

REVIEW

Multiple Endocrine Regulation by Bone Morphogenetic Protein System

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Abstract. Bone morphogenetic proteins (BMPs) were originally identified with regard to their actions to regulate ectopic formation of bone and cartilage and early embryonic development. Subsequently, our research program has investigated a BMP system that exists in the mammalian ovary and plays roles in regulating numerous granulosa cell functions. BMP ligands including BMP-2, -4, -6, -7 and -15 were found to inhibit gonadotropin-dependent progesterone synthesis by granulosa cells, which led to the hypothesis that BMPs are a physiological luteinization inhibitor in growing ovarian follicles during the follicular phase of the ovarian cycle. The physiological importance of the BMP system for normal mammalian reproduction has been further recognized by the discovery of aberrant reproductive phenotypes of female sheep and humans having mutated genes encoding BMP-15. Physiological roles of BMPs in the pituitary, hypothalamus, adrenal and other tissues have also been discovered. Here we discuss recent advances in the understanding of autocrine/paracrine actions of BMPs in the systemic regulation of endocrine function.

Key words: Bone morphogenetic protein, Folliculogenesis, Ovary, Reproduction, Steroidogenesis

BONE MORPHOGENETIC PROTEINS (BMPs) were originally isolated from bone tissues as proteins that induce bone and cartilage formation in ectopic extra-skeletal sites *in vivo* [1]. The amino acid sequences from the corresponding cDNAs revealed that BMP ligands are structurally classified into transforming growth factor (TGF)- β superfamily member. To date, more than 30 members of the TGF- β superfamily have been identified in various species [2, 3]. There is no direct evidence that all molecules designated as BMPs can induce cartilage and/or bone formation, whereas it has been well established that BMPs regulate multiple biological processes including cell proliferation, apoptosis, differentiation and morphogenesis. In this review, the recent advances regarding critical BMP actions in the ovarian folliculogenesis, which has been mostly dis-

covered by Shimasaki's laboratory [4, 5], and in many of extra-gonadal endocrine tissues are introduced.

I) Molecular characteristics and receptor signaling of the BMP system

Ligands of the TGF- β superfamily are initially synthesized as large precursor proteins. The precursor proteins dimerize and then are cleaved by proteolytic processing to produce mature dimeric proteins. The BMPs are distinguished from other members by having seven, rather than nine, conserved cysteine residues in the mature region. Six of the seven common cysteines in the mature protein are linked within the subunit to form a rigid structure called a "cysteine knot." The re-

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Abbreviations: AC, adenylate cyclase; ActRI and ActRII, activin type I and type II receptor; ALK, activin receptor-like kinase; BMP, bone morphogenetic protein; BMPRI and BMPRII, BMP type I and

type II receptor; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; FSH, follicle-stimulating hormone; GDF, growth differentiation factor; GnRH, gonadotropin-releasing hormone; MAPK, mitogen-activated protein kinase; PKA, protein kinase A; P450arom, P450 aromatase; P450scc, P450 steroid side-chain cleavage enzyme; SAPK/JNK, stress-activated protein kinase / Jun-N-terminal kinase (JNK); StAR, steroidogenic acute regulatory protein; TGF- β , transforming growth factor- β

maintaining seventh cysteine is necessary for dimerization through disulphide bonding. BMP-15 and GDF-9 differ from other TGF- β superfamily members in that their mature regions lack cysteine residue to be used for the intra-molecular S-S bonding [6, 7].

Seven type I (ALK-1, -2, -3, -4, -5, -6 and -7) and five type II (ActRII, ActRIIB, AMHR-II, BMPRII and T β RII) receptors for TGF- β superfamily members have been characterized in mammals. Both type I and type II receptors are structurally similar and possess serine/threonine kinase domains in their intracellular domains. Dimeric TGF- β superfamily members bind to a heterotetrameric complex of two type I and two type II receptors. Type I receptors have "GS domain" in the transmembrane domain that is rich in glycine and serine residues. TGF- β and activins first bind type II receptors and the type I receptors are subsequently recruited into a ligand-receptor complex. In the case of BMPs, both type II and type I receptors independently have certain affinity for the ligand and the complex can achieve high affinity binding [8].

Following binding of a BMP to its receptor, the phosphorylated type I receptors activate downstream signaling molecules Smads. The pathway-restricted Smads (Smad1/5/8) are phosphorylated by type I receptors, and then they interact with a common-mediator Smad (Smad4) to form a hetero-oligomeric complex with Smad1/5/8. The complex then translocates to the nucleus, where it binds directly or indirectly to target DNA and induces transcription of specific genes [9]. Other signaling pathways also exist concurrently with Smad signaling 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 or Rac families of small GTP-binding proteins. Extracellular signal-regulated kinase-1/2 (ERK1/2), and stress-activated protein kinase (SAPK)/Jun-N-terminal kinase (JNK), have also been linked to TGF- β signal transduction in some cell types.

II) Roles of ovarian BMP system in female reproductive function

Expression of BMP ligands and BMP receptors in the ovary

Expression of BMP-2, -3, -3b, -4, -6, -7, -15 and GDF-9, and BMP receptor type IA (BMPRIA/ALK-3), type IB (BMPRII/ALK-6) and type II (BMPRII) were

identified in the ovary of various mammals [10]. The various BMP ligands are expressed in cell-specific expression patterns in ovarian cells that undergo dynamic changes during follicular development and corpora luteal morphogenesis [5]. It is therefore possible that the developmental process of folliculogenesis (recruitment, selection and atresia), ovulation, and luteogenesis (luteinization and luteolysis) are accompanied by dramatic spatial and temporal changes in the expression patterns of these BMP genes.

Ligand-dependent BMP actions in granulosa cell functions

BMP-2, -4, -6 and -7 each exerts activities on ovarian steroidogenesis and granulosa cell mitosis in the ovary. BMP-4 and BMP-7 are expressed in theca cells and regulate follicle-stimulating hormone (FSH)-induced estradiol and progesterone production by granulosa cells by increasing FSH-induced estradiol and suppressing progesterone production [4]. An *in vivo* study showed that BMP-7 decreases the number of primordial follicles but increases the number of primary, secondary and antral follicles [11]. Thus, BMP-7 promotes the "recruitment" of primordial follicles into the growing follicle pool, while inhibiting ovulation and progesterone production. Similar to BMP-4 and -7, BMP-6 inhibits FSH-induced progesterone synthesis by granulosa cells [12]. BMP-6 is expressed in the oocytes and granulosa cells of healthy Graafian follicles [10]. BMP-6 inhibits FSH actions by suppressing adenylate cyclase activity [12]. BMP-6 mRNA expression in granulosa but not oocytes rapidly decreases at the time when the dominant follicle is selected [10], implying that BMP-6 is linked to the mechanism of dominant follicle "selection". Unlike BMP-7 action, which induces granulosa cell proliferation, BMP-6 has no significant effect on granulosa cell mitosis. Interestingly, BMP-7 increases FSH-induced estradiol production whereas BMP-6 has no impact on estradiol synthesis [13]. BMP-7 action is in part mediated through a FSH receptor signaling that is independent of cAMP-PKA pathway, *i.e.* BMP-7 actions on FSH-induced estradiol production occur through suppression of ERK1/2 downstream of FSH receptor signaling [13]. Likewise, BMP-2 and -4 enhance FSH-induced p38-MAPK phosphorylation, leading to an increase of FSH-induced estradiol production [14]. Thus, BMP-2, -4, -6 and -7 differentially regulate FSH-induced steroidogenesis by granulosa

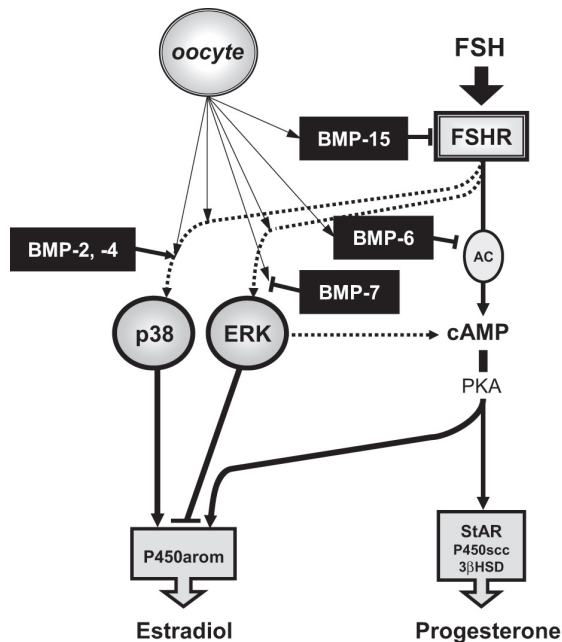


Fig. 1. BMP system regulates steroidogenesis through oocyte-granulosa cell communication in the ovary.

FSH activates estradiol and progesterone production through cAMP-to-PKA pathway in granulosa cells in the ovary. FSH simultaneously stimulates MAPKs, leading to the pathway-specific modulation of FSH-induced steroidogenesis. For instance, BMP-15 inhibits FSH receptor expression while BMP-6 suppresses adenylate cyclase (AC) activity, both of which result in 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 upregulation of estradiol production since ERK1/2 activation is directly linked to inhibition of estradiol. In addition to the regulation of steroidogenesis by granulosa cells, oocyte and/or oocyte-derived factors facilitate FSH-to-MAP kinase and BMP-to-Smad signaling activity, which supports effective control of FSH-induced steroidogenesis by granulosa cells.

cells in ligand-dependent mechanisms (Fig. 1).

Oocyte-derived BMP-15 inhibits FSH receptor expression and stimulates granulosa cell mitosis

Analysis by *in situ* hybridization and immunohistochemistry demonstrated BMP-15 expression exclusively in the oocyte, with its expression increasing in association with follicle growth and development [15]. BMP-15 inhibits FSH action by suppressing FSH receptor expression [16]. Specifically, FSH-induced expression of steroidogenic acute regulatory protein (StAR), P450 steroid side-chain cleavage enzyme (P450scc), 3 β -hydroxysteroid dehydrogenase (HSD),

luteinizing hormone (LH) receptor and inhibin/activin subunits are all inhibited by BMP-15 [16]. The finding that FSH-induced progesterone synthesis are inhibited by BMP-15 demonstrates that BMP-15, like BMP-4, -6, -7 and GDF-9, is part of a group of “luteinization inhibitors” [17]. BMP-15 also stimulates granulosa cell proliferation in a dose-dependent manner [15]. Moreover, BMP-15 stimulates the expression of kit ligand (KL) mRNA in granulosa cells [18].

Oocyte-derived BMP-15 and granulosa-derived KL form a novel negative feedback loop that is functionally linked to granulosa cell proliferation. BMP-15 also stimulates cumulus expansion [19], which is associated with the enhanced expression of epidermal growth factor-like growth factors in cumulus cells. These BMP-15 actions are regulated by a binding protein follistatin [20]. Follistatin is strongly expressed in dominant follicles, but very low or undetectable in atretic follicles [21, 22]. Because BMP-15 is an inhibitor of FSH receptor expression, it can be hypothesized that follistatin regulation of BMP-15 is important for normal folliculogenesis *in vivo*. Furthermore, BMP-Smad signal activities are also regulated by FSH receptor signaling, leading to fine-tuning of the mutual sensitivity of BMPs and FSH [23]. The “communication networks” between oocytes and follicular cells are crucial not only for the growth and maturation of the oocyte, but also for the proper differentiation of somatic follicular cells [24–26]. In this concept, oocyte-secreted factors play a key role in regulating FSH activity and FSH sensitivity during folliculogenesis (Fig. 1).

The significance of BMP-15 in reproductive endocrinology: lessons from sheep and human mutations

As shown above, all these BMPs have been found to be selective inhibitors of progesterone synthesis induced by FSH. This provides strong support for the hypothesis that BMPs are long sought “luteinization inhibitors”.

The Inverdale strain of sheep have been found to carry a single point mutation in the “mature-protein” region of the *bmp15* gene [27]. The heterozygous Inverdale mutants exhibit increased ovulation rates resulting in increased twinning. In the heterozygotes, reduced levels of intact BMP-15 may cause higher levels of FSH receptors in granulosa cells, leading to more developing follicles and high expression levels of LH receptors. Accordingly, the sheep would

exhibit precocious follicle maturation and increased ovulatory follicles at each cycle. On the contrary, homozygous Inverdale females are infertile due to arrested follicle development at the primary follicle stage. The lack of bioactive BMP-15 and its mitotic effects and KL-promoting effects on granulosa cells could account for the arrest of follicle development in the homozygotes [15]. Thus, the reproductive phenotype of Inverdale ewe provides *in vivo* data that support our *in vitro* findings on the role of BMP-15 in regulating follicular development and establishes the importance of this oocyte-secreted factor in mammalian reproduction.

BMP-15 signaling is mediated by binding first to ALK-6 and then recruiting BMPRII to the complex [29]. Another strain of sheep that are highly prolific, Booroola sheep, have been found to carry a mutation in the BMPRII/ALK-6 receptor [30–32], which is a key receptor for BMP-15. Granulosa cells of Booroola are more responsive to FSH than normal granulosa including cAMP production although the FSH binding capacity is not changed [33]. Ovarian follicles of Booroola ewes produce more progesterone induced by FSH than those from wild-type ewes. The enhanced FSH responsiveness of the follicles from Booroola ewes could be explained by the impaired ALK-6 signaling triggered by endogenous BMP-6 and/or BMP-15 [17].

A point mutation in the *gdf9* gene (*FecG^H*) has also been discovered in a different strain of sheep that has a similar phenotype to the Inverdale sheep having a *bmp15* mutation [34]. Interestingly, ewes that are compound heterozygotes for both BMP-15 and GDF-9 mutations have significantly higher ovulation rates than heterozygous carriers of a mutation in only one of the genes [34, 35]. These findings provide evidence for intracellular interactions between BMP-15 and GDF-9 that have important consequences on the secretion of the mature proteins. The *in vitro* observation that these proteins are secreted as homo-dimers when expressed individually and can form hetero-dimers when co-expressed [36, 37] supports this concept [38].

In human, a point mutation in the *bmp15* gene has been discovered in women with infertility due to hypergonadotropic ovarian failure [39]. Recombinant proteins with this mutation lack biological activity, and, importantly, have antagonistic effects toward the wild-type BMP-15 protein. More recent studies have also revealed several point mutations in the *bmp15* and *gdf9* genes, located at the “pro-protein regions” that are

associated with premature ovarian failure [40–42].

Despite the many recent discoveries of the properties and actions of BMP-15 and GDF-9 *in vitro* and the identification of functional effects of BMP-15 and GDF-9 mutations and deletions in various mammals *in vivo*, much work remains to connect the *in vivo* and *in vitro* findings and develop a further understanding of the precise mechanisms by which the interaction of these two factors have such important impacts on female reproductive physiology.

Along these lines, it has recently been found that posttranslational phosphorylation of recombinant BMP-15 and GDF-9 is essential for bioactivity of BMP-15 and GDF-9, and that the dephosphorylated forms of BMP-15 and GDF-9 exhibit antagonistic activity toward not only their phosphorylated counterparts but also toward each other, as well as BMP-7 [43].

III) Roles of the BMP system in the pituitary and hypothalamus

BMP-6, -7 and -15 regulate pituitary gonadotrope function

Locally-produced BMPs play a critical role in the differentiation of the pituitary gonadotrope [44, 45]. During normal mouse pituitary development, BMP-4 is expressed in the ventral diencephalon, while BMP-2 and the BMP binding protein, chordin, are expressed in the ventral condensing mesenchyme. BMP ligands and receptors are expressed in the adult pituitary gland. Specifically, BMP-6, -7 and -15 mRNAs are expressed in the pituitary of mice [18, 46], GDF-9 mRNA in the pituitary of humans [47], BMP-15 and GDF-9 mRNAs in the pituitary of brushtail possums [48] and BMP-15 mRNA in the pituitary of sheep [27]. With regard to BMP receptors, BMPRII, ActRII, ALK-2 and ALK-3 mRNAs are expressed in mice pituitaries [18], ALK-6 mRNA in sheep pituitaries [30], and ActRII and ActRIIB mRNAs in rat pituitaries [49]. Of potential physiological importance are the findings that BMP-6, -7 and -15 can act directly on pituitary gonadotropes to regulate FSH synthesis and secretion.

BMP-4 action is associated with pituitary tumorigenesis

The pituitary BMP system also acts as a regulator of transformation and tumorigenesis of differentiated pituitary cells. Human gonadotropinomas have reduced

expression of the activin/BMP binding protein, follistatin, as compared with non-functioning tumors [50]. In addition, PPAR γ activation is functionally linked to the inhibition of BMP receptor signaling in gonadotrope L β T2 cells [51]. It is interesting that BMP-4 is overexpressed in various lactotrope tumor models and human prolactinomas [52], with the molecular interaction between BMP-4, Smad4 and estrogen receptor (ER) involved in regulating prolactin-promoter activity [53]. We have reported that BMP-4 activates GH and cAMP synthesis induced by forskolin in mouse lactosomatotrope GH3 cells [54]. It is of note, in the presence of a high concentration of somatostatin analogue octreotide, the effects of a dopamine agonist bromocriptine that suppresses BMP-4-Smad1/5/8 signaling are impaired. These findings explain, at least in part, the mechanism of clinical resistance of GH reduction to a combination therapy with octreotide and bromocriptine in a subset of acromegaly patients. Giacomini and colleagues have reported that pituitary-expressed BMP-4 inhibits corticotrope cell proliferation and ACTH production by corticotrope tumor cells, and that BMP-4 expression can be augmented by retinoic acid [55]. Thus, the pituitary BMP system is likely to act as a regulator not only for pituitary differentiation but also for the transformation of differentiated pituitary cells.

Hypothalamic BMP system and gonadotropin-releasing hormone (GnRH) regulation

ActRII and ActRIIB mRNAs are expressed in the rat hypothalamus. These receptors are found in the areas for neuroendocrine regulation including the suprachiasmatic, supraoptic, paraventricular and arcuate nuclei [49]. GnRH synthesis and release are tightly regulated by gonadal steroids, which maintain control through a negative feedback system [56]. In females, estrogens act directly or indirectly on the GnRH neuronal network to modulate the final output of GnRH into the median eminence [57, 58]. An immortalized GnRH-producing GT1-7 cell line is a valuable tool to study the biology of GnRH neurons because of the expression of functional ER [59, 60]. The interaction between BMP receptors and ER is involved in controlling hypothalamic GnRH production and secretion in GT1-7 cells [61]. In this system, BMP-2 and BMP-4 repress ER-induced down-regulation of GnRH transcription by attenuating ER-MAPK signaling. BMP-6 and BMP-7, in turn, increase

GnRH transcription and secretion by stimulating the expression of R-type calcium channel.

IV) Involvement of BMP system in other endocrine tissues

BMP-6 effects on aldosterone induction in the adrenal cortex

Aldosterone production occurs in the adrenal glomerulosa, and is regulated primarily by angiotensin II (Ang II), potassium and, to a lesser degree, adrenocorticotropin (ACTH) [62, 63]. In the presence of the aldosterone stimulators, steroidogenesis in the adrenal cortex is further governed by local autocrine and/or paracrine factors [64]. We have reported the presence of a functional BMP and activin system complete with ligands including BMP-6, activins and their receptors in a human adrenocortical cell line [65, 66]. Further investigation demonstrated that BMP-6 increased Ang II-induced aldosterone production [67]. This BMP-6 action could be involved in the “aldosterone breakthrough” phenomenon. Aldosterone breakthrough is a phenomenon where circulating aldosterone concentrations increase above pretreated levels after long-term therapy with ACE inhibitors [68, 69] or an Ang II type 1 receptor antagonist (ARB) [70]. This phenomenon may lead to important clinical consequences since increased aldosterone in a high salt state may facilitate cardiovascular and renal damages in hypertensive patients [71, 72]. Involvement of various *in vivo* factors such as ACTH, electrolytes, endothelins and Ang II type 2 receptor actions [70, 73] have been proposed. We found that a long-term ARB treatment reverses the reduction of aldosterone synthesis by adrenocortical cells, *i.e.*, “cellular aldosterone breakthrough” [74]. This *in vitro* breakthrough was clearly attenuated by neutralization of endogenous BMP-6 and ALK-2, suggesting that BMP-6 availability in the adrenal cortex *in situ* may be at least in part involved in the occurrence of cellular escape from aldosterone suppression under chronic treatment with ARB.

BMP-4 actions in the adrenal medulla on catecholamine synthesis

The adrenal cortex and medulla functionally interact with each other in a paracrine manner [75,

76]. Endogenous glucocorticoids are known to induce catecholamine biosynthesis by stimulating catecholamine-synthesizing enzymes through the cortico-medullary portal system [77]. We previously reported the presence of the BMP system in the adrenal medulla and a functional crosstalk between glucocorticoid and BMP system in regulating catecholamine synthesis in adrenomedullar PC12 cells [78]. The key components of the BMP system are expressed throughout neural development [79]. For instance, BMP-4 and BMP-7 are expressed in the dorsal aorta and direct sympathetic neuronal differentiation into the adrenergic characteristics [80]. BMP-4 and BMP-7 induce a tyrosine hydroxylase (TH)-immunoreactive adrenergic phenotype in cultures of avian neural crest cells [81, 82]. We also found that BMP-4 and BMP-7 induce catecholamine production in the presence of mineralocorticoid in adrenal medullar cells. Catecholamine biosynthesis in adrenomedullar cells occurs via MR through genomic action and partly through nongenomic action by Rho-SAPK/JNK signaling [83]. Given that the nongenomic pathway was activated by BMP-4, this adrenocortical-medullar interaction via MR and BMPs was hypothesized to be involved in catecholamine regulation [83].

BMP system in the thyroid suppresses TSH receptor signaling

Thyrotropin (TSH) is the principal regulator of thyrocyte growth and hormone production. TSH transduces its signaling through TSH receptors (TSH-R) resulting in stimulation of the cAMP and phospholipase C (PLC) pathways (84). In the presence of TSH, a number of growth factors act as mediators of thyrocyte growth. For instance, insulin and insulin-like growth factor-1 (IGF-1) acting via the IGF-1 receptor promote follicular cell growth synergistically with TSH [85]. TGF- β 1, activin A and BMP-7 proteins are expressed in the follicular epithelium of porcine thyroid sections [86]. The phosphorylation of Smad2 by TGF- β 1 and activin inhibited thyrocyte mitosis, TSH-R expression and TSH-stimulated cAMP responses [86]. BMP-2, -4, -6 and -7 also suppress DNA synthesis, TSH-induced cAMP production and TSH-induced IGF-1 mRNA in porcine thyrocytes [87]. These data suggest that the BMP system in thyrocytes is negative regulator of TSH function as well as thyrocyte mitosis. Considering that BMP-7 also exerts

dose-dependent suppression of DNA synthesis in neoplastic thyroid epithelial cells [88], the thyroid BMP system is likely to play a role in the suppression of thyroid tumorigenesis.

V) BMP functions in cardiovascular and renal tissues

Involvement of BMP-2 and -7 in the progression of pulmonary arterial hypertension

Approximately six percent of the primary arterial hypertension (PAH) cases possess an autosomal dominant pattern of inheritance. Recent research has uncovered a genetic predisposition to familial PAH [89, 90]. Studies involving the screening of the locus of the gene for PAH, mapped to chromosome 2q33 (*PPH1*) have unveiled mutations in the *bmpr2* gene encoding the bone morphogenetic protein type II receptor (BMPRII) in nine out of nineteen [91], or seven out of eight families [92]. Furthermore, the mutation in the *bmpr2* gene can be detected in at least a quarter of apparently sporadic PAH cases [93]. Hence, the identification of heterozygous germline mutations in the *bmpr2* gene in familial and sporadic cases of PAH has proven to be a crucial breakthrough in elucidating underlying pathogenesis of PAH [89]. Subsequent functional studies have provided compelling evidence that PAH cells harboring the *bmpr2* mutations exhibit aberrant function of BMPRII and disrupted BMP signaling [94, 95]. In addition, the interaction between BMP ligands and type I receptors is also critical for the pulmonary arterial smooth muscle cell mitosis in PAH [96]. However, given that almost half of PAH patients do not bear the *bmpr2* mutation, the mechanism of pulmonary arterial smooth muscle cell mitogenesis in PAH lungs remains uncertain.

BMP-4 and -7 ameliorate glomerular mesangial cell proliferation in the kidney

Recent studies have provided evidence that BMPs, in particular BMP-7, play a key role in the pathogenesis of various renal diseases including early experimental diabetic [97] or obstructive [98] nephropathy. BMP-4 and BMP-7 have important roles in renal development than other members of the BMP family, each of which has unique effects on different parts of the developing kidney [99]. In experimental animal models

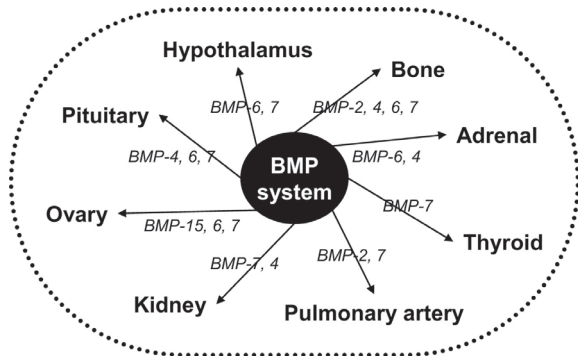


Fig. 2. The BMP system plays cell-specific roles in various endocrine tissues.

Recent data have demonstrated that BMPs are multifunctional regulators in various tissues and cells. In addition to bone, there is evidence that expression of BMP genes is high in the kidney, lung, small intestine, heart and that they can regulate the cellular homeostasis by autocrine/paracrine mechanisms. A variety of physiological roles of BMPs in many endocrine tissues including ovary, adrenal, thyroid, pituitary and hypothalamus have been recently identified. The BMP system composed of BMP ligands, receptors and binding proteins is considered to be crucial regulator for fundamental endocrine functions. The dysregulation of BMP system in some specific tissues is also involved in the pathophysiology of various endocrine disorders.

of obstructive or diabetic nephropathy, BMP-7 reduces glomerular and tubulointerstitial fibrosis and preserves renal function [100, 101]. BMP-7 even resolves glomerular and interstitial fibrosis in rats with diabetic nephropathy [101], which results from inhibition of the TGF- β effects on fibrosis mediators and matrix protein degrading enzymes [102]. BMP-7 antagonizes TGF- β -induced glomerular fibrogenesis and accumulation of collagen, fibronectin and thrombospondin in cultured mesangial cells, suggesting that BMP-7 opposes several profibrogenic activities of TGF- β in mesangial cells [102]. BMP-4 and BMP-7 also antagonize aldosterone-induced mesangial cell proliferation by inhibiting MAPK activation as well as mineralocorticoid receptor (MR) expression [103]. Thus, BMP-4 and -7 seem to exert renoprotective actions leading to amelioration of glomerular sclerosis.

BMP-6 and -7 inhibit breast cancer cell proliferation via estrogen receptor

There has been accumulating evidence that breast cancer tissue expresses all enzymes required for the local biosynthesis of estrogen from circulating precursors, *i.e.* the aromatase pathway that transforms androgens into estrogens and the sulfatase pathway that converts estrone sulfate into estrone [104]. Therapeutic targets for breast cancers include not only the binding of estrogen to ER but also the activity of estrogenic enzymes in the tumor tissues [105]. There have been several reports showing the expression of some of TGF- β superfamily proteins, such as BMP-2 [106], -6 [107], and -7 [108] in breast cancer cells, in which their possible role in breast cancer development and involvement in bone metastasis has been discussed [109, 110]. Furthermore, the involvement of BMP-Smad activation in the progression and dedifferentiation of ER-positive breast cancer was recently reported [111]. BMP-6 and -7 are also found to antagonize estrogen-induced breast cancer cell proliferation by inhibiting p38 phosphorylation and estrogenic enzyme expression [112]. The inhibitory effects of BMPs on the MAPK pathway and/or the expression of ER and estrogenic enzymes could be involved in the suppression of estrogen-induced mitosis of breast cancer cells.

Conclusion

Besides regulating bone formation and bone differentiation [113, 114], considerable recent data have demonstrated that BMPs are multifunctional regulators in a wide array of biological processes in both vertebrates and invertebrates [115]. In addition to bone, there is evidence that expression of BMP genes is high 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 -7) and that they can regulate the cellular homeostasis by paracrine mechanisms [116]. A variety of BMP actions in many endocrine tissues including the ovary, pituitary, thyroid, adrenal and cardiovascular tissues has been gradually identified (Fig. 2). Especially, current research has demonstrated that the BMP system is a critical component of the local regulatory system in the ovary. Now we have recognized that BMP ligands and receptors are powerful regulators of fundamental endocrine functions. Accordingly, further research in this field will greatly advance our understanding of pathophysiology of systemic endocrine regulation and will lead to novel targets for wide-ranging clinical regimens aimed at controlling female reproduction, steroidogen-

esis and endocrine tumorigenesis.

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