Osteoarthritis and Cartilage xxx (2014) 1–6

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

The chondrocyte primary cilium

R. Ruhlen † *, K. Marberry ‡ a

† A.T. Still Research Institute, A.T. Still University, Kirksville, MO, USA
‡ Department of Surgery, A.T. Still University-Kirksville College of Osteopathic Medicine, Kirksville, MO, USA

ARTICLE INFO

Article history:
Received 10 December 2013
Accepted 7 May 2014

Keywords:
Chondrocyte
Cilia
Mechanotransduction
Osteoarthritis

SUMMARY

The presence and role of primary, or non-motile, cilia on chondrocytes has confused cartilage researchers for decades. Initial explanations attributed a vestigial nature to chondrocyte cilia. Evidence is now emerging that supports the role of the chondrocyte primary cilium as a sensory organelle, in particular, in mechanotransduction and as a compartment for signaling pathways. Early electron microscopy images depicted bent cilia aligned with the extracellular matrix (ECM) in a manner that suggested a response to mechanical forces. Molecules known to be mechanotransducers in other cell types, including integrins and proteoglycans, are present on chondrocyte cilia. Further, chondrocytes which lack cilia fail to respond to mechanical forces in the same manner that chondrocytes with intact cilia respond. From a clinical perspective, chondrocytes from osteoarthritic (OA) cartilage have cilia with different characteristics than cilia found on chondrocytes from healthy cartilage. This review examines the evidence supporting the function of chondrocyte cilia and briefly speculates on the involvement of intraflagellar transport (IFT) in the signaling pathway of mechanotransduction through the cilium. Emerging evidence suggests cilia may be a promising target for preventing and treating OA.

Introduction

Articular cartilage is comprised of water, collagen, proteoglycan, and other non-collagenous proteins. The high water content of normal articular cartilage is maintained by the hydrophilic nature of proteoglycans within the collagen framework. Chondrocytes within the cartilage matrix are responsible for forming and maintaining the matrix. Articular cartilage is avascular, so chondrocytes are nourished by synovial fluid diffusion through the matrix by mechanical forces.

To maintain viability, chondrocytes require the right amount of mechanical stimulation: too little or too much loading leads to chondrocyte dysfunction and cartilage degeneration. *In vivo*, articular cartilage experiences routine loading. During walking, forces on the tibial surface are 2–3 times body weight, and peak loading is 4 times body weight[1,2]. Joint loading exerts direct strain on the cartilage matrix as well as on the cells and induces hydrostatic pressure, fluid shear, and changes in osmolarity as synovial fluid is pushed through the tissue[3]. Without loading, cartilage health deteriorates[4], but with excessive or abnormal loading, the joint deteriorates[5]. The degree, frequency, and duration of loading affect whether cartilage thrives or deteriorates. Chondrocytes have been shown to respond to abnormal joint loading by attempting to repair the damaged cartilage—as seen by elevated C-terminal telopeptide of type II collagen in the serum and urine[6]—but eventually apoptosis will occur[7], which suggests a range of compression is necessary for optimal chondrocyte function and to maintain normal articular cartilage. In chondrocytes from osteoarthritic (OA) cartilage, the response to compression is different. In one study, cyclic mechanical stimulation induced membrane hyperpolarization, upregulation of the proteoglycan aggrecan, and downregulation of matrix metalloproteinase 3 in healthy chondrocytes, responses which were absent in chondrocytes from OA cartilage[8]. In fact, the same mechanical stimulation induced the opposite response, membrane depolarization, in OA chondrocytes[8]. This hyperpolarization or depolarization response is thought to be involved in mechanotransduction[8].

How chondrocytes detect and respond to compression and how the mechanotransduction pathway is altered in OA is largely unknown. A recent study has hypothesized that the primary cilium of the chondrocyte plays a role in the detection and transmission of mechanical stimulation[9]. Many factors involved in

* Address correspondence and reprint requests to: R. Ruhlen, A.T. Still Research Institute, A.T. Still University, 800 W. Jefferson St., Kirksville, MO 63501, USA. Tel: 1-660-626-2889; Fax: 1-660-626-2602.
E-mail addresses: rruhlen@atsu.edu (R. Ruhlen), kmarberry@atsu.edu (K. Marberry).
a Kirksville College of Osteopathic Medicine, A.T. Still University, 800 W. Jefferson St., Kirksville, MO 63501, USA. Tel: 1-660-626-2663; Fax: 1-660-626-2636.

http://dx.doi.org/10.1016/j.joca.2014.05.011
1063-4584/

mechanotransduction have also been linked to cilia although not necessarily in chondrocyte cilia. The purpose of this review is to investigate the evidence for the role of chondrocyte cilia in mechanotransduction.

Cilia and chondrocytes

Cilia and a similar organelle, the flagellum, are well known for their role in cellular motility. In the lungs, motile cilia on epithelial cells lining the airways push mucus and foreign particles, including bacteria[10]. Cilia in the Fallopian tubes move the ovum toward the uterus[10]. The role of the primary cilium, an organelle that protrudes from most eukaryotic cells like a short antenna, has been more elusive. While only specialized cells have multiple motile cilia or a flagellum, nearly all eukaryotic cells have a non-motile primary cilium unless undergoing cell division[10]. The axoneme, or central skeleton, of primary cilia consists of a ring of nine pairs of microtubules, known as a 9+0 pattern to distinguish it from the 9+2 pattern of motile cilia that have an additional central pair of microtubules[11]. Because microtubules are enriched in acetylated α-tubulin, cilia can be visualized with acetylated α-tubulin antibodies[12].

With electron microscopy, primary cilia with the 9+0 pattern have been observed on most chondrocytes[13,14]. Primary cilia are found on all murine chondrocytes and on 96% of equine chondrocytes[14], as well as on human and bovine chondrocytes[15,16]. Immunostaining with acetylated α-tubulin identified primary cilia on chondrocytes within cartilage from bovine patellae[16].

For the remainder of this review, the term cilia will refer to primary or non-motile cilia, and motile cilia will be referred to with the adjective. Several theories have been proposed for the role of the primary cilium in chondrocytes.

Early theories of cilia function in chondrocytes

The primary cilium in most eukaryotic, non-motile cells, such as chondrocytes, was once thought to be a vestigial organelle with no identifiable function in normal cells[17]. In another theory, the presence of cilia was thought to indicate motility of chondrocytes, and the characteristic cluster and column formations were thought to be aggregates of motile chondrocytes[15]. However, additional studies failed to support the vestigial nature of cilia, and the difference between motile and primary cilia became better understood such that the presence of a primary cilium is not considered to be evidence of motility. A third theory suggests that cilia regulate mitosis by sequestering the centriole, which localizes to the primary cilium when not in use during mitosis[17]. While it is true that cells with primary cilia tend to be differentiated or in the G_0 phase of the cell cycle, the role of primary cilia in the regulation of mitosis has not been pursued further.

Protein and lipid targeting, secretion, and endocytosis

The Golgi apparatus works closely with intraflagellar transport (IFT)—the process which assembles, disassembles, and maintains cilia—to deliver lipids and proteins to the cilium[18]. The observation of vesicles pinching off the tip of the flagellum of unicellular algae gave rise to the idea that IFT is involved in secretion[19]. IFT has been identified in non-ciliated cells and is associated with exocytosis[19]. Electron microscopy and confocal imaging revealed a close association between cilia and the Golgi apparatus in aortic smooth muscle and in articular chondrocytes, leading to the proposal that the cilium is part of a sensory feedback loop which begins with mechanical transduction and ends with extracellular matrix (ECM) protein secretion[20]. In addition to exocytosis and secretion, the cilia may be involved in endocytosis. In synoviocytes, cilia reside in a dip in the membrane called the ciliary pocket[21]. Cilia in chondrocytes have been shown to reside in similar pockets, and the presence of clathrin-coated vesicles, endosomal proteins, and CD44 in the ciliary pocket indicate endocytosis[21].

Cartilage organization and skeletal patterning

Cilia may direct the columnar organization of chondrocytes, with the columnar and cluster formations representing clonal groups[22]. Mutations in ciliary proteins result in developmental skeletal defects, such as digit patterning, endochondral bone, craniofacial, and dentition abnormalities[12]. Indian hedgehog (Ihh) and Wingless (Wnt) proteins, found in cartilage, are important in skeletal development[12]. Sonic hedgehog (Shh), also present in cartilage, is necessary for proper digit patterning[12]. Receptors for these ligands are present on chondrocyte cilia[12]. Therefore, cilial defects may involve abnormal regulation of Ihh, Wnt, and Shh[12].

Sensory organelle

The role of cilia in mechanotransduction stems from the observation that some of the cilia in chondrocytes appear bent[23]. Observations through transmission electron microscopy, electron tomography, and confocal microscopy allowed visualization of the interaction between cilia bending patterns and the ECM. In the absence of matrix, all cilia were straight, with a wide range of bending patterns observed in cilia from chondrocytes that were within a cartilage matrix (Fig. 1). This observation suggests that cilia bending is a passive response to mechanical forces in the cartilage matrix[23,24].

Cilia are likely involved in multiple, interlinked roles. Currently, chondrocyte cilia are known to be important in processing information about mechanical stimulation and osmotic loading[25]. We will next examine the evidence for the sensory function of cilia, particularly in mechanotransduction.

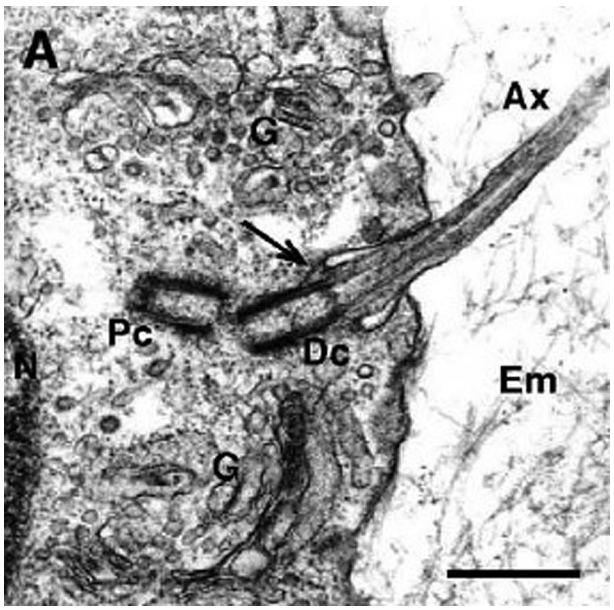

Fig. 1. Structural relationship between the extracellular matrix (Em), the ciliary axoneme (Ax), the distal (Dc) and proximal (Pc) centrioles, and the Golgi apparatus (G). The distal centriole acts as the basal body of the cilium. Arrow = transitional fiber. Bar = 500 nm. Reprinted with permission from Jensen *et al.*[22].

Please cite this article in press as: Ruhlen R, Marberry K, The chondrocyte primary cilium, Osteoarthritis and Cartilage (2014), http://dx.doi.org/10.1016/j.joca.2014.05.011