Minireview

Primary cilium–mediated mechanotransduction in cartilage chondrocytes

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Impact statement

The cellular mechanical environment contributes to both healthy and osteoarthritic articular cartilage. Mechanical stimuli, including compression, dynamic strain, and shear stress, are crucial in determining cell function, fate, and death. The primary cilium in chondrocytes functions as a signaling hub mediating cartilage mechanobiological processes. However, the molecular mechanisms by which how primary cilia mediate mechanotransduction in chondrocytes, particularly in sensing the mechanical cues in the matrix microenvironment, are still not fully understood. Recent progress in the understanding of mechanosensing mechanisms mediated by primary cilia may shed light on targeted precision therapeutics for injured and osteoarthritic cartilage. Moreover, they contribute to developing cell-instructive biomaterials for the future design of functional cartilage tissue engineering.

Abstract

Osteoarthritis (OA) is one of the most prevalent joint disorders associated with the degradation of articular cartilage and an abnormal mechanical microenvironment. Mechanical stimuli, including compression, shear stress, stretching strain, osmotic challenge, and the physical properties of the matrix microenvironment, play pivotal roles in the tissue homeostasis of articular cartilage. The primary cilium, as a mechanosensory and chemosensory organelle, is important for detecting and transmitting both mechanical and biochemical signals in chondrocytes within the matrix microenvironment. Growing evidence indicates that primary cilia are critical for chondrocytes signaling transduction and the matrix homeostasis of articular cartilage. Furthermore, the ability of primary cilium to regulate cellular signaling is dynamic and dependent on the cellular matrix microenvironment. In the current review, we aim to elucidate the key mechanisms by which primary cilia mediate chondrocytes sensing and responding to the matrix mechanical microenvironment. This might have potential therapeutic applications in injuries and OA-associated degeneration of articular cartilage.

Keywords: Chondrocytes, primary cilium, mechanical stimulus, mechanotransduction, matrix microenvironment, mechanobiology

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Introduction

Joint motion or body weight can be endured by articular cartilage (AC) on the joint surface, which is usually exposed to various dynamic mechanical cues. These include compressive and tensile strain, fluid shear stress, osmotic stress, and biomechanical properties of the extracellular matrix (ECM).[1,2] Mechanical signals from the matrix microenvironment regulate intracellular signaling pathways and significantly affect chondrocyte function (Figure 1).[3] The mechanical stimulation of AC affects cell metabolism, matrix synthesis, and cartilage integrity,[4,5] which, therefore, has been used for the development of tissue engineering in many studies.[6,7] Osteoarthritis (OA) is a progressive joint disorder worldwide. OA is complex and multifactorial with biological and biomechanical components.[8] Abnormal mechanical stress disrupts the balance between catabolism and anabolism, ultimately causing the development of OA.[9] However, the mechanical stress–mediated mechanotransduction pathway in OA remains poorly understood.

The AC serves as an important weight-bearing structure. Chondrocytes, as the only cell type within AC, carry out essential cellular metabolism and secrete both synthetic and degradative enzymes. However, the turnover of chondrocytes is limited, thus leading to poor tissue repair.[10] Collagen fibers, non-collagen glycoproteins, and proteoglycans comprise the fundamental ECM framework, which provides mechanical integrity to chondrocytes.[11] The mechanical properties

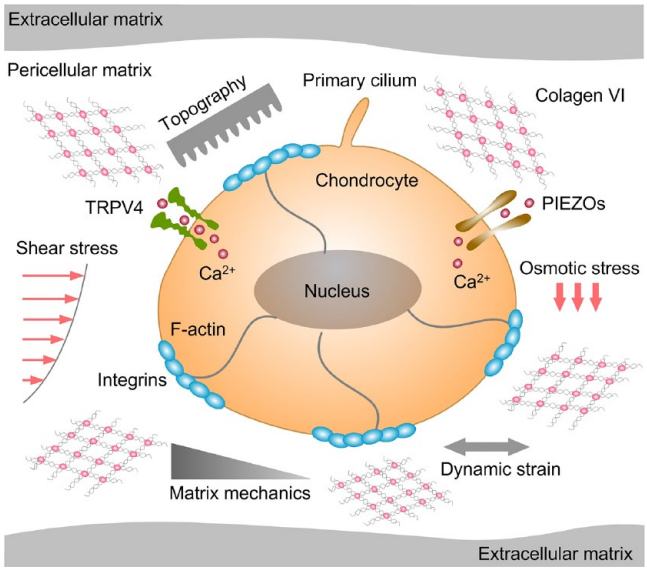

Figure 1. Chondrocyte matrix microenvironment. A single chondrocyte is encapsulated by the pericellular matrix (PCM) and the extracellular matrix (ECM). Collagen VI is widely distributed in the PCM and ECM, providing good properties for chondrocytes. When a chondrocyte is stimulated mechanically (upon shear stress, osmotic stress, dynamic strain, and so on), the primary cilium, ion channels (like TRPV4 and PIEZOs), and integrins all immediately respond to the mechanical microenvironment by changes in calcium signal and F-actin.

of ECM have recently been recognized as important regulators of the mechanobiological behavior of chondrocytes. *In vivo*, chondrocytes experience various types of mechanical loading in the matrix microenvironment. Consequently, the matrix microenvironment plays a vital role in translating extracellular mechanical signals to various mechanical sensors and/or receptors within the cell membrane.[12,13] These sensors and receptors include integrins, ion channels, and primary cilia.[14] They help transduce extracellular mechanical signals into cellular signals in chondrocytes.

Primary cilium–mediated mechanosensitive signaling pathway

The primary cilium is a multifunctional antenna that detects alterations in the matrix microenvironment.[15–17] Primary cilia are located in the cell membrane facing the surrounding ECM[18–20] and are sensory receptors for transducing different biological and mechanical signals intracellularly.[21–23] Clinically, chondrocyte cilia of osteoarthritic cartilage differ from those of healthy cartilage. For example, the lengths of the cilia and the percentage of ciliated cells are bigger in osteoarthritic cartilage than in normal tissue.[24] Recently, the precise process of governing the ciliary length and ciliary resorption has aroused much attention. Some studies have focused on the primary cilia in detecting different mechanical cues by observing the orientation and projection of cilia in the cartilage matrix.[19,20,25–27] For example, primary cilia regulate the matrix environment of the AC.[27–29] In addition, the mechanical loading *in vitro* alters the lengths of the primary cilia.[30] The absence of primary cilia significantly attenuates loading-induced matrix deposition in chondrocytes.[12] These indicate that primary cilia mediate mechanotransduction of chondrocytes, therefore regulating AC health. Here we discuss different mechanotransduction processes involved in primary cilia. These findings shed light on the mechanotransduction mechanism of primary cilia in chondrocytes.

Hedgehog signaling pathway

The activation of primary cilia regulates the Hedgehog (Hh) signaling pathway, which was found first in the *Drosophila melanogaster*. Three mammalian paralogues of the *Drosophila* Hh gene have been identified, including Desert hedgehog (Dhh), Indian hedgehog (Ihh), and Sonic hedgehog (Shh). These paralogues may result from ancient genome duplication events in vertebrates.[31–33] According to a previous study, the expression level of Hh was higher when Ihh/Shh was co-transfected, compared to when Ihh or Shh was transfected alone. No significant difference was observed between Ihh transfection and Shh transfection.[34] In conclusion, Ihh and Shh work together to activate the Hh signaling pathway.

Smoothened (Smo) and Patched (Ptc) are both cilia membrane receptors that mediate the activation of the Hh pathway. Here we present a model for the Hh signaling pathway (Figure 2).[17] In this dynamic model, the binding of Hh morphogenetic protein to Ptc activates the Hh signaling pathway. GLI transcription factors GLI2 and GLI3 together form a complex with a suppressor of fused (SUFU). When Hh signaling is not activated, kinesin family member 7 (KIF7) suppresses GLI's activity. When Hh signaling is activated by the binding of Hh ligands to Ptc, which releases Smo and relocates KIF7. SUFU/Gli complex then moves to the cilium and enhances the Hh-dependent gene transcription.[35]

The Hh signaling depends on primary cilia and regulates the development and maintenance of the AC. For example, Smo ablation accelerates chondrocyte hypertrophy while SUFU ablation inhibits it. Interestingly, ablations of both Smo and SUFU decrease the growth, proliferation, differentiation, and survival of the cell.[36] In addition, OA is associated with abnormal Hh signaling or chondrocyte hypertrophy.[37] A previous study has shown that mechanical cues promote the Hh signaling and ADAMTS-5 expression in OA and exercise-induced muscle hypertrophy in satellite cells.[38,39]

The Hh signaling is involved in primary cilia–mediated mechanotransduction. Cyclic adenosine 3′,5′-monophosphate (cAMP) could be activated by mechanical stress.[40] Meanwhile, cAMP is a regulator of Hh signaling.[41] The activation of cAMP promotes Smo activity in the Hh signaling,[42,43] which suggests that Hh signaling mediates the mechanotransduction. On the other hand, Ihh signaling is not mechanically activated in chondrocytes without primary cilia present. For example, hydrostatic compression enhances the Ihh signaling in chondrocytes in the presence of primary cilia; however, this enhancement is abolished in chondrocytes in the absence of primary cilia.[44]

Calcium signaling

Cytosolic calcium concentration plays a critical role in the physiology and pathology of OA. Mechanic stimulations activate calcium signaling pathways in primary cilia. The maintenance of Ca^{2+} homeostasis is vital for cells because increased Ca^{2+} concentration affects many different kinds of

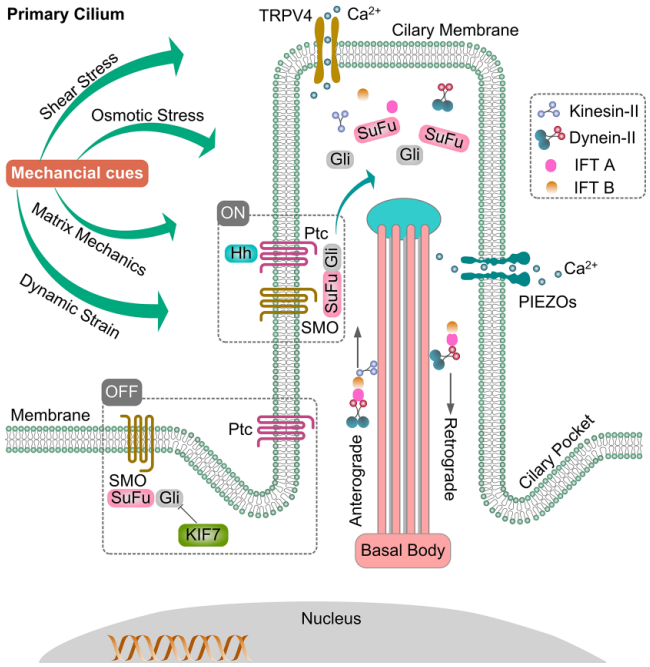

Figure 2. The structure and mechanosensitive signals of the primary cilia. The primary cilium is an independent organelle, acting as a "cell antenna." The ciliary axoneme is composed of microtubules and coated by the ciliary membrane. The IFT system maintains the activity of primary cilia. The IFT-B complex, driven by kinesin-II, carries the desired cargo from the basal body to the tip. In contrast, the IFT-A complex, driven by dynein-II, carries cargo back from the tip to the basal body. Mechanical stimuli, such as shear stress, dynamic strain, or matrix mechanics, can be sensed and transduced by primary cilia. Some force-sensitive ion channels within the ciliary membrane (like TRPV4 and PIEZO channels) transform mechanical signals into calcium signals and participate in mechanical signal transduction. Smo and Ptc receptors in primary cilia mediate the Hh signaling pathway in chondrocytes. When the Hh ligand binds to the Ptc receptor, the Gli complex (SUFU/GLI) is released by Smo and moves toward the ciliary tip; Hh signaling is activated (called "ON"). However, in the absence of the Hh ligand, KIF7 resides at the base of the primary cilium, preventing Gli accumulation in the cilium (called "OFF").

cellular signaling pathways, which leads to the dysfunction or death of cells.[45] The cell detects changes in Ca^{2+} concentration and responds by regulating actin and myosin.[46] Calcium levels regulate actin and myosin in several different ways. For example, intracellular calcium regulates actin arrangement and alters the mechanical properties and migration of cells.[47,48] It also changes the ECM properties and the adhesion between ECM and chondrocytes, thereby affecting chondrocyte migration.[49]

In chondrocytes, cytosolic Ca^{2+} is dramatically increased by the compressive loading.[50] In a hypotonic environment, the cytosolic Ca^{2+} in adult cells oscillates almost threefold more frequently than usual, whereas the duration and magnitude of each cytosolic Ca^{2+} peak are increased in juvenile cells cytosolic Ca^{2+}. However, in a hypertonic condition, both adult and juvenile chondrocytes show slower cytosolic Ca^{2+} oscillations with longer rising and recovery time.[51] Our recent study has shown that the mechanical behavior and calcium signaling of chondrocytes can be modulated by microniche geometry. For example, compared to spheroidal microniches, ellipsoidal microniches promoted the mechanical properties of cells. Ellipsoidal microniches also enhanced the amplitude of cytosolic Ca^{2+} oscillation in chondrocytes while reducing the frequency.[52] However, understanding the role of primary cilia in modulating the calcium signaling of chondrocytes during mechanosensation is needed.

Calcium signaling responds to mechanical loading by instantaneous changes in intracellular and extracellular Ca^{2+}.[53] Cyclic strain suppresses the protein degradation levels of chondrocytes; however, the elimination of primary cilia or inhibition of calcium signaling reverses this effect, leaving cells in an unfavorable environment.[54] Calcium influx mediated by cilia is indicative of chondrocyte mechanotransduction.[55] Under the compressive strain, primary cilia send calcium signals to cells, which triggers a series of intracellular events to maintain and strengthen the AC.[56] More evidence has shown that fluid shear stress bends the primary cilia, which activates Ca^{2+} channels and mechanosensitive receptors on the cilia.[57–59] Wann *et al.*[60] reported that the cilia also regulate adenosine triphosphate (ATP)-induced Ca^{2+} signaling in chondrocytes under compression forces. One study has shown that fluid flow increases Ca^{2+} concentration dependent on Ca^{2+} in the primary cilia in osteocytes.[61] However, some researchers have suggested that primary cilia mediate mechanotransduction through a Ca^{2+}-independent mechanism.[62] In kidney epithelial cells, fluid flow bends primary cilia and increases both ciliary and cytosolic Ca^{2+} levels.[63,64] Despite limited reports on the interaction between cilia and Ca^{2+} levels in chondrocytes, there might be diverse crosstalk.

Mechanosensitive ion channel

In chondrocytes, transient receptor potential vanilloid (TRP) channels, PIEZOs, and voltage-gated calcium channels within the membrane are involved in mechanosensation. Mechanical stimuli activate these ion channels, which rapidly increases cytosolic Ca^{2+} concentration. A previous study has shown that physiologic levels of strain activate TRPV4 channels, while injurious levels of strain activate PIEZO2 channels.[65]

Changes in the Ca^{2+} influx, mechanical stimuli, chemical cues, and temperature can activate non-selective cation TRP channels.[66] The expression of TRPV4 channels was upregulated in osteoarthritic AC. TRPV4-mediated Ca^{2+} influx is shown to cause cell death under excessive stress stimulation.[67] It is also mediated in the regulation of the metabolism of chondrocytes responding to mechanical stress.[68] These studies suggest that TRPV4 channels are promising targets for OA treatment. As a ciliary mechanosensory channel, mechanical loading increases TRPV4 cilium localization and alters cilium length in a histone deacetylase 6 (HDAC6)-dependent manner.[69] TRPV4 knockout animals elevate intraocular pressure and shorten cilia.[70] In mesenchymal stem cells, TRPV4 mediates oscillatory fluid shear mechanotransduction via the primary cilium.[71] In conclusion, TRPV4 mediates mechanotransduction in primary cilia in many cells.

Activated by mechanical forces, PIEZO channels pass calcium ions in various cell types from different species. PIEZO channels are responsible for the Ca^{2+} influx when experiencing a level of strain in chondrocytes.[72] In odontoblasts, the PIEZO1 channel mediates the differentiation by regulating Wnt expression and assembly and disassembly of the cilia.[73]

However, the relationship between PIEZO channels and primary cilia in mechanotransduction in chondrocytes has not been reported.

Primary cilia mediated the mechanotransduction of chondrocytes

During proliferation, cilia appear on the cell surface in G1.[74] Chondrocyte cilia shorten and recover to adapt to different stimuli.[30] *Chlamydomonas flagella* also reduce their flagella length under unfavorable physiological conditions, including low pH and restrictive temperatures. However, this length reduction is reversible and the flagella regain their original steady-state length upon removal of the stimulus.[75] Ciliary axonemes have been found to interdigitate between collagen fibrils and condensed proteoglycans by transmission electron microscopy (TEM) and double-tilt electron tomography.[26] IFT proteins are transported in an advanced anterograde manner toward the ciliary tip. These proteins play a crucial role as they carry all the necessary cargo required for the assembly of axonemes.[76,77] TEM and electron tomography studies have revealed that electron-opaque particles are more located at the tip of the cilia in the distal axoneme, although they are found alongside both the proximal and distal axonemes of the primary cilia. Therefore, the tip region of cilia not only plays a significant role in ciliogenesis but also acts as a crucial transit station for the transport of cargo in both retrograde and anterograde directions.[26]

Structural basis of primary ciliary mechanotransduction

Primary cilia bend in response to mechanical forces. The interactions between the cilia bending pattern and the ECM can be visualized by TEM, electron tomography, and confocal microscopy. Tomography and TEM have shown that the ciliary axoneme interdigitates between collagen fibers and condensed proteoglycans. The transmission of mechanical forces through the matrix macromolecules causes the bending of the primary cilia, indicating their role as mechanosensors.[26] Acetylated and detyrosinated tubulins in the primary cilia contribute to the formation of functional microtubule subsets.[19] In addition, the microtubules within the chondrocyte ciliary axoneme feature a periodic structure of EB1 densities along the axoneme, indicating their high stability. This structure gives the cilia the necessary stiffness to act as biomechanical sensors of the ECM. Moreover, the primary cilium functions as a "cellular cybernetic probe," enabling the transmission of extracellular signals to the centrosome. This, in turn, regulates the stabilization of extracellular macromolecules and facilitates the mechanotransduction processes.[20] However, primary cilia are straight without ECM present, suggesting that the bending pattern in the primary cilia of chondrocytes is a passive response to the mechanical cues in the presence of ECM.[26]

Basics of primary cilium assembly and maintenance: intraflagellar transport (IFT)

Microtubules are the main structure of primary cilia. Since the primary cilium is the sole organelle and cannot synthesize proteins by itself, its formation and maintenance depend on the intraflagellar transport (IFT) system. The IFT system allows proteins to be transported in the microtubules (Figure 2). This system is bidirectional, which means the IFT-A complex can move from the basal body to the distal tip and vice versa with the help of molecular motor proteins. For example, kinesin-II binds to the IFT-B to move the cargo to the distal tip, while dynein-II binds to the IFT-A complex to transport the cargo back to the basal body.[78] Therefore, the IFT system is important for the structure and function of primary cilia. Mutations of the IFT system are associated with the dysfunction of cilia and ciliopathies.[79]

IFT80 and IFT88 both are core components of the IFT-B complex. IFT80 knockout mice have significantly reduced bone density and mechanical strength in fracture-healing tissues.[80] In addition, IFT80-depleted mice showed dysfunctional mechanotransduction due to shorter cilia. IFT88 impacts the deformability and stiffness of cellular cortex actin. IFT88 mutation alters actin-myosin stress fiber assembly and response dynamics of chondrocytes following the cytochalasin D treatment.[81] The upregulation of IFT88 expression enhances the IFT system in chondrocytes by amplifying mechanical stimulation and sensory perception of the extracellular microenvironment.[21–23] However, damage to the IFT88 complex causes abnormal cartilage formation and reduces mechanical retention.[82] In summary, the IFT system is important for primary cilia to transduce mechanical stress.

Primary cilia are involved in mechanotransduction processes

In AC, the orientation of primary cilia has been widely investigated. Based on the interaction of cilia with the matrix, it has been suggested that primary cilia on chondrocytes are important for play mechanotransduction. Current evidence suggests that primary cilia, as an important mechanosensory, are involved in various mechanical signals from the matrix microenvironment.

Compressive and tensile strain. A cartilage growth force response curve indicates that growth remains at the basal rate in the absence of mechanical stimulation. However, mild tension and compression favor growth, and larger compressive stresses rapidly impair growth.[83] Cyclic compressive loading of 1 Hz and 0–15% strain stimulates proteoglycan synthesis in wild type cells, which is completely abrogated in chondrocytes without cilia. This emphasizes the importance of the primary cilia for mechanotransduction in chondrocytes.[60] One study has shown that mechanical loading was found to upregulate the number of primary cilia in the growth plate of chickens and alter the chondrogenesis.[84] In addition, the compressive strain in a three-dimensional (3D) agarose culture model shortens primary cilia, which recover at the uncompressed free-swelling condition.[30] Note that changes in cilia length and incidence may adapt their responses to repeated or prolonged mechanical stimulation during joint activity.[53]

ATP-induced Ca^{2+} signaling has also been shown to be involved in the mechanotransduction of chondrocytes.[60] Under compressive loading (15 kPa), the ATP release rate

increases by almost 10-fold in the chondrocytes.[85] ATP activates the cytosolic Ca^{2+} signaling cascade. It is also documented that the primary cilia act as "mechano-dampeners" in the cartilage,[86] which means primary cilia are more of buffer mediators to protect AC.

Cilia frequency and length are modulated by compressive forces. Mechanical loading significantly reduces the length of cilia as cilia progressively shorten with increasing strain magnitude. In addition, high-stress levels decrease the incidence and length of primary cilia.[87] Cyclic mechanical loading using 0.33 Hz and 0–10% peak strain activates HDAC6 to induce the elongation of primary cilia and the release of nitric oxide (NO) and prostaglandin E_2 (PGE_2).[88] In a high-stress environment, HDAC6 causes primary cilia to disassemble and blocks the activation of Hh signaling, while the inhibition of HDAC6 prevents cilia from disassembly and restores mechanosensitive Hh signaling at 20% cyclic tensile strain.[38] Cellular strain depends on the depth of the AC, with the highest strain intensity on the articular surface.[89] Previous studies have demonstrated that 10% tensile strain at 0.33 Hz regulates chondrocyte depolarization and its gene expression.[90] A recent report shows that the application of LiCl can restore the mechanosensitivity of passaged chondrocytes through primary cilia.[91]

Fluid shear stress. The AC contains a big amount of water. Under mechanical loading, AC expels water out to keep the fluid shear stress close to the membrane. In the absence of mechanical stress, AC intracellularly drives the water back.[92–94] Cartilage responds to various mechanical stimuli, including shear stress, for homeostasis maintenance. Physiologically, the shear stress that ECM and the chondrocytes surrounded by ECM face is 55 kPa and 0.065 Pa, respectively.[95]

Primary cilia are critical for receiving extracellular cues from fluid shear stress. The primary cilia in kidney epithelial cells can transduce urine flow into cellular calcium signaling in mechanotransduction. In different cell types, the calcium signaling caused by fluid shear stress is abolished by chloral hydrate that induces the loss of cilia.[96,97] In addition, fluid shear forces have been shown to activate the mechanical gate complex in primary cilia.[98] The fluid-flow signal also causes NO release and modification of related proteins in chondrocytes.[103] In cartilage tissue, shear stress is associated with matrix degradation, whereas in chondrocytes, it enhances pro-inflammatory factors and pro-apoptosis.[92,100] Another study has suggested that fluid shear activates cAMP signaling and downstream osteogenesis dependent on primary cilia in bone marrow stem/stromal cells (BMSCs).[40]

Osmotic stress. AC compression and relaxation dramatically change water content and osmotic fluctuations. The primary cilia of chondrocytes respond to changes in osmolarity within minutes in a length-altering manner.[101] A previous study found that lower osmotic pressure and IL-1 together increase the length of the primary cilia.[102] However, it is shortened in living cells on intact murine femora *ex vivo*.[101] A recent study indicates that hypo-osmotic challenge blocks the primary cilia elongation mediated by IL-1β.[69] TRPV4 activation mediates the anti-inflammatory response under hypo-osmotic stress. Hypo-osmotic force activates TRPV4 channels within the primary cilia, increasing cytosolic Ca^{2+} concentration.[103] *Trpv4*-deletion chondrocytes could not respond to hypo-osmotic stress, suggesting the role of TRPV4 channels in sensing osmotic stress.[103–105] In renal epithelial cells, primary cilia are also essential for osmotic responses. Ciliated cells have a higher survival rate than non-ciliated cells under osmotic stress.[105]

Hydrostatic pressure. Primary cilia transduce hydrostatic pressure in chondrocytes. Using paralyzed-cilia mutants in *Chlamydomonas*, Yagi and Nishiyama[106] found that excessive hydrostatic pressure restores the beating pattern to wild-type cilia. Moreover, hydrostatic pressure is involved in transducing ECM signals through mechanosensitive ion channels, therefore regulating the membrane potentials. The primary cilia enhance the Ihh signaling pathway in the growth plate chondrocytes upon hydrostatic compression loading.[46] This enhancement is eliminated by chloral hydrate that disrupts the structure of the primary ciliary.[46]

Substrate stiffness. The pericellular matrix (PCM) of chondrocytes is highly viscoelastic.[107] Upon abnormal mechanical stress, PCM degrades as its layers thin, which accordingly changes the fluid flow surrounding PCM. This structural change of PCM then impacts the various sensors or receptors within the membrane in the mechanotransduction of chondrocytes. The PCM also provides an important matrix microenvironment where optimal physical properties (matrix stiffness, viscoelasticity, and geometry) regulate chondrogenesis and maintain homeostasis.

Chondrocytes respond to hydrogel systems with different substrate stiffness. The stiff substrate significantly increases the length of the primary cilia.[108] Substrate stiffness also determines centriole positioning. The centrioles position toward the basal membrane on stiffer substrates, suggesting that substrate stiffness is involved in ciliary localization and cellular responses to external forces. Substrate stiffness is also a critical driver of mechanical signal–related diseases.[112] On polydimethylsiloxane (PDMS) substrate, stiffer substrate activates YAP nuclear expression, which decreases the expression of primary cilia and inhibits inflammatory signaling transduction.[109] Thus, substrate stiffness is an important physical factor for cellular mechanical microenvironment and cells may adapt to the mechanical microenvironment via primary cilia.

3D microenvironment. Primary cilia have a length of 1–2 μm *in situ* in chondrocytes of AC. However, they are longer in isolated cells cultured in a 2D environment. A limitation can be that conventional (monolayer) expansion leads to the dedifferentiation of chondrocytes and therefore the reduction of their mechanosensitivity.[110] However, some techniques for 2D monolayer structures seem highly practical in sensing mechanical stimuli in chondrocytes. To date, some studies have used acrylamide hydrogels to culture cells on different substrates. This improves the fundamental

understanding of the biological particles involved in mechanotransduction. In addition, a 3D microenvironment seems an ideal candidate for cell cultures. Dedifferentiated chondrocytes can restore some mechanosensitivity back to the wild type in a 3D microenvironment. This can be because 3D culture maintains the structure and function of primary cilia. Soave *et al.* reported that LiCl increases primary cilia expression, therefore promoting chondrocyte mechanosensitivity. LiCl application also increases the incidence and length of primary cilia.[91] In another study, using 3D cultures, McGlashan *et al.*[30] found that chondrocyte primary cilia are mechanosensitive and mediated by the length and incidence of cilia under mechanical loads. In addition, 3D culture has been shown as an ideal model to culture primary cilia in urine-derived renal epithelial cells.[111]

Others. Other physiological-related stimuli, including current electricity, pH, and ultrasound, affect the mechanotransduction of primary cilia in chondrocytes, although they are rarely reported. Chondrocyte primary cilia respond to low-intensity ultrasound by changing their length and incidence.[112] Notably, primary cilia play a critical role in low-intensity ultrasound-mediated signal transduction (such as ERK1/2). The flow of Ca^{2+} induces microcurrent in the primary ciliary membrane, causing primary cilia to sense the microenvironment. For example, the cascading Ca^{2+} currents are abolished in the cilia-less beta-cell-specific knockout line.[113] However, more work is needed to elucidate the insights into microcurrent in mediating mechanotransduction in chondrocytes.

Conclusions

Over the past few decades, significant studies have explored a variety of signaling pathways in primary cilia. This review has emphasized the essential signaling pathways for primary cilia–mediated mechanotransduction in chondrocytes. However, understanding the correlation between gene dysfunctions in primary cilia and clinical phenotypes of AC remains a challenging task in the field. Given the complexity of mechanical loads and the specificity of the cellular mechanical microenvironment, primary cilia might mediate the mechanotransduction in different ways. Moreover, the mechanism by which deficient primary cilia cause osteoarthritic AC is not clear. This mechanism could pave the way for the development of small molecules to regulate the pathologies of cilia. The role of primary cilia in sensing the matrix microenvironment of chondrocytes is an emerging field of research. However, primary cilia appear to have different proteins in various tissues. How these tissue-specific proteins in cells sense specific mechanical cues in the matrix microenvironment, particularly in relation to specific diseases, remains a gap in knowledge. Note that multiple mechanical cues and biochemical signaling synergistically regulate cell biological behavior during tissue growth and disease progression. Studying the molecular mechanism of primary cilia–mediated chondrocytes sensing multiple mechanical cues can be challenging. It can be complex to translate *in vitro* findings into functional tissue engineering or clinical treatment.

Researchers have increasingly employed ECM-mimicking hydrogels that display physiological and mechanical properties similar to ECM. Recent studies have utilized two-dimensional (2D) cell-supporting substrates with varying physical properties to elucidate the mechanism underlying primary cilium in chondrocyte sensing mechanical stimuli. This knowledge derived from cells in 2D cultures can be translated to those within 3D environments, where further complexity is likely to arise. More importantly, determining the primary cilia–mediated signaling pathway in the 3D matrix microenvironment could help to better understand etiology related to ciliopathies. Moreover, by harnessing primary cilia–mediated cellular responses to mechanical cues in ECM, the bio-instructive hydrogel can be created and leveraged for regenerative medicine applications and for halting or reversing OA. In addition, the development of imaging technologies that can analyze the ultrastructure and signaling function of the primary ciliary will be crucial for understanding how signaling is initiated from nanoscale complexes within cilia.

The premise of functional cartilage tissue engineering is accurately recapitulating mechanical signatures (fluid shear, hydrostatic pressure, cycling stretching, matrix stiffness, viscoelasticity, stress relaxation, and microniche) in 3D matrix microenvironment reflects what occurs *in vivo*. Designing a new hydrogel mimicking *in vivo* 3D matrix microenvironment to meet emerging challenges in mechanobiology be an important goal for the future. This urgently requires the development of multiple disciplines, including biomaterials, biomechanics, and micro and nanotechnology. In summary, elucidating the regulatory mechanism of primary cilia–mediated mechanotransduction will shed light on the functional tissue engineering of AC defects as well as for developing targeted therapy for AC with ciliopathies.

AUTHORS' CONTRIBUTIONS

YZ and GKT designed and wrote the manuscript. YJZ, XW, and XHW revised the manuscript. XHW and XCW designed and drew the figures. XQ and QZ supervised YZ and revised the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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