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Review

The interplay between biochemical mediators and mechanotransduction in chondrocytes: Unravelling the differential responses in primary knee osteoarthritis

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ABSTRACT

In primary or idiopathic osteoarthritis (OA), it is unclear which factors trigger the shift of articular chondrocyte activity from pro-anabolic to pro-catabolic. In fact, there is a controversy about the aetiology of primary OA, either mechanical or inflammatory. Chondrocytes are mechanosensitive cells, that integrate mechanical stimuli into cellular responses in a process known as mechanotransduction. Mechanotransduction occurs thanks to the activation of mechanosensors, a set of specialized proteins that convert physical cues into intracellular signalling cascades. Moderate levels of mechanical loads maintain normal tissue function and have anti-inflammatory effects. In contrast, mechanical over- or under-loading might lead to cartilage destruction and increased expression of pro-inflammatory cytokines. Simultaneously, mechanotransduction processes can regulate and be regulated by pro- and anti-inflammatory soluble mediators, both local (cells of the same joint, i.e., the chondrocytes themselves, infiltrating macrophages, fibroblasts or osteoclasts) and systemic (from other tissues, e.g., adipokines). Thus, the complex process of mechanotransduction might be altered in OA, so that cartilage-preserving chondrocytes adopt a different sensitivity to mechanical signals, and mechanic stimuli positively transduced in the healthy cartilage may become deleterious under OA conditions. This review aims to provide an overview of how the biochemical exposome of chondrocytes can alter important mechanotransduction processes in these cells. Four principal mechanosensors, i.e., integrins, Ca^{2+} channels, primary cilium and Wnt signalling (canonical and non-canonical) were targeted. For each of these mechanosensors, a brief summary of the response to mechanical loads under healthy or OA conditions is followed by a concise overview of published works that focus on the further regulation of the mechanotransduction pathways by biochemical factors. In conclusion, this paper discusses and explores how biological mediators influence the differential behaviour of chondrocytes under mechanical loads in healthy and primary OA.

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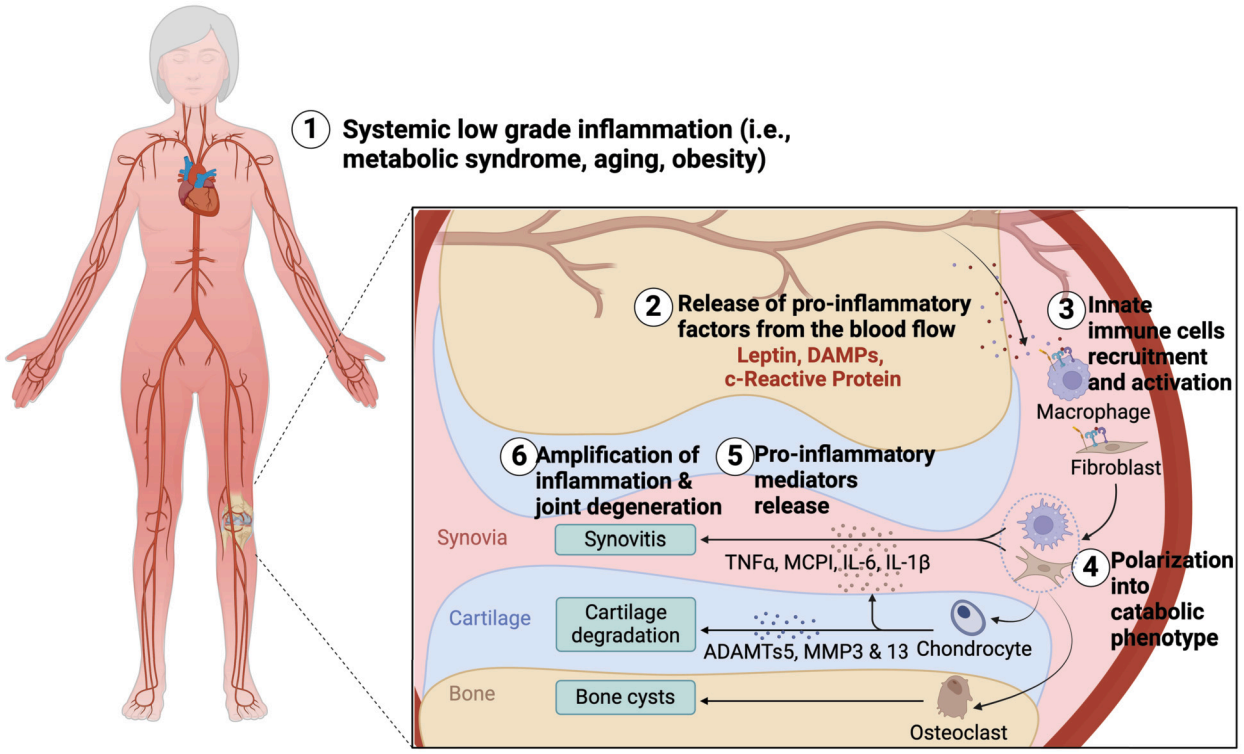

Fig. 1. (1) Metabolic syndromes, obesity, or ageing increase the circulatory levels of pro-inflammatory factors that (2) can infiltrate into joints. (3) They recruit innate immunity cells to the joint which increases (4) the secretion of pro-inflammatory mediators. (5) They diffuse into articular cartilage (AC), activating catabolism in chondrocytes. (6) AC is degraded, and the inflammation perpetuates over time, leading to synovitis, effusion and bone cysts. Adapted from [14,17–19,16]. Created with BioRender.com.

1. Introduction

Osteoarthritis (OA) is the most prevalent arthritic disease and a leading cause of disability worldwide. Women, individuals with prior joint injuries, elders, and obese individuals all have an increased risk of developing OA, as do individuals with certain genetic and biomechanical predisposing factors [1,2]. Furthermore, the increasing global rates of obesity and ageing impose substantial challenges on both the affected individuals, and the healthcare systems [3].

OA can affect any synovial joint, such as the hips, spine, or fingers; however, it is most prevalent in the knee, affecting 22% of men and 31% of women older than 55 years of age, in 2019 [4]. In normal conditions, the knee joint allows smooth movements between adjacent bones thanks to the articular cartilage (AC), a specialized connective tissue that covers each end of the bones. The joint is surrounded by a capsule that defines a cavity filled with a synovial fluid secreted by a specialized membrane, the synovium [5]. The maintenance of the AC is orchestrated by the unique residing cells: the chondrocytes. These cells synthesize and secrete mainly collagen type II (Col-II), and proteoglycans and glycoproteins that assemble to form Aggrecan (Agg), leading to a low turnover tissue. Altogether, Col-II and Agg constitute most of the extracellular matrix (ECM) and provide the essential scaffold for chondrocytes [6–8]. However, the avascular, aneural, and alymphatic nature of AC limits the capacity of chondrocytes to effectively repair the ECM when the tissue becomes damaged, as it happens in OA [9].

In the past decade, there was a gradual but fundamental shift in our perception of the pathological mechanisms underlying knee OA [1]. We no longer view knee OA as a cartilage degenerative disease resulting from abnormal bodily wear and tear, but rather as a multifactorial disorder in which systemic factors are also involved. Specifically, acute or subacute injuries of the AC, often in the context of other risk factors (such as obesity, advanced age, metabolic disorders, and certain genetic factors), can trigger a progressive cycle of chronic pro-inflammatory activity of the joint cells, resulting in incremental cartilage loss and overall joint degeneration over time [10–12].

On the one hand, systemic low-grade inflammation at the body level might play an important role in the pathogenesis of primary OA. The systemic mediators (i.e., leptin, c-Reactive Protein) migrate from the blood flow to the synovial joint, increasing the pro-inflammatory and pro-catabolic activity of all synovium cells (i.e., fibroblasts and synoviocytes), which activates the innate immune system, see Fig. 1 for details [13,2]. Eventually, chondrocytes lose their ability to keep normal maintenance of AC, as they start to secret pro-inflammatory factors and degrading enzymes, which perpetuates pro-inflammatory processes in the joints, including synovitis and effusion [14]. Finally, chondrocytes die and the AC disappears. Non-cartilaginous cells (i.e., osteocytes) also become affected triggering the formation of bone cysts [15–17].

Table 1
PIEZO1/2 responses upon mechanical stimulation.

Chondrocyte source	Mechanical stimuli	Response analysed	Ref.
Femoral condyles of skeletally mature pigs	Custom build Atomic Force Microscopy at >300 nN or 50% strain	Increased Ca^{2+} influx from extracellular reservoirs, except for knock-down of PIEZO1/2	[33]
Distal femur and proximal tibia AC of 5-day-old murine models	1 MPa stress at a frequency of 1 Hz for 1 h	PIEZO1 increases chondrocyte ferroptosis (decrease of GPX4), which ultimately increased oxidative stress	[34]
AC from OA patients	Overloaded zones of cartilage	PIEZO1 increases ferroptotic features and ageing: thickened mitochondrial membrane and mitochondria shrinkage	[34]
AC from OA patients undergoing total knee arthroplasty	(20% amplitude every for 10 min during 24 h)	PIEZO1 inhibition increases the proliferation ratio of chondrocytes	[35]
Femeroal heads, condyles and tibial plateaux of six-day-old mice	Flexcell® Tension PlusTM FX-4000TM at 0.5 Hz for 8 h at different rates of strain at 13% and 18%	Increased expression of PIEZO1/2 for 13% strain and PIEZO2 for 18% strain which leads to higher Ca^{2+} influx.	[28]

On the other hand, chondrocytes are mechanosensitive cells and their responses upon mechanical loads depend on the frequency, rate and amplitude of the loads. Sanchez-Adams et al. [20] summarize how loads might affect chondrocyte metabolism and AC composition. Generally, studies reported that injurious dynamic compression decreased the normal biosynthesis capacity [21] and increased the pro-catabolic activity of chondrocytes [17]. Static loads or strains were related to dose-dependent ECM degradation [22], while dynamic compression in the range of 0.1-1 Hz generally stimulated the synthesis of collagen proteins (measured by the incorporation of proline) and proteoglycans [23]. Interestingly, AC disuse may produce cartilage atrophy and degeneration [24]. Physiologic loads generated by physical exercise also positively influenced the AC and continuous passive motions augmented cartilage repair [7]. However, more recent studies demonstrated that mechanical cues show inconsistencies as per their effect on chondrocyte metabolic activity in healthy or degraded AC. For example, full-thickness osteoarthritic AC, already damaged and stimulated with dynamic physiological compression was not able to up-regulate and restore the expression of Agg [25], in contrast to what Sah et al. [23] reported.

Revealing why previously damaged cartilage responds to mechanical cues differently could provide insights regarding the degenerative process of chondrocytes in primary OA. Previous *in silico* studies with damaged cartilage suggested that in a virtual model for idiopathic OA, collagen degradation could be the starting point of a cascade in AC degeneration [26]. However, it offers a high-level explanation of how chondrocytes respond to physical cues, without considering intracellular details, focusing on tissue-level mechanobiology aspects. So far, we know that mechanical inputs are integrated into chondrocyte metabolism through signalling cascades by a process called direct mechanotransduction, which involves a series of discrete but common steps that convert physical signals into cellular responses. To translate mechanical forces into biochemical signals, chondrocytes have several membrane proteins that are activated upon physical cues called mechanoreceptors [27].

Regarding OA aetiology or AC conditions (i.e., healthy or degraded), the mechanical inputs could be integrated in ways that are beneficial or detrimental to chondrocyte homeostasis. In fact, mechanotransduction processes can regulate and be regulated by soluble factors (i.e., pro- and anti-inflammatory cytokines, growth factors and prostaglandins). Then, understanding how local or even systemic soluble factors could alter the way chondrocytes integrate mechanical loads in these direct mechanotransduction processes can provide insights regarding the degenerative processes of chondrocytes in primary OA.

In the following sections, we summarize how the principal mechanosensors of chondrocytes (i.e., integrins, Ca^{2+} channels, primary cilium) differently respond to mechanical loads. This summary is followed by a concise overview of published works that focus on how biochemical factors regulate specific mechanotransduction pathways, which might help in understanding pathological drivers in primary OA.

2. Calcium channels

Intracellular Ca^{2+} oscillation is one of the earliest molecular responses that chondrocytes exhibit when exposed to mechanical stimuli. Proper regulation of Ca^{2+} is essential for chondrocyte homeostasis and the preservation of the ECM integrity [28]. However, multiple studies have shown that excessive Ca^{2+} overloads, whether from intracellular [29] or extracellular sources [30,31], can lead to cell death. Abnormal Ca^{2+} levels in the cytoplasm can trigger apoptosis, demonstrating the importance of balanced intracellular Ca^{2+} levels in ensuring chondrocyte health and ultimately, AC integrity [32]. See an overview in Fig. 2.

2.1. PIEZO channels

PIEZO channels (i.e., PIEZO1 and PIEZO2) transduce hyper-physiological mechanical strains into increased inwards of Ca^{2+}, leading to degenerative processes in chondrocytes (see Table 1). PIEZO channels expression has been confirmed to be robust in primary chondrocytes, and they both contribute cooperatively to chondrocyte mechanotransduction at high injurious strains, inducing Ca^{2+} influx from extracellular sources [28,33]. Activation of PIEZO1 channels by excessive mechanical stretch leads to abnormal levels of chondrocyte proliferation [35]. Specifically, the same authors in their *in vitro* study with human-derived primary chondrocytes

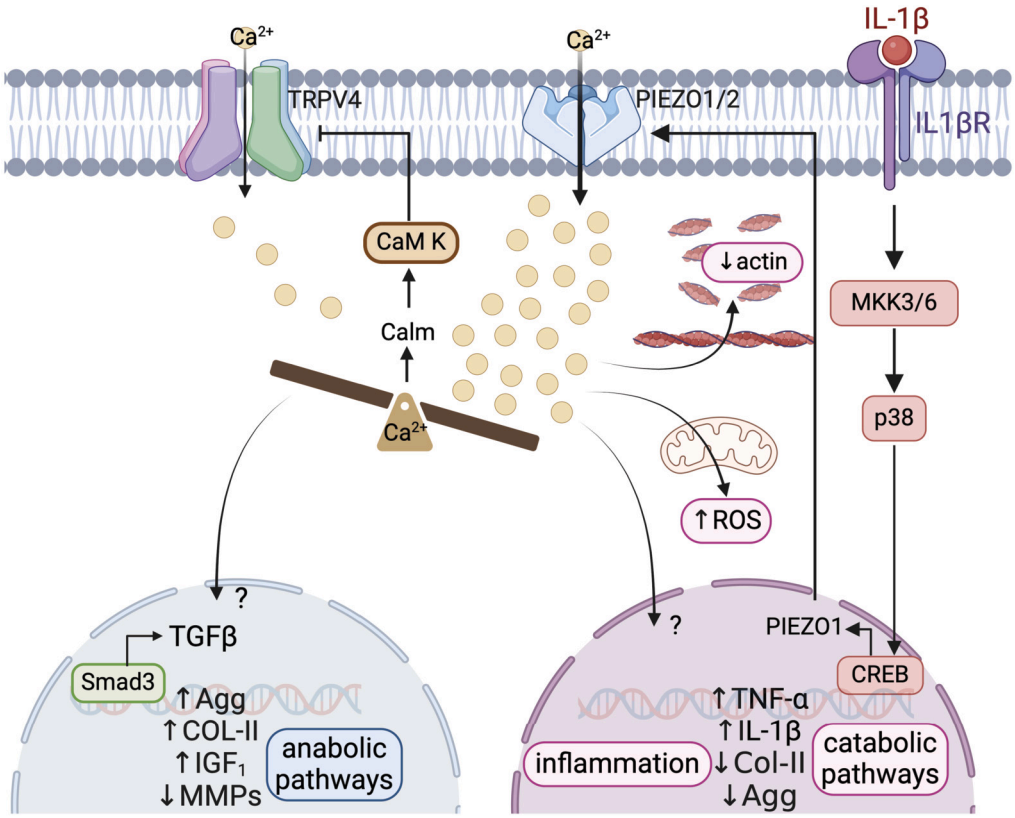

Fig. 2. A proposed example of chondrocyte mechanotransduction pathways for Transient Receptor Potential Vanilloid 4 (TRPV4) and PIEZO channels for Ca^{2+} balance. TRPV4 is opened upon hyperphysiological loads and osmotic changes. Next, an intracellular negative feedback loop makes the chondrocytes inhibit the opening of these channels by calmodulin (Calm). Such amounts of Ca^{2+} are beneficial for chondrocytes as they induce the activation of pro-anabolic pathways. PIEZO1/2 channels are opened upon hyper-physiological mechanical strains and induce a large influx of Ca^{2+} that increases pro-inflammatory and catabolic pathways and reactive oxygen species (ROS), and decreases the stiffness of the cytoskeleton by disrupting the actin filaments. Surrounding increased levels of interleukin 1 beta (IL-1β) increase the activation of this channel. Created with BioRender.com.

demonstrated that PIEZO1 channel activation induces cytoskeleton destruction by activating the kinesin Kif18A, which depolymerizes microtubules. Moreover, they also suggested that changes in the intracellular Ca^{2+} levels might be involved in this response [35]. Another study has shown the implication of PIEZO1 in ferroptosis. Ferroptosis is a type of programmed cell death largely regulated by the phospholipid hydroperoxide-reducing enzyme glutathione peroxidase 4 (GPX4), related to glutathione (GSH) synthesis, which inhibits the ferroptosis process. Increased influx of Ca^{2+} upon activation of PIEZO1 impairs GSH production, so GPX4 levels are reduced and alter ferroptosis in chondrocytes, characterized by mitochondrial dysfunction and exacerbated oxidative stress (e.g., production of reactive oxygen species (ROS) [34].

Local inflammatory factors promote an enlarged accumulation of these channels in the extracellular membrane of chondrocytes. In this sense, Lee et al. [36], demonstrated that interleukin (IL) 1 alpha (IL-1α) could induce an up-regulation of the expression of PIEZO1. Specifically, the IL-1α-IL1 Receptor I complex via Mitogen-activated protein kinase (MAPK) 3/6 (MKK3/6)-p38 MAPK activates cAMP Response Element-Binding Protein 1 (CREB1), increasing the expression of this channel. The higher levels of Ca^{2+} decreased the firmness of the actin cytoskeleton, which results in increased cellular deformation in response to mechanical loading [36].

Parallel to inflammation, endocrine factors influence the expression of PIEZO1 too. On the one hand, oestrogen inhibits stress-mediated PIEZO1 activation and chondrocyte apoptosis by regulating the actin/PIEZO1 axis [37]. This protective role of oestrogen (i.e., inhibition of PIEZO1 channels) could be related to the increased incidence of OA within women after menopause, as oestrogen levels decrease abruptly. On the other hand, the Uricortine 1 (UCN1) depleting antibody and the UCN1 receptor antagonist lead to significant cell death in a human chondrocyte cell line (C-20/A4), demonstrating the pro-survival role of UCN1 [30]. Specifically, UCN1 exerts its chondroprotective effect by maintaining PIEZO1 in a closed conformation through the protein kinase A (PKA) axis [30]. Additionally, UCN1 down-regulates the production of the pro-inflammatory cytokines (i.e., tumour necrosis factor (TNF), interferon-gamma (IFN-γ), IL-6, IL-1β) in inflamed joints of collagen-induced arthritis mice. Also, it increases the levels of anti-inflammatory cytokines such as IL-10 and transforming growth factor beta (TGF-β) in the same animal model [38].

Table 2
TRPV4 responses upon mechanical stimulation.

Chondrocyte source	Mechanical stimuli	Response analysed	Ref.
Porcine articular chondrocytes were enzymatically isolated from the femurs and ulnas of skeletally immature pigs	10% peak-to-peak sinusoidal strain at 1 Hz for 3 h a day.	Increased gene expression of TGF-β3, and decreases the expression of ADAMTS5. The inhibition of TRPV4 prevented the activation of NO synthase and Col2α genes	[39]
Femoral condyles of skeletally mature pigs	Hypo-osmotic (280 mOsm)	Extracellular increase of Ca^{2+}. Increase PGE_2 short-term release	[40]
Chondrocyte cells from mouse chondrocytes (ATDC5 cell line)	Hypo-osmotic swelling (137 mOsm)	Increased conductance of chondrocytes and Ca^{2+} influx	[41]
Porcine stifle articular cartilage joints	Hypo-osmotic 200 mOsm and 0-10% strain for 24 h at 0.33 Hz	Inhibition of the release of NO and PGE_2 upon previous stimulation of IL-1β	[42]
Femoral heads, condyles and tibial plateaux of six-day-old mice	Flexcell® Tension PlusTM FX-4000TM at 0.5 Hz for 8 h at different rates of strain at 3% and 8%	Increased expression of TRPV4 Ca^{2+} influx	[28]

2.2. Transient Receptor Potential Vanilloid 4 (TRPV4)

The Transient Receptor Potential Vanilloid 4 (TRPV4) is also a non-selective cation channel with good selective permeability for calcium ions, sensitive to physiological osmotic and dynamic compression [43,41,39]. However, contrary to PIEZO channels, TRPV4 is induced by physiologic levels of strain and related to anti-inflammatory responses in short-term, moderated modulations [42,28] (see Table 2). TRPV4 mechanosensitivity is suggested to be regulated by distinct mechanisms. For example, TRPV4 can be activated by cell swelling upon arachidonic acid release; can undergo direct channel gating in response to membrane deflection; or can be activated by PIEZO1 upon mechanical activation [42]. In any case, the proteoglycan-rich structure of the AC links mechanical loading to interstitial osmolarity, meaning that TRPV4 can be considered as a mechanosensor, although it is actually activated by osmotic changes [40,42,41]. Specifically, TRPV4 activation is specific to hypo-osmotic loading in response to relative changes in external osmolarity, which leads to intracellular Ca^{2+} concentration fluctuations, subject to Ca^{2+} influx from the ECM and subsequent Ca^{2+} release from intracellular stores [40,39].

TRPV4 mediated signalling enhances the chondrogenic transcription factor Sox9, Col-II and Agg expression in chondroprogenitor cell lines [44]. TRPV4 activation reduces the release of prostaglandin E_2 (PGE_2) and nitric oxide (NO) upon IL-1β stimulation following osmotic and dynamic loading in chondrocyte cultures and cartilage explants [42]. In view of TRPV4's apparent positive role in chondrocyte and cartilage homeostasis, its expression decreases in OA cartilage from patients with total knee replacement [45]. An *in vivo* experiment with a rat model suggests that TRPV4 is involved in less severe forms of OA: mice without TRPV4 showed less OA severity symptoms (i.e., lower Mankin score, less structural degeneration and decreased synovitis) [46]. The specific responses of TRPV4 regarding different loads on chondrocyte metabolism are summarized in Table 2.

A study developed by Woods et al. [47] demonstrated that pharmacological activation of TRPV4 significantly increased the canonical response (i.e., Smad3 activation) of TGF-β with the involvement of the Ca^{2+}/calmodulin axis in bovine articular chondrocytes. Critically, this increase was only observed when TRPV4 was activated after, but not before, TGF-β3 stimulation [47]. The synergy role of TRPV4 on the TGF-β signalling suggests a positive forward loop of mechanical stimulation to enhance chondrocyte anabolism. However, the role of TGF-β is controversial, as its expression increases in the early stages of OA. In fact, a shift towards Smad1/5 signalling occurs, associated with increased cell catabolism. Endoglin, a transmembrane homodimeric glycoprotein, tilts the balance towards Smad1/5 increasing harmful effects of TGF-β on AC chondrocytes [48,49].

Insulin Growth Factor 1 (IGF1) reduces the sensitivity of TRPV4 upon hypo-osmotic swelling. The addition of 300 ng/mL of IGF1 in a monolayer culture of chondrocytes from a mouse cell line (i.e., ACTD5) reduced Ca^{2+} influx. In this study, IGF1 increased actin polymerization which causes the inhibition of TRPV4. This could be understood as a negative feedback loop of IGF1 in chondrocytes to balance the intake of Ca^{2+} upon activation of TRPV4 [50]. IGF1 peaks at 15 years old ($\mu = 300$ ng/mL; $\sigma = 0.3614$) and subsequently decreases to approximately one-third of its peak by the age of 65. Then, the relatively protective role of IGF1 to balance the levels of Ca^{2+} might be reduced in the elders [51].

3. Integrins

Integrins are heterodimeric transmembrane receptors that consist of α and β subunits. They connect the ECM to the cytoskeleton and nuclei [62], and also act as transducers of physical cues [63,52,64]. As summarized in Table 3, specific integrins regulate chondrocyte activity (i.e., proliferation, differentiation, inflammation or anti-inflammation) in response to different mechanical stimuli. Chondrocytes express $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$, but this expression is not consistent between healthy and OA chondrocytes [65]. Chondrocytes from OA tissue have also been shown to express $\alpha_2\beta_1$, $\alpha_4\beta_1$, and $\alpha_6\beta_1$ [66]. While the action of inflammatory mediators on the production of degrading enzymes and structural proteins is well known [14], its influence on cell integrins is less known. For instance, human chondrocytes stimulated with synovial fluid from a patient with rheumatoid arthritis reduced the expression of β_1 in alginate bead chondrocyte cultures and increased the expression of α_1 and β_5 [67]. Mechanical signals

Table 3
Integrin responses upon mechanical stimulation.

Integrin	Chondrocyte source	Mechanical stimuli	Response analysed	Ref.
$\alpha_5\beta_1$	Monolayer chondrocyte cultures derived from the articular cartilage of knee rabbits.	Pulses of 16 kPa at 0.33 Hz for 20 minutes.	Increased membrane hyperpolarization.	[52]
$\alpha_5\beta_1$ and $\alpha_V\beta_3$	3D agarose constructs from chondrocyte of femoral condyles of bovine calves.	2.5% strain at 1 Hz for 24 h.	Increase glycosaminoglycan (GAG) incorporation.	[53]
$\alpha_5\beta_1$	3D agarose constructs with chondrocytes from carpaphalangeal joints from 18 month old cattle.	Sinusoidal 0-15% compressive strain at 1 Hz.	Increase GAG incorporation. Decreased PGE_2 and NO production.	[54]
$\alpha_V\beta_3$	AC from a rat model.	cyclic mechanical stress at 2 Hz.	Increase mRNA levels of MMP-14,9, ADAM5 AND Runx2.	[55]
$\alpha_1\beta_1$	Monoculture chondrocytes from AC from mice.	Hypo-osmotic stress of a 14% and 26%.	Ca^{2+} increased influx in a ligand-dependent manner with TRPV4.	[56]
$\alpha_5\beta_1$	Young and adult bovine AC explants.	Cyclic compressive stress of 1 MPa for 6 and 24 h.	Increased α_5 subunit, but not β_1 on the chondrocyte surface.	[57]
$\alpha_5\beta_1$	Monolayer chondrocyte culture derived from 14-16 week old female rabbit AC.	Cyclic tensile strain at varying magnitudes.	Low tensile strain inhibited NO production in the presence of IL-1β. High tensile strain increased NO production, mediated through NFκB.	[58]
$\alpha_5\beta_1$	Monolayer chondrocyte culture derived from 14-16 week old female rabbit AC.	Cyclic tensile strain at varying magnitudes.	Low tensile strain inhibited NO production in the presence of IL-1β. High tensile strain increased NO production, mediated through NFκB.	[58]
$\alpha_5\beta_1$	Monolayer chondrocyte culture derived from postmortem AC from human knee joints.	Pulses of 16 kPa at 0.33 Hz for 20 minutes.	Release of IL-4, leading to the opening of SK channels and membrane hyperpolarization.	[59]
$\alpha_1\beta_1$	Monolayer culture of immortalized human chondrocyte cell lines (C-28/I2 and T/C-28a2).	Cyclic tensile strain for 48 h.	Moderate tensile strain reduced MMPs and IL-6 mRNA expression and phosphorylation of FAK and STAT3.	[60]
$\alpha_1\beta_1$	Monolayer chondrocyte culture derived from rat knee joints.	Cyclic mechanical strain (7%, 30 cycles/min).	Decreased mRNA of Agg and Col-II. Treatment with IL-4 increased mRNA of Agg and Col-II under mechanical stress.	[61]

mediated by integrins might cross-talk with the signals generated by soluble factors (i.e., growth factors and cytokines), influencing the expression pattern of integrins in the chondrocyte membrane. Ultimately, these differential expressions may help explain the contrasting behaviours exhibited by OA and normal chondrocytes upon mechanical loads.

3.1. *Integrin* $\alpha_5\beta_1$

The most prominently expressed integrin in articular chondrocytes is $\alpha_5\beta_1$, which binds to fibronectin (FN), a fibrillar structural protein of the ECM [68]. It is a key factor in transducing mechanical loads and is involved in cell proliferation, differentiation, and migration [69]. It has also been associated with increased cell survival in 2D cultures of human chondrocytes [70]. Additionally, after cyclic compressive stress, the α_5 subunit expression is up-regulated in chondrocytes, while β_1 subunit expression is less affected by mechanical stress [57]. In response to dynamic compression, $\alpha_5\beta_1$ was found to mediate signalling through the TGF-β3 pathway to increase proteoglycan synthesis and chondrocyte proliferation [54]. $\alpha_5\beta_1$ is considered likely to act as a mechanoreceptor through the interaction with FN, which transmits the mechanical signal from the ECM to the cell surface [71]. However, it has been further proposed that the mechanical activation of this integrin is regulated by IL-1β [54].

As mentioned, mechanical overloading activates the $\alpha_5\beta_1$ integrin, which disrupts the actin cytoskeleton and activates the Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and MAPK3 families [58,72]. Cartilage damage follows due to the release of NO, metalloproteinases (MMPs), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), ROS, PGE_2, and pro-inflammatory cytokines. In contrast, reduced mechanical loading activates the IL-1 β receptor, leading to downstream activation of extracellular signal-regulated protein kinases (ERK1/2) and MMPs that lead to tissue damage [72].

Curiously, this integrin is also involved in the hyperpolarization of chondrocytes, as $\alpha_5\beta_1$ also responds to cyclical strain by increasing the membrane hyperpolarization during monolayer culture of chondrocytes [63,52]. Aside from physical stimulation to induce the hyperpolarization of chondrocytes, IL-4 is essential for the opening of Ca^{2+}-dependent K^+ (SK) channels, involved in chondrocyte hyperpolarization [59]. IL-4 acts in an autocrine/paracrine manner following $\alpha_5\beta_1$ activation, which further activates SK channels through the Protein Kinase C (PKC) pathway [64,59]. This pathway is crucial for chondrocyte homeostasis; specifically, it is involved in increased Agg mRNA expression and a decreased MMP-3 mRNA expression, within 1 hour following cyclic compression. Remarkably, arginine-glycine-aspartate (RGD) peptides and anti-IL-4 antibodies blocked these responses [73]. The related mechanotransduction pathway is depicted in Fig. 3.

Nevertheless, this chondroprotective response is absent in chondrocytes from OA cartilage [74] upon the stimulation of this integrin. Instead of releasing IL-4, OA chondrocytes have been shown to produce IL-1β [72,75]. This differential behaviour between

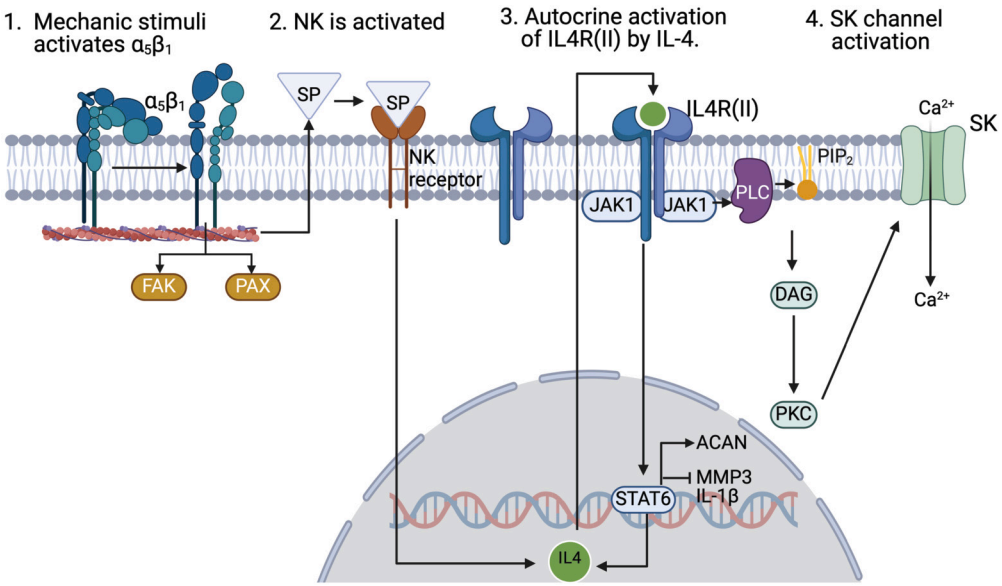

Fig. 3. Schematic view of how 0.33-Hz cyclical strain induces the activation of $\alpha_5\beta_1$ integrin and leads to the expression of IL-4 through the release of substance P (SP). The IL-4 receptor directly activates phospholipase C (PLC) that through diglycerides (DAG) and protein kinase C (PKC) activates small conductance calcium-activated potassium channels (SK) which increase the influx of intracellular Ca^{2+}. Parallelly, the activation of IL-4 induces a pro-anabolic action on chondrocytes as it activates the synthesis of Aggrecan (ACAN) and inhibits the secretion of IL-1β and degrading enzymes (MMP-13). Created with BioRender.com.

OA and healthy cartilage could be explained by the presence of ECM degradation fragments, such as RGD peptides. $\alpha_5\beta_1$ binding to RGD peptides alone, and not to intact FN, increases the catabolic response of chondrocytes from rabbit knees (see Fig. 4). Specifically, RGD peptides increased the expression of MMP-1,3,13 and 9 degrading enzymes. It has also been demonstrated that IL-1β synergies with RGD peptides in the increased expression of MMP-1,3 and 9 [76]; PGE_2 and NO [54]. Further, monolayer cultures of human articular chondrocytes stimulated with FN degradation fragments (FN-f) increased the expression of MMP-13 and pro-inflammatory mediators [77,78]. These actions are mediated by the MAPK pathway (ERK1/2, c-Jun N-terminal kinases (JNK) and p38) [77] and NF-κB [78], respectively. Additionally, FN-f stimulation of $\alpha_5\beta_1$ leads to increased ROS levels in chondrocytes, whereas inhibition of ROS decreases NF-κB and MMPs production [79].

In parallel with ECM degradation fragments, growth factors from the synovial fluid seem to influence the pattern expression of integrins. Chondrocyte culture in monolayer coated with FN increased cell attachment when supplemented with TGF-β. Although Loeser [66] did not directly measure an up-regulation of $\alpha_5\beta_1$ by TGF-β, the increased attachment of chondrocytes on FN-coated cultures suggests that TGF-β regulates $\alpha_5\beta_1$ integrin expression. Further, the pro-anabolic actions of IGF1 require $\alpha_5\beta_1$ to be activated [70].

Interestingly, heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) increases the expression of $\alpha_5\beta_1$. HB-EGF is present in OA cartilage and its expression is increased in OA chondrocytes by FN-f. Although HB-EGF is not required for FN-f stimulation of MMP-13 expression, HB-EGF co-stimulation with FN-f resulted in greater MMP-13 production than FN-f alone. Besides, HB-EGF at 10 ng/ml inhibited Agg expression [80]. Interestingly, inflamed regions of synovium express higher levels of this growth factor compared to normal regions, in patients undergoing knee replacement [81]. Systematic inflammatory processes or obesity are also associated with an up-regulation of HB-EGF, in the blood flow. Accordingly, HB-EGF down-regulation has been reported to offer protection against metabolic syndromes [82]. Metabolic syndromes are considered as a risk factor for OA, and the clinical phenotype of metabolic syndrome-associated OA is defined by the role of systemic and chronic low-grade inflammation [83].

3.2. *Integrin $\alpha_V\beta_3$*

The integrin $\alpha_V\beta_3$ binds to Cartilage Oligomeric Matrix Protein (COMP), FN, vitronectin, and osteopontin as well as RGD peptides [66]. $\alpha_V\beta_3$ is involved in the transduction of dynamic compression, as the inhibition of the integrin in chondrocytes embedded in 3D agarose-based constructs, reduced the synthesis of proteoglycans that was otherwise increased by the same dynamic load [53]. However, $\alpha_V\beta_3$ is involved in the progression of OA in the presence of excessive mechanical stress, triggering downstream inflammatory and degenerative mediators [55].

Similar to $\alpha_5\beta_1$, integrin $\alpha_V\beta_3$ initiates a catabolic response when it comes in contact with degradation products and CD47 through MAPK/ERK signalling, increasing the expression of pro-inflammatory mediators and matrix degradation enzymes [84,85]. See Fig. 4 for details. Downstream from both $\alpha_V\beta_3$ and CD47, ERK is activated and MMPs are up-regulated [84]. Excessive mechanical stimulation also leads to phosphorylation of focal adhesion kinase (FAK) and ERK, which significantly increased the respective expressions of MMP-9, MMP-13, ADAMTS-5, and Runx2, alongside decreased expressions of Col-II and Agg. Chondrocyte pre-treatment of chondrocytes with Cyclo (RGDyK) inhibited $\alpha_V\beta_3$ expression and prevented the increased FAK and ERK phosphorylation [55]. However,

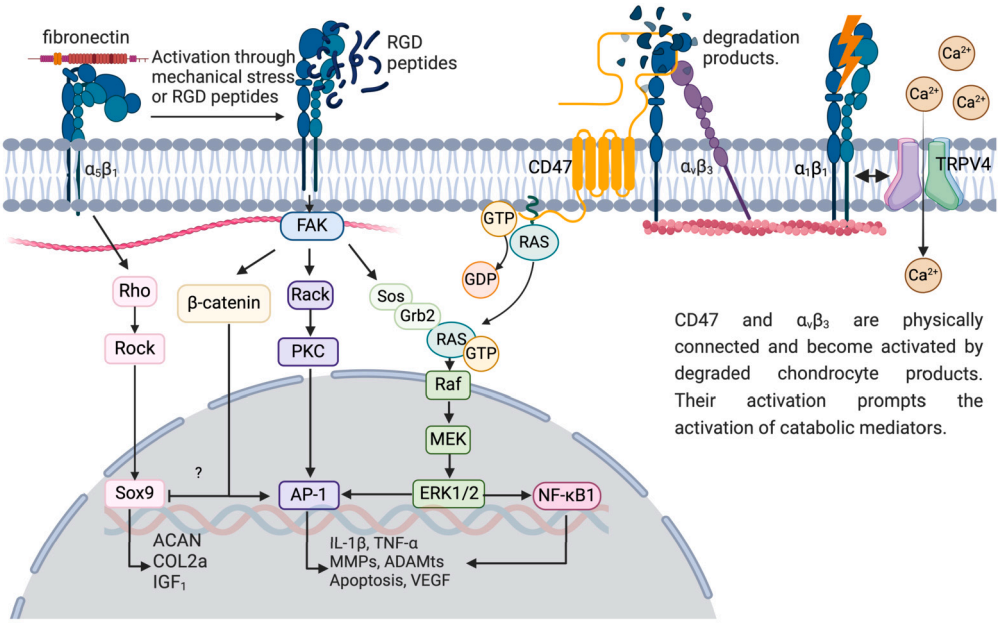

Fig. 4. Integrin regulation by degradation products and its downstream signalling pathways in chondrocytes. **Integrin** $\alpha_5\beta_1$ when bound to fibronectin increases the pro-anabolic pathways of chondrocytes by the activation of Sox9 through Rho-Rock signalling and it is activated upon cyclic dynamic strains. However, RGD peptides that increase upon structural proteins (i.g., Aggrecan (Agg) and Collagen type II (Col-II)) degradation, induce the activation of pro-catabolic pathways through the activation of activation protein 1 (AP-1) and Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factors. Similarly, **integrin** $\alpha_1\beta_3$ upon contact with CD47 and structural protein degradation products activates pro-catabolic events in chondrocytes. **Integrin** $\alpha_1\beta_1$ is involved in sensing hypo-osmotic changes and increasing the membrane potential in a TRPV4-depending fashion. Created with BioRender.com.

the role of $\alpha_V\beta_3$ in chondrocyte inflammation is controversial, as other research suggests that $\alpha_V\beta_3$ could play a protective role by inhibiting IL-1β expression [86,87].

Additionally, $\alpha_V\beta_3$ has been shown to play a role in chondrocyte apoptosis through developmental endothelial locus-1 (DEL1), a protein which increased the severity of OA in knockout mice [88]. Mediated through integrin $\alpha_V\beta_3$, DEL1 protects chondrocytes from apoptosis and anoikis and promotes ERK and protein kinase B (PKB, also known as Akt) activation. While this effect is blocked by RGD peptides and antibodies to $\alpha_V\beta_3$, antibodies to α_V alone had no effect [88]. Hence, the differential effects of the activation of this integrin might be explained by the possible presence of degradation products.

3.3. *Integrin* $\alpha_1\beta_1$

Integrin $\alpha_1\beta_1$ binds to types IV and II collagen and to matrillin-1 [66]. $\alpha_1\beta_1$ plays a crucial role in the transduction of hypo-osmotic stress [89], and increased expression levels of this integrin were found in hypertrophic-like chondrocytes [90]. Under moderate tensile strain, studies showed a β_1-mediated decrease in IL-6 and increased IL-4 and phosphorylation of FAK and ERK1/2 [60,61]. *In vitro* studies have shown that integrin $\alpha_1\beta_1$ are required for hyperpolarization in response to hypo-osmotic changes [56]. According to Servin-Vences et al. [91], α_1 subunit is involved in TRPV4 activation, thus chondrocyte hyperpolarization (Ca^{2+} influx) when cyclic loads are applied. $\alpha_1\beta_1$ appeared crucial in transducing hypo-osmotic stress in chondrocytes, yet likely dependent on TRPV4 [92,56]. Studies have pointed out that deletion of the α_1 subunit inhibits chondrocyte ability to activate TRPV4 and respond to hypo-osmotic stress [56]. Thus, $\alpha_1\beta_1$ plays a key role in the chondrocyte transduction of osmotic stress through cross-talk with TRPV4, independently of matrix binding.

3.4. *Integrins* $\alpha_2\beta_1$ *and* $\alpha_4\beta_1$

Integrin $\alpha_2\beta_1$ binds to type II and IV collagen and to chondroadherin, while $\alpha_4\beta_1$ binds to FN [89]. Although $\alpha_1\beta_1$ also binds Col-II, $\alpha_2\beta_1$ is the preferred receptor [92]. Integrin $\alpha_4\beta_1$ has been implicated in OA cartilage and its expression correlated with the loss of proteoglycans [55,92]. At late stages of OA, α_2 integrin expression is increased and correlates with altered ECM content that occurs due to increased activity of MMPs. Additionally, FN-f and antibodies to this integrin induce the production of MMP-13 through the MAPK pathway [77]. Collagen type X, a marker of cartilage hypertrophy, has also been shown to interact directly through $\alpha_2\beta_1$ [93,94]. However, the up-regulation of these integrins in OA cartilage is still a matter of debate as up to date, no soluble protein has been found to regulate their expression, to date.

Table 4
Primary cilia responses upon mechanical stimulation.

Chondrocyte source	Chondrocyte culture system	Mechanical stimuli	Response analysed	Ref.
Full-depth articular cartilage from bovine metacarpophalangeal joints of 18-month-old steers.	3D agarose constructs at 4×10^6 cells/ml.	Cyclic compression was applied at 0–15% strain at a frequency of 1 Hz for 24 h and 48 h.	*24 h*: Diminished the number of ciliated cells. No incidence on cilia length. *48 h*: Cilia incidence and length decreased, the latter by approximately 30%.	[95]
Chondrocytes from the sterna of 4-d-old mice.	3D agarose constructs.	15% Hydrostatic stress at 1 Hz for 1 h in a sinusoidal fashion.	Agg expression and Ca^{2+} influx increased, the latter mediated by the purinergic-ATP axis.	[96]
Epiphyseal chondrocytes from two-day-old Sprague Dawley rats.	3D chondrocyte pellets 3×10^5 cells/pellet.	1 MPa hydrostatic compression force (1 h on, 1 h off).	Increased Ihh expression.	[97]
Cartilage harvested from the femoral condyles of pigs.	Monolayer culture of chondrocytes at $1 - 1.5 \times 106$ cells/ml.	Hypo-osmotic (280 Osm).	Cilia mediates the influx of Ca^{2+} by TRPV4.	[40]
Articular cartilage from knee joints of 3 days Sprague–Dawley rats.	Monolayer culture.	Cyclic tensile strain at 1 Hz for 22 h with 3 levels of strain.	*1000μ*: Up-regulation of Coll-II, cyclin D1. *2000μ*: The same response, plus IFT88 and ERK up-regulation and mTOR down-regulation *4000μ*: Down-regulation of Coll-II, cyclin D1, reduced cilia length and autophagy.	[98]
Bovine cartilage explants and chondrocytes were obtained from 16-month steers.	5 mm cartilage explants.	Hypo-osmotic (200 Osm).	Inhibits IL-1β mediated NO release via a TRPV4.	[42]

4. Primary cilia

The primary cilia are ideally placed to sense AC deflections, as they are structurally associated with the surrounding ECM [96]. Yet, in contrast to the previously reported mechanosensors, the primary cilium is a complete organelle, which works as microtubule-based antennae to perceive signals and hosts other mechanosensors and signalling pathways (i.e., integrins [99] and TRPV4 [40,42]). The maintenance or depletion of the primary cilia is governed by a process termed intraflagellar transport (IFT), in which protein complexes are moved bidirectionally across the internal structure of the cilium [95]. These protein complexes include key factors of cell growth signalling pathways (e.g., Indian hedgehog (Ihh) [97,100,101] and mammalian target of rapamycin (mTOR)/ERK [98] pathways).

Articular chondrocytes present one non-motile cilium per cell, the incidence and morphological shape of which are sensitive to mechanical cues (see Table 4) and state of AC. In the normal cartilage, the length of the cilium is the lowest in the superficial zone and increases with the distance from the articular surface. In OA tissue, the length of cilia increased nearby the degraded AC surface, resulting in an overall increased proportion of cilia. Besides, the number of cilia per chondrocyte (i.e., incidence) fluctuates in OA tissue according to the mechanical loads transferred to the AC [102]. A study with bovine chondrocytes showed reversible depletion and shortening of cilia upon compressive forces and application time thereof, and cilia incidence remained lower after recovery in a free-swelling state [95]. Mice chondrocytes subject to cyclic tensile strain showed no depletion when stimulated with mild strains, but reduced incidence and length appeared when the chondrocytes were subject to high-intensity strains [98].

Chondrocytes needed the primary cilia to acquire a mechanosensitive synthesis of proteoglycans, related to an increase in the Ca^{2+} influx physiological 15% compressive strain for 1 h in a sinusoidal (1 Hz) fashion [96] and cyclic tensile μ strain for 2 h at 1 Hz [98]. After hypo-osmotic stimulation, cilia also regulate the influx of Ca^{2+} through TRPV4 [40]. A posterior study developed by Fu et al. [42] revealed that hypo-osmotic conditions sensed by cilia and integrated by TRPV4 inhibit the release of IL-1β. Thus, a possible positive feedback mechanism might exist between the primary cilium and TRPV4 channels regulation, as the presence of the primary cilium is essential for TRPV4 activation [40], and TRPV4 activation increases TRPV4 cilia localisation and inhibits pro-inflammatory-related elongations of the cilia [42].

A specific mechanotransduction pathway solely reported in primary cilia is purinergic-ATP signalling, which increases chondrocyte hyperpolarization after hydrostatic compression and ends in an up-regulation of Agg expression [96]. Specifically, the liberation of ATP occurs via the activation of connexin 43 hemichannels after mechanical stimulation, which follows the activation of purine receptors (i.e., P2X and P2Y). This activation initiates a cascade of subsequent events, including calcium signalling, which ultimately modulates chondrocyte function regarding the biomechanical environment [96]. Furthermore, connexin 43 was found on the primary cilium, which suggests the possible involvement of the cilium in the corresponding mechanotransduction pathway [103].

The chondrocyte cilium specifically mediates the activation of the Hedgehog (Hh) pathway through the Ihh ligand of growth plate chondrocytes, when loaded with static hydrostatic stimulation (see Table 4 and Fig. 5 for details) [97]. Besides, the Hh pathway cross-talks with Parathyroid Hormone signalling (PTHrP), which is involved in controlling the chondrocyte phenotype in the growth plate in skeletal development, and in determining the homeostasis that keeps AC healthy. The PTHrP/Ihh feedback loop is a major determinant of the chondrocyte hypertrophic phenotype. While PTHrP inhibits the hypertrophic phenotype, Ihh is considered a hypertrophic marker [104].

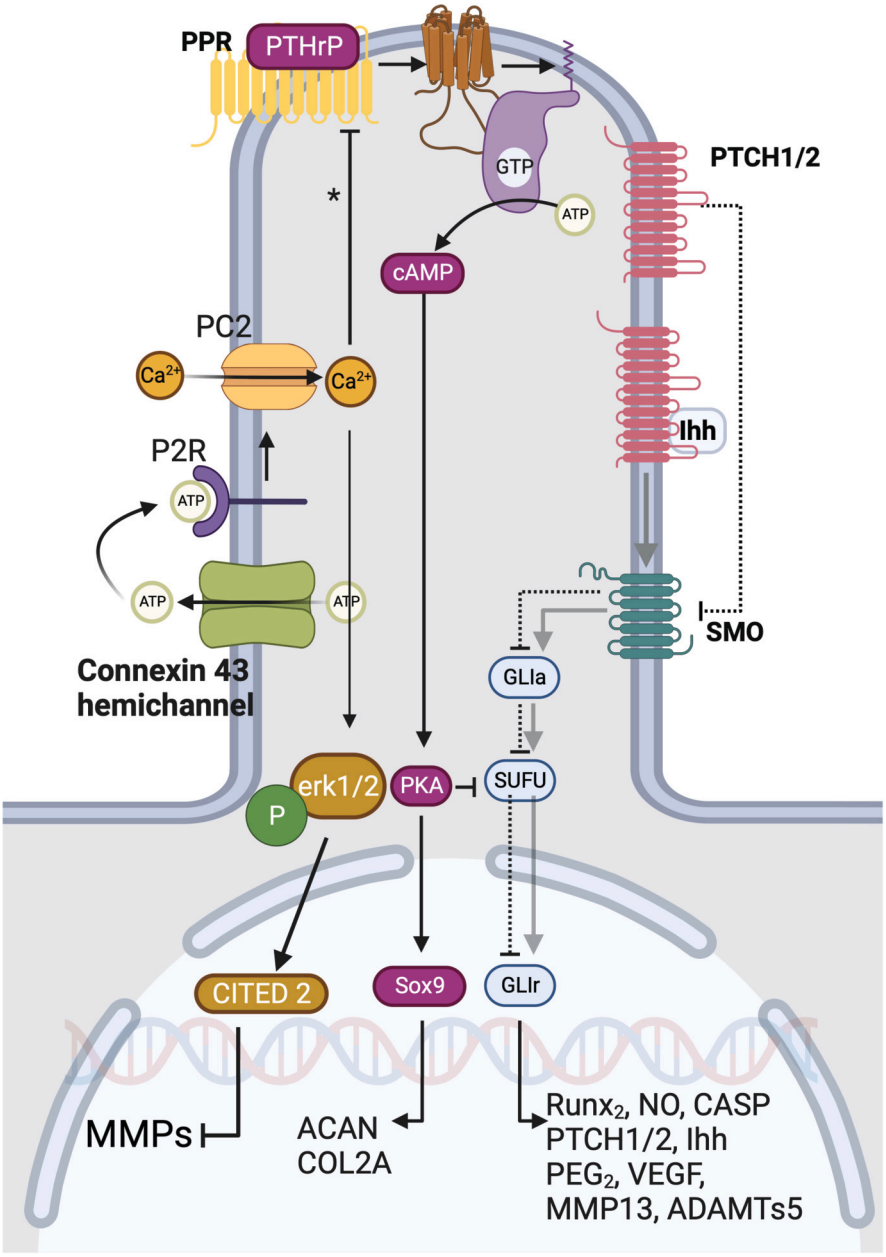

Fig. 5. Primary cilia principal signalling pathways. Connexin 43 hemichannels when open releases ATP from the cell, which binds to the purinergic P2 receptor (P2R) activating the influx of Ca^{2+}. Interestingly, in bone, this pathway (represented with a * in the current figure) down-regulates the activation of the Parathyroid hormone Protein Receptor (PPR). The Parathyroid Hormone-related Protein (PTHrP) favours the activation of Sox9 through protein kinase A (PKA) activation, which at the same time further inhibits the suppressor of fused homolog protein (SUFU), an intracellular mediator of the Ihh pathway. The presence of Indian Hedgehog (Ihh) favours hypertrophy through the activation of Smoothened (SMO) by patched 1/2 (PTCH) receptors and, at the same time, Gli-a, SUFU, and Gli-r are sub-sequentially activated. The latest is a transcription factor that up-regulates the transcription of hypertrophic markers and degrading enzymes. In the absence of the ligand Ihh, this pathway is not active. Created with BioRender.com.

Also, cilia have been reported to be regulated by soluble proteins, such as the case of TGF-β [105]. This growth factor regulates IFT88 gene expression in a post-transcriptional manner. IFT88 is an IFT protein, critical for the maintenance of cilium, chondrocytes and cartilage [105]. These findings suggest that the response of IFT88 upon TGF-β stimulation might be mediated by TGF-β receptor 2, as is the one that induces the polarization of chondrocyte towards a catabolic state and signalling through Smad1/5 (see 3 section). However, the downregulation of IFT by TGF-β was identified by using the ATDC5 murine chondrocytes in monolayer cultures. Therefore, exploring the action of TGF-β in human-derived cells, as well as in 3D cultures might be useful to finally characterize the role of this growth factor.

Parallelly, cilia are also sensitive to, and involved in, the inflammatory processes of chondrocytes. This response is mediated by NF-κB activation upon IL-1β stimulation in monolayer murine chondrocyte cultures [106]. Besides, in monolayer chondrocyte cultures from bovine AC, primary cilia length is increased by IL-1β. This elongation drives the downstream inflammatory response in the form of PGE_2 and NO release [107]. A similar study proved that the elongation of cilium upon IL-1β stimulation is associated with a transient increase in hypoxia-inducible factor 2a (HIF-2a) (a pro-hypertrophic transcription factor) accumulation in human articular chondrocytes [108]. A posterior study suggested that the elongation of cilia, and the downstream actions thereof (i.e., the release of PGE_2 and NO), upon IL-1β stimulation is inhibited by cyclic tensile strain in a TRPV4-dependent fashion [42].

5. The Wnt signalling

Wnts are a family of 19 glycosylated and lipid-modified ligands that bind to the Frizzled (FZD) receptors forming Wnt/FZD complexes that activate the Low-density Lipoprotein-Related Protein 5 or 6 (LRP-5/6) co-receptors. Wnt/FZD/LRP-5/6 leads to the destabilisation of a destruction complex mainly formed by Axin2, Adenomatous Polyposis Coli (APC), Glycogen Synthase Kinase 3 (GSK3), Casein Kinase 1 (CK1) and the E3-ubiquitin ligase beta-transducin repeat-containing protein (β-TrCp). In the absence of an activating stimulus, i.e., a Wnt molecule, this complex ubiquitins β-catenin for ubiquitin-mediated degradation. The disaggregation of the destruction complex following Wnt stimulation promotes the translocation of β-catenin to the nucleus where it binds to different activating DNA regions of Agg, Col-II or IL-β in chondrocytes. FZD, however, have also been reported to associate with other co-receptors, such as the Receptor Tyrosine Kinase-like Orphan Receptor 2 (ROR2) and the Related to Receptor Tyrosine Kinase (RTK), promoting the activation of less characterised signalling cascades. These alternative β-catenin pathways have been grouped under the name of non-canonical Wnt signalling. Their triggering does not promote the accumulation of β-catenin in the nuclei but leads instead to the activation of Calcium Calmodulin Kinase II (CaMKII), PKA and PKC or JNK [109–111] (see Fig. 6 for an illustration of the Wnt signalling). Interestingly, some studies suggest that primary cilia host this signalling pathway [112].

The action of the Wnt signalling in healthy and osteoarthritic chondrocytes is controversial. On the one hand, the over-activation of the canonical Wnt signalling has been related to catabolic actions in AC and is over-expressed in OA patients [113]. Specifically, the activation of the canonical pathway leads to premature hypertrophic-like phenotype development (up-regulation of Collagen type X, MMP-9 and MMP-13) [113]. In a different study, exogenous stimulation of β-catenin by Wnt-3 in monolayer chondrocytes cultures from the elbow and knee of rabbits increased the mRNA expressions of MMP-13; and of ADAMTS-4 and 5, and decreased the mRNA activation of Agg and Col-II [114]. A subsequent study involving human OA cartilage showed that increased ECM deposition correlated with suppressed Wnt activity in chondrocytes. The study also indicated that higher Wnt activity reduced the beneficial effects of the physiological loading regime on chondrocytes [115]. On the other hand, β-catenin inhibition in articular chondrocytes results in AC destruction [116] in an *in vivo* study with mice. Then, tight regulation of Wnt activity is needed to maintain cartilage homeostasis, as both repression and constitutive activation of the β-catenin pathway lead to cartilage breakdown. However, the mechanisms regulating this balanced regulation remain largely unknown. One plausible hypothesis is that Wnt is regulated by endogenous factors, similarly to other mechanoreceptors.

Interestingly, a study from Niu et al. [117] showed that 12% dynamic stretching at 0.05 Hz in ATCD5 chondrocytes increased the nuclear translocation of β-catenin, which is related to an increased mRNA expression of Col-II after one hour of mechanical stimulation. Supplementation of Dickkopf 1 (Dkk1) inhibits the activation of β-catenin by binding to LRP-5/6, under the same 12% dynamic stretching at 0.5 Hz [117]. These findings suggest that Dkk1 exerts a negative feed-forward loop to ensure that β-catenin activation remains in a range that maintains chondrocytes in a healthy state. Accordingly, Dkk1 levels are decreased in plasma and synovial fluid of OA patients compared with healthy individuals [118,119]. Interestingly, increased chondrocyte apoptosis is observed at high concentrations of Dkk1 supplementation (400 ng/ml and not with lower concentrations (100 and 200 ng/ml) [120], which might be related to an excessive inhibition of the Wnt-pathway.

Similar to Dkk1, Wnt-16 activation antagonizes excessive β-catenin activation and protects from AC degradation in [121]. However, Wnt-16 is up-regulated after injury in AC [122]. Then, Wnt-16 might be a weak activator of the canonical Wnt pathway which limits the potent activation of catabolic mediators through the same pathway [122]. This might be related to the fact that Wnt-16 also activates lubricin, an essential joint lubricant that protects chondrocytes from shear stresses and cartilage from mechanically induced breakdown [123].

Finally, sclerostin, produced by osteocytes, decreases the catabolic and hypertrophic actions of Wnt-3 on monolayer chondrocyte cultures of mice. Specifically, it directly reduces the mRNA levels of β-catenin and partially inhibits the effects of this transcription factor on the inhibition of Col-II and Agg; and the activation of MMP-13 and ADAMTS-4. This protective role of sclerostin on chondrocyte metabolism was found to be partially mediated by the inhibition of JNK pathway, a non-canonical event in the Wnt signalling [111], but not the CaMKII/Ca^{2+} event. Additionally, sclerostin inhibits the production of Wnt-5 (a non-canonical trigger of the Wnt signalling) by Wnt-3. A posterior study revealed that the beneficial actions of sclerostin were only active in healthy chondrocytes, inhibiting the pro-catabolic actions of IL-1α on healthy, but not in OA chondrocytes [124].

6. Conclusion

In summary, the progression of chondrocyte pathological changes involves a complex interplay of molecular processes controlled by mechanobiological, autocrine/paracrine, and even endocrine events. A growing body of research has focused on the effects of mechanical cues on the regulation of soluble factors and structural proteins of the ECM. However, the way these soluble factors regulate the direct mechanotransduction events has not been clearly delineated. Not only paracrine/autocrine factors, but also

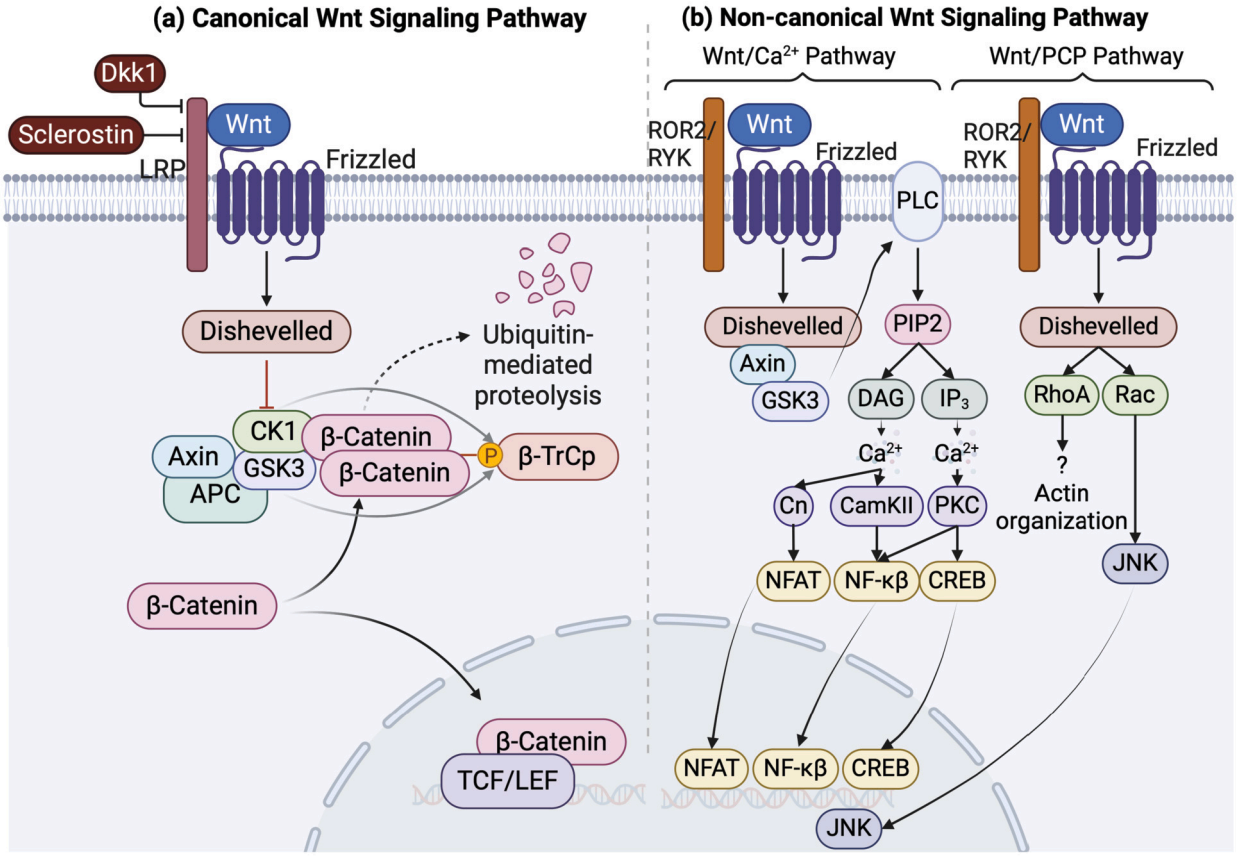

Fig. 6. Wnt signalling pathways. **(a) Canonical (β-catenin dependent) Wnt signaling pathway**. Wnt proteins act as a ligand of Frizzled (FZD) receptors. Upon binding with the co-receptor Lipoprotein-Related Protein 5 or 6 (LRP-5/6), Dishevelled protein is activated and inhibits a degrading complex mainly formed by Axin, Adenomatous Polyposis Coli (APC), Glycogen Synthase Kinase 3 (GSK3), Casein Kinase 1 (CK1) and the E3-ubiquitin ligase beta-transducin repeat-containing protein (β-TrCp). This protein complex targets β-catenin and induces its degradation through ubiquitination and proteases. On the contrary, if Wnt proteins are not present and FZD receptors are not activated, β-catenin accumulates in the cytoplasm and translocates to the nucleus, where it activates a family of transcription factors (e.g., T-cell factor/lymphoid enhancer factor (TCF/LEF)). This same behaviour also occurs when Dickkopf 1 (Dkk1) or sclerostin bind to LRP-5/6, as they inhibit the signal transduction to lower levels and β-catenin is not targeted by the degradation complex. **(b) Non-canonical (β-catenin independent) Wnt signaling pathway**. The same FZD receptor activated by Wnt but bound with other co-receptors (e.g., Receptor Tyrosine Kinase-like Orphan Receptor 2 (ROR2) and Related to Receptor Tyrosine Kinase (RTK)) leads to the activation of the non-canonical pathways, which are mainly two: Wnt/Ca^{2+} and Wnt/Planar Cell Polarity (PCP). The Wnt/Ca^{2+} pathway is characterized by the production of Inositol triphosphate (IP_3) and Diglyceride (DAG) upon activation of Phospholipase C (PLC) acting over Phosphatidylinositol 4,5-biphosphate (PIP2). IP_3 and DAG induce the release of intracellular Ca^{2+}, which activates calmodulin (Calm), Calcium Calmodulin Kinase II (CaMKII) and Protein Kinase C (PKC) to activate transcription factors that will enter the nucleus. The Wnt/PCP pathway activates Rho proteins that intervene in actin organization (i.e., RhoA) or cell proliferation through c-Jun N-terminal kinases (JNK) regulating gene expression. Illustration created with BioRender.com.

endocrine regulation of chondrocytes plays a crucial role in the pathogenesis of OA. Accordingly, OA is eventually seen as a complex multifactorial disorder in which the whole body contributes to the development of joint vulnerability. Thus, apprehending how both local and systemic factors regulate the transduction and integration of mechanical signals by chondrocytes is key to understand better OA pathophysiology.

In this review, we have seen that not only local (i.e., IL-1β, RGD peptides, IL-4, sclerostin and Dkk1) but also systemic hormones (i.e., oestrogen, HB-EGF) regulate the integration of mechanical loads by mechanoreceptors. Remarkably, the relatively new reported role of systemic hormones in the regulation of mechanotransduction events might help to improve our understanding of key predisposing factors to OA such as sex. Women show higher OA incidence than men and shall highly benefit from both early and advanced therapies. We have found that oestrogen is involved in the regulation of Ca^{2+} influx by inhibiting the activation of PIEZO1 channels, commonly activated upon hyperphysiological loads. Hence, women would lose this protective pathway after menopause, usually when OA symptoms start to appear. Besides, we can understand better how metabolic syndromes and systemic inflammation, both common clinical phenotypes of OA patients [83], might alter the chondrocyte response upon mechanical cues. For example, HB-EGF exacerbates the activation of $\alpha_5\beta_1$ [80] and is increased in systematic inflammatory processes or obesity [81], within OA patients. Increased levels of HB-EGF were found actually in the synovial fluid of OA knee joints. Interestingly, the downregulation of this hormone offers protection against metabolic syndromes [82]. Then the activation of $\alpha_5\beta_1$ by HB-EGF arises as a new potential pathophysiologic pathway of primary OA (i.e., without any well-defined predisposing event that triggers the disease).

As per the calcium channels, current findings highlight the local action of the pro-inflammatory mediator IL-1α, which increases PIEZO1 activation and exacerbates the detrimental effects of hyperphysiological mechanical inputs [36]. At the systemic level,

PIEZO1 channels are inhibited by oestrogen [37] and UCN1 [30] hormones. TRPV4 is involved in the activation of pro-chondrogenic genes (i.e., Sox9, Agg and Col-II) [44], the activation of which is eventually induced by TGF-β [47] and IGF1 [50], allowing a balanced influx of Ca^{2+} within chondrocytes (see Fig. 2).

Integrins connect the ECM directly with chondrocytes and their action upon chondrocyte metabolism changes according to the levels of AC degradation. For example, integrin $\alpha_5\beta_1$ by itself [76,74] integrates mechanical cues differently than when it is attached to intact forms of structural proteins, then favouring inflammation [54] and further catabolism [79]. Integrin $\alpha_5\beta_1$ is key to activate the expression of IL-4 in chondrocytes, a cornerstone event in the preservation of chondrocyte homeostasis [64,59]. However, the auto/paracrine role of this anti-inflammatory cytokine is inhibited by RGD peptides, contained in FN-f of the degraded matrix, when bound to this integrin, suggesting the key role of $\alpha_5\beta_1$ integrin in the integration of physiologic mechanical cues, only in healthy cartilage. On top of that, HB-EGF increases the expression of integrin $\alpha_5\beta_1$ and synergies with FN-f to increase the production of MMP-13 [80]. As HB-EGF is a growth factor up-regulated by metabolic syndromes such as obesity [82], it could be another pathological mechanism that promotes the onset of primary OA. Similarly, integrin $\alpha_V\beta_3$ shows different behaviours when bound to degradation products of the AC initiating a downstream catabolic response [66]. While in healthy conditions it up-regulates the synthesis of proteoglycans [53] and down-regulates IL-1β production [86,87], excessive mechanical stress increases inflammation [55] through the activation of the integrin $\alpha_V\beta_3$.

Primary cilia are microtubule-based organelles that host other mechanosensors and signalling pathways. Forty-three hemichannels are only reported to open in this organelle upon mechanical stress [96]. Besides, they specifically host the Hh [97] and parathyroid hormone pathways [104]. Cilium's function in chondrocytes is influenced by various soluble proteins, such as TGF-β, which post-transcriptionally control the IFT88 gene, essential for cilium and cartilage maintenance. The IFT88 response might involve the TGF-β receptor 2, promoting chondrocyte catabolism through Smad1/5 in murine cells [105]. Studying human cells and 3D cultures could clarify TGF-β's role. Concurrently, cilia play a role in chondrocyte inflammation: IL-1β activates NF-κB in these cells [106], extends cilia length [106], and triggers inflammatory agents' release [107]. This elongation also depends on pro-hypertrophic factors [108] and can be inhibited by tensile strain, dependent on TRPV4 activity [42].

The Wnt signalling has emerged as a key pathway for the regulation of chondrocyte homeostasis. While an over-activation of this pathway is detrimental for chondrocytes, complete inactivation also favours hypertrophy and degrading events. Then, a delicate balance of Wnt activation is needed for chondrocyte homeostasis in which sclerostin [124], Wnt-16 [121,122] and Dkk1 [117] would play a key role by endo and paracrine communication effects.

As the field of mechanobiology continues to grow, one of the needs is to establish clear and well-informed guidelines for applying mechanical loads to chondrocytes. The lack of consistent methodologies as seen across Tables 1, 2, 4 currently presents challenges in comparing and synthesizing results across different studies. However, in these mechanobiology experiments, endocrine effects are usually not taken into account, and according to the findings of this review, they act as high-level regulators of mechanotransduction processes. Then, characterizing better their effects on chondrocyte mechanoresponses might leverage the understanding of the pathophysiological mechanisms of OA.

In conclusion, as OA involves the complex actions of endo- and paracrine regulators, researching their actions on chondrocyte metabolism will enrich experimental therapeutic approaches to mitigate and potentially restore cartilage integrity for OA patients.

CRediT authorship contribution statement

Maria Segarra-Queralt drafted the manuscript with the advice of Jérôme Noailly. Maria Segarra-Queralt and Andreu Pascuet-Fontanet helped with the Figure editing. Katherine worked actively in the integrin section. All co-authors revised and approved the final version of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria Segarra Queralt reports financial support was provided by Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) from Generalitat de Catalunya. Jerome Noailly reports financial support was provided by Spain Ministry of Science and Innovation. Jerome Noailly reports financial support was provided by European Research Council. Jerome Noailly and Katherine Crump reports financial support was provided by Marie Skłowdowska Curie International Training Network.

If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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