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

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**Running Title:** Clinical significance linked to mutation in *BMPR2* gene

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**ABSTRACT**

Bone morphogenetic protein type 2 receptor (BMPR2) is one of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily receptors, playing 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 family of proteins-mediated signal transduction leading to expression of target genes implicated in 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, which demonstrates the physiological importance of BMPR2. In this mini review, we will summarize recent findings regarding to 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 be discussed, which is important in the clinical setting for diagnostics and treatment.

## 1. INTRODUCTION

Bone morphogenetic protein type 2 receptor (BMPR2) is one of the members of the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily receptor, which is widely expressed in various tissues and organs, including pulmonary vascular endothelium, pulmonary vascular smooth muscle, cerebellum, hippocampus, thyroid gland, adrenal gland, heart, liver, pancreas and kidney (1-8). It has been reported 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 results in embryonic lethal and conditional *BMPR2* knockout mice in uterine decidua showed that BMPR2 is essential for post-implantation and fertility (9). There are at least 20 BMPs, seven type 1 receptors (ALK1-7), and four type 2 receptors (BMPR2, ACVR1, ACVR1b, and TGF $\beta$ 1) identified to date in mammals (10, 11). Among the 20 BMPs, four 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 cytoplasm by formation of heteromeric receptor complex, disruption of BMP receptors by genetic alterations results in various phenotypic abnormalities. Indeed, causative mutations in *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 has been validated by in vitro functional assay. 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 the 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 identified *BMPR2* variants and resultant physiological disorders with experimentally validated cases.

## 2. FACTORS INVOLVED IN THE BMPR2-MEDIATED SIGNALING CASCADE

### 2. 1. BMPs

The BMPs are part of the TGF- $\beta$  superfamily which is composed of 33 proteins, such as TGF- $\beta$ s, activins, inhibins, nodal, lefty, Growth and differentiation factors (GDFs), anti-müllerian hormone (AMH), and BMPs, in mammals (17). Initially, BMPs were discovered as factors that induce the ectopic formation of cartilage and bone in rats (18). It later turned out that these 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 have shown to possess potent osteogenic activities, enabling generation of ectopic bone formation in vivo (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). Especially, BMP2 is an indispensable factor for osteogenesis and is being studied actively in human clinical uses of bone generation and therapeutic trials of pathogenesis related to bone (21, 22). BMP2 also acts as a major factor in

endochondral 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 endochondral 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 study showed that BMP3 plays a role as a negative controller of bone development (27).

BMPs also play crucial roles in establishment of the basic embryonic body plans, which includes initial vertebrate gastrulation, mesoderm, somite, neural patterning, development of limb and skeleton, and organogenesis (28, 29). It was reported that BMP2-deficient embryo exhibits defects in development of heart, the first organ to be formed during embryogenesis. Further studies have shown 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 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. The roles of BMPs in vascular development 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 pulmonary vascular function by engaging BMP receptors 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 angiogenetic 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.

## 2. 2. BMP receptors

BMPs initiate SMAD protein-mediated signaling by binding to a heterodimeric 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, such as 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 receptor's kinase activity (37). The three type 1 receptors seem to play redundant roles in skeletal development. For example, 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 of the type 2 receptors have resembling 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 dileucine motif located in long C-terminal BMPR2 facilitates faster clathrin-mediated endocytosis than short form. Further study 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 of the BMPR2 might modulate 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 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 the figure 1. To understand pathophysiology of the disease due to the BMPR2 mutations and eventually to develop novel therapeutics, it would be important to understand the different biological functions of BMPR2 in response to different BMP ligands.

### **2. 3. Ligand-receptor oligomerization and signaling**

The BMPs, initially expressed as large inactive precursors, are commonly dimerized either with itself or with a different member of BMPs. The inactive dimeric BMPs are proteolytically cleaved to produce the small mature BMPs, which are

subsequently secreted from cells to conduct biological functions (44). It was reported that BMP heterodimers, such as BMP2/ 5, BMP2/ 6, BMP2/7, BMP2b/7, and BMP4/7, are defined as more potent activators of BMP signaling than homodimers (45). For example, BMP4/7 heterodimer induces stronger activity of mesoderm than BMP4 or BMP7 homodimers in *Xenopus*. Similarly, BMP2/7 heterodimer has higher activity in bone regeneration than BMP2 or BMP7 homodimers in mammals (46). In addition, BMPs bind to various BMP receptors with different affinities. For instance, BMP2 and BMP4 have a higher affinity to BMPR1a and BMPR1b, but relatively low affinity to BMPR2 (47). In contrast, BMP7 preferentially binds to ACVR2a and ACVR2b, whereas it barely interacts with type 1 receptors in general (48). The different binding affinities allow to engage the ligand and receptor complex, which triggers the activation of distinctive signaling pathway (49, 50).

It was reported 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 binds BMPs only weakly 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). Then, the activated type 1 receptor phosphorylates R-SMAD proteins including, SMAD1, SMAD5, and SMAD8, and the 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

corresponding target gene expression. In contrast, 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- $\beta$ , 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, and thus modulate 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, TGF- $\beta$  signaling downstream factor, SMAD2 is activated by Smurfs so that have an opposite reaction to BMP signaling (57).

#### **2. 4. Target gene regulation**

Phosphorylated R-SMAD and SMAD4 complex binds to 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 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). There are three RUNX isotypes (RUNX1, RUNX2, and RUNX3) 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 associating with SMAD signaling contain TBX20 and VEGF. TBX20 plays an important role in cardiac development, and VEGF is the main factor involved in angiogenesis.

### **3. ROLES OF BMPR2 DURING THE EMBRYOGENIC DEVELOPMENT AND BONE DIFFERENTIATION**

#### **3. 1. BMPR2 in embryogenesis**

Although BMPR2 is initially identified as a factor for regulating the chondrogenic and osteogenic differentiation, accumulated studies demonstrated that BMPR2 plays important roles in early embryonic development (65). First, the expression level of BMPR2 is fluctuated during embryonic stages although it was 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 is continuously increased with gradual

embryonic development especially in the anteriormost telencephalon, branchial arches, limb bud, and tail tip mesoderm. In the late stages, *BMPR2* expression is specifically high in neuroectoderm of the mouth anlagen (66). In this regard, *BMPR2* acts as an essential regulator in developing organs and tissues. Consistently, *BMPR2* deficient mice exhibit severe embryonic lethality prior to gastrulation. More specifically, *BMPR2* null murine embryos induce collapse of 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. The epiblast differentiation in embryo and the anterior-posterior (A-P) axis were shown to be abnormal in *BMPR2* knockout mice, although the differentiation of visceral endoderm is relatively normal (65). Taken together, these findings suggest that BMPs-*BMPR2*-mediated signal transduction is critical for diverse tissue and organ development.

### **3. 2. *BMPR2* in vascular development**

In the development of an embryo, BMP signaling enhances endothelial specification, subsequent venous differentiation and angiogenesis, so that vascular homeostasis is maintained (30, 67). Notably, *BMPR2*, 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 poorly expressed in the airway and arterial smooth muscle (68). *BMPR2* is expressed in human microvascular endothelial cells (HMVECs), 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 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 turned out that BMP2-mediated signaling is 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). In a way to promote human pulmonary arterial endothelial cell (HPAEC) survival and proliferation through ERK1/2 activation, BMPR2 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 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 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 (74, 75). Therefore, BMPR2 expression in ECs and SMCs has an important roles in the maintenance of vascular integrity of the pulmonary arteries (76).

### 3. 3. BMPR2 in Osteogenesis

Although biological functions of BMPR2 in bone formation have been observed, the regulatory role of BMPR2 in chondrogenesis and osteogenesis is not yet clearly demonstrated. BMPR2 is involved in osteoblast differentiation and also plays as an important mediator in bone formation and skeletal development during fracture healing, otospongiosis, and osteosclerosis (77-79). In the process of 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. Originally, the differentiation process of osteoblasts is classified into two steps; the first step is the differentiation of MSCs into osteoblast progenitors and the second is the maturation of osteoblast progenitors into osteoblasts presenting the various phenotypes of cells organizing bone (81). For the process of differentiation, one of useful alternative cells is dermal fibroblasts, which are easily available and expandable (82). In the late 1980s, C3H10T1/2, a pluripotent fibroblastic and MSC, was cloned, which can be differentiated 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 using myoblastic C2C12 cells in which treatment of BMP2 inhibits myotube formation from the C2C12. Instead, the cells start to induce ID1 expression so that myoblasts undergo the 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.

#### 4. PATHOGENIC MUTATION OF BMPR2

The importance of the diverse BMPR2 functions have 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 is supposed to result in 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. Here, we will summarize the most up-to-date *BMPR2* mutations in diverse diseases with emphasis on experimentally validated *BMPR2* mutations.

##### 4. 1. 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 in 15% of idiopathic PAH (IPAH) patients,

as well. 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 must be 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 level is 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 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 TGF- $\beta$  signaling which results in hyperproliferation of the SMCs in pulmonary arterioles. Consistent with this idea, it was found that impaired BMP signaling indeed inhibits the smooth muscle cell proliferation. However, underlying molecular mechanism of enhanced TGF- $\beta$  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* mutation cases in PAH patients have been reported, and functional defects of some of those *BMPR2* variants have been validated with patient-driven cells or in vitro functional assay. Most of the experimentally validated *BMPR2* mutations showed impaired SMAD protein-mediated signaling cascade (Figure 2). The *BMPR2* mutations in PAH have been regularly updated and the most recent update can be found in 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 PSMCs from PAH patients with individual *BMPR2* mutation leading to p.W9X, p.C347R, p.C347Y, and p.N903S. Compared to normal PSMCs, they noticed that 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 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, surprisingly they found that the truncated version of *BMPR2*, which lost the first 151 amino acids, is expressed together with the wildtype *BMPR2* from normal allele, which is due to the existence of a downstream Kozak sequence enabling translation re-initiation (91). The truncated version of *BMPR2* is supposed to be an inactive form, as ligand binding domain is lost. Likewise, functional assay of other *BMPR2* variants listed in the figure 2 (upper) have been done with patient-derived PSMCs 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 *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 novel dual fluorescence based assay system of which successful expression will give rise to red and green fluorescence simultaneously in HEK293T cells. Indeed, those *BMPR2* mutations

failed to express green fluorescence indicating that the mRNA is degraded by NMD (94). In the same studies, various missense BMPR2 mutants 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, in vitro functional assay has proved that most of the causative *BMPR2* mutations in PAH lead to impairment of SMAD protein-mediated signal transduction.

#### 4. 2. BMPR2 mutations in cancer

Similar to tumor suppressor roles of TGF- $\beta$ , BMPR1a, and SMAD4 genes are frequently mutated in colon cancers, suggesting that BMPs-mediated signaling also has tumor suppressive functions (95). *BMPR2* gene is also 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 portion of microsatellite instable (MSI) colorectal cancers. First, Kodach et al found impaired expression of BMPR2 in MSI colon cancer cell lines including HCT116, DLD1, SW48, and LOVO, while its expression is 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 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 largely remain elusive.

#### **4. 3. *BMPR2* mutation in obesity**

BMP-mediated signaling has been implicated in controlling adipocyte differentiation and thus it was proposed that *BMPR2* expression might show positive correlation with obesity (98). Indeed, Schleinitz et al found that *BMPR2* mRNA level is significantly high in both visceral and subcutaneous adipose tissue of 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), rs6717924 and rs13426118, were identified. 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).

## 5. CONCLUSION AND PERSPECTIVES

BMPR2, a receptor for the TGF- $\beta$  superfamily, was identified in the 1990s. Since then, the understanding of functional roles of BMPR2 significantly expand our knowledge in the fields of embryonic development, vasculogenesis and osteogenesis. Identification of causative BMPR2 mutations in HPAH and IPAH and other diseases emphasizes the important physiological functions of BMPR2. In particular, it is widely accepted that functional defects of BMPR2 is implicated in the development of PAH. Indeed, most of the BMPR2 mutations identified in PAH are nonsense mutations, the mRNA of which will be degraded by NMD. In addition, in vitro functional assay 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 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 mutation of BMPR2 in patients present with PAH. However, only limited experimental results are currently available to understand pathophysiology of PAH due to the impairment of BMPR2 functions. Understanding the disease at the molecular level is the prerequisite 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. 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 in the bench will be critical for open new ways to diagnosis and cure the disease in return.

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## **CONFLICTS OF INTEREST**

The authors have no conflicting financial interests.

## REFERENCES

1. Harper RL, Reynolds AM, Bonder CS, and Reynolds PN (2016) BMPR2 gene therapy for PAH acts via Smad and non-Smad signalling. *Respirology* 21, 727-733
2. Zhang D, Mehler MF, Song Q, and Kessler JA (1998) Development of bone morphogenetic protein receptors in the nervous system and possible roles in regulating trkC expression. *J Neurosci* 18, 3314-3326
3. Franzen A, Piek E, Westermarck B, ten Dijke P, and Heldin NE (1999) Expression of transforming growth factor-beta1, activin A, and their receptors in thyroid follicle cells: negative regulation of thyrocyte growth and function. *Endocrinology* 140, 4300-4310
4. Rege J, Nishimoto HK, Nishimoto K, Rodgers RJ, Auchus RJ, and Rainey WE (2015) Bone Morphogenetic Protein-4 (BMP4): A Paracrine Regulator of Human Adrenal C19 Steroid Synthesis. *Endocrinology* 156, 2530-2540
5. Wu X, Sagave J, Rutkovskiy A et al (2014) Expression of bone morphogenetic protein 4 and its receptors in the remodeling heart. *Life Sci* 97, 145-154
6. Mayeur C, Leyton PA, Kolodziej SA, Yu B, and Bloch KD (2014) BMP type II receptors have redundant roles in the regulation of hepatic hepcidin gene expression and iron metabolism. *Blood* 124, 2116-2123
7. Dichmann DS, Miller CP, Jensen J, Scott Heller R, and Serup P (2003) Expression and misexpression of members of the FGF and TGFbeta families of growth factors in the developing mouse pancreas. *Dev Dyn* 226, 663-674
8. Bush KT, Sakurai H, Steer DL et al (2004) TGF-beta superfamily members modulate growth, branching, shaping, and patterning of the ureteric bud. *Dev Biol* 266, 285-298
9. Nagashima T, Li Q, Clementi C, Lydon JP, DeMayo FJ, and Matzuk MM (2013) BMPR2 is required for postimplantation uterine function and pregnancy maintenance. *J Clin Invest* 123, 2539-2550

10. Wiley DM and Jin SW (2011) Bone Morphogenetic Protein functions as a context-dependent angiogenic cue in vertebrates. *Semin Cell Dev Biol* 22, 1012-1018
11. Heinecke K, Seher A, Schmitz W, Mueller TD, Sebald W, and Nickel J (2009) Receptor oligomerization and beyond: a case study in bone morphogenetic proteins. *BMC Biol* 7, 59
12. Morrell NW (2006) Pulmonary hypertension due to BMPR2 mutation: a new paradigm for tissue remodeling? *Proc Am Thorac Soc* 3, 680-686
13. Rigelsky CM, Jennings C, Lehtonen R, Minai OA, Eng C, and Aldred MA (2008) BMPR2 mutation in a patient with pulmonary arterial hypertension and suspected hereditary hemorrhagic telangiectasia. *Am J Med Genet A* 146A, 2551-2556
14. Kim IY, Lee DH, Ahn HJ et al (2000) Expression of bone morphogenetic protein receptors type-IA, -IB and -II correlates with tumor grade in human prostate cancer tissues. *Cancer Res* 60, 2840-2844
15. Park SW, Hur SY, Yoo NJ, and Lee SH (2010) Somatic frameshift mutations of bone morphogenic protein receptor 2 gene in gastric and colorectal cancers with microsatellite instability. *APMIS* 118, 824-829
16. Schleinitz D, Kloting N, Bottcher Y et al (2011) Genetic and evolutionary analyses of the human bone morphogenetic protein receptor 2 (BMPR2) in the pathophysiology of obesity. *PLoS One* 6, e16155
17. Wu M, Chen G, and Li YP (2016) TGF-beta and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. *Bone Res* 4, 16009
18. Miyazono K, Kamiya Y, and Morikawa M (2010) Bone morphogenetic protein receptors and signal transduction. *J Biochem* 147, 35-51
19. Kang Q, Sun MH, Cheng H et al (2004) Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene

- delivery. *Gene Ther* 11, 1312-1320
20. Luu HH, Song WX, Luo X et al (2007) Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. *J Orthop Res* 25, 665-677
  21. Razzouk S and Sarkis R (2012) BMP-2: biological challenges to its clinical use. *N Y State Dent J* 78, 37-39
  22. Kanakaris NK and Giannoudis PV (2008) Clinical applications of bone morphogenetic proteins: current evidence. *J Surg Orthop Adv* 17, 133-146
  23. Sun J, Li J, Li C, and Yu Y (2015) Role of bone morphogenetic protein-2 in osteogenic differentiation of mesenchymal stem cells. *Mol Med Rep* 12, 4230-4237
  24. Hyun SJ, Han DK, Choi SH et al (2005) Effect of recombinant human bone morphogenetic protein-2, -4, and -7 on bone formation in rat calvarial defects. *J Periodontol* 76, 1667-1674
  25. King JA, Marker PC, Seung KJ, and Kingsley DM (1994) BMP5 and the molecular, skeletal, and soft-tissue alterations in short ear mice. *Dev Biol* 166, 112-122
  26. Pi CJ, Liang KL, Ke ZY et al (2016) Adenovirus-mediated expression of vascular endothelial growth factor- $\alpha$  potentiates bone morphogenetic protein9-induced osteogenic differentiation and bone formation. *Biol Chem* 397, 765-775
  27. Daluiski A, Engstrand T, Bahamonde ME et al (2001) Bone morphogenetic protein-3 is a negative regulator of bone density. *Nat Genet* 27, 84-88
  28. Hogan BL (1996) Bone morphogenetic proteins in development. *Curr Opin Genet Dev* 6, 432-438
  29. Zhao GQ (2003) Consequences of knocking out BMP signaling in the mouse. *Genesis* 35, 43-56
  30. Zhang H and Bradley A (1996) Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* 122, 2977-2986

31. Winnier G, Blessing M, Labosky PA, and Hogan BL (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev* 9, 2105-2116
32. Jena N, Martin-Seisdedos C, McCue P, and Croce CM (1997) BMP7 null mutation in mice: developmental defects in skeleton, kidney, and eye. *Exp Cell Res* 230, 28-37
33. Kim RY, Robertson EJ, and Solloway MJ (2001) Bmp6 and Bmp7 are required for cushion formation and septation in the developing mouse heart. *Dev Biol* 235, 449-466
34. Tillet E and Bailly S (2014) Emerging roles of BMP9 and BMP10 in hereditary hemorrhagic telangiectasia. *Front Genet* 5, 456
35. Rao SM, Ugale GM, and Warad SB (2013) Bone morphogenetic proteins: periodontal regeneration. *N Am J Med Sci* 5, 161-168
36. Sieber C, Kopf J, Hiepen C, and Knaus P (2009) Recent advances in BMP receptor signaling. *Cytokine Growth Factor Rev* 20, 343-355
37. Lin SJ, Lerch TF, Cook RW, Jardetzky TS, and Woodruff TK (2006) The structural basis of TGF-beta, bone morphogenetic protein, and activin ligand binding. *Reproduction* 132, 179-190
38. van Dinther M, Visser N, de Gorter DJ et al (2010) ALK2 R206H mutation linked to fibrodysplasia ossificans progressiva confers constitutive activity to the BMP type I receptor and sensitizes mesenchymal cells to BMP-induced osteoblast differentiation and bone formation. *J Bone Miner Res* 25, 1208-1215
39. Yoon BS, Ovchinnikov DA, Yoshii I, Mishina Y, Behringer RR, and Lyons KM (2005) Bmpr1a and Bmpr1b have overlapping functions and are essential for chondrogenesis in vivo. *Proc Natl Acad Sci U S A* 102, 5062-5067
40. Amsalem AR, Marom B, Shapira KE et al (2016) Differential regulation of translation and endocytosis of alternatively spliced forms of the type II bone morphogenetic

- protein (BMP) receptor. *Mol Biol Cell* 27, 716-730
41. Lane KB, Machado RD, Pauciulo MW et al (2000) Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet* 26, 81-84
  42. Deng Z, Morse JH, Slager SL et al (2000) Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 67, 737-744
  43. Rosenzweig BL, Imamura T, Okadome T et al (1995) Cloning and characterization of a human type II receptor for bone morphogenetic proteins. *Proc Natl Acad Sci U S A* 92, 7632-7636
  44. Sopory S, Nelsen SM, Degnin C, Wong C, and Christian JL (2006) Regulation of bone morphogenetic protein-4 activity by sequence elements within the prodomain. *J Biol Chem* 281, 34021-34031
  45. Little SC and Mullins MC (2009) Bone morphogenetic protein heterodimers assemble heteromeric type I receptor complexes to pattern the dorsoventral axis. *Nat Cell Biol* 11, 637-643
  46. Sun P, Wang J, Zheng Y, Fan Y, and Gu Z (2012) BMP2/7 heterodimer is a stronger inducer of bone regeneration in peri-implant bone defects model than BMP2 or BMP7 homodimer. *Dent Mater J* 31, 239-248
  47. Knaus P and Sebald W (2001) Cooperativity of binding epitopes and receptor chains in the BMP/TGFbeta superfamily. *Biol Chem* 382, 1189-1195
  48. Greenwald J, Groppe J, Gray P et al (2003) The BMP7/ActRII extracellular domain complex provides new insights into the cooperative nature of receptor assembly. *Mol Cell* 11, 605-617
  49. Gilboa L, Nohe A, Geissendorfer T, Sebald W, Henis YI, and Knaus P (2000) Bone

- morphogenetic protein receptor complexes on the surface of live cells: a new oligomerization mode for serine/threonine kinase receptors. *Mol Biol Cell* 11, 1023-1035
50. Nohe A, Hassel S, Ehrlich M et al (2002) The mode of bone morphogenetic protein (BMP) receptor oligomerization determines different BMP-2 signaling pathways. *J Biol Chem* 277, 5330-5338
51. Wrana JL, Attisano L, Carcamo J et al (1992) TGF beta signals through a heteromeric protein kinase receptor complex. *Cell* 71, 1003-1014
52. Koenig BB, Cook JS, Wolsing DH et al (1994) Characterization and cloning of a receptor for BMP-2 and BMP-4 from NIH 3T3 cells. *Mol Cell Biol* 14, 5961-5974
53. Miyazono K, Maeda S, and Imamura T (2005) BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev* 16, 251-263
54. Hassel S, Eichner A, Yakymovych M, Hellman U, Knaus P, and Souchelnytskyi S (2004) Proteins associated with type II bone morphogenetic protein receptor (BMPRII) and identified by two-dimensional gel electrophoresis and mass spectrometry. *Proteomics* 4, 1346-1358
55. Heldin CH and Moustakas A (2012) Role of Smads in TGFbeta signaling. *Cell Tissue Res* 347, 21-36
56. Murakami G, Watabe T, Takaoka K, Miyazono K, and Imamura T (2003) Cooperative inhibition of bone morphogenetic protein signaling by Smurf1 and inhibitory Smads. *Mol Biol Cell* 14, 2809-2817
57. Zhu H, Kavsak P, Abdollah S, Wrana JL, and Thomsen GH (1999) A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. *Nature* 400, 687-693

58. Ogata T, Wozney JM, Benezra R, and Noda M (1993) Bone morphogenetic protein 2 transiently enhances expression of a gene, Id (inhibitor of differentiation), encoding a helix-loop-helix molecule in osteoblast-like cells. *Proc Natl Acad Sci U S A* 90, 9219-9222
59. Ogata T and Noda M (1991) Expression of Id, a negative regulator of helix-loop-helix DNA binding proteins, is down-regulated at confluence and enhanced by dexamethasone in a mouse osteoblastic cell line, MC3T3E1. *Biochem Biophys Res Commun* 180, 1194-1199
60. Katagiri T, Imada M, Yanai T, Suda T, Takahashi N, and Kamijo R (2002) Identification of a BMP-responsive element in Id1, the gene for inhibition of myogenesis. *Genes Cells* 7, 949-960
61. Ito Y and Miyazono K (2003) RUNX transcription factors as key targets of TGF-beta superfamily signaling. *Curr Opin Genet Dev* 13, 43-47
62. Maeda S, Hayashi M, Komiya S, Imamura T, and Miyazono K (2004) Endogenous TGF-beta signaling suppresses maturation of osteoblastic mesenchymal cells. *EMBO J* 23, 552-563
63. Lee S, Cho HY, Bui HT, and Kang D (2014) The osteogenic or adipogenic lineage commitment of human mesenchymal stem cells is determined by protein kinase C delta. *BMC Cell Biol* 15, 42
64. Satija NK, Gurudutta GU, Sharma S et al (2007) Mesenchymal stem cells: molecular targets for tissue engineering. *Stem Cells Dev* 16, 7-23
65. Beppu H, Kawabata M, Hamamoto T et al (2000) BMP type II receptor is required for gastrulation and early development of mouse embryos. *Dev Biol* 221, 249-258
66. Danesh SM, Villasenor A, Chong D, Soukup C, and Cleaver O (2009) BMP and BMP receptor expression during murine organogenesis. *Gene Expr Patterns* 9, 255-265

67. Dyer LA, Pi X, and Patterson C (2014) The role of BMPs in endothelial cell function and dysfunction. *Trends Endocrinol Metab* 25, 472-480
68. Atkinson C, Stewart S, Upton PD et al (2002) Primary pulmonary hypertension is associated with reduced pulmonary vascular expression of type II bone morphogenetic protein receptor. *Circulation* 105, 1672-1678
69. Finkenzeller G, Hager S, and Stark GB (2012) Effects of bone morphogenetic protein 2 on human umbilical vein endothelial cells. *Microvasc Res* 84, 81-85
70. Langenfeld EM and Langenfeld J (2004) Bone morphogenetic protein-2 stimulates angiogenesis in developing tumors. *Mol Cancer Res* 2, 141-149
71. Wiley DM, Kim JD, Hao J, Hong CC, Bautch VL, and Jin SW (2011) Distinct signalling pathways regulate sprouting angiogenesis from the dorsal aorta and the axial vein. *Nat Cell Biol* 13, 686-692
72. de Jesus Perez VA, Alastalo TP, Wu JC et al (2009) Bone morphogenetic protein 2 induces pulmonary angiogenesis via Wnt-beta-catenin and Wnt-RhoA-Rac1 pathways. *J Cell Biol* 184, 83-99
73. Lee KM, Tsai KY, Wang N, and Ingber DE (1998) Extracellular matrix and pulmonary hypertension: control of vascular smooth muscle cell contractility. *Am J Physiol* 274, H76-82
74. Takahashi H, Goto N, Kojima Y et al (2006) Downregulation of type II bone morphogenetic protein receptor in hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 290, L450-458
75. Takahashi K, Kogaki S, Matsushita T, Nasuno S, Kurotobi S, and Ozono K (2007) Hypoxia induces alteration of bone morphogenetic protein receptor signaling in pulmonary artery endothelial cell. *Pediatr Res* 61, 392-397
76. West J, Harral J, Lane K et al (2008) Mice expressing BMPR2R899X transgene in

- smooth muscle develop pulmonary vascular lesions. *Am J Physiol Lung Cell Mol Physiol* 295, L744-755
77. Onishi T, Ishidou Y, Nagamine T et al (1998) Distinct and overlapping patterns of localization of bone morphogenetic protein (BMP) family members and a BMP type II receptor during fracture healing in rats. *Bone* 22, 605-612
  78. Lehnerdt G, Metz KA, Trellakis S, Jahnke K, and Neumann A (2007) Signaling by way of type IB and II bone morphogenetic protein receptors regulates bone formation in otospongiosis. *Laryngoscope* 117, 812-816
  79. Garimella R, Kacena MA, Tague SE, Wang J, Horowitz MC, and Anderson HC (2007) Expression of bone morphogenetic proteins and their receptors in the bone marrow megakaryocytes of GATA-1(low) mice: a possible role in osteosclerosis. *J Histochem Cytochem* 55, 745-752
  80. Yang C, Yang L, Wan M, and Cao X (2010) Generation of a mouse model with expression of bone morphogenetic protein type II receptor lacking the cytoplasmic domain in osteoblasts. *Ann N Y Acad Sci* 1192, 286-291
  81. Katagiri T, Yamaguchi A, Komaki M et al (1994) Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage. *J Cell Biol* 127, 1755-1766
  82. Hee CK and Nicoll S (2006) Induction of osteoblast differentiation markers in human dermal fibroblasts: potential application to bone tissue engineering. *Conf Proc IEEE Eng Med Biol Soc* 1, 521-524
  83. Davis RL, Weintraub H, and Lassar AB (1987) Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* 51, 987-1000
  84. Wright WE, Sassoon DA, and Lin VK (1989) Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. *Cell* 56, 607-617

85. Edmondson DG and Olson EN (1990) A gene with homology to the myc similarity region of MyoD1 is expressed during myogenesis and is sufficient to activate the muscle differentiation program. *Genes Dev* 4, 1450
86. Olson EN (1990) MyoD family: a paradigm for development? *Genes Dev* 4, 1454-1461
87. Katagiri T, Yamaguchi A, Ikeda T et al (1990) The non-osteogenic mouse pluripotent cell line, C3H10T1/2, is induced to differentiate into osteoblastic cells by recombinant human bone morphogenetic protein-2. *Biochem Biophys Res Commun* 172, 295-299
88. Wu N, Zhao Y, Yin Y, Zhang Y, and Luo J (2010) Identification and analysis of type II TGF-beta receptors in BMP-9-induced osteogenic differentiation of C3H10T1/2 mesenchymal stem cells. *Acta Biochim Biophys Sin (Shanghai)* 42, 699-708
89. Morrell NW (2010) Role of bone morphogenetic protein receptors in the development of pulmonary arterial hypertension. *Adv Exp Med Biol* 661, 251-264
90. Yang J, Davies RJ, Southwood M et al (2008) Mutations in bone morphogenetic protein type II receptor cause dysregulation of Id gene expression in pulmonary artery smooth muscle cells: implications for familial pulmonary arterial hypertension. *Circ Res* 102, 1212-1221
91. Hamid R, Hedges LK, Austin E, Phillips JA, 3rd, Loyd JE, and Cogan JD (2010) Transcripts from a novel BMPR2 termination mutation escape nonsense mediated decay by downstream translation re-initiation: implications for treating pulmonary hypertension. *Clin Genet* 77, 280-286
92. Ihida-Stansbury K, McKean DM, Lane KB et al (2006) Tenascin-C is induced by mutated BMP type II receptors in familial forms of pulmonary arterial hypertension. *Am J Physiol Lung Cell Mol Physiol* 291, L694-702
93. Drake KM, Dunmore BJ, McNelly LN, Morrell NW, and Aldred MA (2013) Correction of nonsense BMPR2 and SMAD9 mutations by ataluren in pulmonary arterial

- hypertension. *Am J Respir Cell Mol Biol* 49, 403-409
94. Nasim MT, Ghouri A, Patel B et al (2008) Stoichiometric imbalance in the receptor complex contributes to dysfunctional BMPR-II mediated signalling in pulmonary arterial hypertension. *Hum Mol Genet* 17, 1683-1694
95. Howe JR, Bair JL, Sayed MG et al (2001) Germline mutations of the gene encoding bone morphogenetic protein receptor 1A in juvenile polyposis. *Nat Genet* 28, 184-187
96. Kim IY, Lee DH, Lee DK et al (2004) Loss of expression of bone morphogenetic protein receptor type II in human prostate cancer cells. *Oncogene* 23, 7651-7659
97. Kodach LL, Wiercinska E, de Miranda NF et al (2008) The bone morphogenetic protein pathway is inactivated in the majority of sporadic colorectal cancers. *Gastroenterology* 134, 1332-1341
98. Schulz TJ and Tseng YH (2009) Emerging role of bone morphogenetic proteins in adipogenesis and energy metabolism. *Cytokine Growth Factor Rev* 20, 523-531

## Figure Legends

**Figure 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 are known to bind to a receptor complex consist 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 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 induce 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.

**Figure 2.** Experimentally verified BMPR2 mutations are indicated on the *BMPR2* gene. BMPR2 domains are indicated. Functional validation performed in patient-derived cells indicated above. Functional validation using in vitro assay system are indicated below.

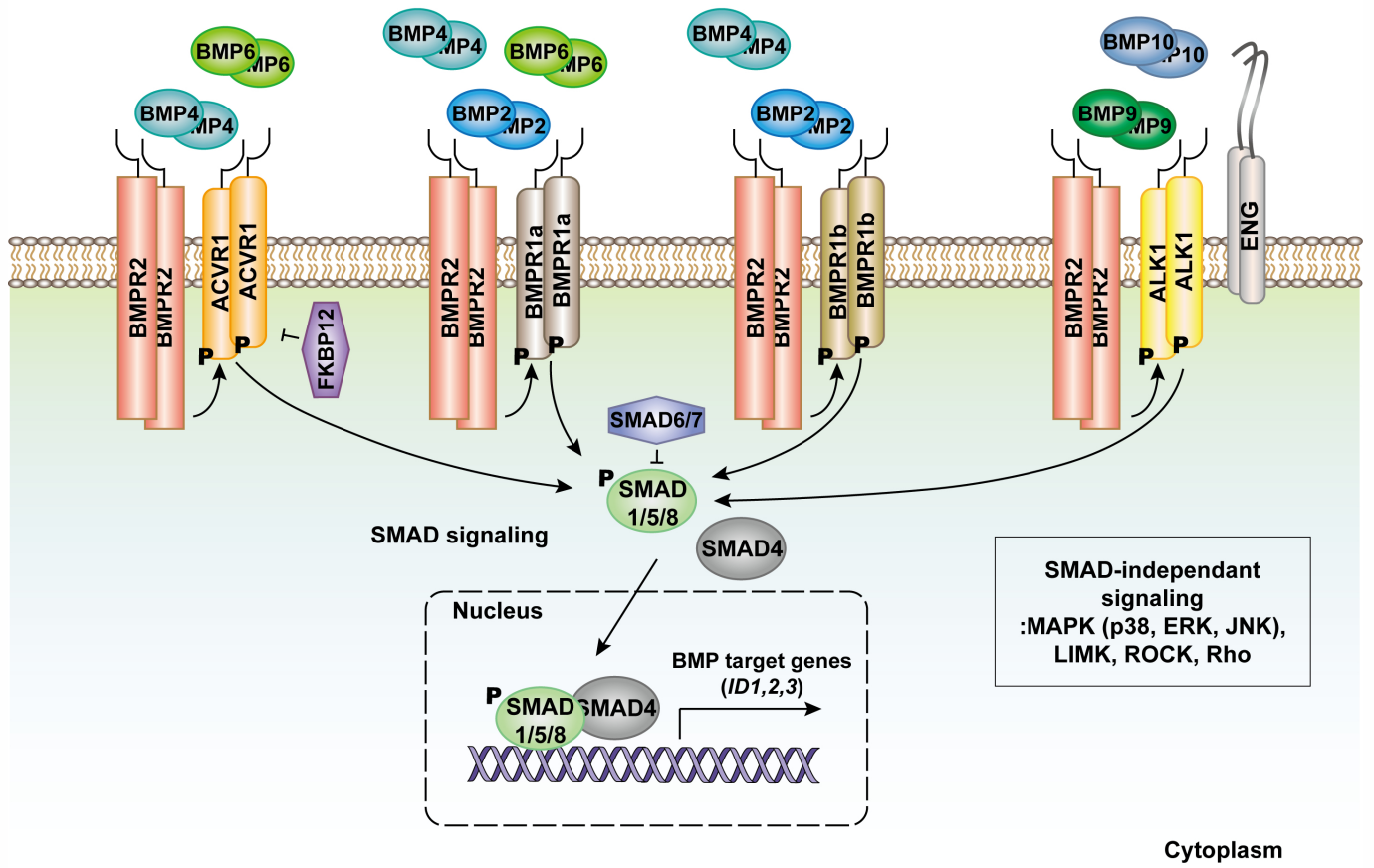


Fig. 1

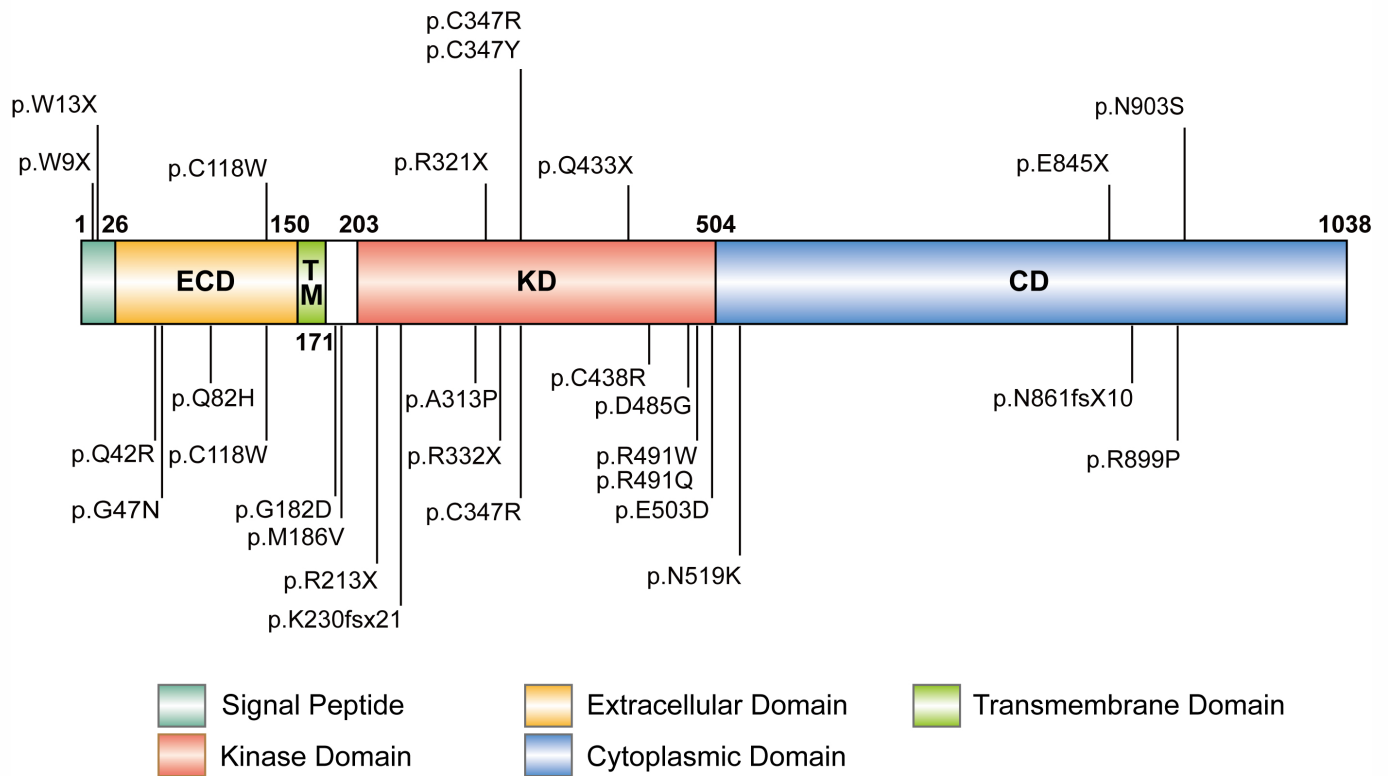


Fig. 2