

The primary cilium as a dual sensor of mechanochemical signals in chondrocytes

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Abstract The primary cilium is an immotile, solitary, and microtubule-based structure that projects from cell surfaces into the extracellular environment. The primary cilium functions as a dual sensor, as mechanosensors and chemosensors. The primary cilia coordinate several essential cell signaling pathways that are mainly involved in cell division and differentiation. A primary cilium malfunction can result in several human diseases. Mechanical loading is sense by mechanosensitive cells in nearly all tissues and organs. With this sensation, the mechanical signal is further transduced into biochemical signals involving pathways such as Akt, PKA, FAK, ERK, and MAPK. In this review, we focus on the fundamental functional and structural features of primary cilia in chondrocytes and chondrogenic cells.

Keywords Chondrocytes · Primary cilia · Mechanotransduction · Chondrogenic progenitor cells

Abbreviations

OA	Osteoarthritis
ECM	Extracellular matrix
FAK	Focal adhesion kinase
MAPK	Mitogen-activated protein kinase

CPCs	Chondrogenic progenitor cells
ERK	Extracellular signal-regulated kinase
MSC	Mesenchymal stem cell
PKA	Protein kinase A
Hh	Hedgehog
IFT	Intraflagellar transport
Wnt	Wingless
EvC	Ellis–van Creveld syndrome
PKD	Polycystic kidney disease
Ihh	Indian Hedgehog

Introduction

The morphological, structural, and material features of the cartilage are genetically programmed but can also be modified by epigenetic factors, such as local tissue stress and strain states [1, 2]. Mechanical stimulation resulting from weight loading, mobilization, and muscle contraction has an important role in bone formation and normal joint cavitation [3, 4]. Paralysis of the skeletal musculature is known to inhibit chondrogenesis in developing limbs [5], which thereby influences the length, mass, and mechanical properties of the forming bone [6–9]. The growth plate and articular cartilage are subjected to massive repeated mechanical forces, and they have a limited capacity for repair. Thus, understanding how articular cartilage is maintained and how mechanical loads are sensed by the chondrocytes is of primary importance.

Sensing of mechanical signals

Mechanosensitivity starts with external or internal mechanical responses, and the mechanical stimuli are transduced by

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the cell into a biochemical outcome. More precisely, this phenomenon is known as mechanochemical signaling or mechanotransduction. Multiple activation mechanisms are simultaneously at play, including the release of autocrine growth factors [10–17] activation of mechanically sensitive kinases, such as Src [18–22] focal adhesion kinase (FAK) [23–25] and extracellular-signal regulated kinase (ERK) [26–32] and initiation of second messenger signaling [33, 34]. Mechanical forces drive many cellular events, including proliferation, differentiation, and gene expression in adult differentiated cells and stem cells [35]. When trying to understand how cells can receive a variety of inputs and translate them into a response, we think of a system, or a cell organelle, that can perform these tasks. Interest in a specialized cell projection organ called the primary cilium has recently emerged. This organ was shown to have the ability to receive and transduce numerous cell signals [36]. Thus, the primary cilium is a good candidate to act as the cell's "control device" for mechanical stimulation because it projects as an "antenna" from the cell into the ECM, and it incorporates integrins, G protein receptors, and calcium channels into the cell membrane.

Mechanosignaling in chondrocytes

Ultrastructural studies have shown that each chondrocyte has such an immotile primary cilium. On chondrocytes, the primary cilia are oriented into the pericellular matrix environment of the chondron, and they interact with collagen types II and IV via receptors [37–42]. A physical and chemical deficiency in the chondroblastic and chondrocytic primary cilia results in skeletal and growth plate abnormalities due to improper ECM secretion [43–49]. Integrins, G proteins, and calcium channels on the primary cilium have all been implicated as mechanoreceptors [19, 50–53]. Numerous genes and pathways have been shown to be differentially regulated as a result of mechanical stimuli; for example, the phosphoinositide 3-kinase/Akt, protein kinase A (PKA) and Mitogen-activated protein kinase (MAPK) pathways [54–56]. It is reasonable to assume that mechanotransduction is a complex multi-component system that allows cells to integrate mechanical stimulations differing in intensity, frequency, duration, and orientation to generate appropriate biological responses, including cartilage formation and regeneration [57] and, especially, growth-plate formation [37, 58]. Mice and humans with mutations in ciliary genes often present with defects in skeletal development. Two human syndromes that include defects in endochondral bone formation were shown to be associated with mutations in ciliary genes. Asphyxiating thoracic dystrophy (Jeune's syndrome) is associated with a missense mutation in IFT80 (part of IFT complex B), and it

presents with skeletal defects resembling those seen in sonic hedgehog homolog depletion. Furthermore, Ptc1 (hh receptor) expression is downregulated in the IFT80 mutant, suggesting that alterations in Hh signaling and Ellis–van Creveld syndrome (EvC) are characterized by numerous skeletal and craniofacial abnormalities. The mutated protein in EvC has been localized to the base of the cilia expressed in chondrocytes and is required for normal Hh signaling. The disruption of EvC in mice resulted in a variety of skeletal abnormalities associated with diminished Ihh signaling [48].

Mice mutated in other ciliary genes also demonstrate alterations in endochondral ossification, resulting in a shortening of the long bones. Conditional deletion of IFT88 or KIF3a produces defects in embryonic endochondral bone formation, observed as early as 15.5 days from gestation [59]. These phenotypes resembled those seen in mice with germline mutations in Ihh [60]. When either Ift88 or KIF3a was deleted at later stages of development using the *col2a*-Cre promoter, the mice demonstrated a progressive loss of the cartilaginous growth plate, resulting in postnatal dwarfism that resembled the phenotype of mice with a conditional deletion of Ihh induced in postnatal cartilage. KIF3a and primary cilia are essential for coordination of chondrocytes maturation and condylar growth. The Ihh signaling pathway is one of the major regulatory pathways that lead to chondrocyte division and differentiation in the growth plate. Hydrostatic compression of the chondrocytic primary cilium upregulates Ihh gene expression [49, 61, 62].

Using electron microscopy, it has been shown that the chondrocyte cilium projects into the ECM and is tightly associated with the Golgi apparatus [39, 63]. Integrins have been shown to be present on the chondrocyte cilium, and integrin-dependent signaling cascades have been described in chondrocyte mechanotransduction [42, 64], suggesting a possible role for the chondrocytic cilia in mechanosensing. Chondrocytes in articular cartilage differ from those in the growth plate in that they are maintained as mature resting cells. Mechanical load is a critical factor in maintaining articular cartilage, but how the load is sensed is not known. Recently, the fate of the primary cilia on articular chondrocytes during the progression of bovine OA has been investigated [47, 65]. Primary cilia were present during all examined stages of OA; however, the proportion of ciliated cells increased and their orientation towards the surface was altered; the significance of this orientation remains unclear [37, 63, 65]. Recently published cyclic compression experiments proved that mechanical loading modulates chondrocyte primary cilia incidence and length. This observation has been made independent from the well-known reduction of cilia appearance during cell division. Axonemal orientation in the cilia of articular

chondrocytes is more pronounced in weight-bearing areas compared to of the cartilage tissue [66, 67].

We have studied the effect of loading on growth plate chondrocytes *in vivo*. Chondrocyte proliferation, differentiation, organization, and the major signaling pathways were found to be modified by loading in a chick model [68]. This demonstrated that the mechanical load affected chondrocytes in the growth plate [69], especially the expression of matrix metalloproteases [70].

The primary cilium

Primary cilia are non-motile sensory organelles that project from cells in many tissues and types of cells, such as kidney tubules, the bile duct, neurons, the endocrine pancreas, the thyroid, smooth muscle cells, and fibroblasts. The complete list of the cells and tissues containing primary cilia can be found at <http://www.bowserlab.org/primarycilia/cilialist.html>. In recent years, cilia have emerged as a hot topic in research, resulting in the creation of numerous databases, including those containing genomic and proteomic data on cilium composition (<http://www.ciliaproteome.org>, <http://www.ciliome.com>) [71–74]. Cilia can be seen as specialized cellular compartments or organelles [36, 75]. They are microtubule-based structures that originate at the basal body and extend into the extracellular space. The basal body is a modified form of the centriole, an organelle well known for its role as a microtubule organizing center of mitotic spindles. The basal body/centriole migrates toward the cell membrane and acts as a template for ciliogenesis and an anchor for the primary cilium. The centriole only moves towards the cell membrane when it is not involved in mitosis; thus, cilium generation occurs during the interphase of the cell. The intraflagellar transport (IFT) system plays a key role in primary cilium formation and maintenance. The IFT system is bidirectional and directs movement along the ciliary axoneme. Because no proteins are synthesized in the primary cilium itself, ciliary proteins are targeted to the basal body and transported to the distal tip through the IFT complex. IFT complexes are trafficked along the axoneme by molecular motor proteins; they are transported by kinesin II in the anterograde direction and by dynein II in the retrograde direction.

Motile and non-motile, primary cilia have microtubule axoneme cores made up of nine sets of microtubule doublets that provide structure and rigidity. However, primary cilia lack the central pair of microtubules (thus are designated 9+0), while other motile machinery includes the inner and outer dynein arms, radial spokes, and central pair

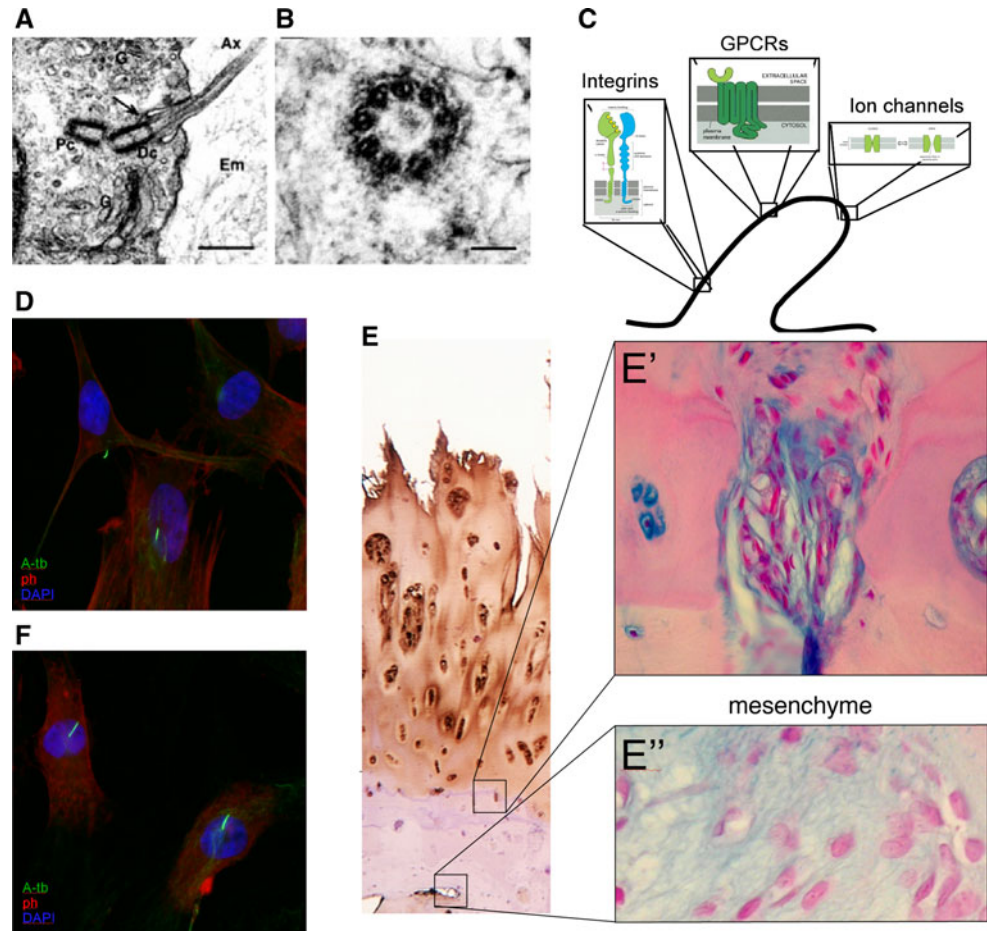
projections (designated 9+2). Unlike the motile cilia, of which there can be many per cell, there is only one primary cilium per cell [76–78].

The function of the primary cilium

To date, there are three hypotheses regarding the functional importance of the primary cilium: first, the primary cilium is a vestigial organ on the cell; second, that it inhibits cell division because it sequesters the centriole; and third, that it is a cellular sensory structure. The first hypothesis has been proven to be incorrect by several experiments. For instance, analyses of mutants, such as the Tg737^{orpk} R^{pw} mouse, have indicated that a functioning primary cilium is essential for normal development and function, not only of the kidney, but also of many other tissues and organs. Hence, ciliary dysfunction might lead to a series of developmental abnormalities and diseases collectively called ciliopathies, including cystic diseases, obesity, and blindness, as well as behavioral, cognitive, and skeletal defects. The second hypothesis seems reasonable because the majority of cells possess primary cilia when they are not undergoing mitosis. Additionally, recent studies have demonstrated the accuracy of the third hypothesis: primary cilia have been shown to be highly involved in cell signaling processes because a number of ion channels, transporter proteins, and downstream effector proteins are associated with the cilium [79–81].

The primary cilium is a few micrometers in length, and it detects and interprets signals from the environment, such as odorants, fluid flow, and protein signaling between cells. Thus, they are spectacularly complex sensors. In ciliary signaling, the receptor protein and the protein that transmits the message into the cell are localized in the cilia. Protein association or dissociation from the cilia controls the signaling pathways, which ultimately trigger responses such as cell division and differentiation [82]. Several independent lines of evidence have demonstrated a role for the primary cilium in Hh signaling [83]. Hh is the master regulator of endochondral ossification in the growth plate, and it determines chondrocyte activity and subsequent bone length [84–86]. Upon Hh stimulation, both Hh receptors, Smo and ptch1, are recruited to the cilium *in vitro* and *in vivo*; Gli2 and Gli3, downstream effectors of Hh, also localize to the cilium in the developing limbs [45, 87]. Other pathways that have been shown to regulate chondrocyte activity but have not yet been linked to the cilium are as follows: bone morphogenic proteins, wingless (Wnts), fibroblast growth factors, and insulin-like growth factors, all of which are essential for normal cartilage formation.

Fig. 1 **a** Electron micrograph of the primary cilium (Ax), the distal (Dc), and proximal (Pc) centriole. *Bar* 500 nm. **b** Cross section of the proximal centriole. *Bar* 100 nm. Reprinted from Jensen et al. (1979) with permission. **c** Schematic presentation of the primary cilium with membrane signaling molecules that were described in it. **d** Human articular chondrocytes and **f** CPCs stained with acetylated α -tubulin (tb, *green*) Abs to detect primary cilia (*arrows*), phalloidin (ph, *red*) and DAPI (DAPI, *blue*). **e** Tissue from the late stage of human OA exhibits surface fissures and cell clusters (the *arrow* indicates the tidemark). Breaks in the tidemark are filled with blood vessels, and the bone marrow is visible underneath the OA tissue. Reprinted from Koelling et al. [92] with permission from the publisher



Cilia function in mesenchymal stem cells and chondrogenic progenitor cells (CPCs)

The effects of mechanical forces on mesenchymal stem cell (MSC) differentiation were examined in a fundamental study of the concept of environmental cell sensing. The study showed that differentiation of MSCs is directed by the stiffness of the culture matrix. On soft collagen gels that mimic the elasticity of brain tissue (0.1–1 kPa), MSCs tend to adhere, spread, and exhibit a neurogenic phenotype. MSCs cultured on tenfold stiffer matrices that mimic muscle elasticity (8–17 kPa) become spindle-shaped, similar to myoblasts. When cultured on matrices that mimic the stiffness of bone osteoid (25–40 kPa), the MSC phenotype becomes osteoblast-like with greater expression of osteogenic genes [88]. This work, along with similar studies, implies that a cell is able to sense its mechanical environment and that mechanical signaling itself can regulate the differentiation of MSCs into different tissues.

More recently, Padmaja Tummala et al. identified the presence of primary cilia on MSCs and determined their role in MSC differentiation. MSCs require primary cilia not only during their differentiation but also to maintain the

phenotypes of differentiated cells [89]. In addition, there is evidence that MSC differentiation into chondrocytes and osteocytes is regulated by mechanical signals [90]. Our research group is working on tissue regeneration to elucidate repair mechanisms, especially in OA (Fig. 1) and rheumatoid arthritis. OA is a chronic degenerative disease characterized by articular cartilage degeneration, and it is multifactorial in origin [89]. Primary cilia are present on chondrocytes, and the percentage of ciliated cells and the lengths of the cilia within OA tissue are higher compared to the normal tissue [65], although the implications of these facts have yet to be elucidated.

We have isolated CPCs from subjects in late-stage OA and characterized their role in the repair of diseased articular cartilage. CPCs have tremendous chondrogenic and regenerative potential. These cells are positive for stem cell markers and exhibit stem cell properties such as clonogenicity, multipotency, and migratory activity. Recently, we identified primary cilia projecting from the surfaces of CPCs using antibodies against acetylated alpha tubulin. Our laboratory is focused on using mechanobiological approaches to investigate the role of primary cilia in differentiation of CPCs into chondrocytes [91, 92].

Conclusions and perspectives

Owing to the involvement of primary cilia in fundamental cellular processes, mutations in primary ciliary proteins result in diverse diseases such as cystic kidney diseases, obesity, and retinal degeneration. Recent studies have presented a comprehensive concept that primary cilia are acting as dual sensors for physical and chemical cues. Therefore, over the past few years, many researchers have been paying attention to primary cilia to understand their role in development and diseases. Here, we have reviewed the basic role of primary cilia in mechanotransduction and their possible impact on cartilaginous tissues. Additionally, our results show that primary cilia project not only from the surface of human osteoarthritic chondrocytes but also from the surface of chondrogenic progenitor cells. One future line of research should be to elucidate the role of the primary cilia in chondrogenic differentiation to enhance the potential of cartilage repair.

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