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Bone Morphogenetic Proteins and Their Antagonists in Skin and Hair Follicle Biology*

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Bone morphogenetic proteins (BMP) are members of the transforming growth factor- β superfamily regulating a large variety of biologic responses in many different cells and tissues during embryonic development and postnatal life. BMP exert their biologic effects via binding to two types of serine/threonine kinase BMP receptors, activation of which leads to phosphorylation and translocation into the nucleus of intracellular signaling molecules, including Smad1, Smad5, and Smad8 ("canonical" BMP signaling pathway). BMP effects are also mediated by activation of the mitogen-activated protein (MAP) kinase pathway ("noncanonical" BMP Signaling pathway). BMP activity is regulated by diffusible BMP antagonists that prevent BMP interactions with BMP receptors thus modulating BMP effects in tissues. During skin development, BMPs its receptors

and antagonists show stringent spatiotemporal expressions patterns to achieve proper regulation of cell proliferation and differentiation in the epidermis and in the hair follicle. In normal postnatal skin, BMP are involved in the control of epidermal homeostasis, hair follicle growth, and melanogenesis. Furthermore, BMP are implicated in a variety of pathobiologic processes in skin, including wound healing, psoriasis, and carcinogenesis. Therefore, BMPs represent new important players in the molecular network regulating homeostasis in normal and diseased skin. Pharmacologic modulation of BMP signaling may be used as a new approach for managing skin and hair disorders. *Key words: BMP/Noggin/proliferation/differentiation/apoptosis J Invest Dermatol 120:36–47, 2003*

Bone morphogenetic proteins (BMP) are the largest family of secreted signaling molecules of the transforming growth factor (TGF)- β superfamily. BMP have been first isolated from bone extracts and displayed ability to induce ectopic bone formation and to heal bone defects in experimental animals (Wozney *et al*, 1988; Reddi, 1998). It was later shown, however, that BMP function as multifunctional regulators of vertebrate development controlling cell proliferation, differentiation, and apoptosis in different body tissues, including skin (reviewed in Hogan, 1996a, b; Massague and Chen, 2000). Furthermore, increased evidence suggests that

during postnatal life, BMP play important parts in normal tissue homeostasis and are also implicated in a variety of pathobiologic processes (reviewed in Myazono *et al*, 2001).

Currently, the BMP family consists of more than 20 secreted proteins that share structural homology exerting their biologic activity via interaction with specific BMP receptors (Massague, 1998; Itoh *et al*, 2000; Myazono *et al*, 2001). While showing a certain structural similarity to other members of TGF- β superfamily, BMP fulfill a large number of distinct specific functions in animals and humans as shown by genetic and experimental studies. In this review, we summarize data on the roles for BMP in the biology of normal and diseased skin. Specifically, we show that BMP function not only as powerful regulators of cutaneous development, but also play important role: in the control of epidermal homeostasis, hair follicle growth, and melanogenesis in normal postnatal skin. Furthermore, we show that BMP are involved in the control of a variety of pathobiologic processes in postnatal skin, including wound healing, psoriasis, and carcinogenesis.

STRUCTURE OF BMP AND THEIR RECEPTORS

BMP are synthesized as precursor molecules, which are cleaved by subtilisin-like proteases to release a carboxy-terminal

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Abbreviations: BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; Shh, Sonic Hedgehog.

Table I. Structure of the selected members in the bone morphogenetic protein family^{a,b}

BMP subfamily	Designation (generic name)	Structure	% identity (human/mouse)
BMP2/4	BMP-2 (BMP-2A)	114 aa	100
	BMP-4 (BMP-2B)	116 aa	98
BMP-3	BMP-3 (Osteogenin)	110 aa	98
	BMP-3B (GDF-10)	110 aa	99
BMP-7/OP-1	BMP-5	138 aa	96
	BMP-6 (Vegetal related-1)	139 aa	96
	BMP-7 (Osteogenic protein-1)	139 aa	98
	BMP-8 (Osteogenic protein-2)	139 aa	94
	BMP-8B (Osteogenic protein-3)	139 aa	100
BMP-9/ BMP-10	BMP-9 (GDF-2)	110 aa	95
	BMP-10	108 aa	100
BMP-11/GDF-8	BMP-11 (GDF-11)	109 aa	100
	GDF-8	109 aa	100
BMP-12/BMP-13/BMP-14	BMP-12 (GDF-7)	104 aa	98
	BMP-13 (GDF-6)	120 aa	99
	BMP-14 (GDF-5)	120 aa	98
BMP-15	BMP-15	125 aa	71

^aTable summarizes data on the molecular structure of BMP family members obtained from the original publications and reviews (Wozney *et al.*, 1988; Feng *et al.*, 1994; Hogan, 1996b; Reddi, 1998; Gamer *et al.*, 1999; Otsuka *et al.*, 2001).

^bBMP-1 is not a member of BMP superfamily and is a cysteine-rich peptidase, which cleaves BMP antagonist chordin (Piccolo *et al.*, 1997).

monomer (Constam and Robertson, 1999). Each BMP monomer consists of approximately 100–140 amino acids (Celeste *et al.*, 1990; Reddi, 1998). Two disulfide-linked monomers form biologically active homo- or heterodimers, which are subsequently released to the extracellular matrix to interact with BMP receptors (BMPR). In mammals, BMP structure is highly conserved and comparison of mature regions of human and mouse BMP reveals in most cases 90–100% homology (Reddi, 1998). Based on amino acid sequence homology, the BMP superfamily is divided into several subgroups (Table I), all of which share a motif containing six cysteine residues and forming a rigid structure (cysteine knot) at the base of every mature monomer (Reddi, 1998).

BMP bind and activate cell signal transduction through a transmembrane receptor complex formed by type I and type II receptors (reviewed in Massague and Chen, 2000; Myazono *et al.*, 2001; Fig 1). Individual type I (BMPR-IA and BMPR-IB) and type II (BMPR-II) receptors have a low affinity for BMP, while the heterotetrameric complex of type I and type II receptors show high-affinity binding to BMP dimers. Both types of receptors contain intracellular serine/threonine kinase domains, and the type II receptor kinases are constitutively active without BMP stimulation. However, type II receptors are not capable of initiating signaling independently. Ligand binding to the heterotetrameric complex of type I and type II receptors results in phosphorylation of the intracellular domain of type I receptor by type II receptor kinases and leads to the transmission of the intracellular signal through “canonical” and “noncanonical” BMP pathways.

BMPR-IA (or activin-like kinase-3, ALK-3) and BMPR-IB (or ALK-6) are both 64 kDa transmembrane glycoproteins that show 98% and 96% homology in humans and mice, respectively (ten Dijke *et al.*, 1994a,b). Human BMPRII is an 80 kDa glycoprotein with 97% homology to murine BMPRII (Beppu *et al.*, 1997). In most cases, BMPR-IA and BMPR-IB form a heterotetrameric complex with BMPRII. There is a possibility, however, for other receptors of TGF- β superfamily (activin receptor type I or ALK-2, activin receptor type II, TGF- β receptor type II) to be a part of the BMPR complex and bind certain BMP (BMP2, BMP6, BMP7) (Itoh *et al.*, 2000; Myazono *et al.*, 2001). Specificity in binding BMPR complex appears to be determined by the type I re-

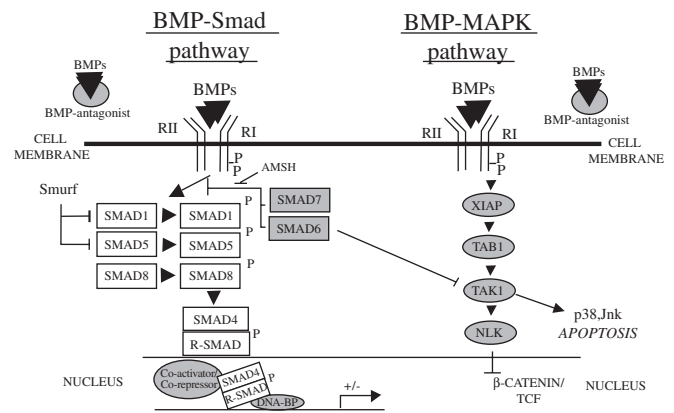


Figure 1. Schematic representation of the “canonical” BMP–Smad pathway and “noncanonical” BMP–MAPK pathway (modified from von Bubnoff and Cho, 2001). BMP interactions with BMPR complex is regulated by diffusible BMP antagonists that prevent BMP binding to BMP receptors. Binding of the free BMP to BMP receptor complex activate either BMP–Smad or BMP–MAPK signal transduction pathways. The BMP–Smad pathway includes recruitment and phosphorylation of R–Smad followed by the formation of their complexes with Co–Smad and translocation into the nucleus to regulate gene transcription. BMP–MAPK pathway via recruitment of XIAP and TAB1 links BMP receptors with TAK1 kinase, which in turn may activate apoptosis via p38/Jnk pathway.

ceptor, while type II receptor is more important for the activation of signal transducing mechanisms (Liu *et al.*, 1995; Massague, 1998). BMP signaling may be blocked by the pseudo-receptor BAMBI (BMP and activin membrane bound inhibitor), a transmembrane protein with extracellular domain similar to that of type I BMP receptors (Onichtchouk *et al.*, 1999).

Similar to many other growth factor families, BMP signaling is regulated on several levels: (i) at the cell surface, by modulating BMP binding to the BMPR complex; (ii) at the cytoplasm, by activating or inhibiting BMP signal transduction pathways; (iii)

in the nucleus, by controlling transcription of BMP target genes. Below, we summarize molecular mechanisms that underlie each level of regulation of BMP signaling in cells.

MODULATION OF BMP ACTIVITY BY SECRETED BMP ANTAGONISTS

The activity of BMP at the cell surface is modulated by a number of secreted BMP antagonists that prevent BMP binding to BMP receptors. BMP antagonists (noggin, chordin, follistatin, cerberus/DAN family of proteins) belong to structurally distinct protein families (reviewed in Massague and Chen, 2000). All of these proteins, however, bind members of the BMP family with affinity higher than the BMPR complex, thus restricting BMP activity to the tissue compartments that are free of BMP antagonists (**Fig 2**). Also, some BMP antagonists are able to bind simultaneously BMP and other growth factors and institute a balance of growth regulators in the tissue (Perrimon and McMahon, 1999).

Noggin is 222 amino acid protein that binds BMP-2 and BMP-4 with affinity 10–15 times higher than BMP receptors and neutralizes the activity of BMP-2 and BMP-4 (Zimmerman *et al*, 1996). Noggin also binds with lower affinity to BMP-7. It was recently shown that noggin, remaining active as BMP-neutralizing protein, has a strong affinity to heparan sulfate proteoglycans, which are abundantly expressed on the surface of all adherent cells and in the extracellular matrix (Paine-Saunders *et al*, 2002). Thus, heparan sulfate proteoglycans may regulate the tissue distribution of the BMP antagonist noggin and restrict cellular responsiveness to BMP. Interestingly, BMP are capable of inducing noggin expression and initiating a negative feedback loop limiting their own activity (Gazzerro *et al*, 1998).

Chordin is a 120 kDa protein, which binds to BMP2 and BMP4 with affinity approximately 10 times lower, than noggin (Piccolo *et al*, 1996; Zimmerman *et al*, 1996). The recently discovered chordin-like protein also interacts with BMP and, in addition, display a weak binding to TGF- β (Nakayama *et al*, 2001). Chordin in the complex with BMP may be cleaved by BMP-1 metalloproteinase with subsequent release of biologically active BMP (Piccolo *et al*, 1997). BMP-1 metalloproteinase, however, cannot degrade noggin (Wardle *et al*, 1999). This suggests that regulation of BMP interactions with BMP receptors is a complex mechanism, including not only neutralization of the extracellular pool of BMP by secreted BMP antagonists, but also

a degradation of selected BMP antagonists by extracellular metalloproteinase.

Follistatin is 35 kDa protein that binds activin, a member of TGF- β superfamily, with high affinity preventing its interaction with activin receptors (Patel, 1998). Activin exerts a large number of biologic responses, which are distinct from BMP effects on tissues (reviewed in Munz *et al*, 2001). Follistatin can bind BMP-2, BMP-4, BMP-7, and BMP-15 with low affinity and subsequently modulate their interactions with the BMPR complex (Fainsod *et al*, 1997; Patel, 1998; Otsuka *et al*, 2001). It has recently been shown that a novel follistatin-like protein can simultaneously bind activin and BMP-2 (Tsuchida *et al*, 2000). Thus, both follistatin and follistatin-like protein, whereas inhibiting interactions of activin and BMP with their corresponding receptors, may coordinate their effects in tissues.

The DAN/cerberus family of proteins includes DAN, gremlin, cerberus, and Caronte, all of which contain a cystein-rich region responsible for binding to BMP (Massague and Chen, 2000). Proteins of this family can neutralize not only BMP activity, but also several other growth factors. Cerberus functions as a multivalent growth factor antagonist that interacts in the extracellular space with BMP, Wnt proteins, and the TGF- β family member Nodal via distinct sites in its molecule (Piccolo *et al*, 1999). This suggests that distinct combinations of locally secreted BMP antagonists may establish a balance or gradient of growth factors and optimize their local concentrations sufficient for induction or suppression of certain biologic effects in different tissue compartments (**Fig 2**).

INTRACELLULAR BMP SIGNALING CASCADE

Increased evidence suggests that BMP after binding to BMP receptor complex activate at least two signal transduction pathways: “canonical” signaling pathway that includes the Smad family of proteins and “noncanonical” BMP–mitogen-activated protein kinase (BMP–MAPK) pathway (**Fig 1**). The “canonical” BMP–Smad signaling pathway has been intensively characterized during the last decade (reviewed in Heldin *et al*, 1997; Massague, 1998; Itoh *et al*, 2000; Myazono *et al*, 2001). In the last few years, it has been shown that many effects of BMP are also mediated by the BMP–MAPK pathway that appears to operate independently of the BMP–Smad pathway (reviewed in von Bubnoff and Cho, 2001). Recent data suggest that the distinct activation of these two pathways depends on the mode of ligand binding to BMP receptors. Binding to preformed receptor complexes induces signal transduction via the BMP–Smad pathway, whereas binding to the BMPR-I with subsequent recruitment of BMPR-II activates the BMP–MAPK pathway (Nohe *et al*, 2002). Below, we review the mechanisms that are involved in BMP signal transduction through the BMP–Smad and BMP–MAPK pathways.

BMP–Smad (“canonical”) pathway Following BMP binding to the heteromeric BMPR complex, BMPR-II phosphorylates the glycine/serine-rich domain of the type I receptors, which in turn phosphorylates specific Smad proteins (**Fig 1**). In mammals, the Smad family consists of eight proteins structurally related to the *Drosophila* Mad (Mother against decapentaplegic) protein (reviewed in Raftery and Sutherland, 1999). Smad1, Smad5, and Smad8 (or receptor-activated Smad, R-Smad) are each phosphorylated by BMPR-I kinases and then form heteromeric complexes with Smad4 (or common-partner Smad, Co-Smad). R-Smad/C-Smad complex subsequently translocates to the nucleus to regulate transcription of BMP responsive genes (**Fig 2**). Smad2 and Smad3, which also belong to the R-Smad subfamily, are activated by ActRI and TGF- β RI kinases and do not appear to be mediators of the BMP signaling pathway (Moustakas *et al*, 2001).

Smad6 and Smad7 (or inhibitory Smad, I-Smad) antagonize the phosphorylation of Smad1, Smad5, and Smad8 by BMPR-I kinases. Also, Smad6 might inhibit BMP signaling by

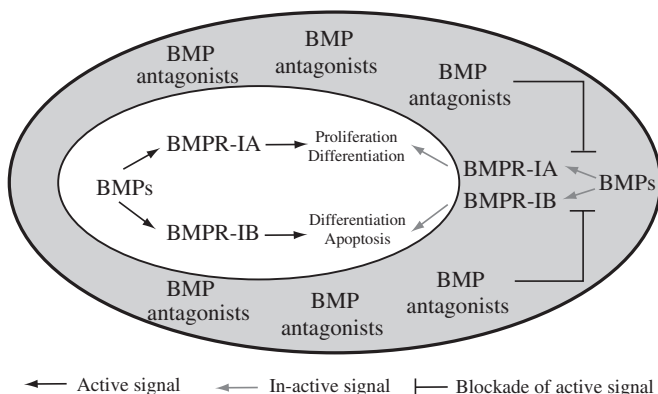


Figure 2. Scheme illustrating the regulation of BMP activity in tissue by the gradient of secreted BMP antagonists. BMP antagonists (noggin, chordin, follistatin, cerberus/DAN family of proteins) bind members of the BMP family with affinity higher than the BMPR complex, thus preventing BMP interaction with BMP receptors (as indicated at the peripheral part of the scheme). Thus, BMP activity is restricted to the tissue compartments that are free of BMP antagonists (as indicated at the central part of the scheme).

competing with Smad4 for binding to Smad1 (Hata *et al*, 1996). Interestingly, Smad1/Smad5 and Smad3 can induce the expression of Smad6 and Smad7, respectively, suggesting a negative feedback loop in "canonical" BMP signaling pathway (Nagarajan *et al*, 1999; Ishida *et al*, 2000). Antagonistic effects of Smad6 in the BMP–Smad pathway may be blocked by the intracellular adaptor protein AMSH, which directly binds Smad6 and interferes with the interactions between Smad 6 and R-Smad (Itoh *et al*, 2001). Basal levels of R-Smad and their availability for BMP signaling is regulated by Smurf1 (Smad ubiquitination regulatory factor-1), a protein that interacts with Smad1 and Smad5 independently of their phosphorylation status to promote their degradation in proteosomes (Zhu *et al*, 1999).

Both R-Smad and Smad4 display domains with similar amino acid sequences at the N- and C-terminal, called MH1 and MH2, respectively. After translocating into the nucleus, the complex of Smad4 and one of the R-Smad (Smad1 or Smad5) binds DNA through MH1 domains that contain specific DNA-binding sequences (GCCG for Smad1 and TGTGC for Smad5) (Kusanagi *et al*, 2000; Li *et al*, 2001). Transcription of BMP target genes is regulated positively or negatively depending on which coactivator(s) or corepressor(s) interact with MH2 or MH1 domains of the R-Smad/Smad4 complex.

During the last few years, many transcriptional regulators that bind Smad in the nucleus have been identified (reviewed in Itoh *et al*, 2000; Myazono *et al*, 2001; ten Dijke *et al*, 2002). The general transcription coactivator p300, OAZ zinc finger transcription factor, and SMIF (Smad4-interacting protein) activate BMP-mediated transcription (Nakashima *et al*, 1999; Pearson *et al*, 1999; Hata *et al*, 2000; Bai *et al*, 2002). Ski protein directly interacts with the MH2 domain of Smad1 and Smad5 and operates as transcriptional repressor of BMP–Smad signaling (Wang *et al*, 2000b). Also, other cofactors, Smad nuclear interacting protein-1 or SNIP1 and anti-proliferative protein Tob, both may negatively regulate BMP–Smad transcription (Kim *et al*, 2000; Yoshida *et al*, 2000).

BMP–MAPK ("noncanonical") pathway It has recently been shown that the activated BMPR complex may interact with intracellular adaptor protein XIAP, which links BMP receptors with TAB1 (TAK1 binding protein) that, in turn, activates TAK1 (TGF- β activated kinase 1) (Yamaguchi *et al*, 1999). TAK1 is a member of MAPK kinase family, whose activity is stimulated by TGF- β 1 and BMP4 (Fig 1). Subsequently, TAK1 activates a distantly related MAP kinase family member Nemo-like kinase (NLK), which has been shown to inhibit phosphorylation of TCF-1/Lef-1 transcription factors and downregulate Wnt/ β -catenin-dependent transcription (Ishitani *et al*, 1999). Also, it was shown that TAK1

activates p38 and JNK pathways, which are involved in BMP-induced apoptosis (Kimura *et al*, 2000; Zhang *et al*, 2000). Interestingly, Smad6 has been shown to bind and inhibit TAK1 activity (Kimura *et al*, 2000), suggesting links between BMP–Smad and BMP–MAPK pathways.

BMP ROLES DURING DEVELOPMENT

Morphologic and genetic studies have demonstrated the essential roles for BMP in embryonic development. BMP, BMP receptors, and R-Smad are broadly expressed in a large variety of developing tissues and organs (Lyons *et al*, 1989, 1990; Bitgood and McMahon, 1995; Takahashi and Ikeda, 1996; Dick *et al*, 1998; Flanders *et al*, 2001). Loss-of-function mutations of *BMP-2*, *BMP-4*, *BMPR-1A*, *Smad1*, *Smad4*, and *Smad5* lead to early embryonic lethality due to multiple defects in developing mesoderm, ectodermal, and endodermal derivatives (Table II; reviewed in (Hogan, 1996a, b; Whitman, 1998; Itoh *et al*, 2000). Constitutive deletion of BMP antagonist *noggin* or double mutation of *noggin* and *chordin* also result in lethality in later stages of embryonic development, in part because of abnormalities in brain and skeletal development (Brunet *et al*, 1998; McMahon *et al*, 1998; Bachiller *et al*, 2000). Genetic ablation of Smad6, one of the inhibitory Smad, leads to defects in cardiac valve formation and other cardiovascular abnormalities (Galvin *et al*, 2000). This suggests that BMP activity during embryonic development is tightly regulated, and both loss and gain of BMP functions result in severe developmental defects.

During embryonic development, BMP regulate multiple cell functions, including proliferation, differentiation, apoptosis, adhesion properties, and migration (reviewed in (Hogan, 1996a, b; Whitman, 1998). As was shown in many developmental structures, BMP activity in the extracellular space is limited by a gradient of locally secreted BMP antagonist(s) preventing BMP binding to BMP receptors (Fig 2). The cellular response to BMP is also dependent on a variety of other factors, including which BMP binds the receptor, the type of receptor (BMPR-IA or BMPR-IB) that is activated by the ligand, the differentiation state of the cell, activity of other growth stimulatory or inhibitory factors, stage of embryogenesis, etc. Below, we briefly summarize the effects of BMP and their antagonists on cell proliferation, differentiation, and apoptosis during development.

BMP and cell proliferation Numerous data suggest that BMP may stimulate or inhibit cell replication during development depending on cell types and circumstances. As it was shown in many models (neural precursor cells, brain cells, osteogenic and chondrogenic progenitors), BMP stimulate proliferation via

Table II. Phenotypes of mice with constitutive deletion of genes encoding BMP, BMP receptors, Smad, or BMP antagonists

Gene targeted	Phenotype	Reference
BMP2	Embryonic lethality (E9.5) due to defects in amnion and heart development	(Zhang and Bradley, 1994)
BMP4	Early embryonic lethality due to the defect in gastrulation and mesoderm formation	(Winnier <i>et al</i> , 1995)
BMP7	Die shortly after birth from renal failure, also show defects in eye development (microphthalmia) and polydactyly	(Dudley <i>et al</i> , 1995; Luo <i>et al</i> , 1995)
BMPR-IA	Early embryonic lethality due to the defect in gastrulation and mesoderm formation	(Mishina <i>et al</i> , 1995)
BMPR-IB	Viable, multiple skeletal defects, and female infertility	(Yi <i>et al</i> , 2000; Yi <i>et al</i> , 2001)
Smad1	Embryonic lethality (E9.5–10.5) due to defects in extra-embryonic tissues (allantois, placenta)	(Lechleider <i>et al</i> , 2001; Tremblay <i>et al</i> , 2001)
Smad4	Embryonic lethality (E7.5–8.5) due to defects in mesoderm formation, gastrulation, and extra-embryonic tissues	(Sirad <i>et al</i> , 1998; Yang <i>et al</i> , 1998)
Smad5	Die between E9.5 and E11.5, show numerous defects in developing amnion, gut, heart, and nervous system	(Chang <i>et al</i> , 1999; Yang <i>et al</i> , 1999)
Smad6	Viable, defects in cardiovascular system (formation of cardiac valves, elevated blood pressure)	(Galvin <i>et al</i> , 2000)
Noggin	Die at E18.5 due to abnormalities in nervous system and skeletal development	(Brunet <i>et al</i> , 1998; McMahon <i>et al</i> , 1998)
Noggin/Chordin	Embryonic lethality due to the defects in forebrain development	(Bachiller <i>et al</i> , 2000)

¹Yar M, Park H-Y, Botchkarev VA, Stewart K, Panova I, Gilchrist BA: Bone morphogenetic protein 4 modulates melanocyte proliferation and melanogenesis. *J Invest Dermatol* 119:337, 2002, in press (Abstr)

activation of BMPR-IA signaling (Arkel and Beddington, 1997; Yamaguchi *et al*, 2000; Panchison *et al*, 2001). Indeed, BMPR-IA knockout mice show reduced cell number in epiblast by 20–30%, compared with wild-type mice (Mishina *et al*, 1995). In developing kidney, low doses of BMP7 increase proliferation of tubule cells, whereas high doses inhibit cell proliferation and stimulate apoptosis (Piscione *et al*, 2001). This suggests that BMP effects on cell proliferation strongly depend on ligand concentration in the close vicinity of the target cell.

Interestingly, as was shown in neural cell progenitors, BMPR-IA activation, promoting cell proliferation, simultaneously induces BMPR-IB expression (Panchison *et al*, 2001). Subsequently, BMPR-IB activation causes mitotic arrest and differentiation or apoptosis in neural progenitor cells (Panchison *et al*, 2001). This mechanism may explain how the sequential actions of BMP receptors control the production and fate of cells during development. It remains to be determined, however, which intracellular signaling pathways are recruited for induction of proliferative and/or anti-proliferative effects of BMP during BMPR-IA and BMPR-IB activation.

BMP and cell differentiation Numerous data suggest that BMP play important parts in initiating and promoting cell differentiation during development. In developing embryo, BMP promote differentiation of a large variety of distinct cell types, including neural progenitors, cardiomyocytes, osteoblasts and chondroblasts, adipocytes, renal tubule cells, hematopoietic cells, etc. In every distinct cell type, BMP induce expression of the lineage-specific transcription factors or markers, such as Cbfa1 and alkaline phosphatase in osteoblasts (Yamaguchi *et al*, 2000), ATF-2 in cardiomyocytes (Monzen *et al*, 2001), or adipon and peroxisome proliferator activated receptor- γ in adipocytes (Chen *et al*, 1998).

Recent studies provide evidence that BMP-induced cell differentiation is mediated by both BMPR-IA and BMPR-IB, requires the recruitment of “canonical” and “noncanonical” BMP signaling pathways and is regulated differently in different cell types. Both BMPR-IA and BMPR-IB are implicated in chondrocyte differentiation (Zou *et al*, 1997; Yi *et al*, 2000). The beginning of neuronal progenitor differentiation in mouse embryo is accompanied by the onset of BMPR-IB expression (Panchison *et al*, 2001). In mesenchymal cell progenitors, activation of BMPR-IA signaling induces adipocyte differentiation, whereas stimulation of BMPR-IB promotes expression of markers specific for osteogenic lineage (Chen *et al*, 1998). In differentiating cardiomyocytes, BMP induce ATF-2 transcription factor via recruitment of Smad1 and TAK1, suggesting that both “canonical” and “noncanonical” pathways cooperate in the initiation of a cardiomyocyte differentiation program in progenitor cells (Monzen *et al*, 2001).

During tooth development, BMP4 induces in cells of enamel knot the expression of the cyclin-dependent kinase inhibitor p21 (Jernvall *et al*, 1998), which is critical for cell transition from proliferation to differentiation (reviewed in Dotto, 2000). Also, *in vitro* studies suggest that growth inhibitory effects of BMP are associated with induction of both p21 and p27, and Smad1 binding sites are recently described on p21 promoter (Franzen and Heldin, 2001; Yamato *et al*, 2001).

Interestingly, BMP also plays a part in regulating cell adhesion-molecule expression during development. In differentiating osteoblasts, BMP2 stimulates expression of N- and E-cadherins, whereas expression of the neural cell adhesion molecule remains unchanged (Hay *et al*, 2000). *In vitro* studies on human fetal chondrocytes suggest that BMP-2 downregulates the expression of $\alpha 3\beta 1$ integrin thus decreasing the ability of cells to attach to the substrate (Nissinen *et al*, 1997). Given that cell differentiation and/or migration are ultimately accompanied by reorganization of the cell adhesion apparatus, differential effects of BMP on the expression of distinct adhesion molecules may represent an important mechanism modulating processes of cell differentiation during development.

BMP and apoptotic cell death BMP have been reported to exert both pro-apoptotic and anti-apoptotic effects during development. BMP mediate apoptosis in a variety of developmental structures: neural tube, neural crest progenitors, mesenchymal cells of interdigital spaces, cells of developing eye, calvarial bone, and tooth enamel knot (Graham *et al*, 1996; Zou and Niswander, 1996; Jernvall *et al*, 1998; McMahon *et al*, 1998; Rice *et al*, 1999; Trousse *et al*, 2001). As was shown for other developmental events associated with the regulation of BMP activity, local expression of noggin prevents BMP-induced apoptosis, which only occurs in areas deprived of noggin (Smith and Graham, 2001).

Several lines of evidence suggest that BMP-mediated apoptosis is initiated via activation of signaling through BMPR-IB (Zou *et al*, 1997; Tang *et al*, 2000; Panchison *et al*, 2001). Retinoic acid serves as an important upstream effector of BMP-induced cell death (Dupe *et al*, 1999; Rodriguez-Leon *et al*, 1999), whereas Msx-2 and p21 are involved as downstream components in BMP-mediated apoptosis (Marazzi *et al*, 1997; Gomes and Kessler, 2001). It has been recently shown that BMP2 induces apoptosis in mouse hybridoma cell line MH-60 via a “noncanonical” signal transduction pathway, which requires activation of TAK1 and p38 kinases (Kimura *et al*, 2000; Zhang *et al*, 2000). Smad6 that directly bind and inhibit TAK1 activity, may negatively regulate BMP-induced apoptosis (Kimura *et al*, 2000).

In myeloma cell lines, BMP-2 induces cell cycle arrest followed by apoptosis associated with upregulation of p21 and hypophosphorylation of the retinoblastoma protein (Kawamura *et al*, 2000). BMP-mediated apoptosis requires the participation of the Bcl-2/Bax family of proteins. BMP-2 induces downregulation of Bcl-x_L expression in myeloma cells and Bax participates in BMP-induced apoptosis in sympathetic neuroblasts (Gomes and Kessler, 2001). Stimulating apoptosis in myeloma cells, BMP-2 and BMP-4 antagonize the growth-promoting activity of interleukin-6 via downregulation of tyrosine phosphorylation and DNA binding activity of the STAT3 transcription factor (Kawamura *et al*, 2000; Hjertner *et al*, 2001).

As was shown in several models, however, BMP may also suppress apoptosis via recruitment of the “canonical” Smad pathway. Smad5-knockout mice show increased levels of mesenchymal apoptosis in those areas, where Smad5 is normally expressed (Yang *et al*, 1999). In differentiating cardiomyocytes, BMP-2 inhibits apoptosis induced by serum deprivation (Izumi *et al*, 2001). Survival-promoting effects of BMP-2 are mediated by Smad1, which induces upregulation of the anti-apoptotic protein Bcl-x_L (Izumi *et al*, 2001). BMP-2 and BMP-4 are also able to suppress tumor necrosis factor- α -induced apoptosis in C2C12 cells, a pluripotent mesenchymal cell line that shows ability to differentiate into osteoblasts after BMP stimulation (Chen *et al*, 2001). Interestingly, anti-apoptotic effects of BMP in this model are not associated with pro-survival activity of nuclear factor- κ B and are most likely mediated by the Smad pathway (Chen *et al*, 2001).

BMP AND BMP ANTAGONISTS IN CUTANEOUS DEVELOPMENT

BMP play pivotal roles in the development of skin and cutaneous appendages. During gastrulation, when ectodermal cells choose between two fates (neural and epidermal), BMP-4 induces epidermal differentiation and inhibits neural development (Wilson and Hemmati-Brivanlou, 1995). During later steps of embryonic development, BMP, BMP antagonists, BMP receptors, and Smad proteins show stringent spatiotemporal expression patterns in skin epithelium and mesenchyme, as well as in neural crest cells that differentiate into melanocytes and components of cutaneous innervation (neurons, glial cells). Genetic and experimental data suggest that BMP interact with members of other growth factor families [Wnt, Sonic Hedgehog (Shh), TGF- β , epidermal growth

factor, fibroblast growth factor, Notch] to control cell proliferation, differentiation, and apoptosis in developing skin and its appendages. Below, we summarize the data on the expression and roles for BMP in the development of skin epithelium and skin appendages.

BMP and epidermal development Members of the BMP family are differentially expressed in embryonic epidermis. *In situ* hybridization and immunohistochemical data suggest that high levels of both BMP-6 transcripts and BMP-6 protein are present in suprabasal layers of embryonic murine epidermis already at E15.5 (Lyons *et al.*, 1989; Wall *et al.*, 1993). Strong expression of BMP-7 mRNA is seen in the basal epidermal layer during the last stages of embryonic development (Takahashi and Ikeda, 1996). This suggests that both BMP-6 and BMP-7 are involved in the control of cell proliferation and differentiation in the epidermis. Expression of BMP-2 and BMP-4 transcripts in developing murine skin are more restricted to hair follicle epithelium and mesenchyme, respectively (Lyons *et al.*, 1989, 1990; Bitgood and McMahon, 1995). Expression of the BMP antagonist *noggin* is seen in mesenchymal cells under the basal membrane of the epidermis at E15.5–17.5, whereas later *noggin* is restricted to the dermal papilla and connective tissue sheath of the hair follicle (Botchkarev *et al.*, 1999).

At E16.5, BMPR-IA protein is expressed in the basal layer of the epidermis, whereas BMPR-IB expression is restricted to keratinocytes of the suprabasal layer (Botchkarev *et al.*, 1999). These data are consistent with results obtained from other developmental models, suggesting that BMPR-IA expression is largely restricted to proliferating cells, whereas the expression of BMPR-IB is associated with the onset of cell differentiation (Panchison *et al.*, 2001). Signal transduction components of “canonical” BMP–Smad pathways Smad1, Smad5, and Smad6 proteins are also abundantly expressed in developing murine epidermis, suggesting a role for BMP signaling in epidermal development (Dick *et al.*, 1998; Flanders *et al.*, 2001).

To study the role of BMP-6 in epidermal development, transgenic mice overexpressing BMP-6 under the control of keratin (K)10 promoter were generated (Blessing *et al.*, 1996). The data obtained from BMP-6 overexpressing mice suggest that the effects of BMP-6 on epidermal development strongly depend on the levels of transgene expression. High expression of BMP-6 transgene in suprabasal epidermal layer inhibits epidermal proliferation and does not markedly change the distribution of differentiation markers (Blessing *et al.*, 1996). Our data obtained from embryonic murine skin explants cultured with high concentrations of BMP4 also suggest that strong stimulation of BMP receptor signaling inhibits epidermal proliferation (Botchkarev *et al.*, 1999). Moderate BMP-6 expression in BMP-6 transgenic mice, however, stimulates proliferation of basal epidermal keratinocytes, leads to aberrant appearance of proliferating cells and K14 expression in suprabasal epidermis, and also results in partial replacement of the normal K1 and K10 expression in suprabasal epidermal keratinocytes by K6 and K16 (Blessing *et al.*, 1996).

Our data obtained from the *noggin* knockout mice are consistent with the data obtained from the BMP-6 transgenic mice (Blessing *et al.*, 1996). Increased BMP signaling in the epidermis of *noggin* knockout mice results in increased proliferation of basal epidermal keratinocytes, downregulation of K10 expression, and ectopic appearance of proliferating cells and K14 expression in suprabasal epidermis (Botchkarev *et al.*, 1999, 2002). These data were recently confirmed by observations that BMP-2 administration into embryonic chicken skin induces localized epidermal hyperproliferation (Scaal *et al.*, 2002).

Taken together, these data suggest that the BMP effect on epidermal proliferation and differentiation during skin development is strikingly concentration dependent. Although the expression patterns for BMP receptors suggest involvement of BMPR-IA in mediating BMP effects on cell proliferation,

whereas BMPR-IB controls cell differentiation, additional genetic and experimental data are required to prove this hypothesis. It also remains to be determined whether BMP affect epidermal development directly or via regulation of other signaling pathways implicated in the control of keratinocyte proliferation and differentiation (reviewed in Eckert *et al.*, 1997; Tomic-Canic *et al.*, 1998; Freedberg *et al.*, 2001).

BMP and development of cutaneous appendages Numerous data suggest that BMP signaling is critically important for the initiation and proper development of ectodermal derivatives, such as tooth, feather, and hair follicle (reviewed in (Chuong, 1998; Chuong and Noveen, 1999; Chuong *et al.*, 2000; Jernvall and Thesleff, 2000; Fuchs *et al.*, 2001; Millar, 2002). In chicken epidermis, local expression of BMP-2 precedes the induction of feather placodes (Noramly and Morgan, 1998). Subsequently, BMP-2 and follistatin expressions are seen in feather placode, whereas BMP-4 expression is restricted to feather mesenchyme (Chuong *et al.*, 1996; Noramly and Morgan, 1998). Interestingly, during feather bud development, only BMP antagonist gremlin is expressed in the interplacode areas, whereas *noggin* and *chordin* are not expressed (Ohayama *et al.*, 2001).

During hair follicle induction in mice, both BMP-2 and BMPR-IA are expressed in hair placode, whereas BMP-4 and *noggin* expression is seen in cells of mesenchymal condensation beneath the placode (Lyons *et al.*, 1989, 1990; St-Jacques *et al.*, 1998; Botchkarev *et al.*, 1999). Similar expression patterns (epithelial for BMP-2 and mesenchymal for BMP-4) are found during mammary gland initiation in mice (Phippard *et al.*, 1996). As was shown in different models, increased BMP signaling inhibits the initiation phase during tooth and feather development (Neubuser *et al.*, 1997; Jung *et al.*, 1998; Noramly and Morgan, 1998). In contrast, downregulation of BMP activity by BMP antagonist *noggin* stimulates feather placode induction (Noramly and Morgan, 1998).

We showed that neutralization of BMP-2 and BMP-4 inhibitory activity by *noggin* stimulates the initiation phase in hair follicle development (Botchkarev *et al.*, 1999, 2002). Interestingly, constitutive deletion of *noggin* selectively affects the inductive process in the secondary (nontylotrich) hair follicles that represent about 90% of hair follicles in mice, whereas primary (tylotrich) hair follicles show increased apoptosis (Botchkarev *et al.*, 1999, 2002).

Expression studies suggest that BMP inhibitory activity on hair follicle induction occurs most likely by downregulation of Lef-1 transcription factor and neural cell adhesion molecule in hair placodes (Botchkarev *et al.*, 1999). Lef-1 is an essential component of Wnt/ β -catenin signaling pathway and it plays critical roles in hair follicle development (reviewed in Fuchs *et al.*, 2001; Millar, 2002). Lef-1 knockout mice are characterized by reduced number of hair follicles and retardation of hair follicle development (Genderen van *et al.*, 1994). Conditional disruption of β -catenin in the epidermis, or K14-driven overexpression of the secreted Wnt antagonist Dickkopf-1 lead to induction failure of both primary and secondary hair follicles (Huelsen *et al.*, 2001; Andl *et al.*, 2002). In contrast, Lef-1 overexpression leads to ectopic hair follicle formation in the oral epithelium, and overexpression of β -catenin, the upstream effector of Lef-1 in the Wnt signaling pathway, induces hair follicle neogenesis in postnatal skin (Zhou *et al.*, 1995; Gat *et al.*, 1998).

Failure of induction of secondary hair follicles observed in grafts of *noggin* null skin transplanted on to SCID mice is accompanied by strong downregulation of β -catenin expression in the epidermis (Botchkarev *et al.*, 2002). These data suggest a cross-talk between BMP and Wnt/ β -catenin/Lef-1 signaling pathways in the control of the initiation phase of hair follicle development, and are consistent with results obtained in other models showing that BMP and Wnt signaling play antagonistic roles during development (Baker *et al.*, 1999). Interestingly, BMP via downregulation of Lef-1 may also inhibit neural cell adhesion molecule expression and cell adhesion in hair placode, as it was

recently shown that Lef-1 could regulate neural cell adhesion molecule promoter activity (Boras and Hamel, 2002).

Increased evidence suggests that BMP signaling is also involved in the control of cell differentiation during hair follicle development. In developing hair follicle, the inductive interactions between keratinocytes of the hair placode and fibroblasts of the dermal papilla lead to the construction of the hair bulb, in which keratinocyte proliferate and differentiate into six distinct cell populations, forming the medulla, cortex, and cuticle of the hair shaft, as well as the cuticle and Huxley and Henle layers of the inner root sheath (Sengel, 1976; Hardy, 1992; Philpott and Paus, 1998). BMP-2, BMP-4, and BMP-1A are broadly expressed in the epithelial and mesenchymal cells of the developing hair bulb, whereas the expression of noggin is restricted to the dermal papilla and follicular connective tissue sheath cells (Botchkarev *et al*, 1999; Kulesa *et al*, 2000).

The role for BMP signaling in cell differentiation in the hair follicle has been addressed in two models. First, it was determined that in *noggin*-null skin grafts transplanted on to SCID mice, long-term excess of BMP leads to developmental arrest of primary hair follicles prior to the onset of hair shaft formation (Botchkarev *et al*, 2002). Second, transgenic mice overexpressing noggin in hair matrix keratinocytes (promoter: *Msx-2* transcription factor) showed lack of external hairs due to the alterations in the proliferation/differentiation transition of matrix keratinocytes and hair shaft synthesis (Kulesa *et al*, 2000). Alterations in hair shaft formation seen in *Msx-2/noggin* transgenic mice are associated with upregulation and ectopic expression of Lef-1 in the hair follicle and with downregulation of *Foxn1* and *Hoxc13* transcription factors (Kulesa *et al*, 2000). Given that Lef-1, *Foxn1*, and *Hoxc13* transcription factors bind selected hair keratin gene promoters (Zhou *et al*, 1995; Prowse *et al*, 1999), these data prove that BMP signaling is causally involved in the control of hair shaft-specific differentiation in the hair follicle. Furthermore, these data suggest that the genetic program of hair shaft formation is tightly controlled by the local balance of noggin and BMP and that either excess or limitation of BMP signaling may affect initiation and maintenance of the hair shaft-specific differentiation in the hair bulb keratinocytes.

BMP and Biology of Postnatal Skin Numerous data suggest that biologic mechanisms regulating cell proliferation, differentiation, and apoptosis in skin during development are ultimately involved in the maintenance of skin homeostasis in postnatal life (reviewed in Fuchs and Raghavan, 2002). BMP fulfill diverse functions in postnatal maintenance of musculoskeletal, reproductive, cardiovascular, respiratory, and nervous systems (reviewed in Myazono *et al*, 2001). Below we review data suggesting that BMP signaling plays important parts in regulating multiple functions (epidermal proliferation and differentiation, hair follicle cycling, melanogenesis, and innervation) in normal postnatal skin (Fig 3).

BMP and epidermal keratinocytes Biochemical studies suggest that BMP-2 transcripts are expressed in proliferating and differentiating murine keratinocytes *in vitro* (Park and Morasso, 2002). Primary mouse keratinocytes induced to differentiate in methylcellulose suspension culture express BMP-6 mRNA and protein (Drosdoff *et al*, 1994). 12-*O*-tetradecanoyl-phorbol-13-acetate-induced differentiation of primary mouse keratinocytes is also accompanied by increased BMP-6 expression (Wach *et al*, 2001). In adult human epidermis, expression of BMP-1A, BMP-1B, and BMP-1C is seen in suprabasal keratinocytes, whereas no BMP receptors are detected in basal epidermal cells (Hwang *et al*, 2001).

In vitro data suggest that, similar to the embryonic skin, BMP signaling is involved in the control of keratinocyte proliferation and differentiation in postnatal skin. In primary mouse keratinocytes BMP-6 inhibits cell growth and DNA synthesis (Drosdoff *et al*, 1994; D'Souza *et al*, 2001). Both BMP-2 and BMP-6 induce differentiation of primary mouse keratinocytes

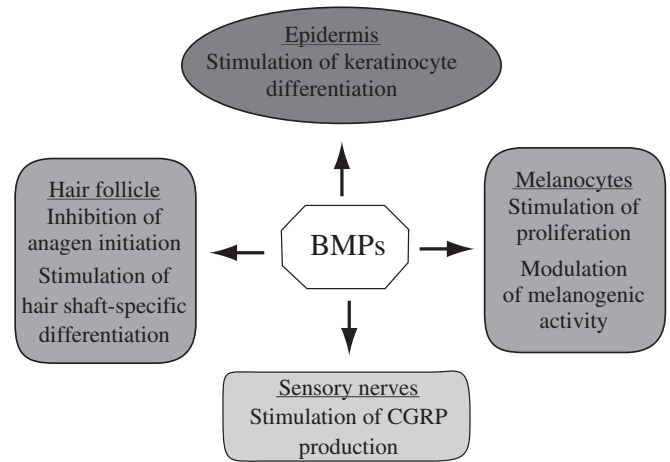


Figure 3. Schematic representation of the BMP effects in postnatal skin. BMP signaling plays important parts in regulating multiple functions in normal postnatal skin. BMP stimulate differentiation of epidermal and hair follicle keratinocytes, inhibit initiation of hair growth, promote melanocyte proliferation and modulate melanogenesis, as well as an increase in calcitonin gene-related peptide expression in sensory neurons innervating skin.

in culture (Drosdoff *et al*, 1994; D'Souza *et al*, 2001; McDonnell *et al*, 2001; Park and Morasso, 2002). In differentiating epidermal keratinocytes, BMP-6 stimulates K1 expression through the Smad pathway, whereas BMP-2 activates the expression of *Dlx-3* transcription factor via recruitment of the Smad1/Smad4 complex (McDonnell *et al*, 2001; Park and Morasso, 2002). BMP-6 also stimulates involucrin expression in cultured murine keratinocytes (D'Souza *et al*, 2001).

It appears that the BMP effects on epidermal proliferation/differentiation are distinct from those of TGF- β proteins, which inhibit both proliferation and differentiation of the epidermal keratinocytes (Dahler *et al*, 2001; reviewed in Freedberg *et al*, 2001). BMP action on epidermal keratinocytes, however, is quite similar to the activin effects, as activin also inhibits keratinocyte proliferation and stimulates their differentiation (Seishima *et al*, 1999). Although the transcriptional targets for BMP signaling in epidermal keratinocytes remain to be elucidated, taken together these data suggest BMP signaling as an important component of the molecular network regulating epidermal proliferation and differentiation in normal postnatal skin.

BMP and postnatal hair follicle growth During postnatal development, the hair follicle shows cyclic activity with periods of relative resting (telogen), active growth and hair shaft production (anagen), and apoptosis-driven regression (catagen) (Paus, 1996; Paus and Cotsarelis, 1999; Cotsarelis and Millar, 2001; Stenn and Paus, 2001). The tightly controlled balance of numerous growth stimulatory and inhibitory factors regulates cyclic activity of the hair follicle. Hair follicle morphogenesis and initiation of a new growth phase in resting postnatal hair follicles have many similar aspects and both are characterized by activation of differentiation programs that lead to the construction of a fiber producing hair bulb. Increased evidence suggests that both processes are regulated by similar mechanisms and require the activity of Wnt/ β -catenin, Shh, and BMP signaling pathways (reviewed in Fuchs *et al*, 2001).

During induced hair cycle in C57BL/6 mice, BMP4, BMP-1A, and noggin show spatiotemporal changes in their expression patterns and are involved in the control of hair follicle telogen-anagen transition (Botchkarev *et al*, 2001). During telogen, BMP4 is produced by both dermal papilla fibroblasts and secondary germ keratinocytes, and most likely interacts with BMP-1A, which is selectively expressed in the secondary germ, thus preventing the onset of anagen development. Activation of hair

growth phase in postnatal telogen hair follicle is associated with upregulation of noggin in follicular epithelium and mesenchyme. Administration of noggin induces active hair growth phase in postnatal telogen mouse skin both *in vivo* and *in situ* (Botchkarev *et al.*, 2001). As noggin binds BMP4 with affinity 10–15 times higher than BMPR-IA (Zimmerman *et al.*, 1996), noggin most likely prevents BMP4 interaction with BMPR-IA expressed in the secondary germ of telogen hair follicle and thus stimulates anagen initiation.

In contrast to noggin, BMP4 plays important inhibitory roles during hair follicle telogen–anagen transition (Botchkarev *et al.*, 2001). BMP4 and BMPR-IA are markedly downregulated in the germinative compartment of early anagen hair follicle, which is characterized by increased keratinocyte proliferation (Wilson *et al.*, 1994). BMP4 administration blocks both depilation-induced anagen development and keratinocyte proliferation in the secondary hair germ of the nontylotrich (secondary) hair follicles. These results are fully consistent with data showing that in embryonic skin BMP excess suppresses induction of secondary hair follicles (Botchkarev *et al.*, 2002).

Interestingly, the anagen-inducing effect of noggin is mediated at least in part by the activation of Shh signaling in the hair follicle. Shh is essential for hair follicle morphogenesis and hair cycle initiation (reviewed in Dlugosz, 1999). Constitutive deletion of Shh results in the arrest of hair follicle morphogenesis at the bud stage (St-Jacques *et al.*, 1998; Chiang *et al.*, 1999). Increased dermal expression of Shh or Shh blockade by neutralizing antibodies alter hair follicle transition from telogen to anagen in postnatal skin (Sato *et al.*, 1999; Wang *et al.*, 2000a). Shh is upregulated in the hair follicle after noggin treatment, whereas BMP4 downregulates Shh (Botchkarev *et al.*, 2001). Furthermore, anagen-inducing activity of noggin is abrogated by anti-Shh antibody *in situ* (Botchkarev *et al.*, unpublished observations). This suggests that noggin-neutralizing BMP4 activity represents an important upstream effector of Shh during initiation of the hair growth phase in telogen hair follicle. These data are consistent with previous observations that BMP4 may antagonize Shh signaling during tooth and limb development (Zuniga *et al.*, 2000).

In actively growing postnatal hair follicles, BMP-4, BMPR-IA, and noggin are expressed in the dermal papilla, hair matrix, as well as in proximal outer and inner root sheaths (Botchkarev *et al.*, 2001). BMP signaling is important for hair shaft specific differentiation (Kulesa *et al.*, 2000). Important downstream component of BMP signaling is Msx-2 transcription factor, which is expressed in hair matrix keratinocytes and mediates BMP effects on the expression of Lef-1, Foxn1, and Hoxc13 transcription factors, which in turn regulate expression of hair keratin genes (reviewed in Fuchs *et al.*, 2001).

Interestingly, Msx-2 transcription factor is also involved in mediating BMP4-induced apoptosis in a variety of embryonic tissues (Marazzi *et al.*, 1997; Chen and Zhao, 1998). Msx-2 overexpressing mice show decreased volume of proximal hair bulb and shrunken hair matrix region with reduced number of hair keratinocyte precursors (Jiang *et al.*, 1999). Thus, by regulating Msx-2 expression BMP may control the size and cell number in actively growing hair follicles, as well as regulate apoptosis-driven hair follicle regression. Indeed, in growing teeth, neutralization of BMP-4 by ectopically applied noggin leads to inhibition of apoptosis, increased tooth size, and the formation of molars instead of incisors (Tucker *et al.*, 1998). In developing hair follicles, increased BMP signaling due to the deletion of the BMP antagonist noggin also leads to the upregulation of apoptosis (Botchkarev *et al.*, 1999). It remains to be determined, however, whether BMP signaling controls the size of postnatal hair follicles by inducing apoptosis.

BMP and functions of neural crest derivatives in postnatal skin BMP and BMP antagonist noggin are intimately involved in the control of development and migration of neural crest cells during embryogenesis (reviewed in Christiansen *et al.*,

2000). In primary quail neural crest cultures, BMP-2 treatment increases melanin synthesis via stimulation of tyrosinase gene expression in melanocyte precursors (Bilodeau *et al.*, 2001). In melanocytes isolated from human newborn skin, however, BMP-4 stimulates proliferation, decreases melanin content, and downregulates tyrosinase message and protein.¹ This suggests that the effects of BMP on melanocytes strongly depend on the latter differentiation stage, expression of BMP receptors, and on other growth factor signaling pathways that may modulate BMP action on melanocytes.

It was also shown that BMP signaling controls cell fate in embryonic neural crest cells and stimulates neural crest progenitor differentiation into autonomic neurons (Christiansen *et al.*, 2000). This suggests that BMP also affects the development and maintenance of postnatal skin innervation. In developing sympathetic neurons, BMP-2 regulates expression of TrkC, the high-affinity receptor for neurotrophin-3 (Kobayashi *et al.*, 1998). Still, it is yet unknown whether BMP signaling is involved in regulating sympathetic neurons that innervate skin targets. It was shown, however, that BMP stimulate survival and responsiveness of sensory neurons to neurotrophic factors by increasing the expression of the high-affinity neurotrophin receptors TrkB and TrkC (Farkas *et al.*, 1999).

Furthermore, it has been shown that neurons in sensory dorsal root ganglia express BMP receptors, and the expression of a sensory neuropeptide calcitonin gene-related peptide in dorsal root ganglion neurons is stimulated by BMP-2, BMP-4, and BMP-6 (Ai *et al.*, 1999). Calcitonin gene-related peptide is an important mediator of the skin neuroimmune network and it controls a variety of functions in skin cells, including inhibition of antigen presentation by Langerhans cells, release of histamine from mast cells and vasodilatation (reviewed in Torii *et al.*, 1997; Levite, 2000). Thus, by regulating calcitonin gene-related peptide expression, BMP may influence cell–cell interactions in skin immune and circulatory systems.

BMP Involvement in Pathologic Processes in Skin Several lines of evidence suggest involvement of factors that control cutaneous development (Shh, Wnt family, TGF- β family, stem cell factor, epidermal growth factor) in a variety of pathologic processes in adult skin, including tissue regeneration, tumor growth, allergic reactions, etc. (reviewed in Loomis, 2001). We summarize here the data showing that BMP are involved in the control of wound healing, psoriasis, and carcinogenesis in adult skin.

BMP and wound healing After skin injury in mice, BMP-6 transcripts and protein are strongly upregulated in the keratinocytes of the newly formed epidermis and in dermal fibroblasts of the wound bed (Kaiser *et al.*, 1998). High levels of BMP-6 mRNA and protein are also detected in chronic wounds of human skin (Kaiser *et al.*, 1998). In BMP-6 overexpressing transgenic mice (promoter: K10), re-epithelialization of skin wounds is significantly delayed and scar formation is enhanced (Kaiser *et al.*, 1998). BMP-2 application to the wound bed of fetal lambs induces scar formation associated with a marked increase of epidermal keratinization and epidermal and dermal thickness (Stelnicki *et al.*, 1998). Epidermal wounds treated with BMP-2 and TGF- β healed with a similar scarring process indicating that both growth factors are important for collagen-producing activity by fibroblasts during the wound healing process (Stelnicki *et al.*, 1998). This suggests that BMP signaling regulates the wound-healing process by changing the proliferative activity and/or differentiation of epidermal keratinocytes and by altering the collagen-producing activity of dermal fibroblasts. It is unknown whether BMP directly stimulate collagen synthesis in dermal fibroblasts during wound healing, as they do so in osteoblasts (reviewed in Yamaguchi *et al.*, 2000), and it remains to be determined whether elevated BMP levels modulate activity of other growth factors,

such as activin and TGF- β (reviewed in Munz *et al*, 2001; Verrecchia and Mauviel, 2002).

BMP and psoriasis Several lines of evidence suggest that BMP plays a part in the pathophysiology of psoriasis. Transgenic mice overexpressing BMP-6 in suprabasal epidermal keratinocytes under the control of K10 promoter develop skin lesions characteristic of psoriasis: parahyperkeratosis and hyperproliferation of the epidermis, alterations of keratin and integrin expression, extensive dermal capillarization, and dermal cell infiltrates (Blessing *et al*, 1996). Long-term treatment of C57BL/6 mice with BMP-2 or BMP-4 also result in thickening of the epidermis with development of psoriasis-like lesions (Botchkarev *et al*, unpublished observations). Elevated BMP signaling may stimulate angiogenesis via upregulation of vascular endothelial growth factor synthesis, as is shown in osteoblasts (Deckers, 2001; Kozawa and Uematsu, 2001). Although the BMP role in psoriasis development remains to be determined, the above data suggest that BMP antagonists could help in psoriasis management.

BMP and skin carcinogenesis Several lines of evidence suggest that BMP signaling may contribute to skin carcinogenesis. Transgenic studies suggest that overexpression of BMP-4 or BMP-6 in murine epidermis is accompanied by increased resistance to 12-*O*-tetradecanoyl-phorbol-13-acetate-induced tumorigenesis (Blessing *et al*, 1995; Wach *et al*, 2001). Furthermore, in chemically induced murine epidermal tumors, Smad1 and Smad5 proteins are strongly downregulated, whereas Smad7 is upregulated (He *et al*, 2001). Importantly, tumor resistance in transgenic mice overexpressing BMP-6 in the suprabasal layer of the epidermis (promoter: K10) is associated with a significant increase in apoptotic cells and downregulation of the transcriptional regulator AP-1, which is seen after 12-*O*-tetradecanoyl-phorbol-13-acetate treatment (Wach *et al*, 2001). This suggests that BMP signaling plays a suppressive role in epithelial tumor formation in murine skin.

Immunohistochemical data show that in human basal cell carcinoma R-Smad expression is decreased (Lange *et al*, 1999). BMP-2 expression is found in the cytoplasm of shadow cells in human pilomatricoma, and BMP-2 may contribute to the differentiation of tumor cells into the bone cells frequently seen in this type of benign skin tumors (Kurokawa *et al*, 2000). The role of BMP signaling in the formation of tumors in human skin remains to be determined. BMP, however, may also suppress tumor formation by antagonizing Shh activity in epithelial cells. Shh, an essential epithelial signal that promotes hair follicle morphogenesis in embryonic skin (St-Jacques *et al*, 1998; Chiang *et al*, 1999), plays important stimulatory roles in the formation of basal cell carcinoma in both humans and mice (reviewed in Oro, 2001). Overexpression of Shh or its downstream effector Gli2 in basal epidermal keratinocytes (promoter: K14) induces the development of basal cell carcinoma (Oro *et al*, 1997; Grachtchouk *et al*, 2000). As was shown in developing teeth and in postnatal hair follicles, however, increased BMP activity both *in vitro* and *in vivo* may downregulate Shh mRNA (Zuniga *et al*, 2000; Botchkarev *et al*, 2001). Thus, the anti-neoplastic activity of BMP in epidermal carcinogenesis appears to be mediated by downregulating AP-1 and Shh pathways.

CONCLUSIONS

During the last decade, tremendous progress has been achieved in delineating the molecular structure and functions of BMP, BMP receptors, and BMP antagonists. While initially identified as growth factors stimulating bone regeneration, BMP are now recognized as powerful regulators of tissue development, homeostasis, and remodeling. BMP activity in developing and adult tissues is modulated and antagonized on different levels (extracel-

lular, cytoplasmic, and nuclear). In normal skin, BMP function as important factors controlling epidermal proliferation and differentiation, hair follicle growth, melanogenesis, and innervation. BMP are also implicated in the regulation of wound healing and hyperproliferative skin disorders, such as psoriasis and in cancer development. BMP effects on the hair follicle suggest that BMP antagonists and agonists may represent a new tool for therapeutic corrections of hair growth disorders. Additional research is required, however, to bridge the gap between our current knowledge of BMP functions in the skin and their potential clinical applications. The progress in this area of research would hopefully lead to the development of multiple applications for using BMP and BMP antagonists in skin and hair growth disorders.

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