2, KFS, KFSL, SGM1, MGC158100, MGC158101	8q22.1	ALK-3, ALK-6	BMPR-II; ActR-IIA	
BMP-14	GDF-5, CDMP-1, OS5, LAP4, SYNS2, MP52	20q11.2	ALK-3, ALK-6	BMPR-II; ActR-IIA, ActR-IIB BMPR-II; ActR-IIA	TGF-βR-III
BMP-15	GDF-9B, ODG2, POF4	Xp11.2	ALK-6		

Table 2. Receptors that BMPs share with other TGF-β superfamily members

Type I receptors (also termed Activin receptor-like kinases, ALK)	Ligands
ALK-1	BMPs
ALK-2 or ActR-IA	BMPs, Activin
ALK-3 or BMPR-IA	BMPs
ALK-4 or ActR-IB	TGF-β, Activin, BMPs
ALK-5 or TβR-I	TGF-β, Activin, BMPs
ALK-6 or BMPR-IB	BMPs, AMH
ALK-7 or ActIR-IC	Activin, BMPs
Type II receptors	**Ligands**
Activin type IIA (ActR-IIA)	Activin, BMPs
Activin type IIB (ActR-IIB)	Activin, BMPs
TGF-β type II receptor (TβR-II)	TGF-β
BMP type II receptor (BMPR-II)	BMPs
BMP type II receptor (shorter form)	BMPs
Anti-Mullerian hormone type II receptor (AMHR-II)	AMH, BMPs

acteristics; white adipose tissue (WAT) serves as an energy reservoir, whereas the brown adipose tissue (BAT) mainly functions in energy expenditure. Both types of adipocytes developmentally arise from mesenchymal stem cells (MSCs), which derive from embryonic stem cells. BMP-2 and BMP-4 promote the differentiation of WAT, whereas BMP-7 directs brown adipogenesis. Obesity is characterized by increase in WAT accumulation in the intra-abdominal visceral area (12). Increased WAT may result from increase in adipocyte size (termed hypertrophy) and/or increase in adipocyte number (termed hyperplasia) (13, 14). The hyperplasia that causes obesity results from increased number or rate of MSCs committing to the adipogenic lineage that subsequently proliferate as preadipocytes and eventually differentiate into mature adipocytes (15). Several lines of evidence indicate that commitment of MSCs into the adipogenic lineage can be strongly influenced by BMP signaling (16, 17). Elevated expression of BMP-4 has been observed precisely during the proliferation to commitment process, in addition to increased level of phosphorylated Smad 1, 5 and 8 following the same pattern (18). Exogenous BMP-4 treatment of embryonic or mesenchymal stem cells during the early proliferative stage promotes differentiation into mature adipocytes (16, 17, 19, 20). Subcutaneous injection of BMP-4-treated pluripotent stem cells into athymic mice even results in the development of adipose tissue that is indistinguishable from endogenous WAT, whereas non-treated cells do not (16). Furthermore, BMP-4 has also been shown to specifically induce white adipogenesis while suppressing brown adipogenesis, as marked by inhibited expression of a BAT marker UCP1 (17). Another BMP ligand that has been shown to promote adipogenesis is BMP-2, despite contradicting results that seem to vary on cell type, dosage and spacitotemporal differences in treatment. Similar to BMP-4, BMP-2 has also been shown to stimulate commitment of progenitors into the white adipogenic lineage, in addition to promoting terminal adipogenic differentiation (21, 22). This can be explained, at least in part, by the ability of BMP-2 to upregulate expression of a major adipogenic marker, PPAR-γ, through activation of Smad1 and subsequent cooperation with Schnurri-2 (23, 24). Accordingly, Shn-2 knockout mice (with consequentially reduced BMP-2 signaling) display reduced WAT mass, suggesting that BMP-2 utilizes the Smad pathway to regulate adipogenesis *in vivo* (24).

Apart from these BMPs, BMP type I receptor (BMPR-IA), ALK-3, has been associated with obesity by favoring differentiation into adipocytes rather than osteoblasts *in vitro* (25). ALK-3 is known to exert a much higher binding affinity to BMP-2 and BMP-4 than to BMP-7 (26), implying different roles in response to BMP-2 and BMP-4 in contrast to BMP-7. Conditional knockout mice with adipocyte-specific deletion of ALK-3 display significant reduction in body weight as well as a trend toward reduced fat mass on both normal and high fat diets (27). In humans, overweight and obese adults display higher mRNA levels of ALK-3 in visceral and subcutaneous adipose tissues, which also correlate with several parameters used to determine health, including percentage body fat as well as fasting plasma glucose and insulin levels (28). In addition, three single nucleotide polymorphisms within the ALK-3 gene were identified as obesity risk alleles, demonstrated by an association with increased body mass index (BMI) in two independent cohorts, as well as correlating with increased adipose ALK-3 mRNA levels (28).

Conversely, role of BMP antagonists in adipocyte differentiation has also been recognized. Treatment of Noggin decreases cytoplasmic triglyceride accumulation and expression of adipogenic marker genes, confirming definitive role of BMPs in adipocyte differentiation (18). In addition, expression of FSTL1 (a Follistatin-like BMP antagonist that binds and inhibits formation of BMP ligand-receptor complexes), which occurs at high levels in undifferentiated primary preadipocytes, declines rapidly during adipocyte differentiation (29). Genetic (ob/ob) and diet-induced obesity mouse models exhibit an increase in subcutaneous and decrease in visceral adipose expression of FSTL3 compared to regular diet controls (30). GDF-3, another well-known antagonist of BMP signaling, also possesses inhibitory effects in adipocyte differentiation. However, these observations should not be defined merely to modulations of BMP signaling, but rather to changes in the systematic interplay between other members of the TGF-beta superfamily, such as Activins and Myostatins.

Finally, the recent discovery of BMP-7 in promoting BAT differentiation and thermogenesis offers a new clinical potential. BMP-7 triggers commitment of MSCs to a brown adipocyte lineage (31, 32), and subcutaneous injection of these cells into athymic mice results in development of adipose tissue containing mostly BAT. BMP-7 knockout embryos display an almost

complete absence of UCP1 and a marked 50-70% decrease in interscapular BAT mass compared with wild-type littermates, whereas the size of WAT and other internal organs, such as the liver, were not altered (17). Tail vein injection of adenovirus expressing BMP-7 increases BAT, without affecting the mass of WAT. Increase in BAT mass results in increased energy expenditure, higher basal body temperature and decreased body weight (17). Thus, BMP-7 promotes phenotypic characteristics and functional activity of mature brown adipocytes, which burns energy and assists in suppressing obesity. In fact, several independent teams using PET-CT (positron emission tomography-computed tomography) have revealed recently that substantial amounts of BAT is also present in humans in the cervical-supraclavicular area (neck and shoulder), particularly in young and lean female subjects (33-37).

Diabetes

In addition to regulating adipogenesis and energy metabolism, BMP signaling has been shown to be involved in glucose homeostasis. More than 5% of the US population is diagnosed with diabetes from having impaired insulin function (38). Type II diabetes is caused by defects in the secretion of insulin and resistance to the action of insulin, which accounts for $>90\%$ of diabetic cases, as opposed to type I diabetes, which is a rarer autoimmune disease that destroys pancreatic β-cells resulting in absolute requirement for exogenous insulin treatment (39, 40). Current therapies for type II diabetes improve insulin action at the level of the liver (metformin) and peripheral tissues (thiazolidinediones), or enhance insulin secretion (sulfonylureas) (41, 42). Discovery of BMP-9 as a first hepatic factor shown to regulate blood glucose concentration over an extended period of time put forward a possible new approach to treating insulin insensitivity while reducing the occurrence of side effects commonly associated with current treatments such as weight gain or hypoglycemia. BMP-9 was identified as a pharmacological and physiological target for regulating glucose metabolism (43), as it is specifically synthesized and released from the liver as a hepatic insulin-sensitizing substance (HISS) and responds to insulin for production and regulation (44, 45). 55% of glucose uptake stimulated by insulin is believed to be through the action of HISS, predominantly in skeletal muscles (46). It has been shown that BMP-9 circulates in the serum and activates Smad5, and upregulates Akt2 expression and, by stipulation, increases glucose uptake in skeletal muscle cells (47, 48). Receptors for BMP-9 have also been identified in hepatocytes and by activating these receptors. BMP-9 inhibits hepatic gluconegenesis and activates enzymes of lipid metabolism, such as malic enzyme and fatty acid synthase (FAS) (49, 50). A single subcutaneous injection of BMP-9 has been shown to reduce glycemia to near-normal levels in normal and diabetic (db/db) mice, whereas rats that received intravenous administration of BMP-9 display improved glucose tolerance and enhanced insulin sensitivity (50). These results suggest that BMP-9 is capable of acting as a systemic factor in an endocrine fashion, in addition to autocrine and paracrine manners.

VASCULAR DISEASES

Embryonic cardiac and vascular development is subject to regulation by BMP signaling as well as other pathways vital for embryogenesis (51). Since many of those pathways are reactivated after vascular injury, BMP signaling must also play crucial roles in vascular homeostasis and disease in adults (52). In fact, a number of BMPs are upregulated at sites of vascular injury, which reinforces the suggestion that they regulate normal vascular homeostasis and disease-associated vascular pathology. Hence, various components of the BMP signaling pathway have been linked to pathophysiology of vascular diseases such as vascular calcification, hereditary hemorrhagic telangiectasia (HHT) and pulmonary hypertension (PH).

Vascular calcification

Vascular calcification is a prominent feature of atherosclerosis and a common consequence of aging, diabetes, hypercholesterolemia and chronic renal insufficiency (53, 54). Vascular calcification can lead to aggravated hypertension and stroke, being a major risk factor for cardiovascular morbidity. Lately, the clinical importance of BMPs and related proteins in the process of vascular calcification has been discovered (55). Expression of BMP-2 and BMP-4 is upregulated in endothelial cells at sites of atherosclerotic plaques (56-58). It has also been shown that an osteogenic program is activated in the aorta of diabetic patients with atherosclerosis, including expression of BMP-2 and the osteoblasts homeobox-containing transcription factor, Msx2 (59). Msx2 has been shown to induce osteogenic over adipogenic differentiation of aortic myofibroblasts (60). BMP-2-Msx2 signaling may contribute to vascular calcification by diverting myofibroblasts capable of differentiating into either osteogenic or adipogenc lineage into the osteogenic lineage. In addition, extracellular modulators that normally inhibit BMP signaling have been associated with vascular injury and disease. Matrix gamma-carboxylated glutamate protein (MGP) is an extracellular matrix component that is produced by vascular smooth-muscle cells (VMSCs) and binds to BMPs (BMP-2 and BMP-4 in particular) to modulate BMP signaling in a concentration-dependent fashion (61, 62). Constitutive activity of MGP and other regulators of bone formation and osteoclastogenesis, along with reduced levels of BMP-2, BMP-4, osteopontin and osteonectin have been found in healthy aortas and early atherosclerotic lesions (63). Alternatively, expression levels of the same genes are upregulated as atherosclerotic plaques calcify. MGP-deficient mice develop widespread vascular calcification from progressively calcifying the tunica media into cartilage tissue (64, 65). Moreover, a poorly gamma-carboxylated form of MGP that cannot bind to BMP-2 was identified in calcified lesions of aortic wall of aging rats (60, 65). In humans, patients with Keutel syndrome, who inherit homozygous germ line mutations in MGP, display stenosis of pe-

ripheral pulmonary vessels (66). Taken together, these results strengthen the hypothesis that BMP signaling promotes vascular calcification. Besides, BMP-7 is a crucial regulator of chronic renal failure, which is a condition that is often accompanied by vascular calcification. Indeed, BMP-7 treatment has been demonstrated to be effective in preventing vascular calcification and reversing the increased osteocalcin expression levels in mouse atherosclerosis models, in addition to regenerating kidney functions in several rodent models of renal disease (67-72). Therefore, BMP-7 alone emerges as a potential therapeutic agent that can decrease vascular calcification, for which there is no adequate therapy at present.

Hereditary hemorrhagic telangiectasia (HHT)

HHT is an autosomal dominant inheritable vascular dysplasia, in which patients develop mucosal and skin telangiectasia, pulmonary, cerebral and hepatic malformations, and hemorrhages associated with these vascular lesions. HHT is associated with mutations in three genes encoding components of the BMP signaling: ENG gene for endoglin, ACVRL1 gene for ALK-1 and SMAD4 gene (73-75). Mutations in endoglin, a co-receptor for ALK-1 results in HHT type 1 (HHT1), whereas mutations in ALK-1 itself have been identified in patients with HHT2 (76). Homozygous ALK-1 null mice die embryonically with widespread arteriovenous malformations (77-79), which is the phenotype also exhibited by endoglin null mice and BMPR-II deficient mice (80-82). These observations suggest that the BMP signaling through the three receptors - endoglin, ALK-1 and BMPR-II play a discrete role in inducing or maintaining embryonic vascular stability different from that of other members of the TGF-beta superfamily. Deletion of ALK-1 in endothelial cells (ECs) alone or heterozygous ALK-1 mutant mice that are aging also develop systemic vascular malformations and symptoms observed in human HHT2 (83-86). In order to inhibit bleeding and other vascular malformations associated with HHT, anti-angiogenic therapies have been successful (87, 88) and several clinical trials using other anti-angiogenesis agents are currently underway with more HHT patients (http://www.hht.org/). This idea implicates that BMP signaling and its close relationship with angiogenesis is a promising therapeutic target.

Pulmonary hypertension (PH)

One of the best studied vascular diseases in relation to BMP signaling is PH. PH is a severe, debilitating and progressive disease with poor prognosis, characterized by increase in blood pressure in the lung vasculature, including pulmonary-artery, -vein, and -capillaries, which eventually leads to heart failure and death (43). It is also a sequel to a variety of cardiovascular and systemic diseases. Within the last 15 years, new pharmacological agents were introduced and entered routine clinical practice, which predominantly address the endothelial and vascular dysfunctions associated with the condition. Nevertheless, these interventions simply delay progression of the disease rather than offer a cure and many patients ultimately exacerbate, requiring new therapeutic approaches. More recently, many novel targets that harness and optimize vasodilation and anti-proliferative effects have been investigated and validated in animal models of PH, including the modulation of BMP signaling.

Pulmonary arterial hypertension (PAH) was the first type of PH to spark the interest in defining a closer relationship between BMP signaling and vascular diseases. Development of PAH arises from vascular remodeling, which involves increased proliferation of vascular muscle cells (VSMCs) and extracellular matrix deposition in the vessels, leading to a decrease in lumen diameter and reduced capacity for vasodilation. These results in increased pulmonary artery pressure and, consequently, sustained pulmonary hypertension. Interestingly, it has been revealed that more than 70% of all familial (hereditary) cases of PAH inherit mutations at the BMPR-II gene (89-92), whereas up to 26% of the sporadic (non-hereditary) cases of PAH have been identified to also have mutations in BMPR-II (93-95). Surprisingly, patients developing PAH after exposure to appetite suppressants fenfluramine and dexfenfluramine also carry BMPR-II mutations (96). These mutations are likely to cause loss of function and/or dominant-negative effects, but there is no clear consensus as to how BMPR-II signaling affects the pulmonary vasculature (97). One hypothesis is that mutations in the cytoplasmic domain of BMPR-II may hinder its interaction with LIM kinase-1 (LIMK-1), which results in constitutive activation of cofilin, an actin depolymerizing factor (43, 94, 98). Various manipulations of BMPR-II expression in mice have provided insights into the mechanisms by which BMPR-II mutations may give rise to pulmonary vascular disease in PAH. Numerous studies suggest that abnormal BMP signaling associated with BMPR-II mutations in patients with PAH results in at least one or a combination of the following events: a shift from contractile to synthetic phenotype of VSMCs, aberrant vascular cell proliferation and apoptosis, changes in expression of actin organization-related genes that may be related to focal adhesions, alterations in inflammationtory cell and cytokine recruitment, and increased collagen and matrix, as well as pulmonary vascular responses to different stimulants (99-103). It appears that normal function of BMPR-II in adult animals is to assist in injury repair process, where impaired ability to terminate the repair process is due to mutations in BMPR-II results in PAH (103).

Although mutations in BMPR-II or dysregulation of the BMP signaling pathway may predispose an individual to PAH, the clinical challenge is to determine whether these new discoveries can be exploited for therapies. Based on emerging understanding of the genetic basis of PAH, various genes and delivery systems have been shown to ameliorate the progression of PAH in animal models. Gene delivery of BMPR-II has achieved significant improvements in PAH animal models, which is encouraging for the development of this technology for human applications (104, 105). Moreover, merely targeting the

BMP signaling pathway alone may not be an efficient therapeutic approach. BMP signaling alone is insufficient to initiate the disease process without additional complications required for the pathogenesis of PAH (89, 106). Thus, up-and-coming approaches look forward to synergistic increase in efficiency through combinations of treatments that target multiple pathways (107).

CANCER

Outcome of BMP signaling is highly contextual throughout development, across different tissues, and thus in cancer. While the TGF-beta molecules represent a well established double-edged sword in carcinogenesis as known as tumor-suppressors in early stages but tumor-promoters in late stages of tumor progression, our current knowledge of BMPs in cancer is far from being clear (108, 109). There is not enough *in vitro* and *in vivo* data that leads to firm conclusions (110-112). Despite *in vitro* and *in vivo* studies linking BMPs to human osteosarcomas, there are no definite pro- or anti-carcinogenic BMPs in the general oncogenic process, and no straightforward mechanism of BMP dysregulation in carcinogenesis has yet been proposed. Most studies to date report that BMP-2, -4 and -7 are over-expressed in various cancers (110). However, changes in gene expression, epigenetic alterations and mutations in genes related to BMPs appear to be mostly a characteristic of a certain type of cell or tissue, not necessarily any direct nor indirect cause of carcinogenesis (110). In terms of their effects on one of the hallmarks of cancer - proliferation, even the same BMPs are described as both growth-stimulators (113-115) and growth-inhibitors (116-120), which likely reflects their complex interactions in developmental processes. Similarly, most of the cancer or anti-cancer effects of BMP signaling seem to depend on dosage, type of cell or tissue and the tumor microenvironment, which can shift a delicate balance between opposing properties of BMPs. Despite the lack of consistent reports of their roles in some events of human cancer development, closer relationships exist between BMPs and various features of carcinogenesis including angiogenesis, metastasis and cancer stem cells.

Angiogenesis

Growth of a tumor requires sustenance in the form of oxygen and nutrients via blood, as well as evacuation of metabolic wastes and carbon dioxide (121). Hence, tumors adopt angiogenesis as a survival mechanism to address these needs and pathological angiogenesis is known as one of the hallmarks of cancer (122). The onset of angiogenesis can occur at any stage of tumor progression depending on the tumor type and microenvironment, as it is orchestrated by a network of various angiogenic factors, including BMPs and their inhibitors (123). In fact, angiogenesis is an active process during embryonic development and female reproductive cycling, most of which BMPs are heavily associated with. Angiogenesis is also re-activated during wound repair and several pathological conditions such as vascular malfunctions and cancer (124-126). The relationship between vascular diseases such as HHT and PAH and dysregulation of the BMP signaling components such as endoglin, ALK-1 and BMPR-II has already been mentioned above. In tumor angiogenesis, BMPs and related proteins either stimulate or inhibit functions of ECs and VSMCs in a context-dependent manner (108). However, the exact role of each BMP and how perturbed signaling may contribute to angiogenesis in cancer and tumor progression remain to be determined in detail (127-130).

BMP-2 and BMP-4 have generally been proposed to be angiogenic factors, promoting tube formation and neovascularization of melanoma cells in the micro-vascular network (110, 131), in human blood endothelial progenitor cells (132) and in lung tumors by associating with VEFG to stimulate angiogenesis (127, 133). As target genes of BMP signaling, myosin-X and cyclooxygenase 2 have been found to play a role in vascularization (134). BMP antagonists have been reported to act as anti-angiogenic factors during tumor angiogenesis. Noggin inhibits BMP-4 induction of BEGFR-2 in embryonic blood vessels (135), whereas Chordin also inhibits BMP-4-induced *in vitro* tube formation of human vascular endothelial cells and malignant melanoma cells (131). Alternatively, BMP-9 and BMP-10 has been identified as the sole physiological ligands of the endothelial receptor ALK-1 in association with BMPR-II, inhibiting bFGF-stimulated proliferation and migration of bovine aortic endothelial cells, as well as preventing angiogenesis induced by VEGF (136, 137). In this view, BMP-9 appears to be a promising target for inhibiting or controlling tumor angiogenesis. However in some other mouse-model studies, inhibition of ALK-1 function impairs VEFG-induced angiogenesis and cancer progression (138). Moreover, a soluble chimeric protein (ALK-1-Fc) that selectively inhibits BMP-9 and BMP-10 mediated Id-1 expression in human umbilical vein endothelial cells has been shown to inhibit cord formation by these cells on Matrigel (139). ALK-1- Fc also reduced VEGF, FGF and BMP-mediated vessel formation, while also inhibiting the growth of B16 melanoma explants in chick chorioallantoic membrane assays (140). In the same study, MCF7 mammary adenocarcinoma grafts in mice treated with ALK-1-Fc also showed reduced tumor burden, which supports the effectiveness of ALK-1-Fc proteins as anti-angiogenic agents capable of inhibiting vascularization. The process of angiogenesis is very complex and relatively poorly understood. Other contributing factors, aside from the involvement of the BMPs, are likely to be the basis for such conflicting reports.

Metastasis

As carcinoma progress to higher pathological grades of malignancy, cancer cells typically develop changes in their morphology and adhesiveness to other cells and to the extracellular matrix (ECM), resulting in local invasion and distant metastasis. Tumors are believed to usurp processes involved in

normal developmental programs and wound healing, such as epithelial to mesenchymal transition (EMT), tissue specific morphogenesis and cellular motility to create a complex microenvironment that sustains its survival and enable invasion. EMT is a normal embryonic process that facilitates cellular migration in gastrulation and tissue patterning (141), which have also been implicated to increase invasive and metastatic potential of cancerous cells in colonizing distant organs. Metastatic cancer cells acquire abilities to invade, to resist apoptosis, and to disseminate through EMT, capable of moving from one organ system to another via blood stream or lymphatic system (142-146).

Several studies support that BMP signaling confers various tumor cells with migratory and invasive properties (147). BMP signaling has been shown to induce EMT in normal and cancerous cell types (148, 149). Consistently, BMP-2 and BMP-4 are shown to enhance motility and invasiveness of prostate cancer cell lines (150), while functional overexpression of BMP-2 in breast cancer cells promotes invasion and ultimately induce tumor growth (151). Stable expression of a dominant-negative BMP receptor inhibited the ability of breast cancer cells to form bone metastasis (152). Moreover, orthotropic implant of tumors with scaffolds coupled with BMP-2, seeded with bone marrow stromal cells promoted metastatic spread of breast cancer cells (153). BMP-4 over-expressing colon cancer cell line displays resistance to serum starvation-induced apoptosis and increased motility and invasion activities, which are inhibited by Noggin (154). BMP-2 enhances migration and invasion of gastric cancer cells by activating the phosphatidylinositol 3-kinase (PI3K) pathway (155). In primary human epithelial ovarian cancer, BMP-4 signaling changes cellular morphology and enhances adhesion, motility and invasion of the cancer cells (149). Exogenous BMP-4 treatment upregulates EMT markers such as SNAIL and SLUG while downregulating E-cadherin, a key cell-to-cell adhesion molecule, loss of which is the best characterized alteration involved in potentiating metastasis. Additionally, the network of alpha smooth muscle actin changes in response to BMP-4 to resemble a mesenchymal cell type (149).

BMP pathway has been shown to promote organ-specific metastasis to the bone particularly in advanced breast and prostate adenocarcinomas, which are the most prevalent cancers in women and men (156-158), respectively. Prostate and breast cancer cells are likely to be predestined to colonize the bone from primary tumor formation even before dissemination, as skeletal metastasis is particularly high in prostate and breast cancer patients: about 75% of prostate cancer and 70% of breast cancer patients have evidence of metastatic bone diseases (159-162). The spine is the most common site of tumor metastasis to bone, presenting significant morbidity to patients (157, 163). Bone metastasis is a devastating complication of these cancers, directly responsible for debilitating bone fractures, severe pain, hypercalcemia, spinal cord nerve compression and consequent paralysis (164, 165). Metastatic tumors drive bone destruction by disrupting a balance of bone homeostasis maintained by osteoblasts, the cells responsible for producing new bone matrix, and osteoclasts, the cells responsible for breaking down the bone matrix (166-168).

Preferential factors for cancer cells to metastasize to bones require sequential or simultaneous interactions among cells, growth factors, cytokines, receptors and the bone (169, 170). First, BMPs appear to select the most invasive tumor cells by stimulating the progenitor cells through an autocrine signaling pathway (171). For example in osteotropic prostate cancer cells, nuclear factor (NF)-kappa B uses PI3K/Akt pathway to activate BMP-2 signaling, which upregulates downstream BMP-2 target genes such as osteopontin, osteocalcin and collagen IA1, implying a predisposition of bone-like features (172). Both osteotropic prostate and breast cancer cells try to resemble osteoblasts by expressing bone matrix proteins as well as alkaline phosphatase-ensuing in osteomimicry which enables higher chances of survival and invasion into the bone tissue (173). Next, BMPs secreted by the tumor cells signal in a paracrine manner to create a reactive stroma through the activation of tumor-associated myofibroblasts, which are known enhancers of tumor cell growth and metastasis (174). Paracrine signaling also influences the bone microenvironment, resulting in a crosstalk between tumor cells and the bone that creates new "pre-neoplastic" niches in the bone for colonization (147, 164, 175). The BMPs produced from the bone may act as chemoattractants, assisting cell detachment by recruiting highly metastatic cells that express the BMP receptors (174). Chemoattractant activity of BMP-2 at a range of 12.5-50 nM has been demonstrated to increase migration of breast cancer cells towards a BMP-2 source in comparison to untreated cells (151). BMP-4 is also one of several factors that increase adhesion of prostate cancer cells to the endothelium of bone marrow (176). Such crosstalk between tumor cells and the bone microenvironment involving BMP signaling seems to generate bone metastasis. Since BMPs are potent regulators of bone morphogenesis, there is an increasing interest to investigate BMPs and their roles in bone metastasis. Therefore, better understanding molecular mechanisms underlying bone metastases will help to develop BMPs and its signaling molecules as new therapeutic targets for better and prolonged life expectation for patients with bone metastases.

Cancer stem cells

BMPs have extensive roles in regulating the biology of stem cells and guiding them to take part in embryonic development/organogenesis and tissue regeneration (177). Disruption of a fine balance between self-renewal and differentiation of stem cells leads to loss of control in terms of cell growth and proliferation that may result in cancer. In fact, stem cells already share characteristics of immortality, pluripotency, EMT plasticity and loss of contact inhibition with cancer cells. Hence, it is not surprising that BMPs also play pivotal roles in

the maintenance and differentiation of cancer stem cells (CSCs). CSCs constitute a cellular subtype within tumors, which is thought to be the origin of tumors and their malignancies. CSCs, like normal stem cells, are believed to initiate tumorgenesis, and drive its growth, invasion and recurrence even after treatments (178, 179). Many patients are believed to respond to clinical treatments with limited effectiveness due to the presence of CSCs. The induction of developmental pathways in the midst of tumor-stromal interactions conceivably promotes an embryonic-like microenvironment that can provide a more welcoming niche for tumor cells to invade. Recent evidences have highlighted the involvement of CSCs in various types of tumors, linking EMT features with those of CSCs in particular (180).

Stem-cell signaling network is composed of cross-talk among overlapping signals between developmental pathways including WNT, FGF, Notch, Hedgehog, TGF-β, and BMP signaling cascades (181, 182). These pathways maintain pluripotent stem cells as well as CSCs by synergistically inducing EMT regulators, such as Snail (SNAI1), Slug (SNAI2), TWIST and ZEB2 (SIP1) at the tumor-stromal microenvironment (183). CSCs acquire more malignant phenotype through accumulation of additional genetic and epigenetic alterations (184). Recently, there have been major advances in understanding the role of BMP signaling in CSCs. However, the underlying mechanisms and processes are poorly understood and the oncogenic mechanisms vary with tumor type, the state of the disease as well as interaction with the tumor microenvironment. For instance, BMP-4 has been demonstrated to induce EMT and acquisition of stem cell-like phenotypes in oral squamous cell carcinoma (179), whereas the same molecule has been shown to be a promising therapeutic agent against CSCs in advanced colorectal tumors by promoting terminal differentiation, apoptosis and chemosensitization of colorectal CSCs (185). Therefore, the putative relationships warrant further investigations to prove more definitive roles of BMPs in the process of tumorigenesis with an emphasis on how aberrant activation of BMP signaling pathways connects the early events with the late events of cancer progression.

Exhaustive research activities are devoted to identifying drugs that interfere with oncogenic signaling by the aforementioned developmental pathways. Like BMPs, the secretion of Hedgehog (Hh) ligands within the tumor-stromal interaction has been shown to promote primary tumor growth (186). Recently, promising clinical data of a compound that disrupts the Hh pathway, GDC-0449 implicates that targeted therapy of developmental signaling pathways has potential for future anti-cancer therapies (187). Dorsomorphin (LDN-193189) is a selective small molecule inhibitor of BMP signaling pathway which specifically blocks ALK1, 2, 3 and 6 signaling through Smad1, 5 and 8 (188). It would be interesting to see how this small molecule influences tumor progression. In 2008, BMP-9 was patented as an anti-cancer therapeutic agent for regulating breast and prostate cancers on the basis of promoting apoptosis of prostate cancers (189, 190). For the time being, recombinant human (rh) BMP-2 and BMP-7 are clinically available for spinal fusions and long bone non-unions. In the meantime, the prevalence of BMP usage has been on the rise with at least 85% of procedures being off-label applications (191). The lack of sufficient knowledge of implications of BMPs in tumoregenesis has potential complications concerning those inpatient procedures. On the other hand, these BMPs would be of particular value in enhancing posterolateral fusion as well as in cages used for anterior reconstruction in patients who undergo routine radiation therapies for tumoregenic lesions in the spine (192). At this time, no reviews and studies report any adverse carcinogenic events in patients who underwent operations using either BMP-2 or BMP-7 (193-195). However, the lack of reported cases may be due to the relatively recent introduction of BMPs to clinical use and extra precautions must be made in determining appropriate dosage and length of retention time in the body.

PERSPECTIVES

Although much remains to be understood about the complexity of BMP signaling in metabolic malfunctions, vascular diseases and cancer, there is emerging evidence from a variety of systems that BMPs possess immense therapeutic potentials. At present, human BMPs are mass produced for clinical applications by recombinant technology and such recombinant human BMPs (rhBMPs) of BMP-2 and BMP-7 are currently used for spinal fusion, fracture healing and dental tissue engineering (196, 197). More recently, transplantation of rhBMPs with autologous stem-cells has also emerged as a promising technique in tissue engineering for the regeneration of various body parts (198). Furthermore, *ex vivo* rhBMP-conditioned cell therapy can be an effective alternative. The approach is theoretically recalcitrant to immunogenic complications, which opens the therapeutic strategy to a wider range of new protein-engineered synthetic BMP ligands. However, clinically effective doses of such rhBMPs (several mg) are much higher than their physiological doses (1-2 ug/kg of bone) (199, 200). Large-scale production still remains costly due to multiple preparation and purification processes and the need for large quantities of rhBMPs leads to higher costs (171). High concentrations also results in increased safety risks due to inflammation, oedema and heterotopic bone formations (201).

In order to address such problems, various genetic engineering approaches are also being explored to design second-generation BMPs. These engineered BMPs seek to acquire improved bioactivity through increased solubility, stability and affinity to receptors, decreased sensitivity to natural BMP inhibitors and better immunogenicity profile (202, 203). Shorter peptides of known active epitopes of BMPs that act just as full-length BMPs are also developed in an effort to reduce production costs (204, 205). Controlling the release of high concentrations of rhBMPs over a prolonged period of time is an-

other major problem for drug delivery. Carriers and scaffolds made of various materials such as collagen sponge, polymer microspheres and lipid-based microtubes have been shown to increase retention time of implanted rhBMPs to overcome short half-life and rapid clearance of the proteins *in vivo* (206-209). Gene therapy is a less expensive method of delivering BMPs to their disease sites in a well-controlled manner (171, 210, 211). Yet, gene therapy has a potential to risk elevated immune response following the use of adeno-, retro- and lentiviruses used to deliver DNA or RNA in gene therapies.

Clinical potentials of next-generation therapeutics such as engineered BMPs (203) and *ex vivo*-conditioned cell therapies offer innovative prospects. These new clinical strategies need to overcome various fundamental limitations such as cost, delivery and possible immunogenic responses. Despite these technical barriers, the emerging future of new biomedical therapeutics targeting BMP signaling is rapidly approaching and promises to be an exciting one.

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