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Review

Multifunctional Bone Morphogenetic Protein System in Endocrinology

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New biological activities of bone morphogenetic proteins (BMPs) in the endocrine system have recently been revealed. The BMP system is composed of approximately 30 ligands and preferential combinations of type I and type II receptors. The BMP system not only induces bone formation but also plays unique tissue-specific roles in various organs. For instance, the ovarian BMP system is a physiological inhibitor of luteinization in growing ovarian follicles. In the ovary, the expression of oocyte-derived BMP-15 is critical for female reproduction. In the pituitary, BMP-4 is a key player for initial development of the anterior pituitary, while it is also functionally involved in some differentiated pituitary tumors, including prolactinoma and Cushing's disease. In the adrenal glands, BMP-6 and BMP-4 modulate aldosterone and catecholamine production, respectively, which contributes to a functional interaction between the cortex and medulla. In the present review, recent advances in BMP biology in the field of endocrinology are described and the possibility for clinical application of BMP activity is discussed.

Key words: bone morphogenetic protein, folliculogenesis, pituitary adenoma, steroidogenesis, transforming growth factor

Bone morphogenetic proteins (BMPs) were identified by Urist [1] as active components in demineralized bone and bone extracts that are capable of inducing bone formation at ectopic sites [2]. Thereafter, a number of new BMP family genes were identified using homology-based cloning. Based on the structure of amino acid sequences from the corresponding cDNAs, BMP ligands are classified into transforming growth factor (TGF)- β superfamily members.

To date, more than 30 members of the TGF- β superfamily have been identified in various species [3, 4]. Not all of the BMP family members induce bone

formation or differentiation, though all BMPs contribute to the regulation of multiple biological processes, including cell proliferation, apoptosis, differentiation and morphogenesis in various tissues in the body [5]. For instance, in contrast to BMP ligands that can elicit bone-forming effects, such as BMP-2, -4, -6 and -7, BMP-3b inhibits the process of osteoblast differentiation and antagonizes the actions of BMP-2 [6].

Accumulating evidence has led to the concept that BMPs formulate a multifunctional regulator system in various biological processes in vertebrates as well as invertebrates [7]. In addition to their expression in bone, BMPs are expressed in the kidney (BMP-3, -4 and -7), lung (BMP-3, -4, -5 and -6), small intestine (BMP-3 and -7), heart (BMP-2, -4, -6 and -7), limb bud (BMP-2, -4, -5 and -7) and teeth (BMP-3, -4 and

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-7), in which they regulate cellular homeostasis by an autocrine/paracrine mechanism [8-11]. Recent studies have shown that the BMP system is a critical component of the local regulatory system in endocrine tissues. Various BMP actions have been observed in numerous tissues, including the ovary, pituitary and adrenal glands [12], and new regulatory roles of BMPs in bone formation and/or bone differentiation have also been shown [13-16].

Seven type-I (activin receptor-like kinase (ALK)-1 to -7) and 5 type II (activin type II receptors (ActRII/IIB), anti-Müllerian hormone receptor (AMHR)-II, BMP type II receptor (BMPRII) and TGF- β type II receptor (T β RII)) receptors for TGF- β superfamily members have been characterized in mammals. Type I and type II receptors are structurally similar and both types possess Ser/Thr-kinase domains in intracellular regions. Type I receptors have a Gly/Ser-rich GS domain in the trans-membrane region. Although TGF- β and activin initially bind to type II receptors and type I receptors are subsequently recruited into the complex, type II as well as type I receptors independently have certain affinity for BMP ligands and the type I/II complex can achieve higher affinity binding [17].

The pathway-restricted Smads (called R-Smads), Smad1/5/8, are activated following the binding of BMP ligands to the receptors. They interact with a common-mediator Smad (Co-Smad), Smad4, to form a complex, which is then translocated into the nucleus for binding to target DNA and the induction of specific gene transcription [18]. Two inhibitory Smads (I-Smads), Smad6/7, act in competition with Smad4 and regulate the target-gene transcription of BMP signaling. Alternative signaling pathways such as TGF- β -activated kinase (TAK-1), a member of the mitogen-activated protein kinase kinase kinase (MAPKKK) family, and members of the Ras/Rac families of small GTP-binding proteins can also be activated. Depending on cell types, extracellular signal-regulated kinase-1/2 (ERK1/2) and stress-activated protein kinase (SAPK)/Jun-N-terminal kinase (JNK) may be involved in TGF- β signal transduction.

Reproductive Roles of the BMP System

Oocyte-derived factors have recently proven to be

of critical importance to ovarian function and female reproductive biology. This is based on the concept of mutual communication of oocytes and surrounding somatic cells that is critical for normal follicular development [19, 20]. Folliculogenesis is regulated by the actions of oocytes, wherein oocyte-derived growth factors act on neighboring follicular cells and integrate various functions of granulosa cells. Local factors expressed in the ovary, including BMPs, growth and differentiation factors (GDFs), activins and inhibins play various autocrine/paracrine roles in female fertility by regulating steroidogenesis as well as mitogenesis in granulosa cells [21-23] (Fig. 1). Expression of mRNA and/or protein for the BMP system components, including ligands, receptors and intracellular signal transduction factors, has been demonstrated in the cells of growing follicles [24, 25]. The biofunctional activity of BMPs in the regulation of ovarian function has been gradually elucidated [22, 26, 27].

The finding that defects of the oocyte-specific growth factors GDF-9 and BMP-15 cause female infertility was an important breakthrough in this field [28, 29]. BMP-15 is exclusively expressed in oocytes [30] and has been shown to induce granulosa cell mitosis and inhibit the actions of FSH by suppressing follicle-stimulating hormone (FSH) receptor (FSH-R) expression [31]. The spontaneously-occurring infertile strain of Inverdale sheep was found to carry a single point mutation in the mature-protein region of the *bmp15* gene [29]. In the heterozygotes of Inverdale mutants, reduced BMP-15 causes higher expression of FSH-R in granulosa cells, leading to more developing follicles and higher expression of luteinizing hormone (LH) receptors (LH-R). The heterozygous mutants therefore exhibit increased ovulation rates, resulting in increased twinning. The aberrant reproductive biology of heterologous mutant Inverdale sheep appears to be based on precocious maturation of follicles and the resultant increased numbers of ovulatory follicles. On the other hand, homozygous Inverdale female sheep are infertile due to arrested follicle development at the primary follicle stage. The lack of bioactive BMP-15 and its mitotic effects lead to arrest of follicle development in homozygotes of the Inverdale strain [30].

Some individuals of another sheep strain, the highly prolific strain Booroola, were found to carry a mutation in the ALK-6 (BMPRII) receptor [32-34],

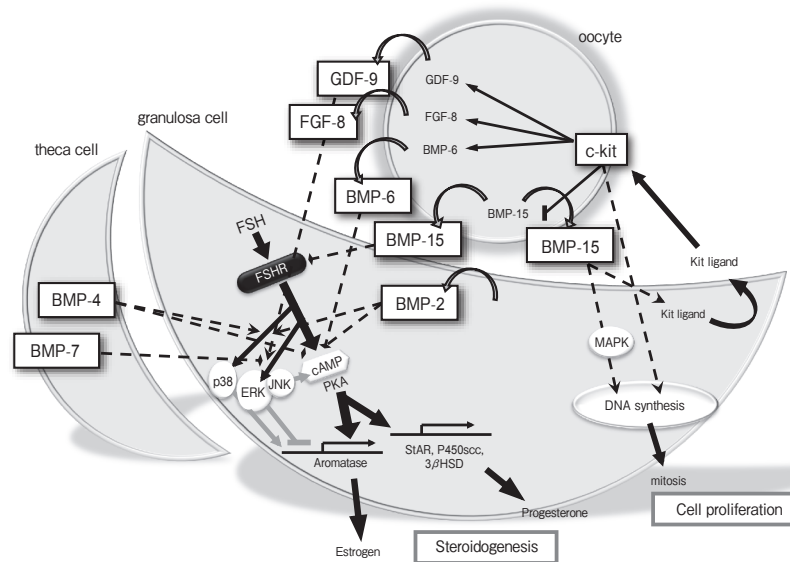


Fig. 1 Reproductive roles of the BMP system in the ovary. FSH activates estradiol and progesterone production through the cAMP-to-PKA pathway in granulosa cells in the ovary. FSH simultaneously stimulates MAPKs, including the ERK and p38 pathways, leading to pathway-specific modulation of FSH-induced steroidogenesis. Oocyte-derived BMP-15 inhibits FSH receptor (FSH-R) expression, while BMP-6 suppresses cAMP synthesis, both of which lead to reduction of progesterone production. BMP-2 and -4 activate FSH-induced p38, leading to stimulation of estradiol production. BMP-7 inhibits FSH-induced ERK1/2 phosphorylation, leading to activation of estradiol production. Oocytes and/or oocyte-derived factors also facilitate FSH-to-MAPKs and BMP-to-Smad signaling activity through oocyte-granulosa cell communication. Oocyte-secreted BMP-15 stimulates granulosa cell mitosis with Kit ligand (KL) expression. KL acts through c-kit on the surface of oocytes to inhibit BMP-15 expression, forming a negative feedback loop. KL-to-c-kit signaling not only increases granulosa cell mitosis but also affects the expression of oocyte factors such as BMPs and FGF-8, leading to the maintenance of steroidogenesis.

which is a key receptor for BMP-15. Ovarian follicles of Booroola sheep produce more progesterone in response to FSH than do those of the wild type [35]. BMP-15 signaling is mediated by binding first to ALK-6 and then recruiting BMPRII to the complex [36]. The enhanced FSH responsiveness of the follicles from Booroola ewes has been proposed to be due to impaired ALK-6 signaling elicited by endogenous BMP-15 and/or BMP-6 [21]. Thus, the reproductive phenotypes of sheep coupled with evidence from *in vitro* findings have established roles of BMP-15 signaling in the regulation of follicular development and also shown the importance of oocyte-secreted factors in the field of mammalian reproduction.

BMP-2, -4, -6 and -7 also exert unique effects on ovarian steroidogenesis and granulosa cell mitosis in the ovary (Fig. 1). BMP-6 (expressed in oocytes and granulosa cells of healthy Graafian follicles [24]) inhibits the actions of FSH by suppressing adenylate cyclase activity [37], leading to the process of domi-

nant follicle “selection.” BMP-4 and -7 (expressed in theca cells) increase FSH-induced estradiol production and suppress progesterone production [38]. BMP-7 promotes the “recruitment” process of primordial follicles into the growing follicle pool [39]. The actions of BMP-7 on FSH-induced estradiol production occur through suppression of ERK1/2 downstream of FSH receptor signaling [40]. BMP-2 and -4 stimulate FSH-induced p38-MAPK phosphorylation, leading to an increase of FSH-induced estradiol production [41]. In addition, some of the BMP actions are extracellularly regulated by the binding protein follistatin [42], while BMP-Smad signal activities are also regulated by FSH receptor signaling, leading to fine-tuning of the mutual sensitivities of BMPs and FSH [43]. Thus, each BMP ligand differentially regulates FSH-induced steroidogenesis by granulosa cells by a ligand-dependent mechanism, but the BMP ligands commonly work as “luteinization inhibitors” [21].

BMP-15 also stimulates granulosa cell prolifera-

tion in a dose-dependent manner [30] via kit ligand (KL)-c-kit interaction between oocytes and granulosa cells [44] (Fig. 1). In addition to the oocyte-to-somatic cell communication, the physiological importance of granulosa-to-oocyte signaling was recognized in naturally occurring mutations at the loci of *c-kit* and *KL* in mice, resulting in developmental abnormalities in oogenesis and folliculogenesis [45, 46]. The *KL* receptor *c-kit* mRNA/protein is localized to oocytes during postnatal ovarian development, while *KL* mRNA expression is localized to granulosa cells in the follicles [45]. Thus, it is possible that *KL* action contributes to the communication from granulosa cells to oocytes via *c-kit* signaling [46, 47]. The functional interaction of the granulosa-derived *KL* and oocyte *c-kit* is also indispensable for normal fertility. Interestingly, *BMP-15* and *KL* are concomitantly expressed in the early stages of follicular development and appear to be involved in granulosa cell mitosis [44] by forming a negative feedback loop between the oocyte and surrounding granulosa cells. The combination of increased *KL* expression and subsequent reduction of *BMP-15* expression contribute to an effective and balanced induction of granulosa cell proliferation [44].

KL-c-kit interaction is not only involved in oocyte maturation and mitotic regulation of granulosa cells but also plays a role in estrogenic regulation through oocyte-granulosa communication (Fig. 1). This bidirectional communication between oocytes and granulosa cells involves oocyte-derived growth factors, including fibroblast growth factor (*FGF*)-8 and *BMP-15/GDF-9*, leading to control of the *FSH*-induced estrogenic capability of granulosa cells. Oocyte-derived *BMP-15* stimulates *KL* expression in rat granulosa cells, leading to stimulation of granulosa cell proliferation [44], while *GDF-9* suppresses *KL* expression in mouse granulosa cells [48]. Therefore, it has been hypothesized that the *KL-c-kit* interaction plays a regulatory role not only in the early process of mitogenesis but also in steroidogenesis by granulosa cells, which is further controlled by oocyte factors. It was recently found that *KL-c-kit* interactions suppressed *FSH*-induced estradiol production and aromatase mRNA expression without affecting *FSH*-induced progesterone production by granulosa cells in the presence of oocytes [49].

The intracellular interaction between *BMP-15* and *GDF-9* is also an interesting issue with regard to the

secretion of active mature proteins. *BMP-15* and *GDF-9* differ from other *TGF- β* members in that their mature regions lack the 7th Cys residue that is responsible for intra-molecular bonding [50, 51]. It is therefore reasonable to expect that *BMP-15* and *GDF-9* form heterodimers [52, 53]. Posttranslational modification by phosphorylation is also critical for bioactivity of *BMP-15* and *GDF-9*, and the dephosphorylated forms of *BMP-15* and *GDF-9* exhibit antagonistic activity to the phosphorylated normal ligands [54]. Moreover, *FGF-8*, another oocyte-derived factor, is associated with the regulation of *BMP* activity. *FGF-8* suppresses *FSH*-induced estrogen production but amplifies *BMP* signaling through oocyte-granulosa cell communication [55]. The interaction between *FGF* and *BMP* systems is critical for the maintenance of *FGF* receptor signaling itself in granulosa cells [56]. The interaction between *FGF-8* and *BMP-15/GDF-9* signaling could be a key for understanding the regulation of *FSH*-induced estrogen production through oocyte-granulosa cell communication via *KL-c-kit* interaction.

BMP Action in Pituitary Tumors

In the pituitary, the *BMP* system plays important roles in development of the anterior pituitary [57]. *BMP-4* is required during the initial process of pituitary organogenesis for forming Rathke's pouch, and inhibition of *BMP-2* by *FGF-8* in turn facilitates the differentiation of corticotrope cells [58, 59]. Suga *et al.* recently reported exciting data showing a role of *BMP-4* in the efficient self-formation of three-dimensional adenohypophysis in an aggregate culture of mouse embryonic stem (ES) cells [60]. A large cell-aggregation culture of mouse ES cells was successfully induced for differentiation into anterior pituitary tissues. In this process, *BMP-4*-treated ES cells increased expression of the Rathke's marker *Pitx2* but inhibited the formation of hypothalamic tissues. Since a *BMP* antagonist inhibited the induction of Rathke, an endogenous *BMP* signal was shown to be critical for development of the anterior pituitary.

Recent studies have further revealed a new role of *BMP-4* in the pathogenesis of the differentiated anterior pituitary [61, 62] (Fig. 2). The *BMP-4* molecule was found to be overexpressed in lactotrope adenomas derived from dopamine D2 receptor null mice and

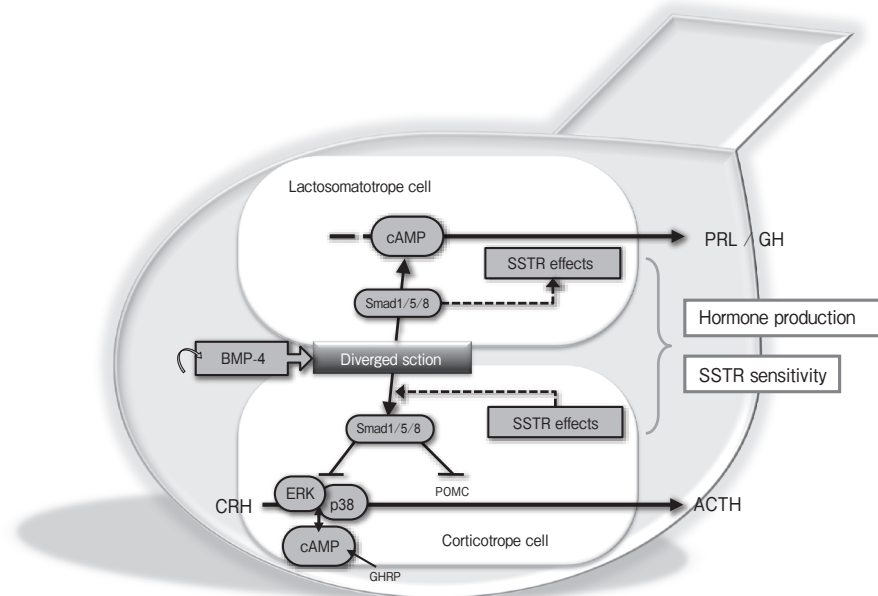


Fig. 2 Roles of the BMP system in pituitary tumors. In lactosomatotrope cells, BMP-4 stimulates the cAMP-PKA pathway via Smad1/5/8 signaling, leading to enhancement of PRL (and/or GH) production. BMP activity is also involved in modulation of SSTR sensitivity in lactosomatotrope cells, in which BMP-4 augments SSTR5 expression but suppresses SSTR2 expression. In corticotrope cells, BMP-4 suppresses CRH-induced MAPK (ERK1/ERK2 and p38-MAPK) activity and POMC transcription via Smad1/5/8 signaling, leading to reduction of ACTH production. The BMP response is upregulated by SSTR analogs through SSTR5 and, to a lesser extent, through SSTR2. GH-releasing peptide (GHRP) also activates POMC transcription and ACTH production in corticotrope cells, although the effect is much less potent than that of CRH.

estrogen-treated female rat pituitary tissues. Increased BMP-4 expression was also detected in human prolactinomas compared with its expression levels in other functioning and nonfunctioning tumor tissues [63]. Biologically, BMP-4 promotes not only lactotrope cell proliferation [63] but also prolactin production in conjunction with Smad-estrogen receptor (ER) interaction [64]. The crosstalk between BMP-4 and estradiol occurs both at the level of prolactin secretion and at the level of its promoter activation [64]. BMP-4 inhibits the transcriptional activity of ER, while estrogen stimulates the transcriptional activity of BMP-4-specific Smad signaling. Given that the expression of a BMP antagonist, noggin, is down-regulated in a prolactinoma mouse model [63], endogenous BMP-4 should be involved in growth promotion and prolactin productivity by lactotropes through the Smad-ER interaction.

Furthermore, we recently found a functional inter-relationship between the BMP system and somatostatin receptor (SSTR) expression in relation to growth

hormone (GH) and prolactin secretion [65, 66] (Fig. 2). Somatostatin acts by binding to 5 subtypes of G protein-coupled receptors that are widely distributed in many endocrine and nonendocrine tissues. The efficacy of somatostatin analogs is linked to the SSTR selectivity profile, in which binding to SSTR2 and SSTR5 is critical for proper function [67]. SSTR2 and SSTR5 are negatively coupled to adenylyl cyclase, the activation of which results in a reduction of intracellular cAMP levels [68]. A unique action of the pituitary BMP system in the modulation of prolactin secretion regulated by somatostatin analogs was uncovered in lactosomatotrope GH3 cells expressing SSTRs. Importantly, BMP-4 reduced SSTR2 expression but increased SSTR5 expression [69]. The effect of the SSTR5-preferring agonist pasireotide, which reduced the prolactin secretion induced by forskolin, was facilitated by the presence of BMP-4 and, in turn, blocked by noggin treatment. Thus, BMP-4 acts to increase prolactin release and, furthermore, the BMP system plays a regulatory role in

the SSTR sensitivity of lactotrope tumor cells [69].

Prolactinomas are the most frequently occurring human functioning pituitary tumors. Administration of dopamine agonists (DA) is the major choice for treatment of general prolactinomas [70]. DA administration suppresses prolactin secretion and cell proliferation by interacting with D2 receptors [71]. D2R agonists are efficient in the majority of cases. However, some cases of prolactinomas fail to attain PRL normalization and reduction in tumor size even with the most potent dopamine agonist, cabergoline [72-74]. In such tumors that are poorly or partially responsive to DA, an alternative medical treatment is needed. Among SSTRs expressed in prolactinomas, SSTR5, but not SSTR2, is predominantly detected [75]. This expression pattern indicates that established somatostatin analogs such as octreotide and lanreotide (SSTR2-preferring agonists) are much less effective for suppressing prolactin secretion from prolactinomas compared with pasireotide (an SSTR5/2-preferring agonist) [76].

An interaction between the BMP-Smad pathway and corticotropin-releasing hormone (CRH) receptor signaling is involved in controlling ACTH production by corticotrope cells in an autocrine/paracrine manner [77, 78] (Fig. 2). In cases of corticotrope tumors, BMP ligands, particularly BMP-4, potentially suppress ACTH production by inhibiting CRH-induced MAPK pathways. In mouse corticotropinoma model AtT20 cells, it was found that somatostatin analogs upregulated BMP-Smad1/5/8 activity, increased the expression of ALK-3/BMPRII and downregulated inhibitory Smad6/7 molecules [77]. Thus, activation of the endogenous BMP system is likely to be involved in the mechanism by which somatostatin analogs suppress CRH-induced ACTH production [77]. Neither BMP-4 nor a somatostatin analog elicited significant suppression of AtT20 cell proliferation. Nevertheless, it was notable that BMP-4 showed a significant inhibition of cell mitosis in the presence of somatostatin analogs [77]. These findings suggest that somatostatin analogs facilitate the actions and/or signal transduction of BMP-4.

SSTR2 is known to be downregulated by excess glucocorticoids, and the inhibition of SSTR2 expression by excess cortisol may cause the ineffectiveness of the SSTR2-preferring analog, octreotide, in patients with pituitary corticotropinomas SSTR2 expression

upregulated by TGF- β in corticotrope cells via Smad4 activation [79]. Overexpression of Smad4 restores SSTR2 expression and reverses the growth inhibition elicited by somatostatin. These findings suggest that Smad4 is associated with the expression and function of SSTR2 in pituitary tumors [79]. Although the direct effects of BMPs on the SSTR expression profile have yet to be elucidated, SSTR action is substantially involved in the bioactivity of endogenous BMP responsiveness leading to ACTH reduction [77]. Considering that somatostatin analogs enhanced BMP-Smad1/5/8 signal intensity in corticotrope cells, activation of the endogenous BMP system may be crucial for the mechanism by which pasireotide and/or octreotide suppress CRH-induced ACTH production in Cushing's tumors.

BMPs in the Adrenal System

Regulation of adrenal function is important for the control of hypertension and its associated organ damage. A functional BMP and activin system complete with ligands including BMP-6 and activin β A/ β B, the receptors ALK-2, ALK-3, ALK-4, ActRII and BMPRII, and the binding protein follistatin exists in human adrenocortical cells [80] (Fig. 3). Activin and BMP-6 cause concentration-dependent increases in aldosterone production with increased expression of StAR, P450_{scc} and CYP11B2. Activin regulates aldosterone synthesis predominantly by modulating the ACTH-cAMP-PKA signaling pathway [81]. On the other hand, BMP-6 contributes to angiotensin II (Ang II)-induced aldosterone production by activating Smad1/5/8 after binding to ALK-2/3 in combination with ActRII [82].

Aldosterone production stimulated by Ang II in human adrenocortical cells was blocked by an Ang II type 1 receptor (AT1R) blocker (ARB) in a concentration-dependent manner [83]. However, the suppressive effects of the ARB on Ang II-induced aldosterone production and CYP11B2 expression were impaired during a chronic course of ARB treatment for approximately 2 weeks. Expression of BMP-6 and its receptors, including ALK-2 and ActRII, was decreased by chronic Ang II exposure. This decrease in expression was reversed by co-treatment with ARB in adrenocortical cells. Given that *in vitro* breakthrough is attenuated by the neutralization of endog-

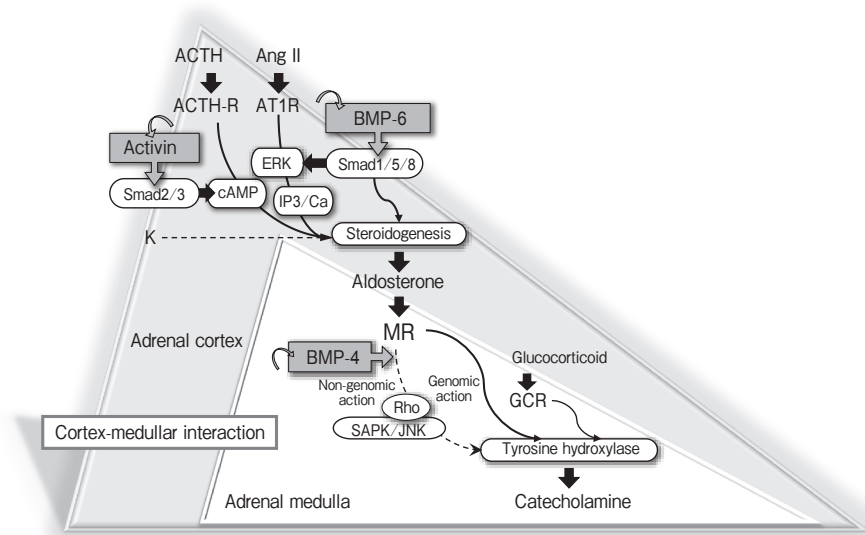


Fig. 3 Roles of the BMP system in the adrenal cortex. In the adrenal cortex, BMP-6 contributes to Ang II-induced aldosterone production by activating Smad1/5/8, which preserves Ang II-induced ERK activation. On the other hand, aldosterone production induced by activin is likely to be regulated via mechanisms that enhance the authentic steroidogenic pathway of ACTH-cAMP-PKA. In the adrenal medulla, aldosterone secreted from the adrenal cortex stimulates catecholamine production and tyrosine hydroxylase (TH) expression by genomic and non-genomic actions through MR-dependent and MR-independent SAPK/JNK signaling. BMP-4 enhances aldosterone-induced catecholamine production and TH mRNA expression via MR by upregulating non-genomic actions through Rho-SAPK/JNK pathways. Adrenal BMPs are important for the interaction between the adrenal cortex and medullary function.

enous BMP-6 and ALK-2, the bioavailability of BMP-6 in the adrenal cortex is likely to be involved in the occurrence of cellular escape from aldosterone suppression under chronic treatment with an ARB.

The physiological role of BMP-6 in regulation of aldosterone *in vivo* was also examined by utilizing rats treated with immunization against BMP-6 [84]. Reduction of urinary aldosterone excretion was caused by BMP-6 inhibition, suggesting that endogenous BMP-6 contributes to the induction of aldosterone production *in vivo*. In contrast, with Ang II treatment, urinary aldosterone and the creatinine-corrected values were not different between the 2 groups, suggesting that BMP-6 activity was impaired under the condition of chronic treatment with Ang II. This phenomenon is the same as that demonstrated in our previous *in vitro* study [83]. The reduction of CYP11B2 mRNA and reduction of the ratio of plasma aldosterone/corticosterone by treatment with BMP-6 immunization further suggested a selective role of BMP-6 in aldosterone production by the zona glomerulosa of the adrenal cortex. Collectively, these findings indicate that BMP-6 is likely to act as one of the modulatory

factors in aldosterone production to maintain systemic aldosterone levels.

The adrenal cortex and medulla functionally interact with each other in an autocrine/paracrine manner [85, 86] (Fig. 3). Endogenous glucocorticoids are known to induce catecholamine biosynthesis by stimulating catecholamine-synthesizing enzymes through the cortico-medullary portal system [87]. The presence of the BMP system in the adrenal medulla and functional crosstalk between glucocorticoid and the BMP system in regulating catecholamine synthesis were found in adrenomedullary cells [88]. The key components of the BMP system are expressed throughout neural development [89]. For instance, BMP-4 and BMP-7 are expressed in the dorsal aorta and direct sympathetic neuronal differentiation into the adrenergic characteristics [90]. BMP-4 and BMP-7 induce a tyrosine hydroxylase (TH)-immunoreactive adrenergic phenotype in cultures of avian neural crest cells [91, 92]. We also found that BMP-4 induces catecholamine production in the presence of mineralocorticoid in adrenomedullary PC12 cells. Catecholamine biosynthesis in adrenomedullary cells occurs via the

mineralocorticoid receptor (MR) through genomic action and partly through non-genomic action by Rho-SAPK/JNK signaling [93]; as well as by glucocorticoid action via glucocorticoid receptor (GCR). Given that the non-genomic pathway was activated by BMP-4, this adrenocortical-medullar interaction via MR and BMPs was hypothesized to be involved in catecholamine regulation [93].

Perspective on Clinical Application of BMPs

Various BMP actions in endocrine tissues, including ovary, pituitary, thyroid, adrenal and cardiovascular tissues, have been gradually discovered. As for ovarian BMPs, it is exciting that a point mutation in the *bmp15* gene has been discovered in infertile women with hypergonadotropic ovarian failure [94]. Recombinant proteins with this mutation lack biological activity but exert antagonistic effects on normal BMP-15 activity. Further studies revealed that several mutations in the *bmp15* and *gdf9* genes localized at the pro-protein coding region were involved in premature ovarian failure (POF) [95–97]. POF is a common cause of infertility in women, which can manifest as primary amenorrhea or secondary amenorrhea after pubertal development. POF is caused by follicle dysfunction and/or depletion, the latter of which is due to accelerated recruitment or increased atresia. Since BMP-15 and GDF-9 play a role both in promoting early follicle growth and restraining dominant/preovulatory follicle development, supplementation of BMP-15 and GDF-9 may overcome the follicle dysfunction or depletion shown in POF. Further work remains to be done to tie these findings together and develop a deeper understanding of the BMP-15 and GDF-9 interaction in female reproduction.

The importance of ovarian BMPs was also found in granulosa cell models exposed to high levels of GH and prolactin. Based on our recent findings, it is likely that endogenous BMPs play roles in inhibiting and/or neutralizing GH/insulin-like growth factor (IGF)-I effects on FSH-induced steroidogenesis in granulosa cells [98]. The suppression by BMP of FSH-induced progesterone synthesis was disturbed by treatments with GH and/or IGF-I. IGF-I in turn showed antagonistic effects on FSH-induced progesterone suppression by BMP-2, -4, -6, -7 and -15. Balanced intensities between the GH-IGF-I axis and BMP system may

be physiologically critical for regulating gonadotropin-induced steroidogenesis in growing follicles. In the case of the prolactin excess observed in prolactinoma patients, prolactin suppresses FSH-induced estradiol production by augmenting FSH-induced MAPK activity with prolactin receptor up-regulation [99]. Prolactin enhances the expression of endogenous BMP-Smad1/5/8 signaling activity via the JAK/STAT pathway, whereas BMPs suppress prolactin receptor expression in granulosa cells. Hence, the BMP system in growing follicles plays a role in helping the ovary to withstand exposure to high concentrations of GH and/or prolactin.

In the pituitary, BMP-4 seems to act as a functional modulator for various agents such as somatostatin and dopamine in an autocrine/paracrine manner, leading to fine-tuning of the sensitivity in lactotropes and corticotropes. For treatment of pituitary adenomas that have recurred after surgery and/or medication-resistant adenomas, alternative therapeutic strategies are clinically required [100]. The currently available medication for corticotropinomas is, in general, less effective for controlling cases that have recurred and/or residual tumors after surgery. As for prolactinomas, cases with low sensitivity to DA occasionally exist. In addition to the use of somatostatin analogs for acromegaly patients, somatostatin analogs may also have potential as a therapeutic agent for resistant cases of other functioning tumors. The functional link between BMP-Smad signaling and SSTR actions may be involved in individual tolerance to somatostatin analogs for various pituitary adenomas. Elucidation of the changes in expression of BMP-4, BMP receptors and SSTRs in functioning pituitary tumors would provide a clue for clarifying this interrelationship.

In terms of the adrenal BMP system, BMP-6 activity may be one of the causes of aldosterone breakthrough or the escape phenomenon observed in hypertensive patients treated with long-term ACE inhibitor and/or ARB therapy. Various factors, such as ACTH, electrolytes, endothelins and Ang II type 2 receptor actions, have been proposed as the cause of aldosterone breakthrough. Among these, the renin-angiotensin-aldosterone (RAA) system is still a key regulator of blood pressure and fluid homeostasis. The breakthrough phenomenon might be associated with important cardiovascular and renal outcomes. It

is therefore important to elucidate the molecular cause of aldosterone breakthrough. Changes in the bioavailability of BMP-6 and cellular BMP responsiveness are, at least in part, involved in the occurrence of cellular escape from aldosterone suppression under the condition of chronic AT1 blockade. Further approaches are necessary to determine the factors that control BMP-6 expression in the adrenal and pathophysiological roles of the adrenocortical BMP-6 in aldosterone breakthrough.

Conclusion

It has gradually been found that the BMP system is a fine regulator of fundamental endocrine activity at various levels. Accordingly, further research in this field will greatly expand our understanding regarding the pathophysiology of classical endocrine regulation modulated by local BMP signaling. Future research will further lead to novel targets for wide-ranging clinical regimens aimed at controlling female reproduction, steroidogenesis and endocrine tumorigenesis.

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