Published in final edited form as:

Ann N Y Acad Sci. 2010 March ; 1192: 422–428. doi:10.1111/j.1749-6632.2009.05243.x.

The osteocyte primary cilium and its role in bone mechanotransduction

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Abstract

Bone is a dynamic tissue that adapts to its local loading environment. Mechanotransduction, the process by which cells convert mechanical forces into biochemical signals, is important for maintaining bone health and homeostasis. It is less clear, however, what the cellular mechanosensor(s) are that sense and initiate these signaling cascades. Primary cilia are solitary, rigid structures that extend from cell body into the extracellular space; as a result, they are prime candidates for mechanosensing in bone. They have been shown to be critical in development, and they have been implicated in mechanosensing in other tissue types including liver and kidney. This review discusses the potential for primary cilia to play an important role in bone mechanotransduction and possible avenues for future study.

Keywords

bone; osteocyte; primary cilia; mechanosensor; mechanotransduction

Introduction

Bone is a dynamic tissue that adapts to meet the physical demands of its external environment. Reduced mechanical loading (disuse) from spaceflight, bed rest, aging, and injury all lead to a decrease bone density and strength. For example, prolonged microgravity associated with spaceflight can cause the loss of bone density at a rate of 1–2% per month.¹ In addition, several studies have shown that individuals disabled by spinal cord injury have observable loss in bone mineral density below the site of injury.² On the other hand, physical activity is known to increase bone size and density. Professional tennis players have greater bone density and bone diameter in their dominant (playing) arm than in their non-dominant arm.³ In addition, professional soccer players have significantly higher bone mineral content and density in their lower limbs compared to age-matched controls.⁴ Taken

together, these studies show that mechanical loading is important in maintaining bone structure.

Bone mechanotransduction

Mechanotransduction, the process by which cells convert external mechanical forces into biochemical responses, is important to maintaining adult bone health and homeostasis. Osteocytes are found in fluid filled voids or “lacunae” within the bone matrix. They form an interconnected network with one another and with osteoblasts at the bone surface via their cell processes that extend through channels called “canaliculi”. Osteocytes make up a vast majority of bone’s cell population and are distributed throughout the bone matrix.^{5, 6} Recently, osteocyte ablation in a mouse model was shown to result in increased intracortical porosity and decreased sensitivity to unloading, supporting their role in mechanotransduction.⁷ Taken together, these data suggest that osteocytes play a major role in mechanotransduction in bone.

Burger et al. described mechanotransduction in terms of a series of stages: (1) mechanical loading must induce a cell-level physical signal that can be sensed by the osteocyte, (2) this physical signal must be transduced by the osteocyte into a biochemical signal, and (3) the biochemical signal must be communicated to effector cells, such as osteoblasts and osteoclasts.⁸ Many mechanisms by which mechanical loading leads to physical signals that can be sensed by bone cells have been proposed, including electric/streaming potentials, substrate strain, and flow-induced shear stress.^{9, 10} Due to its ability to illicit cellular responses at physiological loading levels in *in vitro* studies, the current paradigm is that fluid flow-induced shear stress along the osteocyte body and cell processes is the method by which mechanical loading from daily activity, such as walking or running, affects changes in bone cell metabolism.^{11, 12} Additional evidence for this theory comes from an *in vivo* study by Qin et al., in which oscillatory fluid flow induced bone formation in turkey ulnae in the absence of other mechanical stimulation.¹³ Mechanical loading activates multiple signaling pathways resulting in osteogenic and anti-resorptive responses. Within seconds to minutes after exposure to fluid flow, osteocytes respond with release of NO, ATP, Ca²⁺ and PGE₂.^{14–17} These initial responses lead to downstream effects that mediate bone remodeling, including increases in COX-2 and PGE₂.^{14, 17} In terms of relaying mechanical cues to effector cells, osteocytes communicate with osteoblasts via gap junctions formed at the osteocyte cell process.¹⁸ Osteocytes also regulate osteoclast differentiation and activation.¹⁹ However, dynamic fluid flow results osteocyte-mediated inhibition of osteoclastogenesis and increases in the OPG/RANKL ratio.²⁰ RANKL stimulates osteoclast formation by binding to its receptor on the surface osteoclast precursors, while OPG, a decoy receptor for RANKL, inhibits osteoclastogenesis by competitively binding RANKL.

An important aspect of the osteocyte mechanotransduction process, which is less well understood, is the molecular mechanism(s) by which osteocytes sense mechanical loads and initiate these intracellular signaling cascades. Several molecules have been proposed to have the potential to regulate local bone remodeling in response to physical stimuli, including integrins²¹, connexin hemichannels^{15, 16}, and ion channels²². The primary cilium, a microtubule structure that protrudes from the cell membrane like a cellular antenna, is an

optimal candidate for an extracellular sensor of mechanical loading. It has been implicated in mechanosensing in the kidney and liver.^{23–26} Recently studies have suggested that it is also a mechanosensor in bone.^{17, 27, 28}

Primary cilia structure, formation, and maintenance

Primary cilia are microtubule-based structures that originate at the basal body and extend into the extracellular space (Fig. 1). The basal body is a modified form of the mother centriole, an organelle well known for its role as a microtubule organizing center of mitotic spindles. When not involved in mitosis, the basal body/mother centriole migrates towards the cell membrane and acts as a template of ciliogenesis and an anchor for the primary cilium. Primary cilia are formed and maintained by the process of intraflagellar transport (IFT), a bidirectional system that directs movement along the microtubules of the ciliary axoneme.^{29, 30} Briefly, since primary cilia do not contain machinery for protein synthesis, ciliary proteins are synthesized in the cell body, targeted to the basal body, and transported to the distal tip through IFT. Intraflagellar transport complexes are trafficked along the axoneme by molecular motors: anterograde kinesin-II, consisting of three subunits including KIF3A, and retrograde dynein 2. There are also numerous IFT-associated proteins, including IFT88 (polaris), that are important to primary cilia function.

Primary cilia differ from the better understood motile cilia in many important respects. Both motile and non-motile cilia have a microtubule axoneme core made up of nine sets of microtubules doublets that provide structure and rigidity, however, non-motile primary cilia lack a central pair of microtubules (thus designated as “9+0” cilia) and other motile machinery, including inner and outer dynein arms, radial spokes and central pair projections.³⁰ Unlike motile cilia, there is only one primary cilium per cell. In addition, primary cilia are found in nearly every cell type, exceptions include intercalated cells of kidney collecting duct, hepatocytes, cells that typically exist in suspension (e.g. circulating blood cells), and cells that express motile cilia. (An up-to-date list of cell types with primary cilia can be found at <http://www.bowserlab.org/primarycilia/cilialist.html>.) Motile cilia are found only in specific tissue types, such as the respiratory and reproductive tracts and ventricles of the brain.

Primary cilia in skeletal development

Primary cilia were first described over a century ago, but until recently they were believed by many to be vestigial organelles. Over 30 years ago, primary cilia were first discovered in the rat osteocyte.³¹ It was hypothesized that these cilia play a role in calcium homeostasis and the parathyroid hormone signaling pathway, however, once again no further investigations of the role of primary cilia in the skeleton were reported for several years. Recently, primary cilia have been shown to be important in numerous developmental processes including the establishment of the left-right axis in the embryonic node.³² In addition, ciliary dysfunction results in several ciliopathies with bone abnormalities (reviewed by Haycraft and Serra).³³ Sonic (Shh) and Indian (Ihh) hedgehog signaling are known to be important in skeletal development, particularly in limb patterning and endochondral ossification, respectively.³⁴ It has recently been shown that primary cilia in

the early limb mesenchyme are required for anterior-posterior limb patterning, as deletion of *Kif3a* or *Ift88* in by *Prx1*-promoter driven *Cre* resulted in polydactyly and abnormal Shh signaling.³⁵ Indian hedgehog regulates endochondral bone formation, the developmental process in which bone is formed from a cartilaginous anlagen. *Prx1-Cre* driven conditional deletion of *Ift88* and *Kif3a* results in disrupted Ihh signaling and defects in endochondral ossification, resulting in a shortened limb length.³⁵ In addition, *Kif3a* deletion in chondrocytes resulted in abnormal Ihh signaling and early loss of the post-natal growth plate resulting in dwarfism.³⁶

Primary cilia as mechanosensors in adult bone homeostasis

Much of the study of primary cilia as mechanosensors has occurred in the kidney and liver. Praetorius and Spring found that bending of kidney epithelial cell primary cilia with fluid flow or micropipette manipulation resulted in a dramatic extracellular Ca^{2+} -dependent increase in intracellular Ca^{2+} ; this mechanically-induced response was abolished with cilia removal.^{23, 24} A study by Nauli et al. suggests that the primary cilia-dependent flow-induced increase in Ca^{2+} occurs through the polycystin 1/2 complex.²⁵ The polycystin 1/2 receptor-ion channel complex is made up of polycystin 1 (PC1), a large membrane protein, and polycystin (PC2, also known as TRPP2), a cationic channel that is a part of the transient receptor potential (TRP) channel family. In liver cholangiocytes, it was found that the polycystin 1/2 complex and the stretch-activated ion channel, TRPV4, are localized to the primary cilium and sense fluid shear stress and osmotic pressure, respectively.^{26, 37} Fluid flow within isolated intrahepatic bile duct units causes primary cilia-dependent increases in intracellular Ca^{2+} and suppression of a pharmacologically-stimulated cAMP increase.²⁶

Most studies of primary cilia in bone have focused on its role skeletal development, however recent studies suggest that primary cilia also act as mechanosensors in adult bone homeostasis. Malone, Anderson, et al. found that osteocytes respond to fluid flow with cilia-dependent increases in *Cox-2* gene expression and PGE_2 release.¹⁷ In addition, flow-induced increases in the OPG/RANKL ratio were eliminated with cilia removal through pharmacological agents or siRNA knockdown of *Ift88*.¹⁷ However, these flow-induced cilia-dependent responses were independent of intracellular Ca^{2+} , which suggests that the cilia mechanotransduction signaling pathway in bone differs from the kidney and liver.¹⁷ A recent further study shows that dynamic fluid flow leads to a cilia-dependent decrease in cAMP.³⁸

Although the polycystin 1/2 complex has been implicated in cilia-related mechanosensation in the kidney and liver, its role in bone was unknown. Xiao et al. found that PC1 and PC2 localize to the primary cilium in bone cells.²⁸ Heterozygous *Pkd1* mutant mice, which have a mutation in the gene that encodes for polycystin 1, have decreased bone mineral density, trabecular bone volume, and cortical thickness.²⁸ In addition, there was a decrease in gene expression of the osteoblastic markers, Runx2-II, osterix, and osteocalcin.²⁸ There was also a decrease in the OPG/RANKL ratio in heterozygous *Pkd1* mutant mice.²⁸ Taken together, this study implicates the polycystin 1/2 complex and the primary cilium in mechanotransduction in bone, although a direct study of primary cilia-dependent response to mechanical loading was not conducted.

Future Directions

The primary cilium, which was once unacknowledged except by a few pioneers, has now been found to be important in chemo- and mechano- sensing in numerous cell types, including bone. Studies indicate that it plays a critical role in hedgehog signaling and skeletal development. The primary cilium has also been shown to play a mechanosensory role in bone cells. However, there are many interesting aspects of the role of primary cilia in bone mechanotransduction that are left to be studied.

It will be important to determine the orientation of the primary cilium within the osteocyte lacunae. The pericellular spacing between osteocyte and lacunar wall is approximately 1µm (0.1µm – 2.7µm).³⁹ Studies have found the length of osteocyte primary cilia to be approximately 2–9µm in cell culture.^{17, 28} An image from Xiao and colleagues shows the *in vivo* osteocyte primary cilium from the mouse calvaria to be approximately 3µm in length.²⁸ Some variation is to be expected, however, given that the pericellular space is approximately 1µm, the question of how the primary cilium is situated within the osteocyte lacuna remains open. It is also unclear whether or not there is enough space for the primary cilium to bend with fluid flow, as shown by Malone and Anderson et al.¹⁷ It would also be interesting to determine if there is a specific location and orientation to the primary cilium with regards to osteonal or other organization. Imaging the primary cilium in bone has proven difficult, however, as osteocytes are embedded in a bony matrix and the necessary tissue preparation can destroy the fragile cilium. Also primary cilia are hard to detect due to their size relative to the osteocyte cell body. Possible methods for determining the answers to these questions include serial-section transmission electron, multiphoton, or confocal microscopy.

Another important issue is determining the molecular mechanism by which primary cilia mediate sensing of mechanical stimulation. One possibility is the mechanical activation of ion channels, such as the PC 1/2 complex or TRPV4, which localize to primary cilia and detect fluid flow in the kidney and liver.^{25, 26, 37} The osteocyte primary cilium mediates mechanically induced responses independent of intracellular Ca²⁺ stores release, which differs from that in the kidney and liver. However, the primary cilia-dependent flow-induced decrease in cAMP could be mediated by extracellular Ca²⁺ entry through TRP channels leading to adenylyl cyclase-dependent inhibition of cAMP production as seen in cholangiocytes.²⁶ In addition, integrins, which associate with the extracellular matrix, have been shown to localize to the chondrocyte primary cilium.⁴⁰ This could also be the case in osteocytes, and within the small pericellular space, perhaps small deformations are transmitted to the cilium by integrins localized to the cilia membrane, even if full bending of the cilium does not occur. Nonetheless, deformation of the cilium may be significantly greater than deformation of the cell body at a given loading level. Also, the distal and subdistal appendages of the basal body dock it to the cell membrane and link the basal body/cilium to the cytoskeleton through direct contact with cytoplasmic microtubules.²⁹ This could also serve as a strain deformation amplification mechanism by which a deformation of cilia could be felt within cell body.

Finally, it will also be important to extend the study of primary cilia as mechanosensors beyond the current cell culture methods in order to determine physiological relevance.

Studies of primary cilia as mechanosensors have predominantly been conducted *in vitro*. More recently, *ex vivo* studies in liver ducts have implicated the primary cilia as sensors of fluid flow.^{26, 37} In addition, *Pkd1* heterozygous mice have abnormalities in bone morphology implying that primary cilia play a role in mechanosensation, however the response to mechanical loading has not been directly assessed.²⁸ Primary cilia knockouts are embryonic lethal, however with cre-lox conditional knockout technology it is possible to study how a primary cilia knockout affects the response of a specific tissue, such as bone, to mechanical loading.

Bone is a dynamic tissue that is continually remodeling to meet the demands of its mechanical environment. Mechanical loading results in signaling cascade through process of mechanotransduction. Although there are many interesting things left to be discovered, studies to-date suggest that the osteocyte primary cilium may be an important mechanosensor in bone. Elucidation of cilia orientation as well as cilia-dependent molecular mechanism(s) of mechanosensing will be important for understanding bone mechanotransduction and potentially developing novel classes of effective therapeutics for bone loss.

References

1. Lang T, LeBlanc A, Evans H, et al. Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. *J Bone Miner Res*. 2004; 19:1006–1012. [PubMed: 15125798]
2. Tsuzuku S, Ikegami Y, Yabe K. Bone mineral density differences between paraplegic and quadriplegic patients: a cross-sectional study. *Spinal Cord*. 1999; 37:358–361. [PubMed: 10369173]
3. Krah H, Michaelis U, Pieper HG, et al. Stimulation of bone growth through sports. A radiologic investigation of the upper extremities in professional tennis players. *Am J Sports Med*. 1994; 22:751–757. [PubMed: 7856798]
4. Wittich A, Mautalen CA, Oliveri MB, et al. Professional football (soccer) players have a markedly greater skeletal mineral content, density and size than age- and BMI-matched controls. *Calcif Tissue Int*. 1998; 63:112–117. [PubMed: 9685514]
5. Parfitt AM. The cellular basis of bone turnover and bone loss: a rebuttal of the osteocytic resorption--bone flow theory. *Clin Orthop Relat Res*. 1977:236–247. [PubMed: 912987]
6. Lanyon LE. Osteocytes, strain detection, bone modeling and remodeling. *Calcif Tissue Int*. 1993; 53(Suppl 1):S102–S106. discussion S106–107. [PubMed: 8275362]
7. Tatsumi S, Ishii K, Amizuka N, et al. Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metabolism*. 2007; 5:464–475. [PubMed: 17550781]
8. Burger EH, Klein-Nulend J, van der Plas A, et al. Function of osteocytes in bone--their role in mechanotransduction. *J Nutr*. 1995; 125:2020S–2023S. [PubMed: 7602386]
9. Riddle RC, Donahue HJ. From streaming-potentials to shear stress: 25 years of bone cell mechanotransduction. *J Orthop Res*. 2009; 27:143–149. [PubMed: 18683882]
10. Murray DW, Rushton N. The effect of strain on bone cell prostaglandin E2 release: a new experimental method. *Calcif Tissue Int*. 1990; 47:35–39. [PubMed: 2369689]
11. Weinbaum S, Cowin SC, Zeng Y. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. *J Biomech*. 1994; 27:339–360. [PubMed: 8051194]
12. You J, Yellowley CE, Donahue HJ, et al. Substrate deformation levels associated with routine physical activity are less stimulatory to bone cells relative to loading-induced oscillatory fluid flow. *J Biomech Eng*. 2000; 122:387–393. [PubMed: 11036562]
13. Qin YX, Kaplan T, Saldanha A, et al. Fluid pressure gradients, arising from oscillations in intramedullary pressure, is correlated with the formation of bone and inhibition of intracortical porosity. *J Biomech*. 2003; 36:1427–1437. [PubMed: 14499292]

14. Klein-Nulend J, Semeins CM, Ajubi NE, et al. Pulsating fluid flow increases nitric oxide (NO) synthesis by osteocytes but not periosteal fibroblasts - correlation with prostaglandin upregulation. *Bio Chem Biophys Res Commun.* 1995; 217:640–648.
15. Jiang JX, Cherian PP. Hemichannels formed by connexin 43 play an important role in the release of prostaglandin E(2) by osteocytes in response to mechanical strain. *Cell Commun Adhes.* 2003; 10:259–264. [PubMed: 14681026]
16. Genetos DC, Kephart CJ, Zhang Y, et al. Oscillating fluid flow activation of gap junction hemichannels induces ATP release from MLO-Y4 osteocytes. *J Cell Physiol.* 2007; 212:207–214. [PubMed: 17301958]
17. Malone AM, Anderson CT, Tummala P, et al. Primary cilia mediate mechanosensing in bone cells by a calcium-independent pathway. *Proc Natl Acad Sci USA.* 2007; 104:13325–13330. [PubMed: 17673554]
18. Yellowley CE, Li Z, Zhou Z, et al. Functional gap junctions between osteocytic and osteoblastic cells. *J Bone Miner Res.* 2000; 15:209–217. [PubMed: 10703922]
19. Zhao S, Zhang YK, Harris S, et al. MLO-Y4 osteocyte-like cells support osteoclast formation and activation. *J Bone Miner Res.* 2002; 17:2068–2079. [PubMed: 12412815]
20. You L, Temiyasathit S, Lee P, et al. Osteocytes as mechanosensors in the inhibition of resorption due to mechanical loading. *Bone.* 2008; 42:172–179. [PubMed: 17997378]
21. Litzenger JB, Tang WJ, Castillo AB, et al. Deletion of beta 1 integrins from cortical osteocytes reduces load-induced bone formation. *Cellular and Molecular Bioengineering.* 2009 In press.
22. Rawlinson SC, Pitsillides AA, Lanyon LE. Involvement of different ion channels in osteoblasts' and osteocytes' early responses to mechanical strain. *Bone.* 1996; 19:609–614. [PubMed: 8968027]
23. Praetorius HA, Spring KR. Bending the MDCK cell primary cilium increases intracellular calcium. *J Membr Biol.* 2001; 184:71–79. [PubMed: 11687880]
24. Praetorius HA, Spring KR. Removal of the MDCK cell primary cilium abolishes flow sensing. *J Membr Biol.* 2003; 191:69–76. [PubMed: 12532278]
25. Nauli SM, Alenghat FJ, Luo Y, et al. Polycystins 1 and 2 mediate mechanosensation in the primary cilium of kidney cells. *Nat Genet.* 2003; 33:129–137. [PubMed: 12514735]
26. Masyuk AI, Masyuk TV, Splinter PL, et al. Cholangiocyte cilia detect changes in luminal fluid flow and transmit them into intracellular Ca²⁺ and cAMP signaling. *Gastroenterol.* 2006; 131:911–920.
27. Whitfield JF. Primary cilium--is it an osteocyte's strain-sensing flowmeter? *J Cell Biochem.* 2003; 89:233–237. [PubMed: 12704786]
28. Xiao Z, Zhang S, Mahlios J, et al. Cilia-like structures and polycystin-1 in osteoblasts/osteocytes and associated abnormalities in skeletogenesis and runx2 expression. *Journal of Biological Chemistry.* 2006; 281:30884–30895. [PubMed: 16905538]
29. Pedersen LB, Rosenbaum JL. Intraflagellar transport (IFT) role in ciliary assembly, resorption and signalling. *Curr Top Dev Biol.* 2008; 85:23–61. [PubMed: 19147001]
30. Praetorius HA, Spring KR. A physiological view of the primary cilium. *Annu Rev Physiol.* 2005; 67:515–529. [PubMed: 15709968]
31. Federman M, Nichols G Jr. Bone cell cilia: vestigial or functional organelles? *Calcif Tissue Res.* 1974; 17:81–85. [PubMed: 4451879]
32. Marszalek JR, Ruiz-Lozano P, Roberts E, et al. Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc Natl Acad Sci U S A.* 1999; 96:5043–5048. [PubMed: 10220415]
33. Haycraft CJ, Serra R. Cilia involvement in patterning and maintenance of the skeleton. *Curr Top Dev Biol.* 2008; 85:303–332. [PubMed: 19147010]
34. Ehlen HW, Buelens LA, Vortkamp A. Hedgehog signaling in skeletal development. *Birth Defects Res C Embryo Today.* 2006; 78:267–279. [PubMed: 17061262]
35. Haycraft CJ, Zhang Q, Song B, et al. Intraflagellar transport is essential for endochondral bone formation. *Development.* 2007; 134:307–316. [PubMed: 17166921]

36. Song B, Haycraft CJ, Seo HS, et al. Development of the post-natal growth plate requires intraflagellar transport proteins. *Dev Biol.* 2007; 305:202–216. [PubMed: 17359961]
37. Gradilone SA, Masyuk AI, Splinter PL, et al. Cholangiocyte cilia express TRPV4 and detect changes in luminal tonicity inducing bicarbonate secretion. *Proc Natl Acad Sci U S A.* 2007; 104:19138–19143. [PubMed: 18024594]
38. Kwon RY, Temiyasathit S, Tummala P, et al. Primary cilia mediate intracellular cAMP responses in bone cells exposed to dynamic fluid flow. *Proceedings of the ASME 2008 Summer Bioengineering Conference.* 2008
39. Wassermann F, Yaeger JA. Fine structure of the osteocyte capsule and of the wall of the lacunae in bone. *Z Zellforsch.* 1965; 67:636–652.
40. McGlashan SR, Jensen CG, Poole CA. Localization of extracellular matrix receptors on the chondrocyte primary cilium. *J Histochem Cytochem.* 2006; 54:1005–1014. [PubMed: 16651393]

Fig. 1.

Primary cilia are microtubule-based structures that are found in nearly every cell type, including osteocytes. *Bottom:* Immunofluorescence staining of the osteocyte primary cilium with anti-acetylated α tubulin. *Top:* Schematic showing structure of primary cilium.