Joint Bone Spine 86 (2019) 29–35

Available online at ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com/en

Review

The chondrocyte channelome: A narrative review

Ali Mobasheri[a,b,c,*,1], Csaba Matta[d], Ilona Uzielienè[c], Emma Budd[a,1], Pablo Martín-Vasallo[e], Eiva Bernotiene[c]

[a] Department of Veterinary Pre-Clinical Sciences, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom
[b] Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis, Queen's Medical Centre, Nottingham, United Kingdom
[c] Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania
[d] Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[e] Department of Biochemistry and Molecular Biology, University of La Laguna, Tenerife, Spain

ARTICLE INFO

Article history:
Accepted 31 January 2018
Available online 13 February 2018

Keywords:
Osteoarthritis
Articular cartilage
Chondrocyte
Membranome
Channelome
Ion Channel

ABSTRACT

Chondrocytes are the main cells in the extracellular matrix (ECM) of articular cartilage and possess a highly differentiated phenotype that is the hallmark of the unique physiological functions of this specialised load-bearing connective tissue. The plasma membrane of articular chondrocytes contains a rich and diverse complement of membrane proteins, known as the membranome, which defines the cell surface phenotype of the cells. The membranome is a key target of pharmacological agents and is important for chondrocyte function. It includes channels, transporters, enzymes, receptors, and anchors for intracellular, cytoskeletal and ECM proteins and other macromolecular complexes. The chondrocyte channelome is a sub-compartment of the membranome and includes a complete set of ion channels and porins expressed in these cells. Many of these are multi-functional proteins with "moonlighting" roles, serving as channels, receptors and signalling components of larger molecular assemblies. The aim of this review is to summarise our current knowledge of the fundamental aspects of the chondrocyte channelome, discuss its relevance to cartilage biology and highlight its possible role in the pathogenesis of osteoarthritis (OA). Excessive and inappropriate mechanical loads, an inflammatory micro-environment, alternative splicing of channel components or accumulation of basic calcium phosphate crystals can result in an altered chondrocyte channelome impairing its function. Alterations in Ca^{2+} signalling may lead to defective synthesis of ECM macromolecules and aggravated catabolic responses in chondrocytes, which is an important and relatively unexplored aspect of the complex and poorly understood mechanism of OA development.

1. Introduction

Chondrocytes are the primary resident cells in articular cartilage and as the primary architects of the tissue [1], they are responsible for the synthesis and maintenance of all extracellular matrix (ECM) macromolecules [2]. Fully developed cartilage is hypocellular, avascular, aneural, and alymphatic [3] and nutrition derives predominantly from the joint microcirculation via synovial fluid [4]. Chondrocytes are cytoplasmically isolated within the cartilage ECM and nutrient/waste exchange occurs mainly through diffusion, often over long distances [2,5]. Chondrocytes are unique in their ability to exist in a low oxygen tension micro-environment and most energy requirements are obtained through glycolysis rather than oxidative phosphorylation [2]. Even though chondrocytes possess fully functional mitochondria and all the enzymatic machinery for aerobic respiration, it is their hypoxic microenvironment that induces glycolytic pathways for ATP production [6].

Chondrocytes must also exist under pressure and appear to react to cartilage deformation and to the changes in hydrostatic pressure, extracellular ionic composition and streaming potentials induced by mechanical load [7]. Although the phenotype of chondrocytes is perfectly suited for their unique physiological functions, they possess poor potential for repair and regeneration, predisposing cartilage to degeneration in arthritic conditions such as rheumatoid arthritis (RA) and osteoarthritis (OA), which pose a significant clinical global burden. When the functional ECM is compromised, chondrocytes respond in an attempt to repair the damage but often

* Corresponding author. School of Veterinary Medicine, University of Surrey, Daphne Jackson Road, GU2 7AL Guildford, Surrey, United Kingdom.
E-mail address: a.mobasheri@surrey.ac.uk (A. Mobasheri).
[1] The D-BOARD FP7 Consortium, http://www.d-board.eu and The APPROACH IMI Consortium, https://www.approachproject.eu.

https://doi.org/10.1016/j.jbspin.2018.01.012

the stimulated anabolic activity does not result in effective repair due to the extent of the damage and the structural complexity of the ECM [8]. ECM synthesized following injury and inflammation is often biomechanically inferior and is unable to support the tough mechanical demands of load-bearing synovial joints. In addition to mechanical stress chondrocytes are exposed to a plethora of oxidants, pro-inflammatory cytokines, chemokines and catabolic factors in arthritic joints. The stress associated with being exposed to these factors promotes premature senescence, impairs autophagy and promotes cell death [9]. This is why chondrocytes are intimately involved in OA, the most prevalent disease of synovial joints [10]. Therefore, developing therapeutic strategies that support the anabolic activities of chondrocytes is an important area of research in orthopaedics and rheumatology.

The plasma membrane contains a rich complement of membrane proteins, which defines the cell surface phenotype of the cells, also known as the active surfaceome [11,12] or membranome [13]. Membrane proteins play crucial roles in many physiological and pathological processes, particularly in immune and cancer cells. Membrane proteins are also important for cartilage biology and chondrocyte function. They serve as ion, water and osmolyte channels, ion and molecular transporters, enzymes, receptors, and anchors for intracellular, cytoskeletal and ECM proteins and molecular complexes [14,15]. All living cells display electrical properties and possess a membrane potential. However, it is only muscle and nerve cells that are considered, by convention, to be “excitable”, whereas chondrocytes are cytoplasmically isolated and therefore “non-excitable”. Despite being non-excitable cells, the membranome of chondrocytes contains a diverse collection of channels that form the chondrocyte channelome, a concept that we introduced back in 2010 [16]. The chondrocyte channelome appears to be as complex as one might expect to find in excitable cells, although, in the case of chondrocytes, the function of the channelome is still poorly understood (Fig. 1). Emerging evidence suggests that the membrane potential, conductance and capacitance are altered in disease and this implicates the channelome in OA.

The aim of this narrative review article is to summarise our current knowledge of the chondrocyte channelome and its relevance to cartilage biology and OA and extend this to the clinically relevant overlap between OA and cardiovascular disease. We provide an overview of current research on the chondrocyte channelome and highlight its relation to the chondrocyte proteome and membranome and focus on ion channels that are pathophysiologically relevant in OA. The chondrocyte channelome is important for understanding fundamental aspects of chondrocyte biology and has additional potential as a target for novel and pre-existing pharmacological and biological agents. The chondrocyte channelome is also a potential source of novel biomarkers as some membrane proteins are targets of proteases that may cleave membrane and channel proteins, resulting in cleavage products that may be released into the synovial fluid and some of these may potentially be detectable as biomarkers in synovial fluid and serum. The chondrocyte channelome is also likely to be involved in communication with cells in subchonrral bone and synovium plus other connective tissue and inflammatory cells in the joint. Since many channels are “moonlighting” proteins (i.e. proteins that can perform more than one function) this dramatically expands the functional complexity of the chondrocyte channelome, implicating it in functions way beyond “channel activity”.

2. Ionic and osmotic environment of chondrocytes

Research done in our laboratories and the laboratories of a number of other groups over the last two decades has focused on the role of sodium transport systems in chondrocytes. The reason for this is very simple. The cation content of cartilage is high due to the dense concentration of fixed negative charges on proteoglycans and glycosaminoglycans in the cartilage ECM. Therefore, chondrocytes exist in an unusual ionic and osmotic microenvironment [17]. Their extracellular ionic environment is different from that of most cells, with extracellular Na^+ being 250–350 mm and extracellular osmolality ranging from 350 to 450 mOsm depending on the joint studied and the depth and thickness of the cartilage. The high extracellular [Na^+] creates a steep inward gradient for Na^+ entry into chondrocytes, bringing sharply into focus the important physiological role of sodium channels and Na,K-ATPase in these cells [18].

3. Epithelial sodium channels and Na,K-ATPase

We were the first group to demonstrate the presence of the epithelial sodium channel (ENaC), previously undescribed in chondrocytes [19]. We have shown that human chondrocytes express α and β subunits of ENaC and the abundance of these subunits is altered in arthritic chondrocytes. In cartilage tissue derived from rheumatoid arthritis (RA) patients the quantity of α and β subunits is significantly higher than in control (healthy) cartilage samples. On the other hand, ENaC α and β subunits are absent in the chondrocytes of cartilage derived from OA patients. These early observations indicated that some channels in the channelome are directly influenced by inflammatory and disease processes.

Na,K-ATPase, also known as the “sodium pump”, is the membrane-embedded transport system that maintains the intracellular concentrations of Na^+ and K^+ ions, against steep inward and outward electrochemical gradients and ATP provides energy for this exchange in a phosphorylation-dephosphorylation cycle, known as the Albers-Post cycle. This exchange is responsible for consuming up to 45% of the ATP supply in excitable cells. However, the percentage used by the Na,K-ATPase in chondrocytes has not been estimated. Na,K-ATPase is a heterodimeric protomer composed of an α and β subunit and optionally a third subunit, γ, belonging to the FXYD family of proteins. This is a family of small membrane proteins that share a 35-amino acid signature sequence domain, beginning with the amino acid sequence PFXYD and containing 7 invariant and 6 highly conserved amino acids. FXYD proteins are often found to be associated with the α and β containing protomer [20,21]. Interestingly, the γ subunits and the FXYD proteins also exhibit channel forming properties by themselves. The α subunit contains the binding sites for sodium, potassium and other ions [20,22] and regulatory and pharmacological ligands so far described (cardenolides, bufenolides [23]). Four α (α1 to 4) isoforms, three β (β1 to 3) isoforms and seven FXYD have been, so far, described. α and β isoforms associate promiscuously, any α can form quaternary structure with any β giving rise to isozymes of differentially specific kinetic parameters [24] (i.e. ion affinity, catalytic efficiency) leading to substantial physiological, developmental and pharmacological differences [23]. Chondrocytes express multiple α and β subunits including α1, α2, α3, β1, β2 and β3 isoforms in a differential fashion according to developmental stages and pathologies [19] or in cell lines and primary cultures [25]. In chondrocytes derived from RA and OA joints changes in the ionic and osmotic environment alters sodium and potassium content in the ECM and chondrocytes respond to these changes by altering the active isozyme types present in the plasma membrane [19], in order to maintain the intracellular milieu within physiological conditions. Na,K-ATPase therefore plays an important role in ionic homeostasis in chondrocytes, but its expression in chondrocytes remains to be explored in congenital skeletal diseases and in metabolic diseases, including type II diabetes, hemochromatosis, alkaptonuria, Ehlers-Danlos syndrome and other genetic diseases that implicate sodium transport.

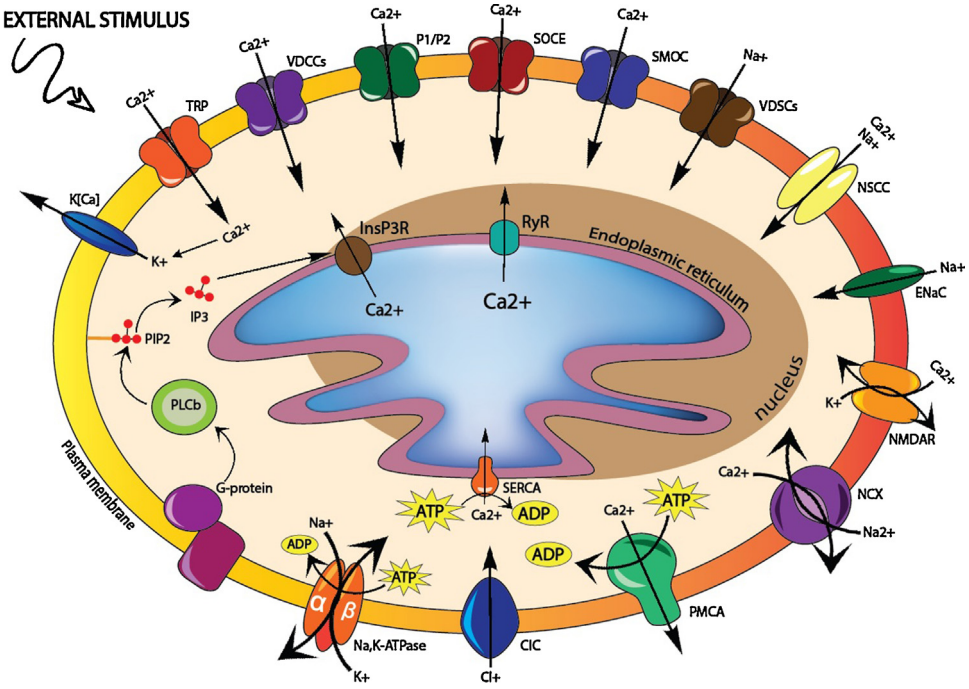

Fig. 1. Schematic representation of the chondrocyte channelome. This figure shows some of the major constituents of the chondrocyte channelome identified to date, with special emphasis on Ca^{2+} channels. Some of these channels show altered expression and function in pathological conditions such as OA. Abbreviations: K[Ca]: Calcium-activated potassium channels; TRP: transient receptor potential; VDCCs: voltage-dependent calcium channel; P1/P2: purinergic receptors; SOCE: store-operated Ca^{2+} channel entry; SMOC: second messenger-operated channels; VDSCs: voltage-dependent sodium channels; NSCC: non-selective cation channel; ENaC: epithelial sodium channel; NMDAR: N-methyl- D-aspartate receptors; NCX: sodium, calcium exchanger; PMCA: plasma membrane calcium ATPase; ClC: chloride channel; Na,K ATPase–sodium pump.

4. Voltage-dependent (activated) calcium channels

Voltage-dependent calcium channels (VDCCs) enable a rapid elevation of cytosolic Ca^{2+} levels and are important for Ca^{2+} signalling in chondrocytes, especially during skeletal development. VDCCs have been classified into the structurally and functionally related families of Ca_V1, Ca_V2, and Ca_V3 [26]. During chondrogenesis VDCCs functionally co-localise with β1 integrins, Na,K-ATPase and ENaC in mechanoreceptor complexes in differentiating mouse limb bud-derived chondrocytes [27]. $Ca_V1.2$ and 3.2 channel subunits were detected in chondrocytes in developing murine embryos and the same L-type Ca^{2+} channels were also identified in the ATDC5 chondrogenic cell line [28]. VDCCs contribute to the elevation of cytosolic Ca^{2+} concentration by insulin-like growth factor-1 in mature articular chondrocytes [29].

Ca^{2+} influx initiated via VDCCs is necessary for signalling pathways regulating ECM synthesis in electrically stimulated articular chondrocytes [30]. The involvement of VDCCs has been demonstrated during in vitro chondrogenesis of micromass cultures established from distal limb buds of 4-day-old chicken embryos; chondrifying cells exhibited large Ca^{2+} transients by exposure to high concentrations of extracellular K^+ [31], implicating the function of VDCCs. Examination of α_1 subunit expression profiles in chicken micromass cultures has identified members of L-type ($Ca_V1.2$ and $Ca_V1.3$); R-type ($Ca_V2.3$); and T-type ($Ca_V3.1$, $Ca_V3.2$, and $Ca_V3.3$) VDCCs [32]. L-type VDCCs play important roles in generating Ca^{2+} influx for oscillations and treatment with nifedipine (10 μM) disrupts the regular pattern of repetitive calcium transients. Furthermore, inhibiting VDCC function with nifedipine abrogates chondrogenesis and almost completely inhibits cell proliferation [32]. Although the above studies have demonstrated the presence of functional α_1 subunits in mature or differentiating chondrocytes, $Ca_V3.2$ was the first α_1 subunit identified as being essential for tracheal chondrogenesis in mice. In mice lacking $Ca_V3.2$ channels, Sox9 expression was shown to be attenuated, accompanied by disturbed tracheal cartilage formation [33]. $Ca_V3.2$ channels were also found to be involved in signalling pathways triggering mechanical load-induced OA. Mice lacking $Ca_V3.2$ $\alpha1$ subunit exhibited significantly lower focal articular cartilage damage than age-matched controls [34]. More work is required to investigate VDCC changes in chondrocytes in early stages of OA and compare expression levels to later stages of OA progression.

5. Ligand-gated $Ca2^+$ entry pathways – Purinergic receptors

Articular chondrocytes express a wide range of both P1 and P2 receptors including A_1, A_{2A} and A_{2B}, as well as different subtypes of P2X ($P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_7$) and P2Y ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$) receptors [35,36]. In a migratory chondroprogenitor cell population (CPC), the mRNA and protein expression of P2X ($P2X_1$, $P2X_4$, $P2X_5$, $P2X_6$ and $P2X_7$, but not $P2X_2$ and $P2X_3$) and all P2Y receptors have been observed and the functionality of $P2Y_1$, $P2Y_2$, $P2Y_4$, as well as $P2X_4$ and $P2X_6$ receptor subtypes have been confirmed [37]. Micromass cell cultures respond to extracellular ATP by elevating intracellular Ca^{2+} levels mainly at the time of chondroblast formation, and administration of ATP to the culture medium stimulated chondrogenesis [38]. The presence of many purinoreceptors, i.e. $P2X_1$, $P2X_4$, $P2X_5$ and $P2X_7$, as well as $P2Y_1$, $P2Y_2$, $P2Y_3$, $P2Y_4$ and $P2Y_5$ has been demonstrated using western blotting in cells of differentiating micromass cultures, the receptor responsible for the rise of Ca^{2+} concentration evoked by extracellular ATP is believed to be the $P2X_4$ receptor [38]. Intracellular ATP oscillations, acting via $P2X_4$ receptors, play a role in pre-chondrogenic condensation [39] and mediate the action of morphogens such as fibroblast growth factor (FGF) and sonic hedgehog (Shh) [40]. Purinergic signalling was also reported to be involved in foetal articular cartilage repair in rat embryos in *vivo* [41]. P2 purinergic receptors activated by extracellular ATP mediate strain-induced mechanotransduction through primary cilia in chondrocytes [42]. The role of P1 and P2 receptors

in OA is less clear and further work is needed to understand the involvement of purinergic signalling in chondrocytes in OA.

6. N-methyl-D-aspartate receptors (NMDAR)

L-glutamate activates both ionotropic (iGluR) and metabotropic (mGluR) receptors. Ionotropic glutamate receptors are ligand-gated cation channels and are classified as either alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors or kainate and N-methyl-D-aspartate receptors (NMDAR), the latter being primarily permeable to Ca^{2+} [43]. Heterotetrameric NMDARs are comprised of two obligatory NR1 subunits and two subunits of four NR2 (NR2A, NR2B, NR2C or NR2D) and/or two NR3 (NR3A or NR3B) subunits [44]. NR1, NR2D and NR3A NMDAR subunits have been shown to be constitutively expressed in developing chondrocytes [45] and mature articular chondrocytes [46] and are active components of mechanotransduction pathways [47]. NR2D-containing NMDARs have also been shown to be important for chondrocyte proliferation [48]. Thus far, there is no published evidence for any direct or mechanistic involvement of glutamate signalling and NMDARs in OA.

7. Transient Receptor Potential (TRP) Channels

Transient receptor potential (TRP) channels play prominent roles in regulating the intracellular Ca^{2+} concentration in non-excitable cells. Mammalian TRP channels are conventionally divided into 6 subfamilies: canonical (TRPC), vanilloid (TRPV), melastatin (TRPM), ankyrin (TRPA), mucolipin (TRPML), polycystin (TRPP), and TRPN ("no mechanoreceptor potential C") [44]. Functioning as non-selective cation channels, members of the TRP subfamilies regulate many basic physiological processes including nociception, temperature, mechano- and osmosensation.

TRP channels appear to be involved in OA. Articular chondrocytes isolated from knee joints of OA patients express various TRP ion channels [49]. Differentiating micromass cultures have been found to express a wide range of TRPV ion channels [50]. TRPA1 mediates acute inflammation and is a potential drug target in OA [51]. The resting membrane potential in chondrocytes is at least partially regulated by TRPV5 [52] and also mediates Ca^{2+} influx and inhibits chondrocyte autophagy in an OA model [53]. TRPV6 may act as a chondroprotective factor in OA as TRPV6 knockout mice exhibited severe osteoarthritic changes, including cartilage fibrillation, eburnation and loss of proteoglycans [54]. Amongst TRP channels, the involvement of TRPV4 in chondrocyte physiology seems to be crucial. TRPV4 is an osmotically active ion channel and it is an important component of chondrocyte mechanotransduction pathways [55–57]. TRPV4 is a positive regulator of Sox9 and pharmacological activation was shown to induce Sox9 reporter activity [58]. A large number of mutations in TRPV4 have been linked to skeletal dysplasias, possibly via increasing follistatin production, which inhibits BMP signalling, leading to skeletal malformations [59].

8. Ca^{2+} Release from internal stores and Store-Operated Ca^{2+} Entry (SOCE)

Ca^{2+} released from intracellular Ca^{2+} stores, primarily from the endoplasmic reticulum (ER), is a main source of cytosolic Ca^{2+} besides influx from the extracellular space across the plasma membrane. Although the existence of store-operated calcium pathways, the primary Ca^{2+} entry to non-excitable cells (via Ca^{2+} release-activated Ca^{2+} channels or CRAC) has long been known, the identification of the molecules that orchestrate SOCE took almost 20 years [60]. STIM1 is a Ca^{2+} sensor protein located in the ER membrane possessing an EF-hand domain that activates SOCE upon Ca^{2+} store depletion, whereas the homologue STIM2 is mainly involved in the maintenance of basal cytosolic and ER Ca^{2+} levels. Upon store depletion, STIM1 redistributes to sub-plasma membrane puncta, where it co-localizes with Orai1 (or Orai2/Orai3) to form the basic subunit required for SOCE. The close apposition of the ER and plasma membrane at the STIM1–Orai1 clusters enables SERCA pumps to rapidly and efficiently refill the ER Ca^{2+} stores [60]. The first study to show the functional expression of STIM1/STIM2 and Orai1 in chondrogenic cells revealed that inhibition of SOCE combined with ER calcium store depletion abolished chondrocyte differentiation and severely diminished proliferation [32]. Functional CRAC channels have also been detected in chondrocyte cell lines [61] and in chondrogenic progenitor cells derived from OA cartilage [37].

9. The Chondrocyte Channelome in OA

As highlighted in the introduction section, OA represents the most common form of a range of degenerative joint diseases that involve active processes by which cartilage and the surrounding tissues (i.e. synovium, peri-articular connective tissues and subchondral bone) respond pathologically to environmental factors. OA is now considered as being far more inflammatory than a disease of simple "wear and tear". However, mechanical loading remains one of the major risk factors in the aetiopathogenesis of OA [62]. Nevertheless, under physiologic conditions, moderate physical activity reduces the risk of cartilage deterioration [63], while mechanical stimuli can improve growth and properties of engineered cartilage tissue [64].

One of the earliest means of chondrocyte response to extracellular mechanical input is a transient increase in intracellular Ca^{2+} levels, occurring within seconds to minutes [65]. At least three types of ion channels that are regulated by different physical signals, including TRPV4 (osmotic and mechanical stress), T-type VDCCs (electrical potential), and mechanical sensitive ion channels (mechanical loading) have been recently demonstrated to play critical roles in controlling the intracellular Ca^{2+} responses of in situ chondrocytes in the loaded cartilage [66]. Ca^{2+} influx via voltage-operated calcium channels and store-operated calcium entry were both reported to regulate intracellular calcium oscillations in chondrogenic cells [26,32].

A positive correlation between Ca^{2+} signalling and matrix synthesis was observed under mechanical load, implying its significant role in mechanotransduction-related anabolic processes [67] while in an altered biochemical and/or biomechanical setting mechanical factors can lead to OA development [62]. Furthermore, joint immobilization has been shown to prevent the development of murine OA and revealed the highly mechanosensitive nature of the protease ADAMTS-5 expression in vivo [68]. There is accumulating evidence to demonstrate that alterations in intracellular Ca^{2+} oscillations are associated with modifications in cartilage ECM synthesis which might be implicated in OA development [44]. One of the causes for chondrocyte dysfunction may be an increase in the Ca^{2+} signalling that occurs during OA, inhibition of Ca^{2+} channels could serve as favourable therapeutic targets for OA [69]. The pro-inflammatory cytokine interleukin 1β (IL-1β) has been shown to modify Ca^{2+} signalling in chondrocytes [70,71]. Application of a combination of pro-inflammatory cytokines including IL-1β, tumour necrosis factor α (TNF-α) plus insulin induced a smaller increase in intracellular Ca^{2+}, while hypotonic shock produced a larger Ca^{2+} increase in chondrocytes from healthy cartilage compared to chondrocytes from OA cartilage, highlighting the need for Ca^{2+} handling for effective adaptation to changes in tonicity even though basal Ca^{2+} was similar in both types of cells [72]. Both

IL-1β [73] and TNF-α [74] were shown to modulate activation and permeability of voltage gated ion channels in neurons. Although such information is not available for chondrocytes, similar activities of inflammatory factors also seem very likely. The available data implies altered calcium signalling responses to distinct loading characteristics and the inflammatory environment of the ECM in OA.

Deletion of TRPV4 4 in mice results in severe OA-like changes, including age and male sex-dependent cartilage fibrillation, eburnation, and loss of proteoglycans [75]. Loss of chondrocyte TRPV4 has been shown to prevent age-related OA development but not mechanical load dependent OA following destabilization of the medial meniscus [76].

Many conditions may influence activity of ion channels and Ca^{2+} signalling during mechanotransduction, which can in turn alter the biosynthetic response. Application of static or dynamic loading, location (zone) of cartilage, intensity, duration of loading, load-induced desensitization or application of superimposed vibrations of different amplitudes and frequency bandwidths may modulate the intracellular calcium levels and switch the cellular responses towards the domination of catabolic events characteristic of OA [67,77,78]. Noteworthy, the intracellular pH and Ca^{2+} response of chondrocytes from OA cartilage was found to be different from the response of chondrocytes isolated from healthy cartilage and dependent on extracellular calcium [72].

10. Crystals modulate calcium signalling in OA

Basic calcium phosphate crystals accumulate in OA cartilage and give rise to numerous manifestations, including OA-like lesions and destructive arthroplasties [79]. Intra-articular injection of calcium phosphate crystals elicit synovial inflammation and cartilage degradation, suggesting a direct pathogenic role in OA [80]. Basic calcium phosphate crystals induce proteoglycan loss and IL-6 expression in human cartilage explants [81]. In a murine OA model related to changes in mechanical loading, increasing deposits of basic calcium phosphate crystals were observed around the joint and correlated with cartilage degradation and IL-6 expression [81]. It has been demonstrated that basic calcium phosphate crystals can induce variation in intracellular Ca^{2+} content and oscillations in chondrocytes, which are mediated through voltage- and non-voltage-dependent Ca^{2+} channels [82]. The changes in intracellular Ca^{2+} content, induced by basic calcium phosphate crystals, were shown to play a pivotal role in catabolic effects on articular cartilage [82].

Modulation of other channels (other than Ca^{2+}) can alter levels of intracellular Ca^{2+} levels and contribute to the development of OA [83]. ClC-7 knockdown or the hypo-osmotic culture conditions, resulting in reduced expression of ClC-7, decreased the acid-sensitive currents in a human chondrocyte cell line, which resulted in enhanced cell death [84]. Thus, downregulation of ClC-7 channels during the hypo-osmotic stress that accompanies OA progression might appear to be one of components of its aetiology.

Expression of a newly identified splice variant in the large conductance Ca^{2+}-activated$K+$(BK) channels, BK channel α subunit (BKαΔe2), was detected in several tissues from humans and mice [85]. This splice variant negatively regulates functional expression of BK channels and modulates essential cellular functions, such as cyclooxygenase-2 gene expression in human chondrocytes.

11. Conclusions

In summary, our knowledge of the chondrocyte is gradually expanding, albeit slowly, compared to most other cells. The study of channels is chondrocytes is clinically relevant. Excessive or inappropriate mechanical loads, an inflammatory microenvironment, alternative splicing of channel components or accumulation of basic calcium phosphate crystals can all result in an altered chondrocyte channelome and changes in membrane potential and conductance. Alterations in Ca^{2+} signalling may further lead to the defective synthesis of ECM macromolecules and contribute to aggravated catabolic responses, which is an important and relatively unexplored concept of the complex mechanism of OA development. Proteomics has highlighted changes in proteins involved in developmental processes in OA [86], and some of these proteins are membrane proteins. Preliminary quantitative proteomic analyses carried out in our laboratory have revealed major changes in the chondrocyte membranome in response to treatment with pro-inflammatory cytokines such as IL-1β and TNF-α (Matta and Mobasheri, unpublished observations). These cytokines downregulate adhesion molecules (integrins and cadherins), intracellular and extracellular chaperones and growth factor receptors but they upregulate receptors for pro-inflammatory cytokines, potentially enhancing the sensitivity of cellular responses to inflammatory signalling. They also increase the abundance of vascular adhesion molecules and zinc transporters that are important for accumulation of zinc, an important co-factor for matrix metalloproteinases. These changes effectively alter the phenotype of chondrocytes, promoting the development of a senescent secretory phenotype.

Many clinically used cardiovascular drugs are also ion channel inhibitors. These are abundantly and protractedly used to treat cardiovascular and neurological diseases and can reach chondrocytes in the joint via the synovial and subchondral microcirculation. A preliminary and pilot study has suggested that certain calcium antagonists may exert beneficial clinical side effects for OA patients [87]. However, the long-term effects of calcium antagonists on chondrocytes and cartilage are unknown and further studies need to be carried out to understand the effects of modulating calcium channels in chondrocytes. From a purely physiological and cell biological perspective, future studies should also focus on further exploration of the roles of connexins, water, osmolytes and putative gas channels in chondrocytes and other cells in the synovial joint in health and disease (i.e. models that recapitulate the inflammatory microenvironment as well as integrating the inappropriate mechanical loads seen in OA joints). We need to fully understand the roles of channels in chondrocytes from healthy joints (i.e. normal chondrocyte function and physiology) before we make any attempt to make deductions or assumptions about their pathophysiological involvement in OA and other joint diseases.

Authors' contributions

All authors have made substantial intellectual contributions to the conception and writing of this review. All authors contributed to manuscript preparation and approved the final version submitted.

Disclosure of interest

This review is the result of a lecture entitled: "The chondrocyte channelome" by Ali Mobasheri, which was presented in the session "Chondrocyte Channels (Role in Mechanotransduction)" or "Channelling the Chondrocyte" at the EULAR 2017 Annual European Congress of Rheumatology held in Madrid, Spain (14–17 June 2017). The authors declare that they have no competing interests. This paper was written by the authors within the scope of their academic and research positions. None of the authors have any relationships that could be construed as biased or inappropriate. The funding bodies were not involved in the study design, data

collection, analysis and interpretation. The decision to submit the paper for publication was not influenced by any the funding bodies.

Acknowledgements

This work was funded by the European Social Fund according to the activity 'Improvement of researchers' qualification by implementing world-class R&D projects' of Measure No. 09.3.3-LMT-K-712 (grant application code: 09.3.3-LMT-K-712-01-0157, agreement No. DOTSUT-215).

The research leading to these results was also funded by the European Commission Framework 7 programme (EU FP7; HEALTH.2012.2.4.5-2, project number 305815; Novel Diagnostics and Biomarkers for Early Identification of Chronic Inflammatory Joint Diseases). This project has also received partial support from the Innovative Medicines Initiative Joint Undertaking under grant agreement No. 115770, resources of which are composed of financial contribution from the European Union's Seventh Framework programme (FP7/2007–2013) and EFPIA companies' in-kind contribution.

C. Matta has been supported by The Bolyai Fellowship of the Hungarian Academy of Sciences, and the European Commission through a Marie Curie Intra-European Fellowship for Career Development grant (project number 625746; acronym: CHONDRION; FP7-PEOPLE-2013-IEF) awarded to A. Mobasheri.

References

[1] Muir H. The chondrocyte, architect of cartilage. Biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. BioEssays 1995;17:1039–48.
[2] Archer CW, Francis-West P. The chondrocyte. Int J Biochem Cell Biol 2003;35:401–4.
[3] Budd E, Nalesso G, Mobasheri A. Extracellular genomic biomarkers of osteoarthritis. Exp Rev Mol Diagn 2018;18:55–74.
[4] Bora Jr FW, Miller G. Joint physiology, cartilage metabolism, and the etiology of osteoarthritis. Hand Clin 1987;3:325–36.
[5] Mardones R, Jofre CM, Minguell JJ. Cell therapy and tissue engineering approaches for cartilage repair and/or regeneration. Int J Stem Cell 2015;8:48–53.
[6] Mobasheri A, Rayman MP, Gualillo O, et al. The role of metabolism in the pathogenesis of osteoarthritis. Nat Rev Rheumatol 2017;13:302–11.
[7] Urban JP. The chondrocyte: a cell under pressure. Bri J Rheumat 1994;33:901–8.
[8] Fukui N, Purple CR, Sandell LJ. Cell biology of osteoarthritis: the chondrocyte's response to injury. Curr Rheumatol Rep 2001;3:496–505.
[9] Musumeci G, Castrogiovanni P, Trovato FM, et al. Biomarkers of chondrocyte apoptosis and autophagy in osteoarthritis. Int J Mol Sci 2015;16:20560–75.
[10] Neogi T, Zhang Y. Epidemiology of osteoarthritis. Rheum Dis Clin N Am 2013;39:1–19.
[11] Neogi T, Felson D, Niu J, et al. Cartilage loss occurs in the same subregions as subchondral bone attrition: a within-knee subregion-matched approach from the Multicenter Osteoarthritis Study. Arthritis Rheum 2009:61.
[12] Bausch-Fluck D, Hofmann A, Bock T, et al. A mass spectrometric-derived cell surface protein atlas. PLoS One 2015;10:e0121314.
[13] Ghosh D, Beavis RC, Wilkins JA. The identification and characterization of membranome components. J Proteome Res 2008;7:1572–83.
[14] Sheng M, Pak DT. Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. Ann Rev Physiol 2000;62:755–78.
[15] Denker SP, Barber DL. Ion transport proteins anchor and regulate the cytoskeleton. Curr Opin Cell Biol 2002;14:214–20.
[16] Barrett-Jolley R, Lewis R, Fallman R, et al. The emerging chondrocyte channelome. Front Physiol 2010;1:135.
[17] Urban JP, Hall AC, Gehl KA. Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. J Cell Physiol 1993;154:262–70.
[18] Mobasheri A, Mobasheri R, Francis MJ, et al. Ion transport in chondrocytes: membrane transporters involved in intracellular ion homeostasis and the regulation of cell volume, free [Ca2+] and pH. Histol Histopathol 1998;13:893–910.
[19] Trujillo E, Alvarez de la Rosa D, Mobasheri A, et al. Sodium transport systems in human chondrocytes. I. Morphological and functional expression of the Na+,K(+)-ATPase alpha and beta subunit isoforms in healthy and arthritic chondrocytes. Histol Histopathol 1999;14:1011–22.
[20] Mobasheri A, Avila J, Cozar-Castellano I, et al. Na+,K+-ATPase isozyme diversity; comparative biochemistry and physiological implications of novel functional interactions. Biosci Rep 2000;20:51–91.
[21] Floyd RV, Wray S, Martin-Vasallo P, et al. Differential cellular expression of FXYD1 (phospholemman) and FXYD2 (gamma subunit of Na,K-ATPase) in normal human tissues: a study using high density human tissue microarrays. Anna Anat 2010;192:7–16.
[22] Vasallo PM, Post RL. Calcium ion as a probe of the monovalent cation center of sodium, potassium ATPase. J Biol Chem 1986;261:16957–62.
[23] Venugopal J, Blanco G. On the many actions of ouabain: pro-cystogenic effects in autosomal dominant polycystic kidney disease. Molecules (Basel, Switzerland) 2017;22.
[24] Blanco G. Na,K-ATPase subunit heterogeneity as a mechanism for tissue-specific ion regulation. Semin Nephrol 2005;25:292–303.
[25] Mobasheri A, Trujillo E, Arteaga M-F, et al. Na(+), K(+)-ATPase subunit composition in a human chondrocyte cell line; evidence for the presence of α1, α3, β1, β2 and β3 Isoforms. Int J Mol Sci 2012;13:5019–34.
[26] Matta C, Zakany R, Mobasheri A. Voltage-dependent calcium channels in chondrocytes: roles in health and disease. Curr Rheum Rep 2015;17:43.
[27] Shakibaei M, Mobasheri A. Beta1-integrins co-localize with Na,K-ATPase, epithelial sodium channels (ENaC) and voltage activated calcium channels (VACC) in mechanoreceptor complexes of mouse limb-bud chondrocytes. Histol Histopathol 2003;18:343–51.
[28] Shao Y, Alicknavitch M, Farach-Carson MC. Expression of voltage sensitive calcium channel (VSCC) L-type Cav1.2 (alpha1C) and T-type Cav3.2 (alpha1H) subunits during mouse bone development. Dev Dyn 2005;234:54–62.
[29] Poiraudeau S, Lieberherr M, Kergosie N, et al. Different mechanisms are involved in intracellular calcium increase by insulin-like growth factors 1 and 2 in articular chondrocytes: voltage-gated calcium channels, and/or phospholipase C coupled to a pertussis-sensitive G-protein. J Cell Biochem 1997;64:414–22.
[30] Xu J, Wang W, Clark CC, et al. Signal transduction in electrically stimulated articular chondrocytes involves translocation of extracellular calcium through voltage-gated channels. Osteoarthritis Cartilage 2009;17:397–405.
[31] Varga Z, Juhász T, Matta C, et al. Switch of voltage-gated K(+) channel expression in the plasma membrane of chondrogenic cells affects cytosolic Ca(2+)-oscillations and cartilage formation. PLoS ONE 2011;6:e27957.
[32] Fodor J, Matta C, Olah T, et al. Store-operated calcium entry and calcium influx via voltage-operated calcium channels regulate intracellular calcium oscillations in chondrogenic cells. Cell Cal 2013;54:1–16.
[33] Lin SS, Tzeng BH, Lee KR, et al. Cav3.2 T-type calcium channel is required for the NFAT-dependent Sox9 expression in tracheal cartilage. Proc Natl Acad Sci U S A 2014;111:E1990–8.
[34] Srinivasan PP, Parajuli A, Price C, et al. Inhibition of T-Type voltage sensitive calcium channel reduces load-induced OA in mice and suppresses the catabolic effect of bone mechanical stress on chondrocytes. PLoS One 2015;10:e0127290.
[35] Knight MM, McGlashan SR, Garcia M, et al. Articular chondrocytes express connexin 43 hemichannels and P2 receptors - a putative mechanoreceptor complex involving the primary cilium? J Anat 2009;214:275–83.
[36] Koolpe M, Pearson D, Benton HP. Expression of both P1 and P2 purine receptor genes by human articular chondrocytes and profile of ligand-mediated prostaglandin E2 release. Arthritis Rheum 1999;42:258–67.
[37] Matta C, Fodor J, Miosge N, et al. Purinergic signalling is required for calcium oscillations in migratory chondrogenic progenitor cells. Pflugers Archiv 2015;467:429–42.
[38] Fodor J, Matta C, Juhasz T, et al. Ionotropic purinergic receptor P2X4 is involved in the regulation of chondrogenesis in chicken micromass cell cultures. Cell calcium 2009;45:421–30.
[39] Kwon HJ, Extracellular ATP. signaling via P2X(4) receptor and cAMP/PKA signaling mediate ATP oscillations essential for prechondrogenic condensation. J Endocrinol 2012;214:337–48.
[40] Kwon HJ, Ohmiya Y, Honma Ki, et al. Synchronized ATP oscillations have a critical role in prechondrogenic condensation during chondrogenesis. Cell Death Dis 2012;3:e278.
[41] Kumahashi N, Ochi M, Kataoka H, et al. Involvement of ATP, increase of intracellular calcium and the early expression of c-fos in the repair of rat fetal articular cartilage. Cell Tissue Res 2004;317:117–28.
[42] He Z, Leong DJ, Zhuo Z, et al. Strain-induced mechanotransduction through primary cilia, extracellular ATP, purinergic calcium signaling, and ERK1/2 transactivates CITED2 and downregulates MMP-1 and MMP-13 gene expression in chondrocytes. Osteoarthritis Cartilage 2016;24:892–901.
[43] Carafoli E. Calcium – a universal carrier of biological signals. Delivered on 3 July 2003 at the special FEBS meeting in Brussels. FEBS J 2005;272:1073–89.
[44] Matta C, Zakany R. Calcium signalling in chondrogenesis: implications for cartilage repair. Front Biosci 2013;5:305–24.
[45] Takahata Y, Takarada T, Osawa M, et al. Differential regulation of cellular maturation in chondrocytes and osteoblasts by glycine. Cell Tissue Res 2008;333:91–103.
[46] Ramage L, Martel MA, Hardingham GE, et al. NMDA receptor expression and activity in osteoarthritic human articular chondrocytes. Osteoarthritis and cartilage 2008;16:1576–84.
[47] Salter DM, Wright MO, Millward-Sadler SJ. NMDA receptor expression and roles in human articular chondrocyte mechanotransduction. Biorheology 2004;41:273–81.
[48] Piepoli T, Mennuni L, Zerbi S, et al. Glutamate signaling in chondrocytes and the potential involvement of NMDA receptors in cell proliferation and inflammatory gene expression. Osteoarthritis Cartilage 2009;17:1076–83.
[49] Gavenis K, Schumacher C, Schneider U, et al. Expression of ion channels of the TRP family in articular chondrocytes from osteoarthritic patients: changes between native and in vitro propagated chondrocytes. Mol Cell Biochem 2009;321:135–43.

[50] Somogyi CS, Matta C, Foldvari Z, et al. Polymodal Transient Receptor Potential Vanilloid (TRPV) Ion channels in chondrogenic cells. Int J Mol Sci 2015;16:18412–38.
[51] Moilanen LJ, Hamalainen M, Nummenmaa E, et al. Monosodium iodoacetate-induced inflammation and joint pain are reduced in TRPA1 deficient mice – potential role of TRPA1 in osteoarthritis. Osteoarthritis Cartilage 2015;23:2017–26.
[52] Lewis R, Asplin KE, Bruce G, et al. The role of the membrane potential in chondrocyte volume regulation. J Cell Physiol 2011;226:2979–86.
[53] Wei Y, Wang Y, Wang Y, et al. Transient Receptor Potential Vanilloid 5 Mediates Ca2+ Influx and Inhibits Chondrocyte Autophagy in a Rat Osteoarthritis Model. Cell Physiol Biochem 2017;42:319–32.
[54] Song T, Ma J, Guo L, et al. Regulation of chondrocyte functions by transient receptor potential cation channel V6 in osteoarthritis. J Cell Physiol 2017;232:3170–81.
[55] Hung CT. Transient receptor potential vanilloid 4 channel as an important modulator of chondrocyte mechanotransduction of osmotic loading. Arthritis Rheum 2010;62:2850–1.
[56] Phan MN, Leddy HA, Votta BJ, et al. Functional characterization of TRPV4 as an osmotically sensitive ion channel in porcine articular chondrocytes. Arthritis Rheum 2009;60:3028–37.
[57] O'Conor CJ, Leddy HA, Benefield HC, et al. TRPV4-mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading. Proc Natl Acad Sci U S A 2014;111:1316–21.
[58] Muramatsu S, Wakabayashi M, Ohno T, et al. Functional gene screening system identified TRPV4 as a regulator of chondrogenic differentiation. J Biol Chem 2007;282:32158–67.
[59] Leddy HA, McNulty AL, Guilak F, et al. Unraveling the mechanism by which TRPV4 mutations cause skeletal dysplasias. Rare Dis 2014;2:e962971.
[60] Frischauf I, Schindl R, Derler I, et al. The STIM/Orai coupling machinery. Channels 2008;2:261–8 [Austin, Tex].
[61] Inayama M, Suzuki Y, Yamada S, et al. Orai1-Orai2 complex is involved in store-operated calcium entry in chondrocyte cell lines. Cell Calcium 2015;57:337–47.
[62] Sanchez-Adams J, Leddy HA, McNulty AL, et al. The mechanobiology of articular cartilage: bearing the burden of osteoarthritis. Curr Rheumatol Rep 2014;16:451.
[63] Racunica TL, Teichtahl AJ, Wang Y, et al. Effect of physical activity on articular knee joint structures in community-based adults. Arthritis Rheum 2007;57:1261–8.
[64] Fitzgerald JB, Jin M, Dean D, et al. Mechanical compression of cartilage explants induces multiple time-dependent gene expression patterns and involves intracellular calcium and cyclic AMP. J Biol Chem 2004;279:19502–11.
[65] Guilak F, Zell RA, Erickson GR, et al. Mechanically induced calcium waves in articular chondrocytes are inhibited by gadolinium and amiloride. J Orthop Res 1999;17:421–9.
[66] Lv M, Zhou Y, Chen X, et al. Calcium signaling of in situ chondrocytes in articular cartilage under compressive loading: Roles of calcium sources and cell membrane ion channels. J Orthop Res 2017, http://dx.doi.org/10.1002/jor.23768.
[67] Weber JF, Waldman SD. Calcium signaling as a novel method to optimize the biosynthetic response of chondrocytes to dynamic mechanical loading. Biomech Mode Mechanobiol 2014;13:1387–97.
[68] Burleigh A, Chanalaris A, Gardiner MD, et al. Joint immobilization prevents murine osteoarthritis and reveals the highly mechanosensitive nature of protease expression in vivo. Arthritis Rheum 2012;64:2278–88.
[69] Berridge MJ. The inositol trisphosphate/calcium signaling pathway in health and disease. Physiol Rev 2016;96:1261–96.
[70] Pritchard S, Guilak F. Effects of interleukin-1 on calcium signaling and the increase of filamentous actin in isolated and in situ articular chondrocytes. Arthritis Rheum 2006;54:2164–74.
[71] Pritchard S, Votta BJ, Kumar S, et al. Interleukin-1 inhibits osmotically-induced calcium signaling and volume regulation in articular chondrocytes. Osteoarthritis Cartilage 2008;16:1466–73.
[72] Sánchez JC, López-Zapata DF. Effects of adipokines and insulin on intracellular pH, calcium concentration, and responses to hypo-osmolarity in human articular chondrocytes from healthy and osteoarthritic cartilage. Cartilage 2015;6:45–54.
[73] Takeda M, Kitagawa J, Takahashi M, et al. Activation of interleukin-1beta receptor suppresses the voltage-gated potassium currents in the small-diameter trigeminal ganglion neurons following peripheral inflammation. Pain 2008;139:594–602.
[74] Czeschik JC, Hagenacker T, Schafers M, et al. TNF-alpha differentially modulates ion channels of nociceptive neurons. Neurosci Lett 2008;434:293–8.
[75] Clark AL, Votta BJ, Kumar S, et al. Chondroprotective role of the osmotically sensitive ion channel transient receptor potential vanilloid 4: age- and sex-dependent progression of osteoarthritis in Trpv4-deficient mice. Arthritis Rheum 2010;62:2973–83.
[76] O'Conor CJ, Ramalingam S, Zelenski NA, et al. Cartilage-Specific knockout of the mechanosensory Ion channel TRPV4 decreases age-related osteoarthritis. Sci Rep 2016;6:29053.
[77] Weber JF, Waldman SD. Stochastic resonance is a method to improve the biosynthetic response of chondrocytes to mechanical stimulation. J Orthop Res 2016;34:231–9.
[78] Diao HJ, Fung HS, Yeung P, et al. Dynamic cyclic compression modulates the chondrogenic phenotype in human chondrocytes from late stage osteoarthritis. Biochem Biophysical Res Commun 2017;486:14–21.
[79] Ea HK, Liote F. Diagnosis and clinical manifestations of calcium pyrophosphate and basic calcium phosphate crystal deposition diseases. Rheum Dis Clin N Am 2014;40:207–29.
[80] Ea HK, Chobaz V, Nguyen C, et al. Pathogenic role of basic calcium phosphate crystals in destructive arthropathies. PLoS One 2013;8:e57352.
[81] Nasi S, So A, Combes C, et al. Interleukin-6 and chondrocyte mineralisation act in tandem to promote experimental osteoarthritis. Ann Rheum Dis 2016;75:1372–9.
[82] Nguyen C, Lieberherr M, Bordat C, et al. Intracellular calcium oscillations in articular chondrocytes induced by basic calcium phosphate crystals lead to cartilage degradation. Osteoarthritis Cartilage 2012;20:1399-408.
[83] Mobasheri A, Lewis R, Ferreira-Mendes A, et al. Potassium channels in articular chondrocytes. Channels 2012;6:416–25.
[84] Kurita T, Yamamura H, Suzuki Y, et al. The ClC-7 Chloride Channel Is Down-regulated by Hypoosmotic Stress in Human Chondrocytes. Mol Pharmacol 2015;88:113–20.
[85] Suzuki Y, Ohya S, Yamamura H, et al. A new splice variant of large conductance Ca(2+)-activated K(+) (BK) channel (subunit alters human chondrocyte functison. J Biol Chem 2016;291:24247–60.
[86] Pitsillides AA, Beier F. Cartilage biology in osteoarthritis – lessons from developmental biology. Nat Rev Rheumatol 2011;7:654–63.
[87] Daniilidis K, Georges P, Tibesku CO, et al. Positive side effects of Ca antagonists for osteoarthritic joints-results of an in vivo pilot study. J Orthop Surgery Res 2015;10:1.