Clinical significance linked to functional defects in bone morphogenetic protein type 2 receptor, BMPR2

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INTRODUCTION

Bone morphogenetic protein type 2 receptor (BMPR2) is one of the transforming growth factor-β (TGF-β) superfamily receptors, performing diverse roles during embryonic development, vasculogenesis, and osteogenesis. Human BMPR2 consists of 1,038 amino acids, and contains functionally conserved extracellular, transmembrane, kinase, and C-terminal cytoplasmic domains. Bone morphogenetic proteins (BMPs) engage the tetrameric complex, composed of BMPR2 and its corresponding type 1 receptors, which initiates SMAD proteins-mediated signal transduction leading to the expression of target genes implicated in the development or differentiation of the embryo, organs and bones. In particular, genetic alterations of BMPR2 gene are associated with several clinical disorders, including representative pulmonary arterial hypertension, cancers, and metabolic diseases, thus demonstrating the physiological importance of BMPR2. In this mini review, we summarize recent findings regarding the molecular basis of BMPR2 functions in BMP signaling, and the versatile roles of BMPR2. In addition, various aspects of experimentally validated pathogenic mutations of BMPR2 and the linked human diseases will also be discussed, which are important in clinical settings for diagnostics and treatment. [BMB Reports 2017; 50(6); 308-317]

KEYWORDS: BMPR2, Embryogenesis, Mutation, Osteogenesis, Pathogenesis

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BMPs

In mammals, BMPs are part of the TGF-β superfamily which is composed of 33 proteins, comprising of TGF-βs, activins, thyroid gland, adrenal gland, heart, liver, pancreas and kidney (1-8). Previous reports state that BMPR2 serves as a type 2 receptor for bone morphogenetic protein (BMP) ligands in mammals, and the engagement of specific BMPs to the BMPR2 and corresponding type 1 receptors plays important roles in osteogenesis, cell growth, cell differentiation, and embryonic development. Knockout of BMPR2 gene is fatal for embryonic development, and conditional BMPR2 knockout mice in uterine decidua revealed that BMPR2 is essential for post-implantation and fertility (9). To date, at least 20 BMPs, seven type 1 receptors (ALK1-7), and four type 2 receptors (BMPR2, ACVR1, ACVR1b, and TGFβR1) have been identified in mammals (10, 11). Among the 20 BMPs, BMP2, 4, 6, and 7 have been reported to engage BMPR2 and its associated type 1 receptors, and the ligand-receptor combination is likely to determine the physiological roles of BMPR2 (12). Since the extracellular signal triggered by BMPs is transmitted into the cytoplasm by formation of heteromeric receptor complex, the disruption of BMP receptors by genetic alterations results in various phenotypic abnormalities. Indeed, causative mutations in the BMPR2 gene have been reported in patients present with pulmonary arterial hypertension (PAH), chronic obstructive pulmonary disease (COPD), hereditary hemorrhagic telangiectasia (HHT), prostatic neoplasms, colorectal cancer, and obesity (12-16). In particular, BMPR2 variants in PAH patients have been extensively identified, and pathogenicity of some BMPR2 mutations have been validated by in vitro functional assays. However, growing evidences have shown that the mutations in BMPR2 gene are also implicated in other diseases, which might be due to the loss of BMPR2 functions in specific combination with the ligands, and its type 1 binding partners. In this review, we summarize functional roles of BMPR2 in diverse molecular pathways. In addition, we discuss the identified BMPR2 variants and the resultant physiological disorders with experimentally validated cases.

FACTORS INVOLVED IN THE BMPR2-MEDIATED SIGNALING CASCADE

BMPs

In mammals, BMPs are part of the TGF-β superfamily which is composed of 33 proteins, comprising of TGF-βs, activins,
inhibins, nodal, lefty, Growth and differentiation factors (GDFs), anti-müllerian hormone (AMH), and BMPs (17). BMPs were initially discovered as factors that induce the ectopic formation of cartilage and bone in rats (18). It was later determined that BMPs in mammals have multiple roles in skeletal development, bone homeostasis, and tissue regeneration by triggering signal transduction via a complex composed of distinct transmembrane serine/threonine kinase receptors, BMPR1 and BMPR2. In addition, BMPs possess potent osteogenic activities, enabling the in vivo generation of ectopic bone formation (19). To date, at least 20 BMPs have been identified in mammals. Among them, BMP2, 4, 6, 7, and 9 are associated with high osteogenic activity (20). BMP2 is especially an indispensable factor for osteogenesis, and is being studied actively in human clinical uses for bone regeneration, and therapeutic trials of pathogenesis related to bone (21, 22). BMP2 also acts as a major factor in endocardial bone development, and induces expression of osteoblastic differentiation markers including alkaline phosphatase (ALP), osteocalcin, and RUNX2 (23). Similarly, BMP4 and BMP7 are responsible for the formation and repair of endocardial bone (24). BMP5 is required for initiation of normal skeletal development, and BMP9, together with vascular endothelial growth factor A (VEGFA), effectively stimulates ectopic bone formation (25, 26). In contrast, BMP3 knockout mice studies showed that BMP3 plays a role as a negative controller of bone development (27).

BMPs also play crucial roles in the establishment of basic embryonic body plans, which includes the initial vertebrate gastrulation, mesoderm, somite, neural patterning, development of limb and skeleton, and organogenesis (28, 29). It was reported that BMP2-deficient embryos exhibit defects in the development of heart, the first organ to be formed during embryogenesis. Further studies showed that the heart defect is due to the lack of interaction between ectodermal and mesodermal cells during development, demonstrating essential roles of BMP2 in organ development (30). Similar studies found that BMP4 exerts the formation of mesoderm during early gastrulation, and BMP7 functions in the organogenesis of heart, kidney, and eye (31-33).

The significant roles of BMPs in vascular development have been emphasized over the years, which have been verified in vascular disorders including HHT and PAH. For example, BMP9 and BMP10, as core cardiac-derived factors, are highly expressed in the heart. Experiments have shown that BMP9 and BMP10 modulate the pulmonary vascular function by engaging BMP receptor complex composed of ALK1 (ACVRL1), BMPR2, and Endoglin (ENG) to activate SMAD1/5/8-mediated signal transduction. Genetic alterations of genes encoding the complex, leading to attenuation of BMP9/10 signaling and thus inducing unbalanced angiogenic responses, have been identified in patients present with HHT or PAH (34). In particular, BMPR2 variants in PAH patients have been extensively identified in the past decade, which will be discussed below.

**BMP receptors**

BMPs initiate SMAD protein-mediated signaling by binding to a hetero-dimeric receptor complex. There are three BMP type 1 receptors, ACVR1 (also known as activin receptor-like kinase 2, ALK2), BMPR1a (ALK3), and BMPR1b (ALK6), and three BMP type 2 receptors, BMPR2, ACVR2a (ActR2a), and ACVR2b (ActR2b). Both type 1 and type 2 BMP receptors share common membrane receptor structures, such as a short extracellular ligand binding domain, a single membrane-spanning domain, and an intracellular serine/threonine kinase domain. The type 1 receptors carry two additional motifs, a glycine/serine-rich region preceding the kinase domain (GS-box) and a short region of eight amino acids (denoted as L45 loop) within its kinase domain (35, 36). An unusual property of type 1 receptors comes from a highly conserved GS motif which regulates the kinase activity of the receptor (37). The three type 1 receptors have redundant roles in skeletal development. BMPR1a and BMPR1b are structurally similar to each other, and have functionally akin features in chondrocyte condensations and developing skeletons. However, ACVR1 possesses a unique function to induce ectopic osteoblastogenesis through the activation of SMAD1/5 signaling (38, 39).

Type 2 BMP receptors are expressed in diverse tissues. Although all type 2 receptors have similar structures, BMPR2 has a unique 508-amino acid long C-terminal tail following the kinase domain. Long and short forms of BMPR2 have been isolated, where the short isoform is the splice variant lacking exon 12, coding most of the C-terminal tail. Similar to the long BMPR2 variant, the short form of BMPR2 is broadly expressed (12). Recently, it was reported that a single di-leucine motif located in the long C-terminal BMPR2 facilitates faster clathrin-mediated endocytosis than the short form. Further studies showed that enhanced expression of the short BMPR2 at plasma membrane led to increased activation of SMAD protein-mediated signaling, suggesting that the C-terminal region may be modulating the activity of BMPR2 (40). The physiological importance of the C-terminal region of BMPR2 has been emphasized by the identification of C-terminal truncation mutations of BMPR2 gene in familial primary pulmonary hypertension (PPH) patients, although the exact molecular function of the C-terminal region of BMPR2 remains elusive (41-43). Different BMPs engage different combinations of BMPR2 and its type 1 receptors, which is summarized in Fig. 1. To understand the pathophysiology of the disease due to BMPR2 mutations and eventually develop novel therapeutics, it would be important to understand the different biological functions of BMPR2 in response to different BMP ligands.

**Ligand-receptor oligomerization and signaling**

The BMPs, initially expressed as large inactive precursors, are commonly dimerized either with itself or with a different
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Fig. 1. Schematic summary of BMPR2 and its associated proteins in the BMP-mediated signaling cascade. Different BMP ligands engage BMPR2 and different corresponding type 1 receptors, such as ACVR1, BMPR1a, BMPR1b and ALK1. BMP4 and BMP6 ligands bind to a receptor complex consisting of BMPR2 and ACVR1; BMP2, BMP4, and BMP6 bind to BMPR2 and BMPR1a; BMP2 and BMP4 bind to BMPR2 and BMPR1b; and BMP9 and BMP10 bind to BMPR2 and ALK1. The activity of ACVR1 is negatively regulated by interaction with FKBP12. Endoglin, as a co-receptor for ALK1, promotes the receptor complex formation. Engagement of BMP ligands triggers SMAD1/5/8 phosphorylation, which then binds to SMAD4. The SMAD complex translocates into the nucleus to induce target gene expression. SMAD6 and SMAD7, which are induced by BMP signaling, bind to type 1 receptors and thus, negatively regulate BMP signaling by negative feedback loop. Some of the BMP target genes, including ID proteins, are indicated. MAPK, LIMK, ROCK, and Rho are activated by SMAD protein-independent manner.

Previous reports indicate that both type 1 and type 2 receptors individually form a dimer when they are associated with the BMP ligands. Therefore, active BMPs engage a tetrameric receptor complex (17). In general, while type 2 receptors are capable of binding BMP ligands on their own, the type 1 receptors are unable to interact with BMPs unless they are associated with corresponding type 2 receptors. BMPR2 weakly binds to BMPs alone, but the interaction is augmented in the presence of type 1 receptors (43). The serine/threonine kinase domain of type 2 receptors is constitutively active, but the activation of type 1 receptor kinase requires physical interaction with type 2 receptors through ligand engagement (51-54). Subsequently, the activated type 1 receptor phosphorylates R-SMAD proteins including SMAD1, SMAD5, and SMAD7, which are phosphorylated SMAD1/5/8 is associated with the common-mediator SMAD (Co-SMAD), SMAD4. The resultant SMAD complex, which functions as a transcription activator, translocalizes to the nucleus and activates the corresponding target gene expression. In contrast, the inhibitory-SMADs (I-SMADs), SMAD6 and SMAD7, are responsible for negative feedback of the signaling pathway (55). SMAD6 efficiently inhibits BMP signaling, and SMAD7 attenuates TGF-β, activin, and BMP signaling. I-SMADs disrupt the association of R-SMAD and Co-SMAD, resulting in inhibition of the expression of target genes. SMAD ubiquitin regulatory factors (Smurfs), an E3 ubiquitin ligase, leads to the ubiquitination and degradation of SMAD proteins, thereby modulating the BMP signals. Smurfs enhance the ubiquitination and degradation of type 1 receptors by interacting with SMAD7, forming a complex with type 1 receptors (56).
Additionally, Smurf1 has shown to control BMP signaling by targeting SMAD1/5 for ubiquitination and proteasomal degradation, which leads to enhanced sensitivity to the TGF-β signaling (57).

**Target gene regulation**
Phosphorylated R-SMAD and SMAD4 complex bind to the promoters of target genes, leading to gene expression. Of the various BMP target genes, ID proteins (inhibitory of differentiation) have been extensively studied. IDs are expressed ubiquitously, although the expression level is down-regulated in differentiating cells. In other studies, Ogata et al. showed that the ID genes are highly expressed in osteoblast lineages, suggesting that IDs play important roles in osteogenesis (58-59). Activated IDs inhibit the transcription activity of the myogenesis factor, MyoD, which in turn suppresses myogenesis (53). Consistently, BMP2 inhibits myogenic differentiation in C2C12 myoblasts by inducing transcription of ID1, resulting in stimulation of osteoblast differentiation (60).

Another well characterized BMP target gene is the runt-related transcription factor (RUNX), which promotes bone formation and hematopoiesis (61). Three RUNX isotypes (RUNX1, RUNX2, and RUNX3) have been identified. RUNX expression is induced by either SMAD-dependent or SMAD-independent manner. As a major transcription factor, RUNX2 is often associated with SMAD1 or SMAD5, and both factors synergistically regulate the transcription of genes required for differentiation of mesenchymal progenitor cells into osteoblasts (61, 62). RUNX2 up-regulates several osteogenic markers including ALP, at the early osteogenic stage, and osteocalcin and osteopontin, at the late stage (63). Osterix, another transcription factor induced by BMP signaling, mediates the differentiation of mesenchymal stem cells (MSCs) into bone cells (64). Other transcription factors associated with SMAD signaling contain TBX20 and VEGF. TBX20 has an important role in cardiac development, and VEGF is the main factor involved in angiogenesis.

**ROLES OF BMPR2 DURING THE EMBRYOGENIC DEVELOPMENT AND BONE DIFFERENTIATION**

**BMPR2 in embryogenesis**
Although initially identified as a factor for regulating the chondrogenic and osteogenic differentiation, accumulated studies demonstrate that BMPR2 plays important roles in early embryonic development (65). The expression levels of BMPR2 fluctuate during embryonic stages, although it is reported that basal level of BMPR2 expression is sustained in most tissues throughout development. BMPR2 expression is relatively low during early stages of heart development, but the expression level continuously increases with gradual embryonic development, especially in the anteriormost telencephalon, branchial arches, limb bud, and tail tip mesoderm. In the later stages, BMPR2 expression is specifically high in neuroectoderm of the mouth anlagen (66). In this regard, BMPR2 acts as an essential regulator in the developing organs and tissues. BMPR2 deficient mice consistently exhibit severe embryonic lethality prior to gastrulation. More specifically, BMPR2 null murine embryos induce collapse of the gastrulation and mesoderm formation, which are similarly observed in BMP4, ALK3, and SMAD4 null embryos. In addition, overexpression of dominant negative BMPR2 brings about abnormal mesoderm formation and patterning. Epiblast differentiation in the embryo and the anterior-posterior (A-P) axis were shown to be abnormal in BMPR2 knockout mice, although the differentiation of visceral endoderm was relatively normal (65). Taken together, these findings suggest that BMPs-BMPR2-mediated signal transduction is critical for diverse tissue and organ development.

**BMPR2 in vascular development**
BMP signaling enhances the endothelial specification, subsequent venous differentiation and angiogenesis during embryonic development, thereby maintaining the vascular homeostasis (30, 67). Notably, BMP2, as a component of the BMP signaling transduction, is prominently expressed in the vascular endothelium and smooth muscle layer of the pulmonary vasculature in normal lung, but is poorly expressed in the airway and arterial smooth muscle (68). BMP2 is expressed in human microvascular endothelial cells (HMECs), human umbilical vein endothelial cells (HUVECs), and aortic endothelial cells, highlighting that BMPR2-mediated signaling cascade plays important roles in vascular development (69).

It was reported that BMP2 is expressed in a variety of cancers. To understand the functional relevance of the BMP2 in cancer cells, BMP2-overexpressed A549 cells were injected in nude mice, and it was observed that BMP2-mediated signaling was involved in tumor angiogenesis (70). In addition, Wiley et al. showed that BMP2-BMPR2-mediated signaling regulates sprouting angiogenesis from the axial vein in zebrafish development, demonstrating that BMPR2-dependent signaling promotes endothelial cell proliferation and angiogenesis (71). To promote human pulmonary arterial endothelial cell (HPAEC) survival and proliferation through ERK1/2 activation, BMP2 is associated with the canonical WNT signaling pathway, which also stimulates non-canonical RhoA-Rac1 pathway to induce endothelial cell migration (72). Defects in BMPR2 cause abnormal vascular remodeling. Pulmonary endothelium in BMPR2 knockout mice has shown to the inclination of PAH, which is characterized by reduced lumen diameter and decreased vasodilation ability resulting from increased proliferation of vascular smooth muscle cells (VSMCs) and excess deposition of extracellular matrix (ECM) in the vessel walls (73). Similarly, BMPR2 mutant mice lacking C-terminal tail in the pulmonary artery smooth muscle cells (PASMCs) have a PAH-like predisposition. Restoration of BMPR2 in mice exposed to chronic hypoxia induces activity of BMP-SMAD1/5 signaling, followed by decline of vascular remodeling, implying a functional imbalance of BMP signaling.
Therefore, BMPR2 expression in ECs and SMCs has important roles in the maintenance of vascular integrity of the pulmonary arteries (76).

**BMPR2 in Osteogenesis**

Although biological functions of BMPR2 have been observed in bone formation, the regulatory role of BMPR2 in chondrogenesis and osteogenesis is not yet clearly demonstrated. BMPR2 is involved in osteoblast differentiation, and is also an important mediator in bone formation and skeletal development during fracture healing, osteopetrosis, and osteosclerosis (77-79). During bone development, BMPR2 and its ligands induce the differentiation of mesenchymal stem cells toward osteoblastic lineage, which promotes the maturation of osteoblasts (80). To elucidate the underlying mechanism of osteogenesis, fibroblast, MSC, and myoblast have been utilized to induce osteogenic differentiation, as naïve osteoblasts are difficult to isolate, manipulate, and expand in vitro. Briefly, the differentiation process of osteoblasts is classified into two steps: first, is the differentiation of MSCs into osteoblast progenitors, and second, is the maturation of osteoblast progenitors into osteoblasts, presenting the various phenotypes of cells organizing bone (81). For the process of differentiation, useful alternative cells are the dermal fibroblasts, which are easily available and expandable (82). In the late 1980s, C3H10T1/2, a pluripotent fibroblastic and MSC, was cloned, which can differentiate into a myogenic lineage by introducing genes of the muscle-specific regulatory factors, including MyoD, myogenin, and Myf-5 (83-86). Since then, Katagiri et al. reported that the C3H10T1/2 cells can also be differentiated into the osteoblast-like cells by stimulation of recombinant human BMP2 protein. They also verified the osteogenic function of BMP2 by using myoblastic C2C12 cells, in which treatment of BMP2 inhibits myotube formation from C2C12. Instead, the cells start to induce ID1 expression so that the myoblasts can undergo differentiation toward osteoblast lineage by BMP2 (81, 87). The osteogenic activity of BMPs is dependent on type 2 receptors. For instance, Wu et al. showed that both BMPR2 and ACVR2 are responsible for osteogenic differentiation of C3H10T1/2 cells with BMP9 treatment (88). Taken together, these findings demonstrate that BMPR2-mediated signal transduction plays a critical role in skeletal development.

**PATHOGENIC MUTATION OF BMPR2**

The importance of the diverse BMPR2 functions has been highlighted by the identification of potential causative BMPR2 gene variants in patients present with diseases, including PAH, cancers and obesity. BMPR2 gene consists of 13 exons, which code for the typical 4 domains described above. Identified genetic alterations of BMPR2 gene lead to missense, nonsense, frameshift, truncation, and splice site mutations, which supposedly results in the loss of BMPR2-mediated signaling with some exceptions. BMPR2 mutations have been extensively determined in patients present with PAH characterized by elevated pulmonary arterial pressure (89). It was reported that PAH patients with BMPR2 mutations usually have a worse prognosis than patients with wildtype BMPR2. Not every BMPR2 mutations are validated functionally, either with the patient-driven cells or with other *in vitro* assays. In this section, we summarize the most up-to-date BMPR2 mutations in diverse diseases, with emphasis on experimentally validated BMPR2 mutations.

**BMPR2 mutations in PAH**

Accumulating body of evidence demonstrates that BMPR2 mutations are strongly associated with hereditary PAH (HPAH). BMPR2 mutations have been determined in 75% of HPAH patients and also in 15% of idiopathic PAH (IPAH) patients. HPAH has been defined as an autosomal-dominant disorder, and thus pathophysiology of the BMPR2 mutation in the PAH can be explained by haploinsufficiency. It is worth noting that only 20% of people who have the BMPR2 mutation develop HPAH, suggesting that there are additional factors, including genetic alterations or environmental agents, required for the development of the disease. Interestingly, it was reported that the wildtype BMPR2 transcripts and protein expression levels are impaired in PAH cells with BMPR2 mutation in the other allele. This observation might explain the reduction of BMPR2 expression in PAH patient-derived cells, although the underlying molecular basis remains elusive. It is still not clear why the BMPR2 mutation carriers eventually develop PAH. One hypothesis is that decreased BMP signal transduction might lead to hyperactivation of the TGF-β signaling, resulting in hyperproliferation of the SMCs in pulmonary arterioles. Consistent with this idea, it was found that activation of BMP signaling indeed inhibited the smooth muscle cell proliferation. However, the underlying molecular mechanism of enhanced TGF-β signaling cascade in response to reduced BMP activity is largely unknown. Potential causative BMPR2 mutations in PAH are basically distributed throughout the BMPR2 region, although more frequent BMPR2 mutations have been identified in key functional domains, such as the ligand binding domain and kinase domain. To date, over 400 different BMPR2 mutations in PAH patients have been reported, and functional defects of some of the BMPR2 variants have been validated with patient-driven cells or *in vitro* functional assays. Most of the experimentally validated BMPR2 mutations show an impaired SMAD protein-mediated signaling cascade (Fig. 2). The BMPR2 mutations in PAH have been regularly updated, and the most recent update is by Machado et al. However, not all mutations are functionally validated using various techniques available in life science. As the functional validation of the BMPR2 mutations is important and informative to understand the pathophysiology of PAH, we will summarize most of the validated BMPR2 mutations in this review.
Yang et al. obtained PASMCs from PAH patients with individual BMPR2 mutation leading to p.W9X, p.C347R, p.C347Y, and p.N903S. Compared to normal PASMCs, they noticed impaired SMAD protein phosphorylation and reduced ID1 and ID2 protein expression, which are downstream targets of SMAD proteins, in BMPR2 mutant cells treated with BMPs (90). Hedges et al. employed patient-derived cultured lymphocytes to validate the function of BMPR2 p.W13X mutant. Initially, the authors presumed that the p.W13X mutant will not be expressed due to the nonsense-mediated RNA decay (NMD). However, they surprisingly found that the truncated version of BMPR2 from normal allele, due to the existence of a downstream Kozak sequence enabling translation re-initiation (91). The truncated version of BMPR2 is an inactive form, as the ligand binding domain is lost. Likewise, the functional assays of other BMPR2 variants listed in Fig. 2 (upper) have been performed with patient-derived PASMCs and PAECs (92, 93). Another way to determine functionality of BMPR2 variants can be accomplished by ectopic expression of BMPR2 mutants in established cell lines to measure the BMPR2-mediated signals using various experimental tools, including immunoblotting, quantitative polymerase chain reaction, reporter assay and immunostaining. For example, to determine NMD of BMPR2 variants, c.2292insA, c.2386delG, c.2620G>T and c.2695C>T, Nasim et al. developed a novel dual fluorescence based assay system; the successful expression of the variant will give rise to red and green fluorescence simultaneously in HEK293T cells. Indeed, the BMPR2 mutations failed to express green fluorescence, thereby indicating that the mRNA is degraded by NMD (94). In the same studies, various missense BMPR2 mutations were expressed in HEK293T cells harboring the luciferase reporter, where the luciferase expression is under the BMP responsive promoter. Using the reporter system, they were able to validate various loss of function missense mutations of BMPR2 (94). Taken together, the in vitro functional assay proved that most of the causative BMPR2 mutations in PAH lead to impairment of the SMAD protein-mediated signal transduction.

**BMPR2 mutations in cancer**

Similar to tumor suppressor roles of TGF-β, BMPR1a and SMAD4 genes are frequently mutated in colon cancers, suggesting that BMPs-mediated signaling also has tumor suppressive functions (95). BMPR2 gene is found to be mutated or down-regulated in cancers. It was reported that BMPR2 expression level is significantly down-regulated in prostate cancer tissues. In addition, expression level of BMPR2 in the prostate cancer cells is negatively correlated with cancer grade (14). Indeed, the reduced expression level of BMPR2 showed statistically significant poor prognosis, such as cancer recurrence and worse 5 year survival (96). BMPR2 expression level is significantly abrogated in large portions of microsatellite unstable (MSI) colorectal cancers. Kodach et al. initially found impaired expression of BMPR2 in MSI colon cancer cell lines including HCT116, DLD1, SW48, and LOVO, while its expression was normal in microsatellite stable (MSS) colon cancer cell lines. Reduced BMPR2 expression level is also confirmed in all the MSI positive colon cancer patient tissues tested in the study. Mutation analysis revealed that HCT116 and LOVO cell lines have BMPR2 mutation in the coding region at 7 adenine tract in exon 12 (c.1742delA), resulting in frameshift and early termination of translation. However, such a mutation has not been found in the cancer patient tissues. Instead, 11 adenine tract in 3’ UTR of BMPR2 gene is mutated in all the colon cancer patient tissues, which results in down-regulation of BMPR2 expression (97). Later, by analyzing public datasets, Park et al. found the...
7 adenine tract mutations in BMPR2 gene in MSI positive colorectal cancer patients (15). Taken together, these findings suggest that genetic alterations of BMPR2 gene and the resultant reduced expression of BMPR2 might be responsible for cancer development, although the underlying mechanism remains largely elusive.

**BMPR2 mutation in obesity**

BMP-mediated signaling has been implicated in controlling adipocyte differentiation, and it was therefore proposed that BMPR2 expression might be positively correlated to obesity (98). Indeed, Schleinitz et al. found that BMPR2 mRNA level is significantly high in both visceral and subcutaneous adipose tissue of the overweight or obese population, compared to the lean population. In an attempt to define genetic alterations in BMPR2 of overweight population, two intronic single nucleotide polymorphisms (SNPs) were identified: rs6717924 and rs13426118. Gene expression analysis showed that allele carrying rs6717924 expresses higher BMPR2 mRNA compared to the other wildtype allele. The authors proposed that transcription factor binding sites surrounding the SNPs might be responsible for the enhanced gene expression, although the molecular basis of the higher BMPR2 expression in allele carrying rs6717924 remains elusive (16).

**CONCLUSION AND PERSPECTIVES**

BMPR2, a receptor for the TGF-β superfamily, was identified in the 1990s. Since then, understanding the functional roles of BMPR2 has significantly expanded our knowledge in the fields of embryonic development, vasculogenesis and osteogenesis. Identification of causative BMPR2 mutations in HPAH, IPAH and other diseases emphasizes the important physiological functions of BMPR2. In particular, it is widely accepted that functional defects of BMPR2 are implicated in the development of PAH. Indeed, most of the BMPR2 mutations identified in PAH are nonsense mutations, the mRNA of which get degraded by NMD. In addition, in vitro functional assays proved that most of the BMPR2 missense mutations lead to defects in SMAD protein-mediated signal transduction. Although haploinsufficiency is the common disease mechanism in PAH, significantly reduced BMPR2 transcripts have been found in patient-derived pulmonary vascular cells, suggesting that not only the BMPR2 mutated allele, but wildtype BMPR2 allele is also not expressed due to the unknown additional defects in the wildtype allele. Considering the low penetrance of HPAH, transcript level of wildtype BMPR2 allele would be the reasonable diagnostic marker for PAH. Questions to be addressed here pertain to understanding the molecular basis of the development of PAH. Advanced sequencing technology allows us to find additional causative mutations of BMPR2 in patients present with PAH. However, only limited experimental results are currently available to understand the pathophysiology of PAH, due to the impairment of BMPR2 functions.

Understanding the disease at the molecular level is the perquisite to overcoming the disease. Other than PAH, potential causative mutations of BMPR2 gene have been determined in cancers and obesity. Since BMPR2 plays important roles in diverse biological pathways, it would possible to identify causative BMPR2 mutations in other diseases also. Analyzing genetic alterations in human diseases is one of the best ways to understand the molecular functions of the genes. Further efforts to characterize the gene at the bench will be critical for introducing new ways to diagnosis and cure the disease in return.

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**CONFLICTS OF INTEREST**

The authors have no conflicting financial interests.

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