Bone 54 (2013) 196–204

Contents lists available at SciVerse ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Review

Emerging role of primary cilia as mechanosensors in osteocytes

An M. Nguyen, Christopher R. Jacobs *

Department of Biomedical Engineering, Columbia University, New York, NY, USA

ARTICLE INFO

Article history:
Received 2 July 2012
Revised 6 October 2012
Accepted 19 November 2012
Available online 28 November 2012

Edited by: Lynda F. Bonewald, Mark E. Johnson, and Michaela Kneissel

Keywords:
Bone
Mechanotransduction
Mechanosensation
Osteocytes
Primary cilia

ABSTRACT

The primary cilium is a solitary, immotile microtubule-based extension present on nearly every mammalian cell. This organelle has established mechanosensory roles in several contexts including kidney, liver, and the embryonic node. Mechanical load deflects the cilium, triggering biochemical responses. Defects in cilium function have been associated with numerous human diseases. Recent research has implicated the primary cilium as a mechanosensor in bone. In this review, we discuss the cilium, the growing evidence for its mechanosensory role in bone, and areas of future study.

This article is part of a Special Issue entitled "The Osteocyte".

Contents

Introduction

The primary cilium is a single, immotile organelle that extends from the cell surface of nearly every mammalian cell. Though cilia were observed in protozoa and described over 300 years ago by Dutch lens maker Antony van Leeuwenhoek [1], primary cilia were first observed in mammalian cells just over a century ago in 1898 by Swiss anatomist Karl Zimmermann [2]. After observing it in several cell types, including rabbit kidney cells and human pancreatic cells, Zimmermann hypothesized a sensory role for the primary cilium. For over a century, this hypothesis remained largely ignored and this poorly understood organelle was believed to be of little functional importance [3]. Recently, renewed interest in this organelle has led to

* Corresponding author at: Columbia University, Department of Biomedical Engineering, Cell and Molecular Biomechanics Lab, 500 W 120th Street, 351 Engineering Terrace, MC 8904, New York, NY 10027, USA. Fax: +1 212 854 8725.
E-mail address: christopher.jacobs@columbia.edu (C.R. Jacobs).

8756-3282/$ – see front matter
http://dx.doi.org/10.1016/j.bone.2012.11.016

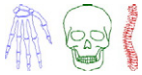
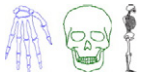
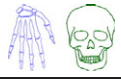
numerous studies and insights into the primary cilium structure and function.

In this review, we discuss primary cilia in the context of bone. It was not until 2006 when Xiao et al. revealed for the first time primary cilia in bone, providing both *in situ* and *in vitro* evidence [4]. This review begins with a brief overview of ciliopathies and primary cilia biology. The remainder of this review discusses recent advances in primary cilia research in bone, focusing on (1) the proposed role of osteocyte primary cilia, (2) related signaling pathways, and (3) new research tools and techniques. We wrap up the review by exploring future areas of research and unanswered questions.

Ciliopathies

Defects in primary cilia structure and function have been implicated in many disorders. The set of conditions related to these defects are known as ciliopathies. Because of the ubiquity of the primary cilium, defects can result in multisystemic dysfunction. Hallmarks of ciliopathies include renal disease, retinal degeneration, and cerebral anomalies [5]. The role of primary cilia in renal disease has been extensively studied. Murcia et al. provided the first clue in linking primary cilia to polycystic kidney disease in 2000 [6]. Using the *Oak Ridge Polycystic Kidney* mouse model for polycystic kidney disease, the authors found mutations in the *Tg737* gene led to left–right axis defects and primary cilia defects. The authors named the protein encoded by the *Tg737* gene polaris. In addition to polaris, other ciliary proteins, such as Kif3a, have also been associated with polycystic kidney disease [7]. On the other end of the spectrum, proteins, such as polycystin-1 and polycystin-2, linked to polycystic kidney disease have more recently been found to localize to the cilium [8]. A less studied but still prevalent feature of ciliopathies is skeletal abnormalities. The most common skeletal abnormalities observed include congenital defects of the extremities, craniofacial defects, and ossification disorders. Table 1 lists human ciliopathies with observed skeletal manifestations.

Table 1
Human ciliopathies with skeletal abnormalities. The blue hand indicates abnormalities in the extremities, including polydactyly and phalangeal cone-shaped epiphyses. The green skull denotes craniofacial defects and the red spine denotes spinal abnormalities, including scoliosis and kyphosis. The black skeleton indicates short stature or skeletal dysplasia.

Ciliopathy	Skeletal features	Reference
Alström syndrome		[105]
Asphyxiating thoracic dystrophy		[106]
Bardet–Biedl syndrome		[96,107]
Ellis–van Creveld syndrome		[108]
Joubert syndrome		[109]
Mainzer–Saldino syndrome		[110,111]
Meckel–Gruber syndrome		[112]
Nephronophthisis		[113,114]
Oral–facial–digital syndrome		[115,116]
Polycystic kidney disease		[117]
Senior–Loken syndrome		[118]
Simpson Golabi Behmel syndrome		[119]

Primary cilia biology

The primary cilium is a microtubule-based structure similar to that of motile cilia and flagella in eukaryotic cells (Fig. 1). All three consist of an axoneme of nine microtubule doublets extending from the basal body into the extracellular space. The microtubule doublets serve as the basis for structure and rigidity. In contrast to the motile cilium and flagellum, the primary cilium lacks two central microtubules, resulting in a $9+0$ arrangement. The primary cilium also lacks other axonemal components, including radial spokes, dynein arms and nexin links [9]. These missing components are thought to reinforce the axoneme and increase stiffness, providing a possible explanation for the one order of magnitude increase in flexural rigidity observed in motile cilia compared to primary cilia.

The primary cilium is formed and maintained through a process called intraflagellar transport (IFT). IFT is bidirectional trafficking system that transports proteins along the ciliary axoneme. Because primary cilia do not have the machinery to form proteins, all ciliary proteins are formed elsewhere in the cell and transported to the cilium through IFT. Kinesin-2 is responsible for anterograde movement while dynein serves as the retrograde motor. Kif3a and polaris, two proteins previously discussed for their implications in polycystic kidney disease, play integral roles in IFT. The anterograde motor kinesin-2 is formed by Kif3a and Kif3b proteins [10]. Polaris forms part of a multi-protein IFT complex used in carrying other proteins [11,12]. Not surprisingly, polaris is also known as Ift88.

Though primary cilia are ubiquitous, found on nearly all human cell types except those of myeloid and lymphoid lineages [13,14], primary cilia are not a constant presence on these cells. Instead, the occurrence of primary cilia is cell cycle-dependent and a dynamic process of assembly and resorption. Assembly of the cilium occurs in the interphase with the most cilia found on non-proliferating G0–G1 cells [14,15]. The mother centriole converts to the basal body anchoring the cilium as IFT and kinesin-2 extend its length. Resorption of the cilium occurs prior to mitosis and entry of the cell cycle.

Proposed mechanosensory roles of primary cilia in osteocytes *in vivo*

It was not until a century after Zimmermann's observations that the primary cilium's role as a mechanosensor was established. Roth et al. demonstrated that the primary cilia of kidney cells bend in response to fluid flow [16]. Schwartz et al. then modeled this bending and proposed a sensory role as flow sensors in kidney cells [9]. Praetorius and Spring later supported this hypothesis with experimental evidence, demonstrating that cilia were mechanically sensitive to flow and served as part of a calcium signaling system [17]. Though evidence for the mechanosensory role of primary cilia is limited in bone compared to the kidney, primary cilia in osteocytes have been implicated in mechanosensing *in vivo*.

Bone formation

Using Cre-lox technology to develop conditional knockout mice of *Kif3a*, two studies have shown decreased bone formation in mice with the *Kif3a* deletion. A conditional knockout of *Kif3a* is necessary because