

Review

Bone morphogenetic proteins and their receptor signaling in prostate cancer

L. Ye¹, J.M. Lewis-Russell¹, H.G. Kyanaston² and W.G. Jiang¹

¹Metastasis & Angiogenesis Research Group, Department of Surgery, Wales College of Medicine, Heath Park, Cardiff and

²Department of Urology, University Hospital of Wales, Heath Park, Cardiff, UK

Summary. Bone morphogenetic proteins (BMPs) belong to the TGF- β superfamily and are vital bone inductive factors. BMPs also play important roles during embryonic development and the postnatal homeostasis of various organs and tissues, by controlling cellular differentiation, proliferation and apoptosis. Prostate cancer is the most common cancer in men in Western countries, with a high incidence of bone metastasis. Once bony metastasis developed, the condition is incurable, and contributes significant disease specific morbidity and mortality. However, the mechanisms underlying the development of bone metastasis remain unclear. BMPs have been implicated in the development of both primary and secondary tumors, particularly skeletal metastasis. Aberrations in BMPs signaling have also been identified in various neoplasms. Recently studies have also suggested a pivotal role in bone metastasis for Noggin, which is a BMP antagonist. In this review, we discuss the current knowledge of BMPs signaling, abnormalities which have been identified and their involvement in tumour progression, and particularly in the development of bone metastasis in prostate cancer.

Key words: Bone morphogenetic protein, Prostate cancer, Cancer biology, Bone metastasis

Introduction

Prostate cancer is the most commonly diagnosed male cancer in UK, US and most other Western countries. More than 30,000 new cases are diagnosed each year in the UK alone. It is also the second leading cause of mortality from cancer after lung cancer in men in UK, accounting for 10,100 deaths each year

(CancerStats, 2005).

Bone is the most common metastatic site of prostate cancer, and approximately 90% of patients with advanced prostate cancer have skeletal metastases (Bubendorf et al., 2000). Morbidity from bone metastasis is the most common complication in patients with prostate cancer, and problems include partial paralysis from spinal cord compression injuries, hypercalcemia, pathologic fractures, and bone pain. There are a few effective treatments available for treating skeletal metastases, and none is curative. The molecular and cellular mechanisms leading to the development of bone metastasis in prostate cancer are currently under intensive investigation. Some progress has been made in this area, but most key issues remain unclear. For example, is there a specific genetic predisposition that makes the prostate cancer cells more prone to spreading to bone; what factors are involved in the development of bone metastasis at the level of the local microenvironment; what interactions are there between metastatic prostate cancer cells and bone marrow endothelial cells, osteoblasts, and osteoclasts that assist metastatic prostate cancer cells to develop to their full metastatic potential? These are important questions for our understanding of bone metastases, and it is just here that the Bone Morphogenetic Protein family may play a key role. BMPs belong to the TGF- β superfamily, whose members play crucial roles in embryogenesis and organogenesis by controlling cell growth and differentiation. As they are powerful osteogenic factors enriched in bone matrix, there is an increasing interest in the role which BMPs may play in bone metastasis.

Bone morphogenetic proteins and their signaling pathway

Bone morphogenetic protein

Bone morphogenetic proteins (BMPs) are members of the transforming growth factor-beta (TGF- β)

Offprint requests to: Dr. Lin Ye, Metastasis & Angiogenesis Research Group, University Department of Surgery, Wales College of Medicine, Cardiff University, Heath Park, Cardiff, CF14 4XN, UK. e-mail: YeL@cf.ac.uk

superfamily, which was first named by Urist (Urist 1965). BMP proteins were first purified and cloned in late 1980s (Wozney et al., 1988; Celeste et al., 1990; Lee 1990; Ozkaynak et al., 1990), to date, more than 20 BMPs have been identified in humans (Table 1).

Bone morphogenetic proteins are synthesized as large precursor molecules, consisting of an amino-terminal pro-region and a carboxy-terminal ligand which contains seven conserved cysteines (Wozney et al., 1988, 1990; Ozkaynak et al., 1990). Each BMP ligand has seven conserved cysteines, in which six cysteines construct a cysteine knot, and the seventh cysteine contributes to the dimerisation (Butler and Dodd, 2003). Presently, little is known with regard to the processing of the precursor molecule or secretion of BMPs. But it has been shown that some of the proprotein convertases (PCs), such as furin, proprotein convertase subtilisin/kexin type 6 (PCSK6) and proprotein convertase subtilisin/kexin type 5 (PCSK5) which belong to a subtilisin-like proprotein convertase family, can proteolytically activate BMP precursors at the sequence of R-X-K/R-R or R-X-X-R (Cui et al., 1998; Constam and Robertson, 1999; Tsuji et al., 1999; Hashimoto et al., 2005). The pro-region of the precursor BMP protein controls the stability of the processed mature protein, and the amino acid motif adjacent to the cleavage site determines the efficiency of cleavage (Constam and Robertson, 1999). Amongst the BMPs,

growth differentiation factor-9 (GDF9) and BMP15(GDF9B) may be an exception and have only six cysteines in mature ligand without the seventh cysteine. This characteristic of BMP15 and GDF9 may help to define its ligand binding property to its receptors (Mazerbourg et al., 2006). The pro-region of some BMPs remains noncovalently associated with the mature ligand even after secretion from the cell, for example: GDF-8 and BMP-9 (Lee and McPherron, 2001; Brown et al., 2005).

Once processed and activated, BMP proteins are biologically active both as homodimer, and as heterodimer molecules, in which two chains are connected by disulfide bonds. Interestingly, the heterodimers of BMP4/7, BMP2/6, BMP2/7 and BMP7/GDF7 are more effective than when they form homodimers (Aono et al., 1995; Israel et al., 1996; Suzuki et al., 1997; Butler et al., 2003).

BMP receptors

BMP signals are mediated by receptors which are dedicated to TGF- β signaling, and include type I and type II serine/threonine kinase receptors. Seven type I receptors and five type II have been identified in humans (Table 2). Six of the type I receptors and three of the type II receptors are responsible for BMP signaling. BMPR1A, BMPR1B and BMPR2 are specific for the

Table 1. Members of bone morphogenetic protein and growth differentiation factors (identified in humans).

Official Symbol	Alternative Name	Gene Location in homo sapiens	Year of identification
BMP2	BMP2A	20p12	1988
BMP3		4p14-q21	1988
BMP4	ZYME; BMP2B; BMP2B1	14q22-q23	1988
BMP5	MGC34244	6p	1990
BMP6	VGR; VGR1	6p24-p23	1990
BMP7	OP-1	20	1990
BMP8A		1p35-p32	2002
BMP8B	BMP8; OP2	1p35-p32	1992
BMP10		2p13.3	1999
BMP15	GDF9B, ODG2	Xp11.2	1998
GDF1		19p12	1991
GDF2	BMP-9; BMP9	10q11.22	1994
GDF3		12p13.1	2000
GDF4		?(Reserved)	?
GDF5	CDMP1	20q11.2	1994
GDF6		8q22.1	1999
GDF7	BMP12	2p24-2p23	1998
GDF8	MSTN, myostatin	2q32.1	1997
GDF9		5q23-5q33.1	1993
GDF10	BMP-3b	10q11.22	1995
GDF11	BMP-11	12q13.13	1998
GDF15	PLAB, MIC-1, PDF, MIC1, NAG-1, PTGFB	19p13.1-13.2	1997

Data collected from HGNC and Entrez Gene. Based on literature published BMP2 (Wozney et al., 1988); BMP3 (Wozney et al., 1988); BMP4 (Wozney et al., 1988); BMP5 (Celeste et al., 1990); BMP6 (Celeste et al., 1990); BMP7 (Ozkaynak et al., 1990); BMP8A (Strausberg et al., 2002); BMP8B (Ozkaynak et al., 1992); BMP10 (Neuhaus et al., 1999); BMP15 (Dube et al., 1998); GDF1 (Lee 1990); GDF2 (Celeste et al., 1994); GDF3 (McPherron and Lee, 1993); GDF5 (Hotten et al., 1994); GDF6 (Davidson et al., 1999); GDF7 (Lee et al., 1998); GDF8 (McPherron et al., 1997); GDF9 (McPherron and Lee, 1993); GDF10 (Cunningham et al., 1995); GDF11 (Gamer et al., 1999); GDF15 (Yokoyama-Kobayashi et al., 1997).

BMP and prostate cancer

BMPs; whilst ACVRL1, ACVR1, ACVR1B, ACVR2B, and ACVR2A are also the receptors for activin; TGFBR1(ALK5) is known as the type I receptor for TGF- β 1, 2 and 3 (Fig. 1).

Both types of the BMP serine/threonine kinase receptors consist of an N-terminal extracellular ligand binding domain, a transmembrane region and a C-terminal serine/threonine kinase domain. The structure of the extracellular ligand binding domain of both receptors is similar. It has a three-finger toxin fold, with each finger formed by a pair of anti-parallel β strands (Greenwald et al., 1999; Kirsch et al., 2000). The intracellular region of the type I receptors, but not type II receptors, contains a highly conserved GS domain enriched in glycines and serines. The region is located in the intracellular juxtamembrane region of the receptor (Attisano et al., 1994; Wrana et al., 1994). Type II receptors recruit type I receptors by phosphorylating the GS domain of type I receptor during signal transduction.

BMP signal transduction

Smad proteins are important signaling molecules downstream of the BMP receptors. Eight Smads have been identified in humans, and comprise three subgroups: pathway restricted Smads (receptor regulated Smads) (referred to as R-Smads which include Smad 1, 2, 3, 5 and 8) (Table 3), common mediator Smad (Co-Smad, Smad4), and inhibitory Smads (I-Smads, Smad 6 and 7) (Shi et al., 2003; Nohe et al., 2004). R-Smads 1, 5 and 8 are substrates of the type I receptors (ALKs 1, 2, 3 and 6), whereas R-Smads 2 and 3 are substrates of the type I receptors (ALKs 4, 5 and 7) (Kretschmar and Massague, 1998; Chen and Massague, 1999; Jornvall et al., 2001; Shi and Massague, 2003). All Smad proteins share considerable homology in their primary sequences. R-Smads and Co-Smad contain two highly conserved Mad homology domains: the Mad homology 1 (MH1) domain in the amino-terminal part and the Mad homology MH2 domain in the carboxy-terminal part.

The MH1 domain can bind to specific DNA sequences, and the MH2 domains are responsible for homo- and heteromeric complex formation. R-Smads contain the carboxy-terminal SSXS motif which is phosphorylated by the Type I receptor during signal transduction of BMPs (Nohe et al., 2004).

There are two categories of signaling pathway for BMPs and BMP receptors: the Smad dependent pathway and the Smad independent pathway (Fig. 2). Type I and

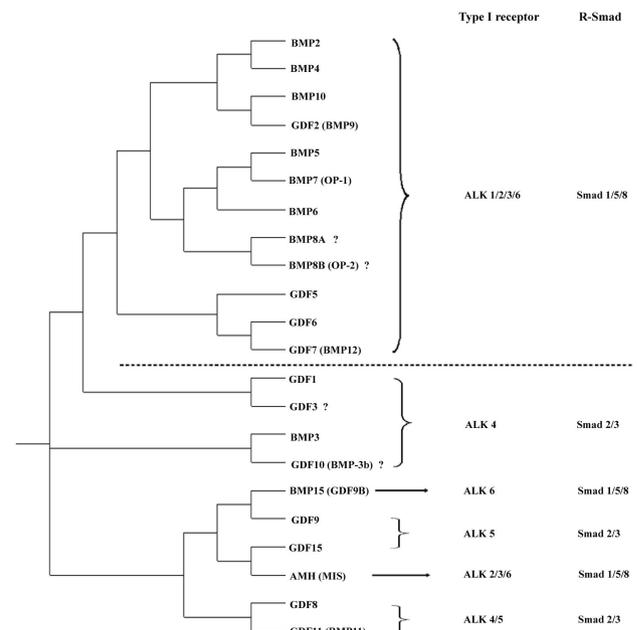


Fig. 1. Dendrogram tree and key downstream signaling molecules of BMP/GDF. Phylogenetic analyses was performed using the ClustalW (<http://www.ebi.ac.uk/clustalw/>), and the dendrogram tree was drawn by using the Treeview (Version 1.6.6, <http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>). ? is unknown.

Table 2. Transmembrane serine/threonine kinase receptors.

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

Transmembrane serine/threonine kinase receptors. There are seven Type I and five Type II transmembrane serine/threonine kinase receptors identified in humans. Six Type I receptors and three Type II receptors that have been found involved in the signal transduction of BMPs, which are bold italic in the table. ACVRL1, activin A receptor type II-like 1; ACVR1, activin A receptor, type I; BMPR1A, bone morphogenetic protein receptor, type IA; ACVR1B, activin A receptor, type IB; TGFBR1, transforming growth factor, beta receptor I; BMPR1B, bone morphogenetic protein receptor, type IB; ACVR1C, activin A receptor, type IC; TGFBR2, transforming growth factor, beta receptor II; TGFBR3, transforming growth factor, beta receptor III; BMPR2, bone morphogenetic protein receptor, type II; ACVR2B, activin A receptor, type IIB; ACVR2A, activin A receptor, type IIA.

type II BMP receptors are required for both pathways. When a dimeric ligand of BMPs binds to two different types of serine-threonine kinase transmembrane receptors, the Type-II receptors then transphosphorylates the GS domain of the Type-I receptors (Moustakas and Helding, 2002). This forms a ligand-receptor complex consisting of a dimer of each ligand, Type-I and Type-II receptors. If the BMP ligand binds simultaneously to the preformed hetero-oligomeric complexes (PFC), this leads to activation of the Smad dependent pathway (Nohe et al., 2002, 2004). It includes recruitment of the pathway-restricted Smads (R-Smads, Smad1, 2, 3, 5 or 8), and regulates the transcription of target genes, this is known as the Smad dependent pathway.

Unlike other members of TGF- β superfamily, BMPs have a higher affinity for the Type-I receptors, rather than the Type II receptors. Thus, BMP ligand can also bind to ALK3 or ALK6, and then recruits BMPRII into a hetero-oligomeric complex (BMP-induced signaling complexes, BISC), this leads to the activation of the Smad independent pathway (Nohe et al., 2002, 2004). During intracellular signal transduction, the X-linked inhibitor of apoptosis protein (XIAP) functions as an adaptor protein bridging between the Type I receptor and TGF- β activated binding protein (TAB1/2/3), which is an activator of the MAPKKK TGF- β activated tyrosine kinase 1 (TAK1) (Yamaguchi et al., 1995, 1999; Shibuya et al., 1996). The activation of TAK1 can lead to activation of p38, a mitogen-activated protein kinase (MAPK) (Moriguchi et al., 1996; Kimura et al., 2000; Nohe et al., 2002). TAK1 can also activate Jun N-terminal kinases (JNKs), NF-kappaB (NF- κ B) and Nemo-like kinase (NLK) (Shirakabe et al., 1997; Ishitani et al., 1999; Lee et al., 2002).

Regulation of BMPs' signaling

The regulation of BMP signaling may occur extracellularly during the process of ligand binding to the receptors, or intracellularly during signal transduction (Table 4). Recent evidence suggests that BMPs can also regulate their function through a negative feedback loop, in which the pseudoreceptor, Inhibitory Smads (Smad 6 and 7) antagonists of BMPs, are likely to be involved (Canalis et al., 2003).

Regulation of BMP signaling by extracellular events

Perhaps the most investigated molecules that are able to influence BMP signalling extracellularly are the BMP antagonists. More than 10 BMP antagonists have been identified to date. BMP antagonists exert their influence over BMP and BMP receptors in two ways: direct competition between the antagonists and BMPs, and regulation of expression of the antagonists by BMPs themselves. The antagonists can bind to BMP receptors competitively, and block/inhibit the effect of the BMPs. For example, competition between Noggin and BMP4 regulates dorsalization during *Xenopus* development

Table 3. Receptors and R-Smads involved in BMP signaling.

Official Symbol	Type II receptor	Type I receptor	R-Smad
BMP2	BMPRII ActRIIA	ALK 3/6	Smad 1/5/8
BMP3	ActRIIA	ALK 4	Smad 2/3
BMP4	BMPRII ActRIIA	ALK 3/6	Smad 1/5/8
BMP5		ALK 3	Smad 1/8
BMP6	ActRIIA ActRIIB BMPRII	ALK 1/2/3/6	Smad 1/5
BMP7	BMPRII ActRIIA	ALK 2/3/6	Smad 1/5/8
BMP8A			
BMP8B			
BMP10	BMPRII ActRIIA	ALK 3/6	Smad 1/5/8
BMP15	BMPRII	ALK 6	Smad 1/5/8
GDF1	ActRIIB	ALK 4	Smad 2/3
GDF2	ActRIIA ActRIIB BMPRII	ALK 1	Smad 1/5
GDF3			
GDF5	BMPRII ActRIIA	ALK 3/6	Smad 1/5/8
GDF6	BMPRII ActRIIA	ALK 3/6	
GDF7	BMPRII ActRIIA	ALK 3/6	
GDF8	ActRIIB	ALK4/5	Smad 2/3
GDF9	BMPRII	ALK5	Smad 2/3
GDF10			
GDF11	ActRIIA ActRIIB	ALK4	Smad 2/3
GDF15			Smad 2/3

Receptors and R-Smads involved in BMP signaling. BMP2 (Koenig et al., 1994; Yamaji et al., 1994; Liu et al., 1995; Namiki et al., 1997); BMP3 (Daluiski et al., 2001); BMP4 (Koenig et al., 1994; ten Dijke et al., 1994; Yamaji et al., 1994; Nohno et al., 1995; Rosenzweig et al., 1995; Aoki et al., 2001); BMP5 (Beck et al., 2001; Zuzarte-Luis et al., 2004); BMP6 (Ebisawa et al., 1999; Ahmed et al., 2001; Aoki et al., 2001); BMP7 (ten Dijke et al., 1994; Liu et al., 1995; Rosenzweig et al., 1995; Aoki et al., 2001); BMP10 (Mazerbourg et al., 2005); BMP15 (Moore et al., 2003); GDF1 (Cheng et al., 2003); GDF2 (Brown et al., 2005; Lopez-Coviella et al., 2006); GDF5 (Nishitoh et al., 1996; Aoki et al., 2001; Nakahara et al., 2003; Sammar et al., 2004; Chen et al., 2006); GDF6 (Mazerbourg et al., 2005); GDF7 (Mazerbourg et al., 2005); GDF8 (Rebbapragada et al., 2003); GDF9 (Mazerbourg and Hsueh 2006); GDF11 (McPherron et al., 1999; Oh et al., 2002; Andersson et al., 2006); GDF15 (Xu et al., 2006).

BMP and prostate cancer

(Re'em-Kalma et al., 1995). On the other hand, noggin expression in osteoblasts can be induced by BMP2, 4 and 6. Therefore, the BMPs are able to modulate their effect via a negative feed back loop by upregulation of the expression of their antagonist (Gazzerro et al., 1998).

Besides the BMP antagonists, there are other possible mechanisms by which BMP signaling is regulated extracellularly. One of these extracellular mechanisms is the expression of co-receptors or

dominant negative non-signaling pseudoreceptors in a cell. The pseudoreceptor, BMP and activin membrane bound inhibitor (BAMBI), is a transmembrane protein which has an extracellular domain similar to that of the Type I BMP receptor. However, the pseudoreceptor lacks the intracellular serine/threonine kinase domain. BAMBI binds to ligand competitively, and then interferes with the signaling of the BMPs and other TGF- β molecules (Onichtchouk et al., 1999). BMP4 can

Table 4. Regulatory factors of BMP signalling.

Location	Category	Official Symbol	Target
Extracellular	Antagonist	Noggin	BMP2, 4, 6 and 7; GDF5 and GDF6
		Chordin	BMP4, 7
		Chordin like 2(CHL2)	BMP2, 4, 5, 6, 7 and GDF5
		Follistatin	BMP6, 7, 11 and 15, GDF8 and 9
		Ventropin	BMP4
		FLRG	BMP2
		Twisted gastrulation(Tsg)	BMP2, 4
		Gremlin(DRM)	BMP2, 4 and 7
		Dan	BMP2, 4 and GDF5
		Cerberus	BMP2, 4
		(PRDC)	BMP2, 4
		Sclerostin(SOST)	BMP6 and 7
		Caronate	BMP2, 4 and 7
		(DAND5)	BMP4
			Enhancer
Membrane	Pseudoreceptor	BAMBI	BMP4
	Co-receptor	Dragon	BMP2, 4
		RGMa	BMP2, 4
Intracellular	Inhibitory Smads	Smad6 and 7	R-Smad, Co-Smad
		Ski	Smad 2, 3 and 4
	Smad binding protein	SKIL/SnoN	Smad 2, 4
		Tob	Smad 1, 5 and 8
		AMSH	Smad6
	Ubiquitination and degradation of Smad	Smurf1 and 2	Smad 1, 5, 6 and 7
		NEDD4-2	Smad 2
Deubiquitination of Smad	UCH37	Smad7	

Regulatory factors on BMP signaling. The regulation of BMP signaling can happen during the process of ligand binds to receptor, and the intracellular signal transduction. Dan, Cerberus, Gremlin, PRDC, Sclerostin, Caronate and DAND5 blong to DAN/Cerberus family. Based on literatures published: Noggin (Re'em-Kalma et al., 1995; Chang and Hemmati-Brivanlou, 1999; Haudenschild et al., 2004; Pera et al., 2004; Zhu et al., 2006); Chordin (Sasal et al., 1995; Piccolo et al., 1996; Dale et al., 1999); Kielin/Chordin like (Lin et al., 2005); Chordin like 2, CHL2 (Nakayama et al., 2004); Follistatin (Fainsod et al., 1997; Iemura et al., 1998; Otsuka et al., 2001; Balemans and Van Hul, 2002; Pierre et al., 2005); Sclerostin (Kusu et al., 2003); Ventropin (Sakuta et al., 2001); FLRG (Tsuchida et al., 2000); Gremlin (Hsu et al., 1998; Merino et al., 1999); Twisted gastrulation (Chang et al., 2001; Ross et al., 2001); Dan (Hsu et al., 1998; Dionne et al., 2001; Gerlach-Bank et al., 2004); Cerberus (Piccolo et al., 1999); Protein related to DAN and Cerberus, PRDC (Sudo et al., 2004); Dan domain family member 5, DAND5 (Marques et al., 2004); Caronate (Yokouchi et al., 1999); BAMBI (Onichtchouk et al., 1999; Grotewold et al., 2001); Dragon (Samad et al., 2005); RGMa (Babitt et al., 2005); Ski (Luo et al., 1999); Ski like, SKIL/SnoN (Stroschein et al., 1999; Vignais 2000); Tob (Yoshida et al., 2000); SH3 domain of STAM, AMSH (Itoh et al., 2001); Smad ubiquitination regulatory factor, Smurf (Arora et al., 2001; Murakami et al., 2003); Neural precursor cell expressed, developmentally down-regulated 4-2, NEDD4-2 (Kuratomi et al., 2005); ubiquitin C-terminal hydrolase, UCH37 (Wicks et al., 2005).

also induce the expression of BAMBI in mouse embryonic fibroblasts (Grotewold et al., 2001), in doing so, BMP4 creates a negative feedback loop to regulating BMP function.

Recent evidence suggest that, like other members of the TGF- β family, there are co-receptors for the BMP ligand, which enhance the signaling of BMPs. DRAGON is the first co-receptor reported for BMP, and is a glycosylphosphatidylinositol-anchored member of the repulsive guidance molecule family. DRAGON binds directly to BMP2 and BMP4, but not BMP7 or other TGF- β ligands. The interaction between DRAGON and BMPs enhances the signaling and ultimately leads to a stronger biological response from the cell. Interestingly, this enhanced effect due to the DRAGON/BMP interaction can be reduced by the BMP2/4 antagonsit, Noggin (Samad et al., 2005). A homologue of the Dragon, repulsive guidance molecule (RGMa), has been identified as another co-receptor for BMPs (Babitt et al., 2005).

Regulation of intracellular signaling by BMP/BMP receptors

Inhibitory Smads

Once the BMP ligand binds to the BMP receptors, inhibitory Smad (Smad 6 and 7), Smad binding protein (Ski and Tob) and Smad ubiquitin regulatory factor (Smurf 1 and 2) can also regulate the intracellular signal

transduction of BMPs.

Smad 6 and 7 inhibit signal transduction of BMPs, by interference with the activation of Smad 1 and 5, which are phosphorylated by the BMP Type I receptor. BMP6/7 also inhibit the heterodimerization between Smad1/5 and Smad 4 (Hayashi et al., 1997; Imamura et al., 1997). There are also evidences to suggest that BMPs can adjust their signaling by up-regulating the expression of Smad 6 and 7 (Takase et al., 1998; Ishisaki et al., 1999).

Smad binding proteins

Smad binding proteins suppress BMP signaling by associating with the MH2 binding domain of Smads. Sloan-Kettering retrovirus (Ski) binds Smad 1, 2, 3, 5 and 4 and inhibits BMP signaling (Luo et al., 1999; Wang et al., 2000; Xu et al., 2000). The transducer of ErbB-2 (Tob) is probably associated with the MH2 domain of Smad 1, 5, 6, 7 and 8 (Yoshida et al., 2000, 2003).

Molecules that facilitate degradation of the Smads

Smurf 1 and 2 modulate TGF- β signaling by selectively targeting the receptors and Smad proteins for degradation and ubiquitination (Arora and Warrior, 2001). Smurf 1 can directly interact with Smad 1/5, and facilitate their degradation (Zhu et al., 1999). It can also indirectly interact with the BMP Type I receptor through

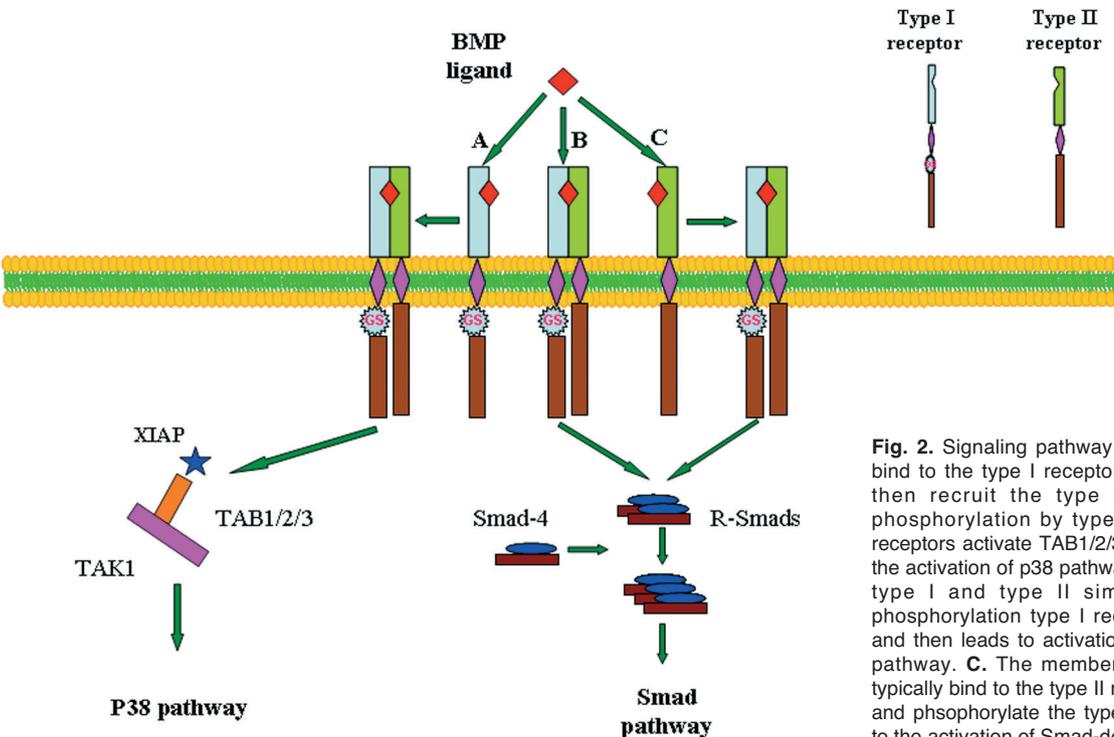


Fig. 2. Signaling pathway of BMPs. **A.** BMP ligand bind to the type I receptors with higher affinity and then recruit the type II receptors, after the phosphorylation by type II receptors, the type I receptors activate TAB1/2/3 through XIAP, leading to the activation of p38 pathway. **B.** BMPs ligand bind to type I and type II simultaneously, after the phosphorylation type I receptors recruit R-Smads, and then leads to activation of the Smad-dependent pathway. **C.** The members of TGF- β superfamily, typically bind to the type II receptors firstly, then recruit and phsophorylate the type I receptors, thus leading to the activation of Smad-dependent pathway.

BMP and prostate cancer

I-Smad 6 and 7, and induce ubiquitination and degradation of the receptors (Murakami et al., 2003). TNF has been shown to inhibit osteoblastic bone formation through upregulation of Smurf 1 and 2 (Kaneki et al., 2006).

NEDD4-2 (neural precursor cell expressed, developmentally down-regulated 4-2) was recently found to be a direct binding partner of Smad7 (Kuratori et al., 2005). NEDD4-2 is structurally similar to Smurfs 1 and 2 (Smad ubiquitin regulatory factors). It can interact with Type I receptor via Smad 7, and induce its degradation. It can also bind to Smad 2 and 3 in the ligand-dependent manner, and degrade Smad 2, but not Smad 3. Overexpression of NEDD4-2 inhibits the transcriptional activity induced by TGF- β and BMPs. Wicks and Haros et al. recently reported a novel ubiquitin: C-terminal hydrolase (UCH37). UCH37 is a deubiquitinating enzyme that can potentially reverse Smurf-mediated ubiquitination. It forms a stable complex with Smad 7, which deubiquitinates and stabilizes the type I TGF-beta receptor (Wicks et al., 2005). However, its role in BMP signaling remains unclear.

The associated molecule with the SH3 domain of STAM (AMSH) is a direct binding partner for Smad6 and has been found to inhibit the interaction between Smad6 and the activated BMP type I receptor, thereby allowing more efficient BMP receptor-induced phosphorylation of R-Smads. In addition, AMSH was found to interfere with the interaction between Smad6 and the activated R-Smad. Thus, AMSH promotes BMP signaling by negatively regulating the function of I-Smads (Itoh et al., 2001).

The expression of BMPs in prostate cancer

Bone metastasis is the most common metastatic site for prostate cancer. The interaction between prostatic tumour cells and bone contributes significantly to this organ tropism and the predominant osteoblastic

characteristics of prostate metastases. As a group of the most powerful bone inductive factors, the expression of the BMPs in prostate cancer has stimulated much interest since the early 1990s (Table 5).

Expression of BMPs in prostate cancer

The mRNA level of BMP 1-6 has been examined in prostatic tissue with benign hyperplasia, non-metastatic prostatic carcinoma and in metastatic prostatic carcinoma. BMP-6 expression was detected in the prostate tissues of over 50% of patients with clinically defined metastatic prostate adenocarcinoma, but was not detected in non-metastatic or benign prostate samples (Bentley et al., 1992). The initial finding suggests BMP-6 may have an important role to play in progression and the development of bone metastasis in prostate cancer. Therefore, both mRNA and the protein level of BMP-6 were assessed in subsequent studies. An elevated level of BMP-6 is associated with the higher grades of primary tumour and with advanced prostate cancer with metastasis. It also may contribute to the progression of prostate cancer in the absence of androgens (Barnes et al., 1995; Hamdy et al., 1997; Tamada et al., 2001).

In contrast to BMP6 which has an elevated level in more aggressive prostate cancers, BMP2/4, BMP7 and GDF15 are expressed predominantly in normal prostate tissue, and their expression tends to be lower or absent during progression of prostate cancer (Harris et al., 1994; Thomas et al., 2001; Horvath et al., 2004; Masuda et al., 2004). Expression of GDF15 is higher in the androgen-sensitive LNCaP cells than in the androgen-insensitive PC-3 and DU-145 cells. The level of GDF15 in prostate cancer cells was seen to rise after cells were exposed to androgens (Kakehi et al., 2004). Similarly, an androgen-dependent expression of BMP-7 was revealed in a mouse model (Thomas et al., 1998). This latter feature was subsequently identified in humans: the expression of BMP-7 in prostate epithelial cells increases in response to dihydrotestosterone (DHT)

Table 5. Level of expression of BMP signaling in prostate cancer.

Features	BMP-2	BMP-3	BMP-4	BMP-5	BMP-6	BMP-7	PLAB (GDF15, MIC)	BMPRIA	BMPRIB	BMPRII	Smad4	Smad8
Normal	+	+	++		±	++	++	++	++	++	++	++
Malignant	±	+	+	+	+	+	±	±	+	-	±	-
Malignant												
Lower grade	±				+	+	±	+	+	+	+	+
Higher grade	-				++	±	-	±	±	±	±	±
Primary tumour (localized)					+							
Primary tumour (metastatic)				++								
Primary tumour					+	+	±					
Bone metastasis				++	++	++						

Level of expression of BMP signaling in prostate cancer. Based on the literature published: BMP2 (Harris et al., 1994; Kim et al., 2000; Horvath et al., 2004); BMP4 (Harris et al., 1994); BMP5; BMP6 (Bentley et al., 1992; Harris et al., 1994; Barnes et al., 1995; Hamdy et al., 1997; Tamada et al., 2001); BMP7 (Masuda et al., 2004); PLAB (Thomas et al., 2001; Kakehi et al., 2004); BMPR1A (Kim et al., 2000); BMPR1B (Kim et al., 2000); BMPR2 (Kim et al., 2000; Kim et al., 2004); Smad4 (Horvath et al., 2004); Smad8 (Horvath et al., 2004).

(Masuda et al., 2004). Taken together, the loss or reduction in expression of BMP7 and GDF15 may be due to a shift in the tumour cell phenotype from androgen dependent to androgen independent in prostate cancer cells.

More interestingly, GDF15 and BMP-7, expression of which has been reduced or lost in primary tumours, can be re-expressed in metastatic bone lesions (Thomas et al., 2001; Masuda et al., 2003). This suggests that selected BMPs may play differential roles in primary tumours and secondary tumours.

BMPs signaling molecules in prostate cancer

The expression of BMPRIA, BMPRIIB, and BMPRII in human prostate cancer tissues has also been investigated and was found to correlate with tumour grade. Using immunohistochemistry and Western blot analyses, it was shown that there was frequent loss of expression of these three receptors in high-grade prostate cancer. But it appears that only the loss of expression of BMPRII has a correlation with poor prognosis in prostate cancer patients (Kim et al., 2000, 2004).

Intracellular signaling molecules downstream of the BMP receptors have also been shown to alter in prostate cancer. The level of Smad 4 and Smad 8 in the nuclei is thought to be associated with the development of prostate cancer, and loss of Smad 4 is related to progression to a more aggressive phenotype (Horvath et al., 2004).

Taken together, these findings support the hypothesis that the BMPs and their signaling pathways play a significant role in the osteoinductive activity of prostate cancer during metastases. The pattern of expression of BMPs may be important in the pathogenesis of osteoblastic-type metastases in prostate cancer.

The biological function of BMPs in prostate cancer

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

Effects of BMPs on growth and proliferation of prostate cancer cells

As shown in Figure 1, BMPs can be classified into sub-groups according to the homology of their protein sequence. An anti-proliferation function has been found in two of these BMP subgroups.

BMP-2 and 4 inhibit the growth of the androgen-sensitive prostate cancer cell line LNCaP, but not the

androgen-insensitive PC-3. The inhibitory effect of BMP-2/4 on cell proliferation is related to the activation of Smad 1, up-regulation of the cyclin-dependent kinase inhibitor (CDKI) p21 (CIP1/WAF1), and phosphorylation of retinoblastoma (Rb) (Brubaker et al., 2004). Further investigation shows that BMP-2 decreases the phosphorylation of retinoblastoma (Rb) protein induced by treatment with DHT. DHT promotes proliferation of LNCaP cells through induction of cyclin A and cyclin-dependent kinase 2 (CDK2) and the transcription factor E2F-1, which can be inhibited by co-treatment with BMP-2. This suggests that BMP-2 inhibits DHT-induced growth of LNCaP cells through a decrease in E2F protein expression and suppression of E2F activity by hypophosphorylation of Rb (Tomari et al., 2005).

On the other hand, another subgroup, BMP-6 and 7, are more likely to inhibit the proliferation of both androgen-sensitive and androgen-insensitive prostate cancer cells. BMP-6 inhibits the proliferation of both LNCaP and DU-145 cells, by up-regulation of several cyclin-dependent kinase inhibitors such as p21/CIP, p18 and p19. The inhibitory effect on cell growth by this subgroup can be either prevented by the application of the recombinant Noggin (Haudenschield et al., 2004), or by up-regulation of Noggin by BMP-6 itself, as discussed earlier in this article. Under certain culture conditions (1% Fetal bovine serum in culture medium), BMP-7 inhibits the proliferation of the LNCaP, PC-3 and DU-145 cells in a concentration-dependent manner (5-500ng/ml), possibly by the up-regulation of the cyclin-dependent kinase inhibitor (CDKI) p21^{CIP1/WAF1}. Interestingly, BMP7 can also induce a concentration-dependent biphasic effect on the proliferation of LNCaP in the absence of exogenous androgen. It promotes proliferation at lower concentrations (20 ng/ml), but inhibits proliferation at higher concentrations (80 ng/ml). Exogenous androgen can prevent this biphasic effect (Miyazaki et al., 2004). If the FBS concentration in the culture medium is reduced to 0.5%, the anti-proliferation effect of rh-BMP7 at a concentration of 50 ng/ml was only seen in the cell line representing benign prostatic epithelial hyperplasia (BPH-1), but not in the invasive PC-3 and DU-145 cells (Yang et al., 2005). Under *in vitro* conditions in which 10% FBS was supplied in the culture medium, BMP 6 did not alter the rate of cell growth of C4-2B or LuCaP 23.1 (Dai et al., 2005). C4-2B is a variant of LNCaP with a propensity for bone metastasis (Hsieh et al., 1993; Wu et al., 1994; Chen et al., 1998).

The nature of the diverse, sometimes contrasting effects of different BMPs is interesting, but the underlying mechanism(s) remain unclear. While BMPs themselves may hold some of the answers, BMP receptors are probably also determining factors in distinguishing between the pro- or anti-proliferation effects seen in prostate cancer cells. There is a good possibility that further understanding of the expression pattern of BMPRIA, BMPRIIB, and BMPRII and their

relationship to the development and progression of prostate cancer may provide more information on this phenomenon. For example, Kim et al. demonstrated that transfection of a domain negative BMP-RII (BMP-RIIDN) into PC-3 cells (PC3M), resulted in a growth rate 10 times higher than that in control cells in the murine tumour model (Kim et al., 2004). But once the PC-3 cells express a constitutively active BMPRII (c.a.-BMPRII) in a tetracycline (Tet)-regulated manner, the Tet/doxycycline-regulated expression of the c.a.-BMPRII results in the inhibition of both the *in vitro* cell proliferation and the tumour growth *in vivo* (Miyazaki et al., 2004).

A biphasic effect on the proliferation of LNCaP can be induced by rh-BMP2 under appropriate hormonal conditions. A decrease in cell proliferation in response to rhBMP2 was elicited in the presence of an androgen, which was thought to be the result of up-regulation of BMPRII expression. Conversely, an increase in cell growth was seen in the absence of androgen (Ide et al., 1997). Similar biphasic effects were also revealed in LNCaP on exposure to rh-BMP7 (Miyazaki et al., 2004). This may partially help to explain some other results where identical inhibition by BMP-2 and -4 on cell growth was not seen on another androgen-sensitive prostate cancer cell line: LuCaP 23.1 - because of the absence of exogenous androgen (Dai et al., 2005).

BMPs and apoptosis

Apoptosis or programmed cell death is the key event for physiological growth control and regulation of tissue homeostasis. Aberration in the rate of apoptosis plays a crucial role during oncogenesis and subsequent progression. BMPs have been shown to regulate the apoptosis of malignant cells. The apoptotic response to BMPs is dependent upon cell type and, within the same cell type, is dependent on phenotype, hormone and growth factors status, and *in vitro* culture condition. For example, BMP2 induces apoptosis in medulloblastoma cells and colonic epithelial cells (Hallahan et al., 2003; Hardwick et al., 2004), but prevents apoptosis in breast cancer cells (MCF-7) (Clement et al., 2000). On the other hand, in the myeloma cell lines, BMP4 can inhibit DNA synthesis and induce apoptosis in two IL-6 dependent myeloma cells (OH-2 and IH-1), but not the IL-6 independent ANBL-6 cells (Hjertner et al., 2001).

BMPs regulate the expression of the molecules involved in the procedure of apoptosis, forming a basis for the previous observations. BMPs can directly affect apoptosis by regulating the transcription of the caspases. For example, BMP2 partially prevents an increase in caspase-3 mRNA level in MCF-7 cells as the result of withdrawal of serum in cell culture (serum free culture condition) (Clement et al., 2000). The expression of the apoptosis mediators DRP-1 death kinase and ZIP kinase may be regulated by BMPs through the Type I receptor, as demonstrated by expressing constitutively active (ca)BMP type I receptors in the cells (Korchynski et

al., 2003). Senescent cells as the result of BMP4 treatment had lower ERK activation, VEGF expression, and Bcl2 expression than wild-type cells. This is consistent with a less proliferative, less angiogenic phenotype that has an increased susceptibility to death by apoptosis (Buckley et al., 2004).

BMPs alter apoptosis through several signaling pathways. BMP2 activates p38 mitogen-activated protein kinase (MAPK) pathway in medulloblastoma cells leading to apoptosis, which can be prevented by Noggin (Hallahan et al., 2003). BMP2 can also rescue MCF-7 cells from hypoxic cell death via activation of the MAPK ERK1/2 and the basic helix-loop-helix transcription factors Id-1 pathways (Raida et al., 2005). Anti-Mullerian hormone (AMH) is a member of the TGF- β family, which can trigger different pathways leading to inhibition of cell growth and apoptosis in various cell lines: it activates the ERK1/2 pathway in both breast and prostate cancer cell lines, but enhances p16 and modulates the E2Fs in ovarian cancer cell lines (Ha et al., 2000). GDF-15 is indispensable for the pro-apoptotic activity of several apoptosis-inducing agents including the retinoid-related molecules (CD437 and ST1926) (Xu et al., 2006).

Signaling mediated by BMPRII is believed to contribute to apoptosis during the development of the embryonic limb (Zou and Niswander, 1996). BMP4 induces apoptosis of myeloma cells through ALK3 and ALK6, BMP5 acts partially by ALK3, whereas BMP6 and 7 rely on ALK2 (Ro et al., 2004).

Recent studies have begun to uncover the role of BMPs in apoptosis in prostate cancer cells. BMP7 can stabilize the level of survivin in prostate cancer cells (LNCaP and C4-2B), and restore the activity of c-jun NH2-terminal kinase (JNK), both of which contribute to the anti-apoptotic activity of BMP7 (Yang et al., 2005, 2006).

Influence of BMPs on cellular motility and invasion

Invasion and metastasis are the major causes of cancer related mortality. The motility and invasiveness of cancer cells are some of the important determining factors regarding the metastatic spread of a tumour. Recent evidence demonstrates that BMPs also regulate cellular motility and the invasiveness of some malignant cells, including lung cancer cells (A549 and H7249), malignant melanoma cells, and breast cancer cells (MCF-7) (Langenfeld et al., 2003; Clement et al., 2005; Rothhammer et al., 2005). Similar to the diverse effects on cell growth, the effect on cell motility also appears to be dependent on the particular BMP and the type of tumour cell. For example, recombinant human BMP-2 can inhibit the invasion and metastasis of a rat brain glioma cells under certain conditions (Zhang et al., 2002).

In the case of prostate cancer cells, studies have revealed that the motility and invasiveness of prostate cancer cells can be increased by the BMPs. BMP-2 and

BMP-7 promote the migration and invasion of osteoblastic prostate cancer cells (LAPC-4 and LAPC-9) in a dose-dependent manner, but BMP-4 does not have this effect (Feeley et al., 2005). BMP-2 and BMP-6 can increase the *in vitro* invasive ability of the prostate cancer cell lines C4-2B and LuCaP (Dai et al., 2005). BMP-2 and, to a lesser extent, BMP-4 will stimulate PC-3 cell migration and invasion in a dose-dependent fashion, an effect which Noggin can subsequently inhibit (Feeley et al., 2006).

Further details of the underlying pathways and precise mechanisms of BMP induced cell migration and invasion await further investigation. However, in addition to the receptors implicated in the previous discussion, BMP2 has been proven to activate the P38 MAPK and JNK signaling pathway to induce the expression of tenascin-W in tumour stroma, an effect that was thought to contribute to the stimulation of migration of tumour cells. Tenascin-W belongs to a family of extracellular matrix glycoproteins with distinctive expression patterns. Higher expression in the stroma around tumours is associated with a high potential for development of metastases (Scherberich et al., 2005).

Epithelial homeostasis and epithelial-mesenchymal transdifferentiation

The BMP pathway is important in epithelial homeostasis. Inactivating mutations of the Smad4 and BMPR-IA genes account for up to 50% the juvenile polyposis syndrome (JPS). This is an autosomal hamartoma syndrome with high risk of developing gastrointestinal polyps and colorectal cancer (Radtke and Clever, 2005). The concurrent inactivation of the BMPs (BMP-3b and BMP-6) with activation of the Ras signalling pathways is important in lung carcinogenesis (Kraunz et al., 2005). These suggest that an aberration of BMP signaling in the epithelia can result in a disturbance of epithelial homeostasis, which may lead to carcinogenesis.

Epithelial-mesenchymal transdifferentiation/transformation (EMT) is an important event during the development of certain pathological processes including cancer. In normal development, BMP-2 acts synergistically with TGF- β 3 in the initiation of EMT during the generation of the endocardial cushion (Nakajima et al., 2000). The application of the BMP antagonist, Noggin, disrupts the EMT induced by BMPs during the development of the chicken heart (Romano and Runyan, 2000). The EMT induced by BMP-7 contributes to the repair of tubular injury in a fibrotic kidney (Zeisberg et al., 2003, 2005).

An aberration of BMP signaling will not only impair epithelial homeostasis which may lead to carcinogenesis, it may also induce EMT in prostate cancer cells leading to a more aggressive phenotype. In the bone metastasis-derived PC-3 prostate cancer cell line, BMP7 has been shown to induce epithelial-mesenchymal trans-

differentiation with classical changes in morphology, and the promotion of both motility and invasiveness in prostate cancer cells (Yang et al., 2005).

Our understanding of the mechanisms underlying BMP-induced EMT is poor. The induction of Inhibitor of differentiation factors (ID-1, ID-2 and ID-3), and activation of the proto-oncogene phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signaling pathway by some BMPs (BMP-2 and BMP-7) has been implicated in BMP-induced EMT, but further exploration is required (Ogata et al., 1993; Kowanetz et al., 2004; Langenfeld et al., 2005).

Taken together, these findings suggest that BMPs are able to modulate the biological behaviour of prostate tumour cells in diverse ways and in a cell specific manner, and point to certain mechanisms by which these secreted signaling molecules may contribute to prostate cancer growth and metastasis.

BMP and bone metastasis

Bone is the most common site of metastasis for certain tumour types. Prostate cancer, breast cancer and lung cancer all preferentially spread to bone (Mundy, 2002). Bone metastases can generally be categorised as osteoclastic (osteolytic) leading to bone destruction, or osteoblastic (osteosclerotic) leading to bone formation. The osteoblastic lesion is the predominant form of bone metastasis formed by prostate cancer, and is always associated with a significant increase in tumour-induced osteolysis (Demers et al., 1995, 2003; Garnero et al., 2000). There are a number of factors in the bone microenvironment known to contribute to the development of bone metastasis (Fig. 3). Well documented factors include endothelin-1, BMPs, prostaglandins, TNF α , IL-1, IL-6, PTHrP, PDGF and RANKL (Yin et al., 2005; Yoneda and Hiraga, 2005). Some interesting patterns have been reported recently, by comparing the expression profile of certain genes in two prostate cancer cell lines that develop different bone metastasis: PC-3 which induces osteoclastic lesions *in vivo*, and LAPC-9 which produces osteoblastic lesions. PC-3 cells expressed RANKL, IL-1, and TNF-alpha, which are associated with osteoclastogenesis. In contrast, LAPC-9 cells showed expression of osteoprotegerin, which blocks osteoclast production, results in minimal amounts of TNF-alpha and undetectable levels of RANKL or IL-1. Both these cells also secreted BMP-2, -4, -6, and IL-6, which are associated with bone formation (Lee et al., 2003).

The role of BMPs in bone malignancies has been investigated in several primary and secondary malignant deposits in bone. BMP-2 is expressed in more than half of osteosarcomas, but not in chondrosarcomas or Ewing's sarcomas (Yoshikawa et al., 1994). The expression of the BMP type II receptor was found to correlate with the presence of metastases in osteosarcomas, which implicates the BMP pathway in tumor progression. BMP-2, -4, and -6 also are expressed

BMP and prostate cancer

in osteosarcoma and other sarcoma tissues, indicating a potential for autocrine or paracrine growth stimulation in these tumors (Guo et al., 1999).

Adaptable expression of BMPs in bone metastases from prostate cancer

As discussed earlier, a specific expression pattern of BMPs (BMP-2, 4, 7 and GDF15) is associated with disease progression in primary prostate cancer. The role of BMP-2, 4, 6 and 7 in bone metastases from clinical prostate cancer has also been investigated. It was found that BMP-7 and GDF15 are re-expressed in bone metastasis (Masuda et al., 2003), suggesting that these molecules contribute to the development of metastatic bone lesions (Fig. 3). This extends their pivotal roles in primary tumor development to include secondary tumour formation in the bones.

On the other hand, the expression of BMP-6 is consistently elevated in both primary and secondary tumours of prostate cancer (Autzen et al., 1998; De Pinieux et al., 2001). The elevated expression of BMP-6 within the primary tumor may be due to selective demethylation of its gene promoter during disease progression, which results in tumour cells with a more aggressive phenotype (Tamada et al., 2001).

Role of BMPs in the crosstalk between cancer cells and the bone micro-environment

From the moment a metastatic cell settles in the bone, there is constant interaction between the tumour cell and its residing microenvironment. Host factors from the bone environment and factors generated by the cancer cell exhibit a reciprocal influence over each other, the BMPs secreted by the cancer cells would certainly

influence remodeling of the bone, including osteoblastic and osteoclastic activity. However, it remains unclear as to how exactly the local factors participate in the regulation of BMP expression in the prostate cancer cells.

BMPs that are normally enriched in the bone environment, not only promote the motility and invasion of cancer cells, they are also able to induce the expression of other growth factors, which enhances the vicious circle of bone-tumour-bone interactions. A few links have been documented in recent years. For example, BMP-2 is able to stimulate a 2.7-fold increase in osteoprotegerin (OPG) expression in PC-3 cells which inhibits osteoclastogenesis (Brubaker et al., 2004), and BMP-7 induces VEGF protein and mRNA expression in C4-2B cells, which contributes to the pro-osteoblastic activity of C4-2B cells.

BMPs and angiogenesis

Angiogenesis is an important event during the development and progression of both primary and secondary tumours. It has been demonstrated that BMPs, including BMP-2, 4, 6, 7 and GDF5, are capable of inducing angiogenesis. This may be one of the ways in which they contribute to the process of bone formation (Yamashita et al., 1997; Mori et al., 1998; Yeh and Lee, 1999; Glienke et al., 2000). The experimental evidence suggests that BMPs promote angiogenesis indirectly through up-regulation of the expression of VEGF in both cancer cells and osteoblasts: since Noggin, the BMP antagonist, produces the same effect as anti-VEGF antibody: it diminishes the pro-osteoblastic activity of osteoblast cells which is induced by conditioned medium from C4-2B cells (Yeh and Lee, 1999; Deckers et al., 2002; Dai et al., 2004). Also, the early stage of bone

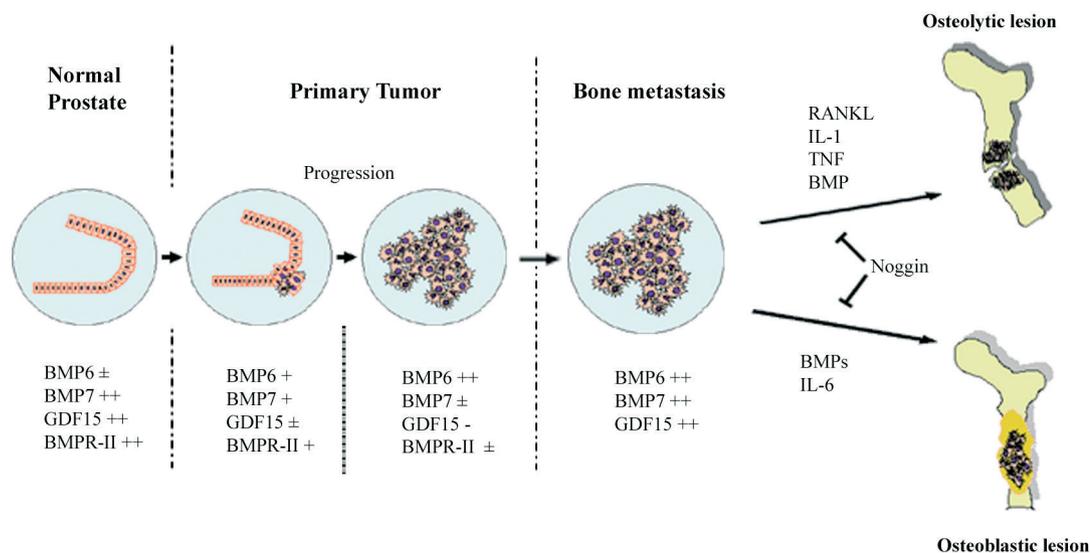


Figure 3. BMPs in the bone metastasis of prostate cancer.

induction by rhBMP-2 can be blocked by the anti-angiogenic agent(TNP-470) (Mori et al., 1998). This evidence indicates that the control of angiogenesis is, to some extent, integrated with the influence which BMPs have over osteoblastic activity.

Dai et al. have demonstrated that it is possible for BMP-7 to promote osteosclerosis through VEGF in the skeletal metastases from prostate cancer (Dai et al., 2004). This angiogenesis induced by BMPs can also be synergized by basic fibroblast growth factor (bFGF) and TGF- β 1 (Ramoshebi and Ripamonti, 2000), which suggests that the angiogenesis induced by BMPs is a vital event during the initial stage of bone metastasis development.

Regulation of the expression of BMPs

BMP and androgen

In prostate cancer, androgens play an important part in the carcinogenesis, progression and metastasis of the disease, and controlling the level of circulating androgens constitutes the only effective therapy in advanced disease. Androgen-deprivation therapy (ADT) by orchidectomy or an Leutenising hormone releasing hormone (LHRH) agonist is the mainstay of treatment for advanced prostate cancer and is becoming more accepted as an effective treatment for locally advanced disease (Labrie, 2004). Prostate cells are highly hormone-sensitive until late in the disease. Depriving androgen-sensitive cells of testosterone results in tumour cell apoptosis, a consequent reduction in tumour size and a delay in symptomatic progression. In most patients, the development of androgen resistance to hormonal therapy is the harbinger of disease specific mortality (Klotz, 2000).

Androgens can induce the expression of some BMPs, BMP receptors and intracellular signaling molecules. With regard to the receptors, androgens induce the expression of BMPR-IB mRNA, but not the expression of BMPR-IA and BMPR-II mRNAs in the androgen-sensitive human prostate cancer cell line LNCaP. As discussed above, rh-BMP-2 induces a biphasic effect on the proliferation of LNCaP. In the presence of an androgen, there is a decrease in cell proliferation in response to rhBMP2. This is thought to be the result of up-regulation of BMPR-IB expression. Conversely, an increase in cell growth is seen in the absence of androgen. Thus, the induction of BMPR-IB expression by an androgen appears to result in a signal which inhibits cell proliferation in response to stimulation by BMPs (Ide et al., 1997).

Turning to the proteins themselves, the level of BMP-7 mRNA can be affected by manipulating the level of androgens in a murine model. Orchidectomy resulted in a decrease in the expression of BMP-7 and administration of testosterone or dihydrotestosterone caused an increase in the expression level (Thomas et al., 1998). However, androgen deprivation appears to

have no effect on BMP-6 production in the normal rat prostate, suggesting an alternative and androgen-independent gene regulation for this particular protein (Barnes et al., 1995).

BMP and DNA methylation

During cancer progression, besides the known inactivation of tumor suppressor genes by hypermethylation, activation of BMP-6 by selective demethylation occurs and may also contribute to the shift to a more aggressive phenotype in prostate cancer (Tamada et al., 2001). Aberrant methylation of BMP-2 has also been recorded, and the resultant loss of BMP-2 expression has been implicated in the carcinogenesis of gastric tumours (Wen et al., 2006).

Kitazawa et al, also noted abnormal methylation in an unusual case of desmoid-type fibromatosis which had features of metaplastic bone development. They observed that hypermethylation of the BMP and activin membrane-bound inhibitor (BAMBI) promoter had caused a decreased expression of the BAMBI gene, which resulted in an enhanced responsiveness to BMP signaling and thus the abnormal bone formation (Kitazawa et al., 2005).

Other regulatory factors

There are several other growth factors involved in the regulation of the BMPs. Nacamuli et al. demonstrated that BMP3 expression can be controlled by recombinant human fibroblast growth factor in calvarial osteoblasts (Nacamuli et al., 2005). EGF can also influence BMP expression. The expression of BMP6 has been shown to be reduced in breast cancer tissues, a reduction accompanied by a concurrent reduction in EGF receptor expression. The relation between BMP6 expression and EGF was further confirmed by the inductive expression of BMP6 in breast cancer cells (MCF-7) in vitro by EGF through EGF receptor activation (Clement et al., 1999). Retinoid induces expression of BMP2 in the retinoid-sensitive cell lines (Hallahan et al., 2003), and Rapamycin induced BMP4, and reduced follistatin expression in PC3 cells, which contributes to its anticancer effect (van der Poel et al., 2003).

Conclusion

The phenotypic pattern of BMPs and their signaling pathways in prostate cancer are different in the primary tumor compared to that in secondary deposits in bone. Most BMPs and receptors are detectable at a relatively higher level in normal prostate tissue. Their expression decreases in a manner which correlates with progression of the primary tumor, except BMP6, which shows an increase. The expression of BMP7, GDF15 and BMPR-IB can be induced by exposure to androgens in the androgen-sensitive prostate cancer cell lines and the

prostate epithelial cell lines (Ide et al., 1997; Thomas et al., 1998; Tamada et al., 2001; Kakehi et al., 2004). Aberrant expression of BMP and BMP associated molecules have also been shown to have prognostic value (Kim et al., 2004). The modification of the BMP phenotype is clearly closely involved with the development and progression of primary prostate tumors, and it also contributes to the onset and development of bone metastases. For example, BMP6 remains highly expressed in both primary and metastatic bone lesions, whereas BMP7 and GDF15, which are expressed at low levels in normal prostate and in primary prostate tumours, are re-expressed at a higher level in skeletal metastatic lesions (Thomas et al., 2001; Masuda et al., 2003). BMPs not only contribute to the development of an osteoblastic-type lesion in bone metastases from prostate cancer, they are also partially responsible for the osteolytic appearance of occasional lesions. In the *in vivo* bone tumor model, exposure to Noggin, an antagonist of BMPs, can reduce the size of bone lesions by decreasing both osteoblastic and osteolytic processes. These findings collectively indicate a promising therapeutic value for BMPs and their antagonists in the management of bone metastases.

BMPs clearly play profound roles in the development, progression, and metastasis of prostate cancer. If the mechanisms underlying the adaptation of the BMP phenotype in both primary tumor and bone metastasis can be elucidated, it will provide a greater understanding of the pathogenesis of prostate cancer and may provide clues for novel therapies for managing advanced disease.

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BMP and prostate cancer

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