Review

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# Are cellular mechanosensors potential therapeutic targets in osteoarthritis?

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The role of mechanical factors in driving osteoarthritis is undisputed, but historically this was largely explained by chronic attrition of the articulating surfaces. The finding that mice deficient in matrix-degrading enzymes were protected from experimental osteoarthritis (OA) suggested an alternative explanation: that mechanosensitive pathways drive the enzymes responsible for cartilage breakdown. Mechanical factors are also important for joint homeostasis and are therefore both good and bad for the joint. Several mechanosensing pathways have been identified in a variety of cell types *in vitro* and *in vivo*. Here, we review those pathways with demonstrable roles in chondrocyte mechanotransduction including ion channels, integrins, the primary cilium and the pericellular and intracellular matrices. At least two of these pathways, involving release of FGF2 from the pericellular matrix and activation of TRPV4 are chondroprotective in OA models *in vivo*. We discuss the potential for modulating selective mechanosensing pathways for therapeutic benefit in OA.

**Keywords:** animal models • fibroblast growth factor 2 • ion channels • mechanotransduction • osteoarthritis • pericellular matrix • primary cilium

## Evidence for abnormal joint mechanics in the development of osteoarthritis

Osteoarthritis (OA), the most prevalent form of arthritis, is one of the biggest healthcare challenges of current western societies. It is a heterogeneous condition for which no disease-modifying treatments exist. The lack of drug targets is largely due to a poor understanding of disease pathogenesis, primarily driven by academic neglect and the mistaken assumption that the processes of tissue destruction are due to an inevitable wearing down of tissues with increased load and age. The notion that mechanical factors are essential etiological agents in disease is undisputed. Indeed, absence of mechanical load, by experimental joint immobilization or paralysis, prevents the development and halts progression of OA. This and the evidence for mechanical factors in driving human OA is comprehensively reviewed elsewhere [1]. Mechanical risk is linked to both an increase load traversing a normal joint, for instance by occupational overuse and obesity, or normal loading of a joint that has lost its mechanoprotective properties (Box 1). Perhaps the best studied example of the latter is the increased risk of OA seen in individuals who have sustained acute trauma to the knee joint resulting in joint instability. These individuals have a risk of developing OA in the region of 50% within 10 years, even at young age [2–4]. The increased risk of OA seen with age and obesity may also be due partly to the loss of mechanoprotective mechanisms relating to muscle support around the joint and gait reflexes, which are lost with age and inactivity.

Confusing the matter further is the observation that mechanical loading can also be good for the joint. Complete immobilization of the normal joint, such as is seen following spinal cord injury, leads to a loss of volume of the cartilage [5,6]. This so-called tissue atrophy is quite distinct from the loss of tissue

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**Box 1. Abnormal joint mechanics in osteoarthritis.**

**Increased load on a normal joint**
- Occupational overuse, for example, cotton pickers (hand osteoarthritis [OA]), coal miners (back OA), farmers (hip OA)
- Obesity
- Acute articular cartilage trauma (e.g., intra-articular fracture)
- Joint malalignment

**Normal load on a weakened joint**
- Acute destabilizing injuries (e.g., cruciate/meniscal tears)
- Loss of gait reflexes with age
- Chondrodysplasias (weak cartilage matrix)
- Joint damage due to previous inflammatory arthritis
- Loss of joint support through muscle weakness (age and so on)

Disease is associated with increased load on a normal joint or normal load on a joint that has lost its mechanoprotective mechanisms.

that occurs in OA as it is not associated with a loss of the integrity of the articulating surface; moreover, it is reversible upon remobilization [1,7,8]. Overloading cartilage that is weakened by atrophy may prevent cartilage volume recovery and predispose to OA [8]. When individuals with modest OA were given a moderate exercise regime, an increase in cartilage volume was measured by MRI [9], suggesting that even joints that are damaged can respond positively to the right type of load. Taken together, these data suggest that there are good and bad pathways driving mechanosensitive joint tissue responses.

## Evidence that mechanotransduction pathways drive OA in preclinical models

The use of animal models of OA has been essential for studying early events in disease as human studies are limited by lack of access to tissue samples from patients, and the inability to identify patients at very early stages of their disease. The best validated models of OA are those that are induced by surgical joint destabilization, typically by cutting the medial meniscus or the cruciate ligaments. Surgical models are very good models of post-traumatic OA in humans; there is modest inflammatory change in the joint immediately following surgery, but this subsides and there then follows insidious, robust degradation of the cartilage, remodeling of subchondral bone, osteophyte formation and late pain [10–12]. Cartilage degradation in the mouse is dependent upon the induction of specific cartilage-degrading enzymes, specifically the aggrecan-degrading enzyme, a disintegrin and metalloproteinase with thrombospondin motif-5 (ADAMTS5) and the collagenase, matrix metalloproteinase 13 (MMP13) [10,13]. Genetic deletion of either of these leads to significant cartilage protection following joint destabilization. The induction and control of each of these enzymes is complex and likely involves a number of transcriptional, as well as post-transcriptional, mechanisms including extracellular processing to the active enzyme form and reuptake by cell surface receptors [14,15]. Nonetheless, a small fold induction of Adamts5 mRNA is detected immediately (6 h) following joint destabilization in the mouse [16]. Moreover, this induction is mechanosensitive as joint immobilization (by prolonged anesthesia) following surgery abrogates the induction of this and other inflammatory response genes [16].

Another interesting observation emerged from the above studies: while the majority of genes induced in the joint following destabilization were mechanosensitive (they were suppressed if the mouse was kept under anesthesia following joint destabilization), partial immobilization of the joint by sciatic neurectomy (where the mouse is able to bear weight through a fully extended knee joint, but is unable to flex the knee) showed differential effects on gene expression. This indicated that different types of joint loading (compression vs dynamic flexion) activated different mechanosensing pathways and induced different sets of genes. As mice that had sciatic neurectomy failed to induce proteases in the joint and were protected from developing destabilization-induced OA, it suggests that the pathways activated in response to dynamic flexion of the joint are likely to be critical for disease promotion [16].

## Cellular responses to mechanical stimulation of joint cells

The OA community has been divided for a number of years on the issue of how mechanical load drives OA; the historical view was that mechanical attrition of the articular surfaces over time, much like the wearing down of the rubber tyre of a car, could account for the tissue breakdown. An alternative argument, supported by the mechanobiologists, is that the cells of the joint are adapted to receive and respond to changes in the mechanical environment leading to the switching on of specific mechanosensitive pathways and downstream biological consequences. This theory has been probed extensively *in vitro* where joint cells (osteocytes, osteoblasts, chondrocytes and tenocytes) either isolated or in their native matrix, can be stimulated by compression, stretching or cutting. Such *in vitro* studies have demonstrated a diverse number of cellular responses. Some of the studies suggest that tissue-resident cells are able to sense the magnitude,

mode and frequency of mechanical stress and respond to it differentially. This has been well demonstrated in the chondrocyte, where mechanical insult can have a dual effect on cartilage homeostasis and inflammation. For instance, low magnitude cyclic tensile stress is able to exert anti-inflammatory effects on articular chondrocytes through reduced oxidative stress and inhibition of nitric oxide production. It is also able to counteract the catabolic and inflammatory effects of TNF and IL-1β on chondrocytes [17,18]. On the other hand, high magnitude loads promote inflammation by increasing nitric oxide production and thereby oxidative stress in the cartilage [19,20]. The magnitude of load is unlikely to be the only factor controlling biological variability; clear differences in signaling pathway activation can be demonstrated by the type of load; for example, shear versus compressive stress, as well as whether the cells are stimulated in their native matrix or isolated [21–24]. Cyclic load rather than static load is typically described as promoting anabolic responses in chondrocytes, although careful scrutiny of the literature shows that this does not hold true for all load magnitudes and changes according to the system in which this is examined (reviewed in [25]). Our own work, as well as others, has demonstrated that crude cutting injury to cartilage activates a number of intracellular signaling pathways including the MAPKs (ERK, JNK and p38), PI3K, NF-κB and the Src kinases, which lead to changes in gene regulation, some of which have predicted procatabolic actions and others anticatabolic/repair promoting [21,23,26–28].

## Mechanisms of mechanosensing in chondrocytes

Essentially all organisms from bacteria to humans need to be able to react to environmental stresses, including changes in temperature, oxygen levels and mechanical insult. To be able to translate mechanical forces and tissue deformation into biochemical signals that change cell behavior, a number of sensors of mechanical stress are in place. These include cell surface receptors, such as integrins and ion channels, cell organelles (e.g., the primary cilium), the cytoskeleton and the pericellular matrix (PCM; Figure 1). The responses of cells to mechanical stimuli may be separated into acute or rapid responses that occur within seconds to minutes, and longer-term changes that occur over hours. The rapid responses include the activation of a variety of intracellular signaling pathways often induced by changes in electrophysiological membrane potentials and intracellular ion concentrations induced by the opening of membrane ion channels. Investigation of mechanosensing mechanisms in chondrocytes has largely stemmed from those pathways of mechanisms identified in other cell types. Some of these appear to have clear functional importance in chondrocytes both *in vitro* and increasingly *in vivo*. This article will focus principally on these well-characterized mechanosensing mechanisms in chondrocytes involving TRP ion channels, integrins, the primary cilium, the PCM and its links to the cell cytoskeleton.

### Ion channels

Ion channels form a very large family of membrane pore-forming proteins. They can be classified according to their mechanism of gating (e.g., voltage, ligand, mechanical or light) or by the type of ion they permit ($K^+$, $Cl^-$, $Na^{2+}$, $Ca^{2+}$ or nonselective). A number of ion channels are expressed in normal and OA chondrocytes [29] and their activation can be induced by cyclic mechanical stimulation of isolated chondrocytes [30–32]. Mechanically gated ion channels are capable of inducing extremely rapid mechanosensory transduction often resulting in a rapid influx in $Ca^{2+}$, which triggers mobilization of further intracellular $Ca^{2+}$ into the cytosol, primarily from the endoplasmatic reticulum via G protein-coupled receptor-mediated activation of phospholipase C (reviewed in [33]).

TRPV4 has an increasingly recognized functional role in chondrocyte mechanosensation. There are 33 identified TRP ion-channel genes in mammals. The family is further divided into seven subfamilies based on sequence similarities (TRPC, TRPV, TRPM, TRPA, TRPN, TRPP and TRPML). These $Ca^{2+}$-permeable, nonselective cation channels are involved in the sensing of basic physiological cues including temperature, pH and mechanical stress [34,35]. TRPV4 is expressed highly in cartilage and skin where it functions as a mechanosensor [36,37]. Indeed, TRPV4 knockout mice show reduced sensitivity to nociceptive pressure in the tail, but respond normally to gentle touch [37]. Further confirming TRPV4's role as a mechanosensor is its activation by hypoosmotic stimuli when expressed in human cell lines and porcine articular chondrocytes [36]. TRPV4 regulates volume changes and prostaglandin ($PGE_2$) expression in response to osmotic stimuli [36]. Mechanically loaded chondrocytes embedded within agarose increase TGF-β and suppress Adamts5 mRNA expression in a TRPV4-dependent fashion, possibly by mechanically driven pericellular hypoosmotic stress [38]. This potential anticatabolic role is supported by the finding that TRPV4-null mice develop accelerated OA with a high-fat diet and following surgical joint destabilization [39,40]. Loss of function of TRPV4 in humans is associated with chondrodysplasia and arthropathy [41,42].

Two other TRP proteins, TRPA1 and TRPP2 deserve mention as these have demonstrable

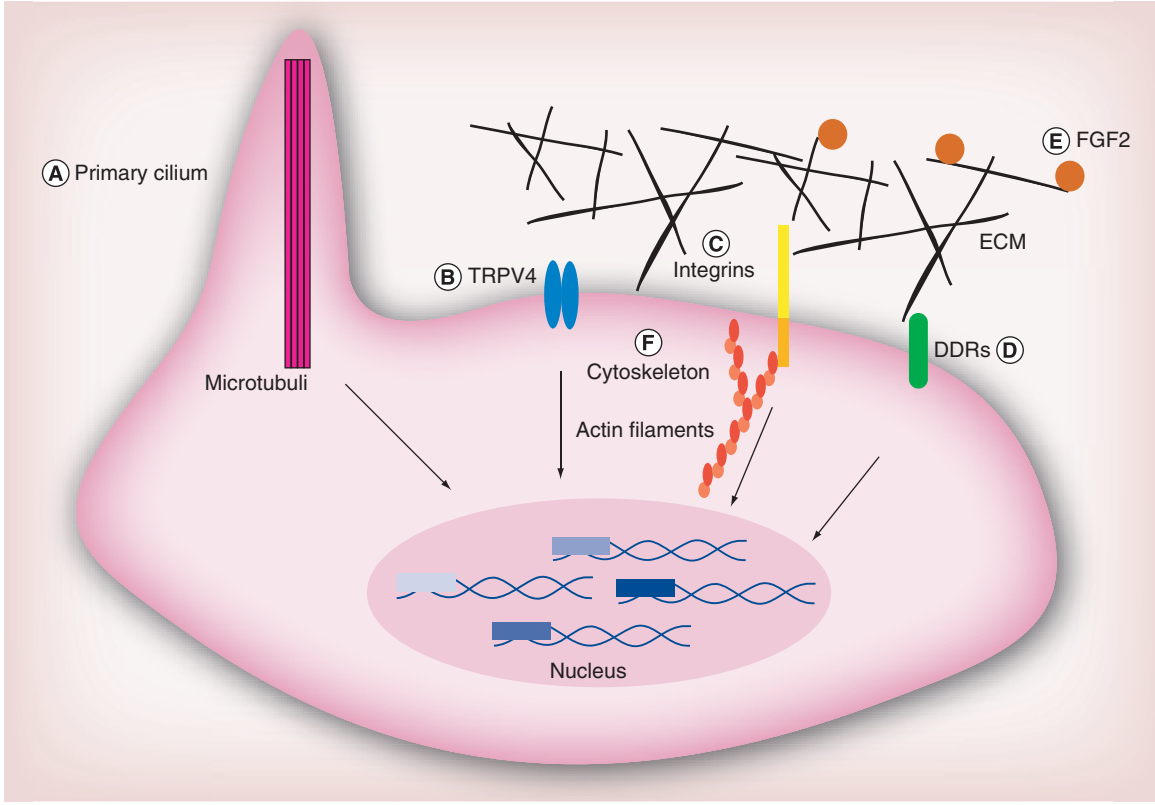


**Figure 1. Principal mechanisms of cellular mechanosensing in chondrocytes. (A)** Primary cilium (for detail see Figure 2); **(B)** ion channels; best described is TRPV4; **(C)** integrins, mediating interactions with extracellular matrix proteins and connecting extracellular matrix with intracellular cytoskeleton; **(D)** DDRs; these are collagen-binding tyrosine kinase receptors with putative mechanosensing roles; **(E)** extracellular matrix, specifically the pericellular matrix with roles in controlling local osmolarity and release of sequestered growth factors in response to mechanical loading; **(F)** cytoskeleton, creating combination of tension and compression (according to the principles of tensegrity). This has a putative functional role in mechanotransduction, by permissive action on enzyme/substrate interactions within the cytoplasm.
DDR: Discoidin domain receptor.

mechanosensing properties in other cell types. Mice deficient for TRPA1 are less sensitive to pain-induced mechanical stimulation, a function also described for its *Drosophila* ortholog [43,44]. TRPP2 was suggested as a mechanosensor in the cilia of renal epithelia [45]. TRPP2 is mutated in patients suffering from polycystic kidney disease [46] and has been found to co-localize with TRPV4 in the kidney. Knockdown of TRPV4 reduced shear stress induced $Ca^{2+}$-flow, implicating TRPV4 as part of the mechanosensing complex in renal epithelia [47].

The degenerin/epithelial Na channel (DEG/ENaC) channel family is a relatively recently defined family of nonvoltage gated Na channels with emerging roles in mechanotransduction. This family of $Na^+$ ion-selective channels shows a broad tissue expression pattern and seems to be activated by a diverse range of stimuli. Most evidence for their role as mechanosensors is derived from studies in *Caenorhabditis elegans* and mutants of several of these are touch insensitive [48]. ENaC expression has been found in avian and human cartilage, a rat osteoblast cell line, primary human bone marrow stromal cells and chondrocyte-like cells [49–51]. ENaC expression is altered in OA; compared with chondrocytes from healthy cartilage, OA chondrocytes show no expression of ENaC, while in cartilage from rheumatoid arthritis, ENaC expression is upregulated [51]. Although these studies might implicate ENaC channels in chondrocyte mechanosensing and OA, more functional studies are required to substantiate this claim.

Another candidate family is the two-pore domain $K^+$ channels ($K_{2P}$) consisting of TREK-1 (TWIK-related $K^+$ channel), TREK-2 and TRAAK. These family members have been shown to be gated mechanically in the COS (kidney-derived) fibroblast cell line *in vitro* [17,19,52–54]. TREK-1 knockout mice are more sensitive to mechanical stimuli of the skin, suggesting that TREK-1 regulates the depolarization of a so far undefined mechanosensitive channel or is involved

in the recovery from depolarization. Besides the skin, TREK-1 has a potential mechanosensitive role in the vasculature, as vasodilation from mechanical insult is decreased in TREK-1$^{-/-}$ mice [19,21,55]. These newer families of ion channels have not been explored as mechanoreceptors in chondrocytes.

## Integrins

Integrins are the major mammalian receptors for cell adhesion to extracellular matrix. They are heterodimeric transmembrane glycoproteins that comprise an α- and β-subunit [56,57], of which at least 18 α- and eight β-subunits combine to form more than 24 specific integrin 'receptors'. Both subunits have a large modular extracellular domain, followed by a single transmembrane helix and a short cytoplasmic domain that mediates interactions with the cytoskeleton.

Integrins have been implicated in mechanosensing in a number of cell types [58,59]. Mechanisms of integrin signaling involve changes in integrin conformation, which results in exposure of novel binding sites for improved ligand and signaling molecule binding affinities. Such changes can be modulated by extracellular ('outside-in') and intracellular ('inside-out') interactions, including factors such as matrix stiffness, cyclic mechanical loading and cooperation with other mechanosensing receptors (e.g., ion channels) [60–62]. Integrin clustering leads to the recruitment of kinases, such as integrin-linked kinase and adapter proteins to the cytoplasmic tail of the β-subunit, promoting association with the actin cytoskeleton [63,64]. The resulting complex is termed the focal adhesion complex (FAC). So far, two major kinase families have been shown to be recruited and activated in the FAC. One of the earliest events is the recruitment of focal adhesion kinase, which enables the activation of tyrosine kinases, such as Src and Fyn, to phosphorylate the FAC components paxillin and tensin [23]. The second kinase activated in the FAC is protein kinase C, which is one mediator of mechanically induced upregulation of proteoglycan production in chondrocytes [24,65].

In cartilage α1β1, α5β1, αVβ1 and α10β1 are the major integrins expressed in normal adult human articular cartilage [66,67], although expression may be altered in OA cartilage [68,69]. α5β1 has been shown to be critical for membrane hyperpolarization and tyrosine phosphorylation following mechanical stretching of chondrocytes [23,32], a response that is dependent upon release of soluble IL4 [70]. Anabolic responses of chondrocytes encapsulated in agarose in response to cyclic load was dependent upon αVβ3 and β1 integrins [71]. OA chondrocytes have altered integrin-dependent responses to mechanical loading *in vitro* and fail to mount an anabolic response (aggrecan synthesis) following cyclic load [72].

*In vivo*, the β1 integrin subunit has demonstrable effects on musculoskeletal development. It leads to disorganization of growth plate chondrocytes and abnormal formation of the PCM. Loss of β1 integrin does not appear to lead to increased cartilage degradation [73,74]. On the other hand, deletion of the α1 integrin is associated with increased spontaneous cartilage degradation with age [75]. It is not clear whether these phenotypes are specifically related to a mechanosensing failure.

The intracellular cytoskeleton is considered to be an essential component of integrin-mediated mechanotransduction responses. The cytoskeleton is a dynamic structure that provides a combination of tension and compressive forces that determine cell shape and phenotype, according to the principles of tensegrity [76]. Of the three major cytoskeletal networks (F-actin, tubulin and intermediate filaments), the F-actin cytoskeleton is most strongly remodeled in response to mechanical load [76,77]. Hydrostatic pressure, osmotic changes, as well as dynamic compression cause F-actin remodeling in articular chondrocytes [78–80]. Treatment of chondrocytes with thymosin β4, an inhibitor of F-actin polymerization, increases MMP9 expression and activation thereby enhancing cartilage remodelling following mechanical stress [81]. Whether the cytoskeleton is directly involved in the cellular response to mechanotransduction is unknown, but it is postulated that tension within the cytoskeleton could change thermodynamic or kinetic parameters to alter the association of enzymes with their substrates [82].

## The primary cilium

While cell surface proteins are currently the most extensively studied mechanosensors, the cellular organelle, the primary cilium, is also emerging as an important mechanotransducer in several cell types including chondrocytes. First described by Swiss anatomist Karl Zimmermann in 1898, the immotile primary cilium is a microtubule-based structure similar to motile cilia and flagella and one is present on most mammalian cells [83], including chondrocytes [84,85]. The formation of the primary cilium is a dynamic process with constant assembly and resorption taking place. All ciliary proteins are transported through an active process termed intraflagellar transport (IFT). Naturally occurring and experimental mutation of motors and trafficking proteins of IFT has illuminated the fundamental roles of the primary cilium in cell- and mechano-biology.

Evidence for the role of primary cilia as mechanosensors first arose from studies in kidney

epithelia [86,87]. Recently, evidence has emerged that human mesenchymal stem cells rely upon the primary cilium for both the response to soluble osteogenic factors induced by mechanical stress [88] and fluid flow itself [89]. A mechanosensory role for the primary cilium in bone *in vivo* was suggested by experiments in which conditional deletion of the IFT protein Kif3a in osteoblasts, led to abnormalities in osteoblast differentiation and compression-induced bone formation [90,91]. Osteocytes, in fluid-filled lacunae, also respond to hydrostatic compression and flow in a manner that is dependent upon the primary cilium [92]. The mechanism by which the cilium drives mechanosensitive responses in bone may involve adenyl cyclases that convert ATP to cAMP, and reside in primary cilia [93]. AC6 has been shown to be responsible for the reduction of intracellular cAMP upon shear stress [94] and to mediate loading-induced bone adaptation *in vivo* [95].

Several studies have suggested the importance of the primary cilium in cartilage physiology [96–100] and more recently in its role in mechanotransduction. Compressive forces applied to chondrocytes stimulate a purinergic response in the form of ATP release, possibly through the purine channel connexin 43 [101]. Interestingly, it seems that primary cilia in chondrocytes are not the initial sensors of mechanical stress, but rather are required for detection of released ATP following mechanical load [102]. Indian hedgehog signaling, regulated by the primary cilium, is mechanically activated in a primary cilia-dependent fashion [103,104]. Moreover, pathological mechanical forces further modulate hedgehog signaling through active disassembly of the primary cilia architecture [105]. Hedgehog signaling is both upregulated in OA and capable of modulating murine OA following surgical joint destabilization [106]. Taken together, a model emerges, where primary cilia orchestrate the response to mechanically induced ATP release, and are required for and regulate hedgehog pathway activation (Figure 2).

## The extracellular matrix

The extracellular matrix of cartilage is exquisitely adapted to receive, transmit and amplify mechanical forces coming from the joint surface to the chondrocyte. The PCM in particular appears to play a critical role in these processes. The PCM is structurally and biochemically quite distinct from the type II collagen/aggrecan rich bulk extracellular matrix [107–109]. Its two principal components are the nonfibrillar type VI collagen, and the heparan sulfate proteoglycan, perlecan, with other proteins frequently associated with basement membranes [110]. These account for the low elastic modulus (stiffness) of the PCM compared with the bulk matrix and probably allows it to undergo selective deformation upon mechanical tissue compression [111–113]. Accumulation of PCM in culture appears to determine TRPV4-dependent mechanoresponsiveness [38], indicating that the PCM influences cell surface mechanoreceptor function in some way (possibly by controlling local osmolarity). In OA, the PCM expands and its constituent proteins are more highly expressed, although its mechanical integrity is reduced [114–116].

The PCM is also able to sequester chondrocyte regulatory molecules, which are released upon tissue injury. The best described of these is FGF2, which is bound to the heparan sulfate chains of perlecan. Pericellular FGF2 is released in response to cutting-induced tissue injury, as well as cyclic mechanical loading [28,117,118]. Released FGF2 then activates chondrocytes by binding to one of four tyrosine kinase FGF receptors. Recent studies suggest that the predominating FGF receptor is critical for determining the biological outcome, with FGFR1 mediating more procatabolic responses and FGFR3 driving more protective pathways [119–121]. The significance of FGF2-mediated responses *in vivo* is demonstrated by the finding that FGF2-null animals develop accelerated joint degeneration spontaneously with age and following surgical joint destabilization [122]. FGF2 also drives the induction of a number of mechanosensitive genes *in vivo* following induction of OA [123].

Chondrocytes also express membrane-associated proteoglycans and glycoproteins, known as the glycocalyx. In other cell types, best described in the endothelium, the glycocalyx is thought to have several roles including limiting cell adhesion between luminal and resident cells, controlling transmigration of immune cells and mediating fluid flow-induced mechanoregulation. The latter is dependent upon interactions between membrane-associated proteoglycans and the intracellular cytoskeleton [124]. Stimulation of endothelial cells by shear stress is able to upregulate MMP13 mRNA in a heparan sulfate-dependent manner, suggesting that that glycocalyx may mediate catabolic responses to shear stress [125]. To what extent the glycocalyx of chondrocytes is involved in mechanotransduction is unclear, although cell loss of the cell surface heparan sulfate proteoglycan, syndecan 4, is associated with protection from experimental OA [126].

## Other putative mechanosensing pathways

The discoidin domain receptors (DDRs) are receptor tyrosine kinases that recognize collagens as their ligands. Although there is little published to show a direct mechanosensing role of DDRs, they are good candidate molecules as they connect the cell with the extracellular matrix, they are induced in a

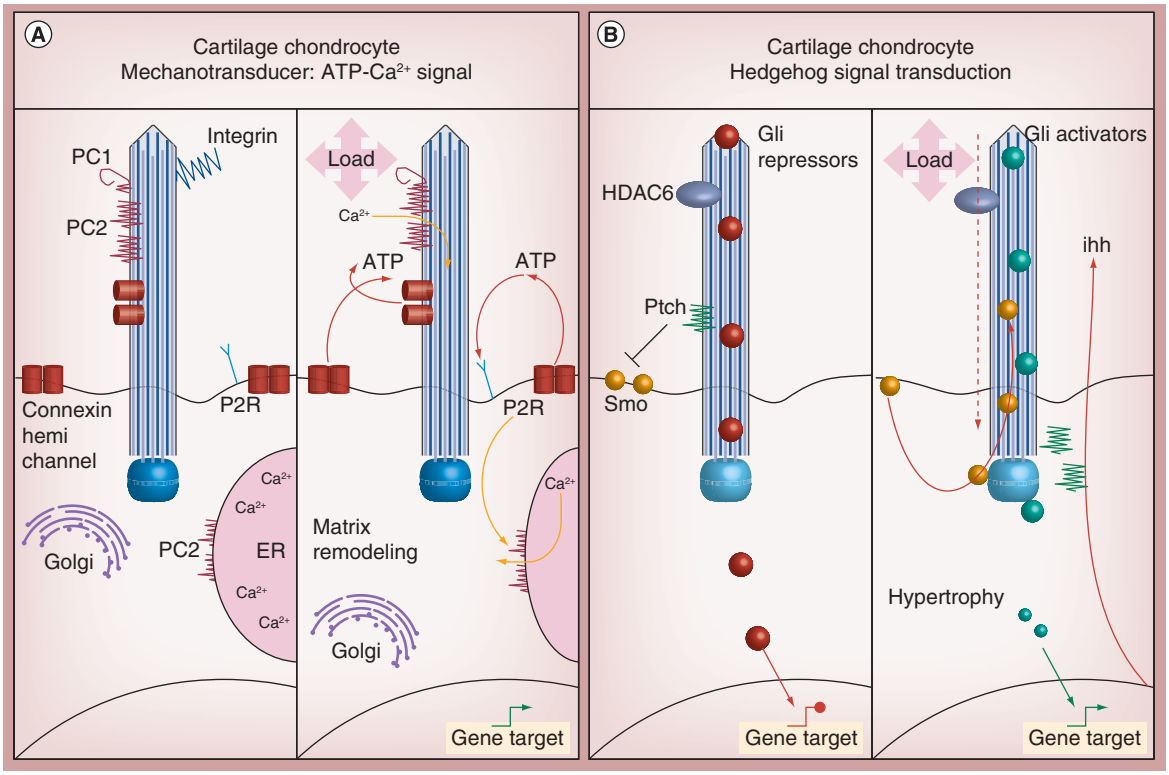


**Figure 2. Mechanosensitive signaling by the primary cilium.** In chondrocytes, the primary cilium is likely bound up by dense matrix, possibly physically attached by integrins expressed on its surface. The Golgi apparatus is often polarized on the side of the cell expressing the cilium. The cilium expresses connexin hemichannels and polycystins, although they are also expressed elsewhere on the cell. **(A)** ATP-signaling: early responses to mechanical loading such as mechanically-induced ATP release through hemichannels, are not dependent on the cilium. In other words, in chondrocytes the cilium is not the initial sensor. Released ATP binds P2R and activates intracellular calcium signaling, a process which is dependent upon the cilium. This is likely through cilial polycystins or cilial influence over cellular polycystins. **(B)** Ihh gene expression and pathway activation occurs upon mechanical loading, a process that is not dependent upon the cilium. This signal is, however, transduced through the cilium as the receptor Ptch, leaves the cilium upon ihh activation, releasing inhibition over Smo. Smo then enters the cilium and converts Gli repressors into Gli activators, which are transcriptional regulators. Higher levels of mechanical loading have been shown to actively reduce cilia length through HDAC6 and further regulate hedgehog signaling and downstream ADAMTS5. In cartilage, hedgehog pathway activation is associated with chondrocyte hypertrophy and progression of osteoarthritis.
ER: Endoplasmic reticulum; ihh: Indian hedgehog; P2R: Purinergic receptors; Ptch: Patched; Smo: Smoothened.

mechanosensitive manner and have a role in regulating matrix degradation *in vivo* following joint destabilization [127–129]. The DDR family comprises two distinct members, DDR1 and DDR2, which both undergo tyrosine autophosphorylation upon collagen binding with some ligand selectivity [130–132]. Tyrosine phosphorylation occurring upon activation allows the recruitment of Src homology domain, SH2- and SH3-containing proteins. In the case of DDR1, this leads to the activation of the PI3K/Akt and Ras/ERK cascade, while DDR2 appears mainly to activate Src kinase [130].

The importance of DDRs as collagen receptors in chondrocytes is evident in DDR knockout mice. These animals exhibit skeletal abnormalities, show impaired inflammatory and fibrotic responses and are protected from OA development [133].

## Targeting mechanotransduction pathways in OA

The studies above suggest that one could target mechanotransduction pathways in two broad ways: either promote the pathways that are driving anti-catabolic and/or repair processes in the joint or block the pathways that drive pathogenic protease induction.

Strategies for promoting FGF2 signaling have been explored. FGF2 has long been considered a good candidate molecule for improving outcome in OA as it is an important chondrogenic factor *in vitro* and is often used in combination with other cytokines for expanding and differentiating chondrocytes for autologous cartilage repair strategies. Being released from the damaged matrix, it also makes it a likely

candidate molecule for signaling to intrinsic progenitor cells to initiate the process of repair. *In vitro* FGF2 has conflicting roles that are likely rationalized by the finding that deleterious effects of FGF2 are mediated mainly through FGFR1 and beneficial effects through FGFR3. Although FGF2 is promiscuous and can signal through FGFR1 and FGFR3, other FGF ligands (e.g., FGF18) are more selective in their action and preferentially bind and signal through FGFR3. Studies of intra-articular FGF18 have been successful in preclinical models [134] and clinical trial results are awaited. The exciting possibility that the ratio of FGF receptors is dynamic and modifiable by growth factor stimulation is also another potential strategy for improving disease outcome [121,135].

Mechanical stimulation through TRPV4 also appears to promote chondroprotective responses, so TRPV4 activators could be used to promote anabolism and anticatabolic responses. TRP channel agonists are putative targets in a number of other diseases and several pharmacological agents are already in development [136].

The specialized apparatus of the primary cilium may be an interesting potential target in OA, especially as the percentage of ciliated chondrocytes increases with disease severity [137] and cilia length is modulated in disease and following IL-1 stimulation [137,138]. *In vitro* mechanical loading also stimulates cilia disassembly [139] and activates cilia-dependent hedgehog signaling and Adamts5 expression [105]. Modulating cilia trafficking, especially by targeting molecules unique to the 'ciliome', will be a good starting point for establishing cilia-directed therapies.

Manipulating such pathways provides us with potentially highly novel and tractable targets. Established *in vivo* model systems allows us to test molecules arising from *in vitro* studies and to screen potential pathways with relative ease. Their success will depend upon a number of current unknowns such as whether these pathways are important for progression, as well as induction of disease, whether these pathways have critical remodeling roles in adult cartilage and whether they are essential in other important connective tissue mechanosensing responses.

## Conclusion

How cells respond to a change in their mechanical environment has been studied for a number of years, but we are only just beginning to understand which of these pathways are critical for driving both the beneficial and detrimental processes that drive joint homeostasis and disease. The change in mechanical environment may be mediated by loss of mechanical protection or increased load going through the joint, but may also be dynamically altered as the extracellular matrix changes as disease progresses. The ability to study selective pathways in models of disease has allowed us to determine the *in vivo* significance of such pathways and to consider these molecules as potential therapeutic targets for patients with OA.

## Future perspective

This is a radical rethinking of how we view mechanical factors in OA pathogenesis. The next few years are likely to see the targeting of specific mechanosensing pathways in *in vivo* models of disease. Validating these pathways in preclinical models will pave the way for validation of pathways in humans with disease and ultimately targeting these as therapies in those most at risk or at early stages of disease.

### Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial

### Executive summary

**Mechanics in human osteoarthritis**

- Abnormal mechanical loading is the most important etiological factor in the development of osteoarthritis (OA) and this is likely due to direct mechanosensing by the cells of the joint.

**Biological responses to mechanical loading *in vitro***

- Cells *in vitro* are able to modify their biological response according to the magnitude, mode (compression vs shear stress) and frequency of applied load.

**Biological responses to mechanical load *in vivo***

- Following joint destabilization in rodent models, gene induction is highly mechanosensitive and different joint movements likely activate different mechanosensing pathways.

**Mechanisms of mechanotransduction in joint cells**

- There are a number of well-described mechanisms by which cells sense a change in their mechanical environment. The best validated of these in chondrocytes include cell surface ion channels, specifically TRPV4, integrins, the primary cilium and release of sequestered molecules from the pericellular matrix.

**Mechanotransduction pathways as targets for OA**

- Described mechanotransduction pathways deliver both anabolic, as well as potentially disease-promoting pathways. Selective stimulation/blockade of these pathways represent potential therapeutic strategies.

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