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Physiological mechanisms and therapeutic potential of bone mechanosensing

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Abstract

Skeletal loading is an important physiological regulator of bone mass. Theoretically, mechanical forces or administration of drugs that activate bone mechanosensors would be a novel treatment for osteoporotic disorders, particularly age-related osteoporosis and other bone loss caused by skeletal unloading. Uncertainty regarding the identity of the molecular targets that sense and transduce mechanical forces in bone, however, has limited the therapeutic exploitation of mechanosensing pathways to control bone mass. Recently, two evolutionally conserved mechanosensing pathways have been shown to function as “physical environment” sensors in cells of the osteoblasts lineage. Indeed, polycystin-1 (Pkd1, or PC1) and polycystin-2 (Pkd2, or PC2, or TRPP2), which form a flow sensing receptor channel complex, and TAZ (transcriptional coactivator with PDZ-binding motif, or WWTR1), which responds to the extracellular matrix microenvironment act in concert to reciprocally regulate osteoblastogenesis and adipogenesis through co-activating Runx2 and a co-repressing PPAR γ activities. Interactions of polycystins and TAZ with other putative mechanosensing mechanism, such as primary cilia, integrins and hemichannels, may create multifaceted mechanosensing networks in bone. Moreover, modulation of polycystins and TAZ interactions identify novel molecular targets to develop small molecules that mimic the effects of mechanical loading on bone.

Keywords

Bone mechanosensing; Extracellular matrix stiffness; Senile osteoporosis; Polycystin complex; TAZ; Osteoblastogenesis; Adipogenesis

1 Introduction

Physical cues in the bone microenvironment are critically important in maintaining skeletal homeostasis. Defective bone mechanosensing may underlie age-related osteoporosis, as well as bone loss due to immobility, inactivity and/or sarcopenia, and microgravity conditions [1,

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2]. Skeletal unloading is associated with mesenchymal precursors in bone switching from an osteoblastic to adipogenic fate. The hallmark of age-related decline in bone mass is the replacement of bone marrow with adipose tissue in both men and women [3] caused by a decrease in osteoblast-mediated bone formation and increase in adipogenesis [4]. Immobilization increases marrow fat [5], and microgravity decreases osteogenesis and increases adipogenesis; whereas exercise, exposure to low-magnitude mechanical forces and pulsed electromagnetic fields, and mechanical loading of osteoprogenitor cells have opposite effects to inhibit bone marrow adipogenesis and stimulate osteoblast-mediated bone formation [6–15]. Mechanical forces also modulate bone development and influence fracture healing. Treatments for stimulating osteoblast-mediated bone formation without increasing bone resorption or adipogenesis to increase bone mass in age-related osteoporosis, are lacking.

Knowledge gaps in the pathogenesis of age-related bone loss, particularly knowledge of the factors controlling the balance between osteoblastogenesis and adipogenesis, are major obstacles to developing effective treatments of these disorders. Many questions and controversies about the events underlying age-related osteoporosis remain, such as the cell types responsible for the defective bone formation (i.e., mesenchymal precursors, mature osteoblasts, or osteocytes); the exact biological processes (i.e., lineage commitment versus transdifferentiation) responsible for the inverse relationship between adipocytes and osteoblasts observed in age-related osteoporosis, and the cellular and molecular pathways that mediate the “effects-of-aging” on osteoblastogenesis and adipogenesis. Moreover, the specific physical cues and molecular identity of the mechanosensors in the bone microenvironment responsible for maintaining bone mass are uncertain.

Elucidating these mechanosensing and mechanotransduction pathways in bone is fundamental to the understanding of bone physiology. In addition, development of drugs and specific mechanical forces to activate these targets in bone may provide new treatments for senile osteoporosis, accelerate fracture healing, and/or prevent bone loss caused by skeletal unloading, such as during immobilization and space flight.

2 Molecular mechanisms of bone mechanosensing

There are many competing ideas regarding the cellular and molecular components that constitute the bone mechanosensing network [7–11]. The osteoblast lineage, which is responsible for generating new bone, consists of a pluripotent mesenchymal stem cell population in the bone marrow that give rise to pre-osteoblasts that develop into mature osteoblasts that line the bone surfaces and generates new bone. A subset of mature osteoblasts become embedded in bone matrix to form osteocytes that act to coordinate the activities of osteoblasts and osteoclasts via the release of local regulators of bone remodeling, such as Sost and RANKL. Osteocytes also regulate systemic calcium and phosphate homeostasis through the release of the hormone FGF23.

Osteocytes are thought to be the major cell type responsible for sensing mechanical stress in bone [16–19]. Osteocytes form a complex, interconnected network in bone and show greater sensitivity to mechanical stress than osteoblasts [20–22]. The wide distribution of osteocytes

throughout the bone matrix and their high degree of interconnectivity via gap-junction-coupled cell processes and canaliculi networks bone make them ideal for a mechanosensing role [17, 23–28]. Osteocytes also communicate to cell surface osteoblasts through paracrine factors (i.e., RANKL, FGF23, PGE2, NO, and IGF-1) as well as through direct connections [19, 28–30] that regulate both osteoblast [23, 31–33] and osteoclast [24, 34–36] activities.

The skeletal networks that regulate bone mass are likely to be complex. How osteocytes and other cells in the osteoblast lineage sense physical forces is not certain. Many possible molecular mediators have been proposed, including primary cilia and polycystins [37], extracellular matrix stiffness and alterations in cytoskeletal dynamics that regulate members of the YAP/TAZ transcription co-activators [38, 39], $\alpha\text{v}\beta 3$ integrin receptors [40–42], connexins/gap junctions, hemi-channels [43, 44], and stretch-activated ion channels [45–48]. There are also multiple physical forces in the bone microenvironment, including flow, stretch deformation, cell-cell and cell-matrix interactions, and spatial confinement in physical niches, such as osteocytes embedded in bone that also may activate distinct mechanosensors.

Comparative analysis of prototypic mechanosensing molecular mechanisms in different tissues and use of mouse genetic approaches have identified several molecular candidates for the physiologically relevant bone mechanosensor. However, at present none of these yet meet the essential criteria for a druggable bone mechanosensing target, but polycystins, primary cilia and transcriptional co-activator with a PDZ-binding domain (TAZ) are emerging as leading candidates that integrate the response to flow and extracellular matrix environment in bone.

3 Role of polycystins in bone mechanosensing

The primary cilium/polycystin complex is a prototypic mechanosensor for fluid flow in kidney and other tissues [37, 49–53], and may constitute a *bona fide* mechanosensing organelle in bone [37, 49, 54–60]. PC1 (Pkd1, or PC1) is a transmembrane receptor belonging to adhesion G protein-coupled receptors family (adhesion-GPCR), which contains unique GPS (the **G**PCR **p**roteolysis **s**ite) and GAIN (the *G*PCR-*a*utoproteolysis *i*nducing) domains in the extracellular region [61, 62]. Polycystin 2 (Pkd2, or PC2; or TRPP2) is a calcium channel. PC1 is coupled to PC2, through its C-terminal domain [63]. The PC1 and PC2 complex co-localizes to primary cilia [49, 50, 55, 57]. PC1 and PC2 have the structural properties to sense a wide range of physical forces [64–66].

PC1 and PC2 are also expressed in the skeleton [67] in mesenchymal derived cells, including osteoblasts and osteocytes [37, 68–72]. There is emerging evidence that PC1 and PC2 form a receptor channel complex that acts as a “physical environment sensor” in cells within the osteoblast lineage to regulate bone mass [73] (Fig. 1a).

Recent studies using mouse genetic approaches show that polycystins regulate bone mass, osteoblastogenesis and adipogenesis. Selective ablation of *Pkd1* and *Pkd2* in osteoblasts in mice results in osteopenia [37, 59, 67, 73–77]. *Col1a1*(3.6)-, *Osteocalcin* (*Oc*)-, and *Dmp1*-Cre, have been crossed with *Pkd1*^{fllox/fllox} or *Pkd1*^{fllox/null} mice [78] to create conditional

deletion of *Pkd1* in mesenchymal precursors [59], mature osteoblasts [76], and osteocytes [73], respectively. Regardless of which stage in the osteoblast lineage that *Pkd1* was deleted, significant reductions in bone mass were observed due to decreased osteoblast-mediated bone formation (Fig. 2a, c, e) *in vivo* and impaired osteoblast differentiation in primary osteoblasts cultures derived from the conditional *Pkd1* knockout mice (Fig. 2g, i) [59, 73, 76]. Conditional deletion of *Pkd1* in the osteoblast lineage also resulted in increased bone marrow fat *in vivo* and enhanced adipogenesis *ex vivo*. This reciprocal relationship between osteoblasts and adipocytes was observed in *Pkd1*-deficient pre-osteoblasts, mature osteoblasts or osteocytes, albeit the ratio of *PPARγ/Runx2* was ~4-fold in *Pkd1^{Col1a1(3.6)-cKO}* and *Pkd1^{Oc-cko}* but 1.5-fold in *Pkd1^{Dmp1-cko}* osteoblasts. These findings implicate a role of PC1 in osteoblast/adipocyte lineage determination.

Similarly, conditional deletion of *Pkd2* in mature osteoblasts by crossing *Oc-Cre* with *Pkd2^{fllox/-}* mice resulted in a reduction in bone mineral density, trabecular bone volume, cortical thickness and mineral apposition rate *in vivo* (Fig. 2b, d, f) and impaired osteoblast differentiation *ex vivo* (Fig. 2h, j) and decreased expression of osteoblast-related genes, including, *Runx2*, *Osteocalcin*, *Osteopontin*, *Bone Sialoprotein*, *Sost* and *FGF23* (Fig. 2f). Significant reductions in both serum concentrations and bone mRNA expression of *FGF23*, *RankL* and *TRAP* were also observed in *Oc-Cre;Pkd2^{fllox/null}* compared to controls [79]. In contrast, loss of *Pkd2* in the osteoblast lineage resulted in a concordant impairment of adipogenesis. *Pkd2^{Oc-cko}* mice exhibited diminished *PPARγ* and adipocyte markers expression and reduced bone marrow fat *in vivo*. Osteoblast cultures derived from *Pkd2^{Oc-cko}* mice had a decrease in both osteoblast and adipocyte markers, including decrements in *Runx2* and *PPARγ*. These findings indicate that the function of PC1 and PC2 can be uncoupled with regards to adipocyte differentiation. Conditional deletion of *Pkd1* and *Pkd2* in osteoblast resulted in decrease expression of RANKL, leading to reductions in osteoclast-mediated bone resorption and decreased bone expression and serum levels of *Trap* [76] (Fig. 2e). Finally, immortalized osteoblastic cell lines of *Pkd1*- and *Pkd2*-deficient mice and shRNA knockdown of *Pkd1* in both MG-63 osteoblastic [60] have reduced basal intracellular calcium a significantly attenuated response to fluid shear stress [55, 79].

Polycystins also mediate the response to mechanical loading as evidenced by the differential response to *in vivo* ulna loading of wild-type and *Pkd1*-deficient (*Pkd1^{Dmp1-cko}*) mice [73]. There was a significant decrease in periosteal mineral apposition rate in *Pkd1^{Dmp1-cko}* ($0.58 \pm 0.14 \mu\text{m/d}$), which have selective deletion of *Pkd1* in osteocytes, compared to control mice ($1.68 \pm 0.34 \mu\text{m/d}$) after applied loads. Mechanical loading stimulated *Cox-2*, *c-Jun*, *Wnt10b*, *Axin2*, and *Runx2* expression in wild-type controls, but these mechanoresponsive genes were significantly attenuated in loaded *Pkd1^{Dmp1-cko}* mice. Immortalized wild-type osteoblasts exposed to 6.24 dyn/cm^2 pulsatile laminar fluid flow exhibited the expected rise in intracellular calcium. In contrast, exposure of *Pkd1* or *Pkd2*-deficient osteoblasts to an identical flow stimuli resulted in a significant attenuation of the calcium response curve in the heterozygous cells and complete loss of calcium influx in *Pkd1^{-/-}* and *Pkd2^{-/-}* osteoblasts. These findings have been confirmed by others [68, 80, 81].

In addition, PC1 deficiency prevented unloading-induced bone loss *in vivo* using tail suspension model. Micro-CT analysis showed that unloading reduced trabecular bone

volume and cortical bone thickness in wild-type mice by about 24 and 13 %, respectively, but had no effect to reduce bone mass further in *Pkd1*^{Dmp1-cKO} mice. *Dmp1*-Cre mediated deletion of *Pkd1* in osteocytes establish the role of osteocytes in regulating osteoblast differentiation and mechanosensing [73]. Both pre-osteoblasts and mature osteoblasts also respond to fluid flow *in vitro* [7–9] and the conditional deletion of *Pkd1* and *Pkd2* in pre-osteoblasts and mature osteoblasts inhibits flow-induced increases in intracellular calcium [73, 79]. Thus, each of these cell types within the osteoblast lineage has the inherent potential to respond to mechanical forces through polycystins.

In spite of the incontrovertible evidence that PC1 and PC2 play an important direct role in skeletogenesis and post-natal bone functions in mice [68, 82–85], the role of polycystins in human bone homeostasis is controversial, because skeletal haploinsufficient ADPKD patients do not have a well characterized bone phenotype. Recent human genome wide associative studies (GWAS) link the PC2 with osteoporosis [82], and craniofacial abnormalities have been reported in ADPKD patients [82], suggesting a functional role of polycystins in the human skeleton. In addition, ADPKD patients have more than double the amount of osteoid and reduction in cortical bone volume than patients with chronic glomerulonephritis [86] and have earlier alterations in the osteocyte-derived hormone FGF23 [87]. Indeed, embryonic lethal biallelic mutations of PKD1 and PDK2 in humans result in severe skeletal abnormalities [85, 88], similar to knockout animal models. Human GWAS studies also link PKD2 with osteoporosis (OP) [68] and with abnormal shape of craniofacial bones in patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) [82].

4 Role of primary cilia in bone mechanosensing

Polycystins are closely linked to primary cilia. Indeed, other gene mutations involving components of primary cilia that lead to renal cytogenesis and skeletal dysplasia suggest that polycystins and primary cilia are involved in common mechanosensing networks.

Several ciliopathies have polycystic kidney disease and a bone phenotype. Deletion of *Tg737* gene (encoding Polaris/ IFT88 protein) results in loss of cilia and abnormal development of the appendicular skeleton [89–91]. The hereditary ciliopathies Oral-facial-digital (OFD) and Bardet–Biedl syndrome (BBS) have both skeletal abnormalities and renal cystic disease [92–94]. Recessive cystic kidney diseases caused by mutations in NPHP6, MKS1 and MKS3 are also associated bone changes [95, 96].

There is also direct experimental evidence that primary cilia/polycystin complex mediates the mechanosensing response in osteocytes in mouse bone [73, 74, 97]. First, primary cilia are present in osteoblasts and osteocytes *in vitro* [37, 98–100] and *in vivo* [37, 101]. Immunofluorescent staining of calvaria *in vivo* and isolated osteoblast cultures *in vitro* [37, 98–100] and transmission electron microscopy in rat tibial cortical bones [101] respectively found a single primary cilium in each osteoblast and osteocyte. In osteocytes, the mother centrioles form short primary cilia oriented toward the long axis of weight-bearing bones. Second, primary cilia functions as a flow sensor in osteoblasts and osteocytes *in vitro* [60, 74, 102]. In these studies, disruption of *Kif3a* or *Tg737*, or *Pkd1* impaired ciliogenesis and

resulted in loss of flow-induced increase in $[Ca^{2+}]_i$ and cAMP signaling in cilia-deficient osteoblasts and osteocytes *ex vivo*. Third, conditional *Kif3a* null mice (*Kif3a*^{Oc-CKO}) develop osteopenia and impaired mechanosensing due to disruption of primary cilia formation in osteoblasts [74, 103]. The fact that polycystins co-localize with primary cilia in osteocytes and osteoblasts [99, 102–105], and the bone phenotypes are similar in conditional deletion of *Pkd1*, *Pkd2* and *Kif3a* in bone, suggest that polycystins and primary cilia have co-dependent functions. *Kif3a*^{Oc-CKO} mice had reductions of primary cilia number and length and developed osteopenia in association with impaired osteoblast function *in vivo* and *in vitro* [74]. *Col1a1(2.3)-Cre;Kif3a*^{flox/flox} mice, similar to *Dmp1-Cre;Pkd1*^{flox/null} mice, exhibit decreased formation of new bone in response to mechanical ulnar loading compared to control mice [73, 103].

The relationship between primary cilia and polycystins, however, is complex; as evidenced by the fact that superimposed *Kif3a* haploinsufficiency paradoxically reversed the skeletal abnormalities in heterozygous *Pkd1*^{+/-} mice [75]. This may be explained by the fact that primary cilia are coupled to a broader array of intracellular signal pathways that overlap those of polycystins, including hedgehog/Gli and AC6/cAMP signaling, which have been linked to mechanosensing responses in osteoblasts and osteocytes in bone [74, 100, 102, 106]. The recent creation of an inducible Cilia^{GFP} mouse model to visualize cilia in live tissues will aid future studies of ciliary mechanosensing function in osteoblasts and osteocytes in bone [107].

5 Emerging role of TAZ in bone mechanosensing

Inactivating mutation of TAZ (transcriptional co-activator with a PDZ-binding domain; also known as WW domain containing transcription regulator 1, or WWTR1) also results in renal cysts and bone abnormalities. *Taz* knockout and conditional deletion of *Taz* from the kidney result in cystic kidney disease in mice [108, 109], similar to polycystin inactivation, suggesting that *Pkd1*, *Pkd2* and *Taz* participate in common pathways. TAZ and YAP (Yes-associated protein; or YAP1) are downstream effectors of the Hippo pathway that regulate mesenchymal lineage specification. TAZ and YAP are also regulated by extracellular mechanical stimuli that bypass classical Hippo signaling cascades and involves cytoskeletal-dependent nuclear shuffling in response to alterations in extracellular matrix stiffness (Fig. 1b).

There is emerging evidence that TAZ plays a role in mechanosensing and lineage determination in osteoblasts. First, TAZ translocates to the nucleus, where it coactivates Runx2 to stimulate osteoblastogenesis [110] and purportedly represses PPAR γ activity to inhibit adipogenesis [111]. Second, transgenic overexpression of *Taz* in osteoblasts in mice leads to increased osteoblast-mediated bone formation and decreased bone marrow adipogenesis [112]; depletion of *Taz* in zebra fish impairs bone development [113] and *Taz*^{-/-} mice have small stature and ossification defects [108]. Third, TAZ is regulated by mechanical and cytoskeletal signals in response to alterations in extracellular matrix elasticity [38, 39]. Soft matrix inhibits TAZ to enhance adipocyte differentiation; stiff matrix and low shear stress stimulate TAZ and osteoblastogenesis [38, 39, 114]. Fourth, TAZ enhances TGF- β -dependent signaling and inhibits Wnt-signaling [113]. Fifth, TAZ binds to

PC1-CTT to facilitate nuclear translocation [115] and to PC2-CTT, leading to PC2 degradation [116]. Thus, TAZ is involved in mechanosensing pathways linking “extracellular matrix stiffness” to osteoblastogenesis and adipogenesis [38] through its function as a co-activator for Runx2 and a co-repressor for PPAR γ activity [111, 113].

There may also be cross talk between polycystins and YAP/TAZ mechanosensing pathways. Mechanical stimuli induce cleavage of the PC1 C-terminal tail which translocates to the nucleus to regulate gene transcription [117]. Additional studies suggest that TAZ interacts with the PC-1 C-terminal tail and participates in the nuclear translocation [118]. Both TAZ and YAP were significantly decreased in *Pkd2*-deficient mouse bone and immortalized osteoblasts [79]. Interactions between polycystins and TAZ may explain the inverse relationship between osteoblastogenesis and adipogenesis and may integrate two types of mechanical stimuli, namely flow and extracellular matrix stiffness (Fig. 3). Consistent with this hypothesis, decreased TAZ and YAP message and protein levels, increased phosphorylation of YAP and TAZ, and reduced TAZ-mediated activation of the TEAD reporter activities, are present in *Pkd1*-deficient osteoblasts (unpublished observations), consistent with polycystins regulation of TAZ at multiple levels (i.e., both transcription and nuclear translocation of *Taz*).

6 Role of other mechanosensors in bone

Many other cell surface molecules, including integrin receptors [40–42], LRP5 [119–122], estrogen receptor α [123–125], and hemichannels [43, 44, 126], as well as other putative mechanosensing gene products [36, 38, 45–48, 127–131] have been proposed to mediate the response to skeletal loading in osteocytes and osteoblasts, but in contrast to polycystins and Taz, the ability of many of these molecules to modify mechanosensing responses has been demonstrated only in cell culture models *in vitro*, and confirmation of their mechanosensing properties *in vivo*, as well as a conceptual/ structural frame work for how these molecules sense forces are generally lacking.

6.1 Role of connexin 43, gap junctions (GJ), and hemichannels in bone mechanosensing

Connexin 43 (Cx43) belongs to a family of proteins that form gap junction (GJ). Cx43 is the predominant GJ protein in osteoblastic cells and bone [2] and has been implicated in effects of mechanical stimulation to induce calcium wave propagation in adjacent cells [132, 133] and release anabolic factors, such as PGE₂, ATP, and nitric oxide (NO) [134–136]. *In vitro* studies suggest that gap junctional intercellular communication sensitizes bone cells to mechanical signals [132, 137–139].

In spite of the compelling data supporting a role of gap junctions in mediating the anabolic response of bone to mechanical load [140, 141], recent *in vivo* studies have failed to provide unequivocal support for this hypothesis. One study found that Cx43 deficient mice have an decreased anabolic response to mechanical load [43], whereas another study found that Cx43 deficient mice have an increased anabolic response to mechanical load [142, 143]. Both studies, however, found that Cx43 deficient mice are protected against the catabolic effects of mechanical unloading [144–146]. Cx43 deficient mice also have increased bone resorption and TRAP-positive osteoclasts relative to wild-type control [142, 147] and Cx43

deficient MLO-Y4 cells display an increase in the RANKL/OPG ratio compared to control MLO-Y4 cells [142, 148].

6.2 Role of $\alpha 5 \beta 1$ integrin receptors in bone mechanosensing

Integrins, comprised of heterodimers of α and β subunits, are major receptors/transducers that connect the cytoskeleton to the extracellular matrix and interact with plasma membrane proteins such as metalloproteases, receptors, transporters, and channels mainly through the extracellular domain of their α subunits. The integrin $\alpha 5$ subunit may act as a tethering protein that, when perturbed by shear stress, opens hemichannels in osteocytes, allowing the release of prostaglandin E2 (PGE2) and ATP. Mechanical forces applied to $\alpha 5 \beta 1$ integrin receptors trigger intracellular calcium signaling via ultra-rapid activation of TRPV4 ion channels [40, 149].

Several observations suggest that integrins mediate the effects of mechanical stress in osteocytes [150–154]. First, integrin $\alpha 5 \beta 1$ and $\alpha v \beta 3$ are present in osteocytes along the cell processes and body and associated with hemichannels mechanotransduction [155, 156]. Second, mechanical stress *in vitro* activates $\alpha 5 \beta 1$ integrin in osteoblasts and osteocytes, which induces opening of hemichannels, thereby allowing the release of anabolic factors and triggering a signaling cascade [150, 151, 154, 157]. $\alpha v \beta 3$ may mediate mechanotransduction via DMP1 in osteocytes [40, 41, 155]. Third, activation of integrin $\alpha 5 \beta 1$ or the extracellular matrix (ECM)-integrin $\alpha 5 \beta 1$ interactions via RGD binding have been linked to the control of osteoblastogenesis through activation of TRPV4 ion channels and FAK/RhoA-ROCK/ERK [40–42, 158–160]. Fourth, integrin $\alpha 5 \beta 1$ affects bone formation in osteoblasts and osteocytes *in vivo* [161–164].

On the other hand, there is lack of direct evidence that selective deletion of $\alpha 5 \beta 1$ integrins in the osteoblast lineage impairs mechanosensing responses *in vivo* [163]. Local administration of integrin agonists promotes bone regeneration [153]. For instance, lentivirus-mediated expression of the $\alpha 5$ integrin subunit in human adult mesenchymal stromal cells promotes bone repair in mouse cranial and long-bone defects [165]. Moreover, low intensity pulsed ultrasound (LIPUS) enhances fracture healing via activation of integrin $\alpha 5 \beta 1$ signaling in osteoblasts and osteocytes in bone [157, 166]. Studies of potential cross-talk between integrins, polycystins and TAZ may reveal additional integration of mechanosensing pathways.

7 Integration of mechanosensing signaling pathways to regulate osteoblastogenesis and adipogenesis

These mechanosensing pathways are not mutually exclusive and their integrated functions may provide flexibility and diversity in how cells in the osteoblast lineage respond to physical cue in the bone microenvironment. For example, primary cilia, PC1/PC2 and integrins are coupled to multiple intracellular signal pathways and cytoskeletal responses, which have been linked to mechanosensing responses in osteoblasts/osteocytes in bone [129, 167, 168], whereas TAZ acts as a co-factor in downstream transcriptional activation and Cx43 propagates calcium signaling to neighboring cells.

There is strong evidence that implicate effects of mechanical stimulation to increase intracellular calcium and activate Runx2-mediated osteoblastogenesis. Indeed, compound heterozygous *Pkd1*^{+/-}; *Runx2*^{+/-} mice that show additive effects to reduce bone mass, implicate Runx2 in a common pathway with PC1 [67]. Complementary *in vitro* studies suggest that flow induced activation the PC1-PC2 complex regulates Runx2 gene transcription through intracellular calcium and nuclear factor 1 (NFI) family of transcription proteins [67], of which Nfic has been shown to play an essential role in inversely regulating osteoblast differentiation and bone marrow adipogenesis [169, 170].

Wnt signaling is also a possible point of integration for multiple mechanosensing pathways. The canonical Wnt/ β -catenin pathway is another anabolic pathway that is activated in osteocytes in response to mechanical loading [128, 129, 131]. Mechanical stimulation leads to decreased sclerostin, an inhibitor of the Wnt pathway [36, 167, 171]. The Wnt co-receptor LRP5 [119–122] is involved for mechanosensing responses.

There is also cross-talk between the Wnt signaling cascade and PC1. Mechanical stimuli induced cleavage PC1 to release the PC1-CTT inhibits glycogen synthase kinase-3 β and leads to stabilization of β -catenin [76, 117, 172–174]. The canonical Wnt signaling pathway directly regulates Runx2 via β -catenin/TCF1 regulatory sites in the Runx2 promoter [175]. Upregulation of Wnt/ β -catenin is severely impaired in response to mechanical loading in bone from *Dmp1*-Cre-mediated conditional *Pkd1* null mice [73]. *Pkd1*^{Oc-cko} mice have suppressed activity of the phosphatidylinositol 3-kinase-Akt-GSK β - β -catenin signaling pathway in osteoblasts [76]. The expression of *Axin2*, a direct down-stream gene of Wnt/ β -catenin, was significantly lower in bone and osteoblasts from conditional *Kif3a*^{Oc-CKO}-null mice compared with *Kif3a*^{flox/+} controls [74], consistent with a role of primary cilia coupling to Wnt/ β -catenin signaling [176–180]. An integrative model for potential interactions between polycystins, primary cilia, TAZ and Wnt signaling is shown in Fig. 3.

Cross-talk with other mechanosensing pathways also exists. For example, integrins, which act as fibronectin receptors, also modulate Wnt activity, and Syndecan-4, a heparan sulphate proteoglycan, is able to regulate canonical and non-canonical Wnt pathways [181–183]. Expression of β -catenin protein, a molecule implicated in mechanotransduction, was higher in bones from *Dmp1*-Cre; *Cx43*^{flox/flox} mice, compared to littermate controls. In addition, MLO-Y4 osteocytic cells knocked-down for Cx43 exhibited higher β -catenin protein expression and enhanced response to mechanical stimulation [143].

A recent study has shown Wnt/ β -catenin signaling activates TAZ [184, 185], whereas TAZ has been shown to inhibit Wnt-signaling [113], thereby creating a potential negative feedback loop. TAZ would also be expected to augment Runx2- and inhibit PPAR γ mediated effects of PC1, as well as potentially uncouple PC1 interactions with PC2 by TAZ binding to PC2 and promoting its degradation. In addition, integrin-mediated mechanical response of osteoblast lineage cells also regulates TAZ signaling, resulting in another point of integration of distinct mechanosensing mechanisms [186, 187].

Anabolic signals that are released within seconds after loading in osteocytes include nitric oxide (NO), prostaglandins, and other small molecules such as ATP [188–190]. NO, a short-

lived free radical inhibits resorption and promotes bone formation in osteoblasts. *In vivo* studies have shown that new bone formation induced by loading can be blocked by the prostaglandin inhibitor, indomethacin, and agonists of the prostaglandin receptors have been shown to increase new bone formation. Runx2 has also been linked to fluid shear stress induction of COX-2 in osteoblasts [191]. In addition, the P2X₇ nucleotide receptor mediates the mechanosensing effects of ATP on bone [47]. Prostaglandin production can be stimulated by the cilia-PC1/PC2 complex [192]. Load-induced stimulation of *Cox-2* is impaired in osteoblasts lacking *Kif3a* or *Pkd1* *in vivo* [73, 74]. PC2 was reported to be involved in the NO production in responding to fluid shear stress in MLO-Y4 cells and vascular endothelial cells [193, 194]. Recent data indicate that primary cilia activation by fluid shear stress leads to *COX2* mRNA expression and PGE₂ release in osteoblasts [195, 196]. In MLO-Y4 osteocyte-like cells there is evidence that PGE₂, ATP, and NO are released through Cx43 hemichannels in response to loading [134–136]; working in concert with $\alpha 5\beta 1$ integrin, which may tether to open hemichannels in osteocytes [150–153] to allow the release of PGE₂ and ATP.

8 Druggability of mechanosensing mechanisms to treat bone diseases

Insights into how mechanical cues are sensed and transduced in bone and a structural understanding of polycystins, integrins, TAZ and connexin interactions will facilitate the development of ways to manipulate these pathways in age-related osteoporosis, skeletal unloading and disuse osteoporosis, and fracture healing, as well as have broader applications in other diseases caused by mutations in *Pkd1* and *Pkd2*. At present there are no specific “ligands” to activate PC1, however, triptolide, a diterpenoid epoxide, is an agonist for PC2 [197].

A variety of mechanical forces have been found to influence bone cells *in vitro*, such as fluid flow, compressive strain, substrate stretch, gravity force, low-intensity pulsed ultrasound, vibration, magnetic bead twisting, atomic force, and high-energy acoustic waves. Simple aerobic exercise, such as walking, jogging, and running, could provide an anabolic stimulus for older bone and prevent bone loss in senior population [198, 199] and advanced resistive exercise device prevents bone loss during spaceflight [200, 201]. Low-intensity pulsed ultrasound (LIPUS) is used clinically to accelerate the fracture healing process [166, 202], with variable success [203]. Finally, whole-body vibration (WBV) has been reported to have the positive effect on BMD and bone strength [199, 204], similar to that of low magnitude mechanical signals (LMMS) in both animal [205, 206] and human [207, 208] studies. WBV activates the osteoblasts while reducing the activity of the osteoclasts directly by mechanical strain [209, 210], or indirectly through amplification of the signal by intramedullary pressure or fluid flow in bone tissue [211, 212].

The lack of a specific molecular mechanosensor has been a critical barrier to progress in this field. Unraveling the structural basis and molecular interactions between these pathways may permit identification of small molecules to target specific components of these pathways and/or allow refinement and optimization of methods (i.e., device development) to apply physical forces to a specific mechanosensor molecule to increase bone mass.

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Fig. 1. Integration of **a** the polycystin-1 (PC1; Pkd1) and polycystin-2 (PC2; Pkd2) flow sensing complex, and **b** the TAZ, extracellular matrix, and cytoskeletal dynamics mechanosensing pathway in the regulation of osteoblastogenesis and adipogenesis in response to mechanical cues in the bone microenvironment

gene dosage-related manner. Primary Obs derived from *Pkd1*^{Colla1(3.6)-cko}, *Pkd1*^{Oc-cKO}, and *Pkd1*^{Dmp1-cKO} mice exhibited increased adipogenesis

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Fig. 3.
Schematic of integration of flow activation of PC1-PC2 and ECM stiffness activation of TAZ signaling pathways to regulate osteoblastogenesis and adipogenesis