

# Functional Diversity of Ciliary Proteins in Bone Development and Disease

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## Abstract

**Purpose of Review** The primary cilium is a non-motile microtubule-based organelle that senses a diverse range of extracellular signals. While recent studies highlight the importance of ciliary-dependent developmental signals, including Hedgehog, Wnt, and platelet-derived growth factor, it is not well understood whether and how bone morphogenetic protein (BMP) signaling, a key regulator of skeletogenesis, is involved in cilia-related bone developmental aspects and in the etiology of skeletal disorders.

**Recent Findings** Increasing evidence suggests that osteoblast- or osteocyte-specific deletion of ciliary proteins leads to diverse skeletal malformations, reinforcing the idea that primary cilia are indispensable for regulating bone development and maintenance. Furthermore, it became evident that ciliary proteins not only contribute to ciliogenesis but also orchestrate cellular trafficking.

**Summary** This review summarizes the current understanding of ciliary proteins in bone development and discusses the potential role of BMP signaling in primary cilia, enabling us to unravel the potential pathogenesis of skeletal ciliopathies.

**Keywords** Cilia · Bone disease · Bone morphogenetic protein · Growth factor signaling

## Introduction

Bone is a dynamic tissue responsible for the architectural framework of the body that supports other connective tissues and protects internal organs in vertebrates. Bone also exerts important functions as an internal organ, such as calcium and phosphate storage and bone marrow support. Like other tissues, bone is being constantly remodeled. The highly coordinated physiological remodeling process involves bone resorption by osteoclasts and subsequent bone formation by osteoblasts. Osteocytes, the bone matrix-embedded descendants of osteoblasts, are also known to contribute to this process [1]. Whereas bone remodeling primarily occurs to repair damage and to allow adaptation to the mechanical environment in a site-specific manner, it also occurs to maintain mineral homeostasis in a non-site-specific manner [2]. In order to orchestrate bone remodeling, bone cells need to recognize the extracellular environment. However, the precise mechanisms to sense mechanical and chemical changes have yet to be elucidated.

The primary cilium is a non-motile microtubule-based organelle present on the surface of most mammalian cell types. It is observed primarily in quiescent or differentiated cells [3], although certain differentiated cell lineages such as lymphocytes, hepatocytes, adipocytes, and skeletal muscle cells lack primary cilia [4]. Primary cilia are known to transduce a diverse range of extracellular signals into cellular responses via various signaling pathways (e.g. Hedgehog [Hh], Wnt, and platelet-derived growth factor [PDGF]) that regulate cell proliferation, differentiation, migration, and polarity, and are thereby crucial in tissue morphogenesis [5–9]. Although the mechanisms involved in the formation of primary cilia are

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unclear, there is increasing evidence suggesting that cilia formation and functions depend on a specialized protein transport process known as intraflagellar transport (IFT), which is mediated by a large multiprotein complex [10]. Because proteins are not synthesized within the cilia, they must be transported from the cytoplasm to build and maintain the primary cilia [4]. Any defect in the formation, maintenance, or functioning of primary cilia results in ciliopathies, a class of genetic multi-organ diseases that share clinical features and affect nearly every major tissue, including the kidney, brain, retina, liver, and bone. Their broad phenotypic spectrum highlights the critical role of primary cilia in development and homeostasis [11]. One of the remarkable phenotypes of ciliopathies is abnormal bone development, observed in Jeune syndrome, short-rib polydactyly, orofacial digital syndrome, and Ellis-van Creveld syndrome [12].

Because of the emerging interest in primary cilia in the field of skeletal development and disease, it is critical to understand their role in bone development and maintenance. This review summarizes recent advances in the understanding of the function of ciliary proteins in bone cells. Among growth factor signaling pathways, BMP is known as one of the most important signaling axes during bone development, maintenance, and disease, and its potential roles in ciliogenesis are also discussed.

### Primary Cilia and Cell Cycle

Recent studies have suggested that primary cilium formation and cell cycle progression may reciprocally influence one another, although the exact mechanism remains to be elucidated [3]. In normal cells, primary cilia form during the quiescent state (G0/G1 phase) and begin to disassemble as cells re-enter the cell cycle [13]. One of the key components that link primary cilia and cell cycle is the centrosome. The centrosome, where spindle fibers appear during the mitotic phase, migrates to the cell surface, whereupon the mother centriole becomes the basal body of primary cilia [14]. Primary cilia cannot be formed during the mitotic phase, presumably because the basal body needs to be released from the cell surface before being translocated to the nucleolus to act as a mitotic component. This indicates that the conversion between the centriole and basal body of primary cilia is not only reversible, but also cell cycle-dependent [14]. In fact, centrosome dynamics are precisely synchronized with the cell cycle, therefore ciliogenesis and cell cycle are thought to be mutually exclusive events [15]. Moreover, many proteins known to be important for centrosome functions are also required for ciliogenesis [16, 17]. Hence, inhibiting the activity of ciliary proteins can promote cell cycle progression (IFT88/Polaris), disrupt the cell cycle (IFT27), or have no effect (OFD2) [3]. These findings

thus indicate diverse cilia-independent functions for these ciliary proteins (discussed later).

### Primary Cilia in Bone Cells

Although the existence of primary cilia in osteoblasts was first reported in the 1970s [18, 19], their possible contribution to bone homeostasis has only been recently proposed [20]. The significance of primary cilia in bone development and disease is widely recognized, but their incidence in bone cells is still controversial. A recent study reported that only a small fraction of bone cells (4.0% of osteocytes and 4.6% of bone-lining cells) possess primary cilia [21•], which is in accordance with a previous study [18]. However, it has also been reported that the majority of rat osteocytes (94%) possess acetylated, tubulin-positive primary cilia [22]. Such discrepancies between the two reports can be attributed to differences in the detection methods, animal species, location, or age of the animals analyzed.

Even though osteocytes are vital for regulating bone homeostasis, these cells do not divide further and most likely remain in the G0/G1 phase in vivo. In addition, osteocyte-specific disruption of ciliary proteins such as *Pkd1* (*Pkd1<sup>Dmp1-cre</sup>*) shows considerable phenotypic alterations [23]. As a result, it has been speculated that the majority of osteocytes possess primary cilia in vivo, but further studies are required to confirm these speculations.

Recent studies have reported that primary cilia are also present in mesenchymal stem cells (MSCs), the progenitors of osteoblasts [21, 24, 25]. To investigate their functional significance in these cells, IFT88/Polaris siRNA was used to inhibit primary cilia formation in MSCs, inducing a significant decrease in mRNA levels of all three (i.e., osteoblast, chondrocyte, and adipocyte) lineage-specific transcription factors [24]. MSCs transfected with the IFT88/Polaris siRNA and cultured in both osteogenic and adipogenic media lost cell adhesion, suggesting that primary cilia are required for osteogenic and adipogenic differentiation [24].

Osteoclasts, on the other hand, are derived from hematopoietic lineage cells [26], which are believed to lack primary cilia [4, 27]. However, a recent study reported the presence of primary cilia in such hematopoietic lineages, including peripheral blood mononuclear cells and acute myeloid leukemia cell lines [28•]. Although their presence in osteoclasts is still controversial, osteocytic primary cilia might play a role in bone resorption. Receptor activator of nuclear factor  $\kappa$ B ligand (RANKL) and its antagonist osteoprotegerin (OPG), which are secreted by osteocytes, directly regulate osteoclast differentiation, and it has been reported that primary cilia regulate the mechanical stress-induced RANKL/OPG expression in osteocytes [29], thereby suggesting that they indirectly regulate osteoclastogenesis.

Several studies have reported that disruption of IFT proteins (e.g., IFT80, IFT88/Polaris), IFT motor proteins (e.g. KIF3A), and other ciliary proteins (e.g. EVC, polycystin) results in impaired osteoblast differentiation and bone formation [10]. Targeted deletion of *Pkd1* in osteoblasts (*Pkd1*<sup>Oc-cKO</sup>) induces abnormalities in Runx2-mediated osteoblast development and increases adipogenesis in the bone marrow, resulting in osteopenia [30, 31]. Similarly, selective deletion of *Kif3a* in osteoblasts (*Kif3a*<sup>Oc-cKO</sup>) disrupts primary cilia formation and function, also resulting in osteopenia [32]. In addition, *Kif3a*-deficient osteoblasts exhibit a reduction in response to fluid flow shear stress in vitro and impaired cilia-related pathways, such as Hh and Wnt signaling [32]. Remarkably, while deletion of *Pkd1* alone leads to skeletal abnormalities, abrogation of both *Pkd1* and *Kif3a* compensates for the skeletal phenotype. This indicates that crosstalk between *Pkd1* and *Kif3a* might play a counterbalancing role in bone formation through the differential regulation of osteogenesis and adipogenesis [30].

Targeted deletion of *IFT80* in osteoblast precursor cells (*IFT80*<sup>Osx-cKO</sup>) results in significantly decreased bone mass due to impaired osteoblast differentiation [33••]. Deletion of IFT80 not only blocks canonical Hh-Gli signaling, but also stimulates cilia-independent non-canonical Hh-G $\alpha$ i-RhoA signaling, resulting in excess stress fibers and subsequent osteoblast differentiation. Inhibition of non-canonical Hh signaling disrupts the formation of stress fibers and enhances cilia formation in IFT80-deficient osteoblasts. Therefore, IFT80 regulates osteoblast differentiation by balancing canonical Hh-Gli signaling and non-canonical Hh-G $\alpha$ i-RhoA signaling.

### Primary Cilia as a Mechanosensor in Osteocytes

Osteocyte-specific deletion of *Pkd1* (*Pkd1*<sup>Dmp1-cre</sup>) results in impaired sensory functions and disrupts mechanical stress-regulated skeletal homeostasis [23]. A recent study demonstrated that mechanical loading stimulates the homing of bone marrow cells to the bone surface, enhances osteogenic differentiation, and increases bone mass. In response to mechanical stimulation, deletion of *Kif3a* in the recruited osteoblasts significantly decreased the amount of bone formation [34••]. In response to oscillatory fluid flow-induced shear stress, the osteoblast and osteocyte cell lines MC3T3-E1 and MLO-Y4 displayed upregulated gene expression for osteopontin (Opn) and prostaglandin-E2 (PGE2), respectively, both of which are known to respond well to mechanical stimuli [29]. However, treatment with chloral hydrate or gene silencing by IFT88/Polaris siRNA reduced the number of ciliary cells and downregulated Opn and PGE2 gene expression. Interestingly, unlike in kidney cells, primary cilium-mediated cellular responses are independent of Ca<sup>2+</sup> flux and stretch-activated ion channels [29], and a recent report further confirmed that

primary cilia are not Ca<sup>2+</sup>-responsive mechanosensors in many cell types [35].

### Primary Cilia and BMP Signaling

Despite many studies showing the critical importance of ciliary proteins in bone cells, the underlying signaling pathways remain elusive. Most of the current studies focus on investigating the ciliary-dependent Hh, PDGF, and Wnt pathways and on clarifying the significance of these signals during skeletogenesis [9, 10]. However, it is not known whether BMP, one of the most critical factors regulating bone development, is involved in cilia-mediated signal transduction. While it still remains unclear if BMP signaling is transduced by primary cilia in bone cells, recent studies have highlighted a potential link between transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling and primary cilia in retinal pigment epithelial cells [36] and fibroblasts [8]. Since BMP belongs to the TGF- $\beta$  super-family [37], it would be reasonable to predict that BMP signaling may also be regulated by primary cilia, but the mechanism of how BMP is exactly transduced by primary cilia remains to be determined. Of note, BMP receptors are subject to endocytosis and trafficking when signaling is activated, and this regulatory mechanism is critical for the fine-tuning of BMP signaling response [38]. Because TGF- $\beta$  signaling is transduced by primary cilia via endocytosis [8], ciliary-dependent BMP signaling may be orchestrated by the endocytosis machinery, which is likely critical for normal bone development and homeostasis.

Meanwhile, BMP signaling may also be required for primary cilia formation in bone cells. For example, deficiency of *Acvr1*, the gene encoding BMP type I receptor ACVR1, in mouse embryonic fibroblasts has been shown to result in severe defects in the formation of nodal primary cilia [39]. BMP signaling via ACVR1 positively regulates phosphorylation of cyclin-dependent kinase inhibitor p27<sup>KiP1</sup>, which in turn maintains the cells in quiescence and induces the formation of primary cilia. Moreover, BMP7 has been shown to inhibit the proliferation of human anaplastic thyroid carcinoma cells via stabilization of p21<sup>CIP1</sup> and p27<sup>KiP1</sup> [40]. In accordance with these findings, several mouse models of polycystic kidney disease, such as transgenic mice expressing *Cux1* and *Cys1*, showed downregulation of p27<sup>KiP1</sup> stability and its subsequent cell proliferation [41, 42]. In murine embryonic fibroblasts, a recombinant BMP2 and BMP4/7 fusion protein induced cell cycle arrest and stabilization of p27<sup>KiP1</sup>, thereby arresting the cell cycle in the G0/G1 phase [43]. In addition, BMP4-induced G0/G1 arrest mediates osteoblast differentiation through increased expression of p21<sup>CIP1</sup> and p27<sup>KiP1</sup>, which are associated with BMPR-IA/ALK3-specific Smad1/5 phosphorylation [44]. Furthermore, BMPs are known to stimulate osteoblastic differentiation from their precursors

[45, 46]; this supports the notion that BMPs are involved in the regulation of cell cycle, which is presumably important for ciliogenesis in osteoblasts.

In an Oak Ridge polycystic kidney (ORPK) mouse model, non-ciliated aortic endothelial cells acquired the ability to transdifferentiate into osteogenic cells in a BMP-dependent manner [47]. In addition, endothelial cells lacking primary cilia are sensitized to undergo BMP-induced osteogenic differentiation along with the activation of  $\beta$ -catenin [47]. These results suggest that primary cilia play an important role in BMP signaling by integrating it with other signaling pathways such as Wnt. Unfortunately, the currently available data on the role of BMP signaling are very limited, and it is unclear whether BMP signals act as a “ciliogenic” growth factor or are actually transduced by primary cilia in bone cells. Further studies are necessary to clarify these aspects, and the molecular pathogenesis of BMP-related disorders, including fibrodysplasia ossificans progressiva, [48•] will hopefully be fully uncovered in the future.

### Cilium-Independent Functions of Ciliary Proteins

Recent studies have revealed that ciliary proteins are also found at certain non-cilia sites, where they have discrete roles in cellular function [49]. Interestingly, the disruption of each ciliary

component results in a different phenotype, which suggests that these components are involved in processes other than cilium formation and functions. Phenotypic differences of ciliary protein disruption in bone cells are summarized in Table 1.

We have recently reported that a neural crest-specific deletion of IFT20 in mice compromises ciliogenesis and collagen intracellular trafficking, leading to osteopenia in the craniofacial region. These IFT20-mutant mice exhibited normal osteogenic differentiation potential, but the process of mineralization was severely attenuated due to the dysfunctional transport of procollagen from the endoplasmic reticulum (ER) to the Golgi apparatus. Hence, these data suggest that IFT20 is crucial for regulating not only ciliogenesis but also intracellular collagen transport [50••]. Additionally, previous studies demonstrated that non-ciliated T lymphocytes express several IFT proteins, as well as the IFT-dependent kinesin-2 motors, which are required for interaction of T cells with antigen-presenting cells and subsequent cytokine exocytosis [51]. In the non-ciliated T lymphocytes, IFT20 and other IFT proteins associate with both the centrosome and the Golgi apparatus, with a similar localization as that seen in ciliated cells [52]. These studies clearly demonstrated that IFT proteins are involved not only in cilia assembly/disassembly, but also in non-ciliary functions such as exocytosis.

Accumulating evidence indicates that many ciliary proteins exhibit more than one function in osteoblasts [50••] and in

**Table 1** Phenotypic differences of ciliary protein disruption in bone cells

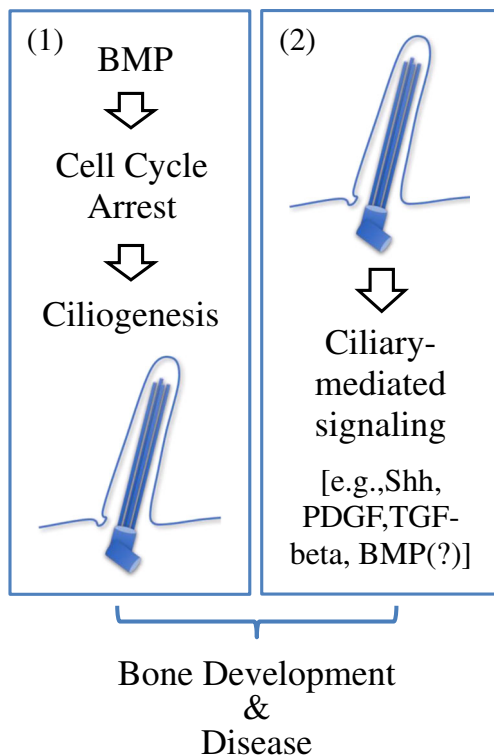
Mouse model	Target cells	Cilia phenotype	Bone phenotype	Signaling pathway	References
Kif3a <sup>Prx1-cre</sup>	Early limb mesenchyme	Cilia loss	Shortening of limb bone	Hh	[57, 58]
Kif3a <sup>Wnt1-cre</sup>	Cranial neural crest cells	Cilia loss	Craniofacial abnormalities, both endochondral and intramembranous skeletal structures	Hh	[57, 59]
Kif3a <sup>Col<math>\alpha</math>1(1)2.3-cre</sup>	Osteoblasts	n/d	Normal development but impaired mechano-response	n/d	[60]
Kif3a <sup>Oc-cre</sup>	Mature osteoblasts	Reduced cilia number	Osteopenia	Hh and Wnt	[32]
Ift80 <sup>Prx1-cre</sup>	Early limb mesenchyme	Cilia loss	Defect in endochondral bone	Shh and Ihh	[58]
Ift80 <sup>Osx-cre</sup>	Osteoblast progenitors	Cilia loss	Decreased bone mass	Canonical Hh-Gli and non-canonical Hh-G $\alpha$ i-RhoA	[33••]
IFT88 <sup>Oc-cre</sup>	Mature osteoblasts	n/d	Decreased bone density in mixed background mouse. Increased bone volume in C57BL/6	n/d	[61]
Ift88 <sup>Tg737Rpw</sup> (ORPK)	Global	Malformed cilia	Claniofacial abnormalities including cleft palate	PDGF	[47]
Pkd1 <sup>Oc-cre</sup>	Mature osteoblasts	Reduced cilia number	Reduced bone mineral density	Hh and Wnt	[30, 31]
Pkd1 <sup>Dmp1-cre</sup>	Osteocytes	n/d	Impaired mechano-response	n/d	[23]
IFT20 <sup>Wnt1-cre</sup>	Cranial neural crest cells	Cilia loss	Impaired intracellular collagen transport	PDGF	[50••]

n/d not determined

other cell types as well [49, 51, 53–55]. The contradicting findings based on the disruption of each ciliary protein are then most likely due to the multifunctional role and localization pattern of these proteins. Although ciliopathies comprise a group of genetic disorders associated with the disruption of cilium functions or defective expression of ciliary proteins, they display different phenotypes in distinct organs and at different stages of life. Therefore, the true etiology of ciliopathies may require re-evaluation and further investigation.

## Conclusion

It has become increasingly evident that primary cilia act in a diverse range of cellular events involved in bone development and maintenance. A better understanding of cilia function can provide insights into the extracellular niche and environment in bone cells. The complexity of skeletal ciliopathies is due to the fact that ciliary proteins not only contribute to ciliogenesis but are also involved in mitosis and cellular trafficking. Therefore, it may be important to revise the notion that the phenotypes of skeletal ciliopathies are simply attributable to altered ciliary-dependent signals.



**Fig. 1** The role of BMP signaling in bone development and disease. BMP signaling is critical for regulating cell cycle arrest required for the primary cilia formation [1]. Along with Shh, PDGF, and TGF-beta signaling, BMP signaling may also be transduced by primary cilium to regulate bone development [2]

In this review, we highlighted a possible link between BMP, ciliogenesis and ciliary-dependent BMP signaling (Fig. 1). The functional output of BMP signaling relies on extensive crosstalk with other signaling pathways, including Hh, Wnt, Notch, mammalian target of rapamycin (mTOR), and receptor tyrosine kinases, which have been reported to be coordinated by the cilia/centrosome axis [56]. Defining the underlying mechanisms of BMP and primary cilia will contribute to the understanding of the interactions of BMP and other developmental signals critical for controlling bone development. Further studies are necessary to clarify the cilium-dependent and cilium-independent mechanisms of action of ciliary proteins, and to understand the precise pathogenesis of skeletal ciliopathies.

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## Compliance with Ethical Standards

**Conflict of Interest** Masaru Kaku and Yoshihiro Komatsu declare no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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