Potassium channels in articular chondrocytes

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Chondrocytes are the resident cells of cartilage, which synthesize and maintain the extracellular matrix, and the range of known potassium channels expressed by these unique cells is continually increasing. Since chondrocytes are non-excitable, and do not need to be repolarized following action potentials, the function of potassium channels in these cells has, until recently, remained completely unknown. However, recent advances in both traditional physiology and "omic" technologies have enhanced our knowledge and understanding of the chondrocyte channelome. A large number of potassium channels have been identified and a number of putative, but credible, functions have been proposed. Members of each of the potassium channel sub-families (calcium activated, inward rectifier, voltage-gated and tandem pore) have all been identified. Mechanotransduction, cell volume regulation, apoptosis and chondrogenesis all appear to involve potassium channels. Since evidence suggests that potassium channel gene transcription is altered in osteoarthritis, future studies are needed that investigate potassium channels as potential cellular biomarkers and therapeutic targets for treatment of degenerative joint conditions.

Introduction

Articular cartilage is subjected to extraordinary stresses due to loading pressures resulting from everyday physical activity.[1] Chondrocytes are the only cells found in the extracellular matrix (ECM) of cartilage and have the ability to detect and respond to the changes caused by these mechanical loads by altering their metabolic state. The ECM consists of type II collagen fibers and aggregating proteoglycans, which give cartilage its tensile strength and rigidity, enabling it to resist stresses. This mechanotransduction results in changes of matrix synthesis and

degradation rates.[2,3] Mechanically induced cell membrane deformation is one of a number of possible pathways for mechanotransduction and involves a number of membrane proteins (**Fig. 1**).[4-7] For example, the non-selective transient receptor potential vanilloid V4 (TRPV4) is a widely expressed sensor of membrane stretch.[8] Since this channel is also found in chondrocytes, it is thought to be key to mechanotransduction in these cells too.[9,10] Changes in ionic and osmotic pressure, ion transport, fluid flow and electrical current across the chondrocyte membrane are all important mechanotransduction phenomena in cartilage. With inappropriate mechanical loading of the joint, as occurs with traumatic injury, ligament instability, bone misalignment or excessive weight bearing, cartilage exhibits manifestations characteristic of osteoarthritis (OA). The composition of cartilage reflects the net response of the chondrocytes to the prevailing loading pattern, with cartilage proteoglycan content highest in heavily loaded regions and removal of load leading to cartilage thinning and proteoglycan loss.[2] Breakdown of cartilage matrix in OA involves degradation of ECM macromolecules and decreased expression of chondrocyte matrix proteins necessary for normal joint function. OA cartilage often contains increased amounts of type I collagen and has increased synthesis of proteoglycans characteristic of immature cartilage.[11,12] Over the last decade, the focus of research on chondrocyte mechanotransduction has shifted from the biochemical responses of the ECM to the chondrocyte plasma membrane and its complement of ion channels. In particular, recent evidence has focused on potassium ion channels. Since OA is strongly associated with aging, it would also be of great interest to understand how those signaling and regulatory pathways change over a lifetime. This knowledge may be important for formulating therapeutic strategies for the rational design of pharmaceutical compounds capable of modulating the metabolic and biosynthetic activities of chondrocytes. Combined immunohistochemical and physiological investigations of chondrocytes have shown the expression of a number of membrane channels,[13] including aquaporin water channels[14-16] as well as ENaC,[17] NMDA,[18-20] calcium,[21-24] chloride[25,26] and sodium ion channels.[27,28] The most widely reported ion channels of chondrocytes are, however, the potassium channels. While it was soon
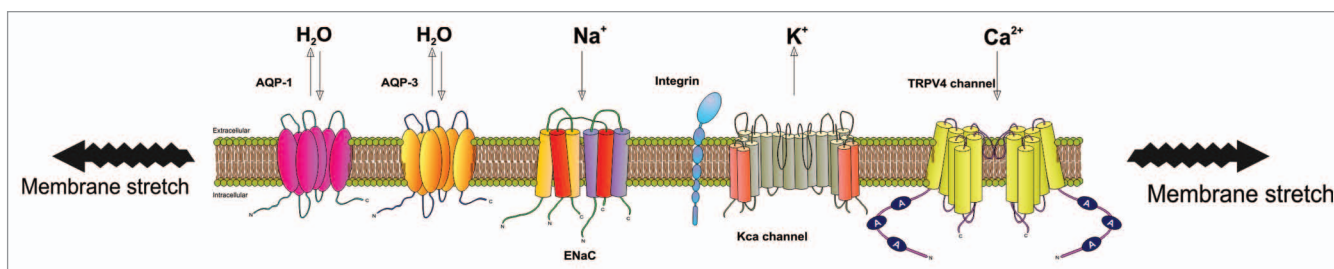
Figure 1. Schematic illustration of plasma membrane proteins, ion and water channels potentially involved in mechano-electrochemical responses in chondrocytes under mechanical load. This model incorporates β-1 integrins along with ion channels identified in chondrocytes and chondrocyte-like cells. Aquaporin 1 (AQP1) and aquaporin 3 (AQP3) mediating water transport,[14-16] epithelial sodium channels (ENaC) mediating sodium and/or cation influx,[17,144,145] calcium-activated potassium channels[84] and transient receptor potential vanilloid type four (TRPV4) channels, mediating cation influx, with a six times greater permeability for calcium over sodium.[10,47] It has been hypothesized that these channels respond to membrane stretch upon compression of cartilage. The diagram illustrates schematics of complete AQP and ENaC channels, but cross-section of TRPV4 and a hypothetical K_{Ca} channel.

demonstrated that potassium ion channel modulation affected chondrocyte biosynthetic activity, little was known about potential mechanisms that may be involved. In this review, we discuss the latest developments in chondrocyte potassium channel physiology and show how several potential roles in chondrocyte cellular signaling, survival and function are now emerging.

An Overview of the Potassium Channel Superfamily

The human genome contains over 70 different potassium channel genes and, arguably, potassium channels are the largest family of membrane ion channels. Diversity is increased further by alternative splicing of α-subunit genes, and by the presence of both homomeric and hetero-tetrameric assemblies of the various α-subunits. Despite the high selectivity most potassium channels have for K^+ ions over other ions, many retain a high permeability and allow passive flow of large quantities of K^+ ions along their electrochemical gradient. In many cell types studied to date, potassium ion channels play a role in stabilizing the membrane potential, and in excitable cells they are important in returning the membrane potential to its resting state following an action potential; "repolarization." In nerves, the many different types of calcium and voltage-sensitive potassium channels allow neurons to exhibit complex firing patterns, with varying frequencies and depths of after-hyperpolarizations. Potassium ion channels also have roles in modulation of neurotransmitter release, hormone secretion, epithelial electrolyte transport, cell proliferation, regulation of cellular volume, apoptosis, tumor progression and maintenance of potassium homeostasis.[29-34] Diseases involving potassium channels (channelopathies) are not uncommon and include hyperinsulinemia, a cardiac dysrhythmia called long QT syndrome and certain epilepsies.[35-40] Potassium channels are already the targets of a number of medicines for diseases including hypertension, diabetes and angina, but are likely to become targets of further drugs in the future as more evidence of potassium channel involvement in tumor progression emerges.[41-45]

The tertiary structure of a typical potassium channel includes a central ring of pore-forming α-subunits associated with between one and four accessory proteins. Each α-subunit possesses a pore loop (P-domain), which lines a specialized part of the pore called the "selectivity filter." As the name suggests, the selectivity filter confers the ability of potassium channels to discriminate between different ions.[46] However, the number of transmembrane domains in each of these α-subunits varies considerably and is the basis for dividing the potassium channel super-family into three distinct groups.[47] The first of these groups consists of potassium channels with six transmembrane (6TM, or 7TM in the case of K_{Ca}) domains in each of their α-subunits. Four such subunits assemble to form functional voltage-gated (Kv) and Ca^{2+}-activated K^+ channels (K_{Ca}).[32,48-50] The second group is made up of K^+ channels that have four transmembrane (4TM) domains per subunit, these "tandem pore" channels TWIK, TREK, TASK, TALK, THIK and TRESK are thought to equate to "leak" channels. They contain two pore domains in each α-subunit and the functional channel probably forms as a dimer.[29,51] Finally the 2TM domain inward rectifier family, itself a diverse family of channels including the "energy sensing," ATP-sensitive K^+ (K_{ATP}) channel.[31,52-54]

Potassium Channels in Chondrocytes

This review will focus on recent experiments that characterize both the identity and function of potassium channels in isolated, cultured and chondrocytes in situ within cartilage. A wide range of potassium channels have now been identified, including members of most potassium channel subfamilies and we are also now in a position to hypothesize about their roles in metabolic regulation, mechanotransduction, cell volume regulation, apoptosis and cell proliferation.

Voltage-Gated (Kv) Potassium Channels

A number of investigators including our group have identified functional voltage-gated (Kv) channels in chondrocytes.[26,55-62] Since chondrocytes are non-excitable cells (i.e., do not conduct action potentials), the role of these channels is not obvious. One common hypothesis is that the Kv conductance contributes to a resting membrane potential (RMP).[56,58] Evidence would suggest that there are several different species of Kv channels expressed

in chondrocytes, possibly changing with chondrocyte development and maturation.[59] Initial studies showed that chondrocytes express a Kv-like conductance with relatively positive midpoints of voltage activation (ranging from -12 to +25 mV),[55,56,61,62] similar to that expected for Kv1 or 4 subfamilies.[30] The pharmacology of the chondrocyte Kv current is clearly not consistent with any one Kv ion channel subtype; suggesting that a mixed population may be present.[26,55-59,62] RT-PCR and immunohistochemical data support this conclusion. Kv4.1 and Kv1.3 were detected in maturing chondrocytes derived from chicken mesenchymal stem cells.[59] Although Varga et al. (*ibid*) failed to detect plasma membrane expression of Kv1.3, it is interesting to note that the recombinant Kv1.3 activation, inactivation and current-voltage profiles,[63] are somewhat similar to those of the Kv current observed in native chondrocytes.[56,62] In a recent mouse articular chondrocyte study, combining quantitative RT-PCR and electrophysiology, high-abundance of Kv1.6 transcript was detected.[58] In purely descriptive terms, the chondrocyte Kv current has consistently been shown to inactivate relatively slowly. Significant inactivation generally becomes apparent only above approximately -10 mV.[56,58,62] The tertiary structure of Kv channel subunits can consist of heteromultimers[64] and the chondrocyte voltage-gated potassium current profile does not perfectly fit any one pure Kv subtype; we would again suggest chondrocyte Kv channels may also be heteromultimeric.

Calcium-Activated Potassium Channels

Ca^{2+}-activated potassium (K_{Ca}) ion channels have also been identified in chondrocytes by several groups.[65-75] Our own recent work has identified the large K_{Ca} (BK) channel at high density in equine chondrocytes.[69] While these channels are clearly activated by low levels of intracellular calcium and inhibited by low concentrations of tetraethylammonium (TEA), indicative of BK, they are only weakly inhibited by the selective BK inhibitor toxin iberiotoxin (*ibid*). Interestingly, co-expression of the accessory β-subunit has been shown to reduce the efficacy of iberiotoxin.[76] We, and others, have identified immunostaining for both the α- (KCNMA1)[60,69] and β- (KCNMNB1) subunits of BK channels, particularly in the superficial zone of cartilage.[69] KCNMA1 expression has also been confirmed with RT-PCR.[60,75] Interestingly, although BK channels are clearly identifiable in "normal" cartilage, their expression appears changed in OA cartilage,[34] suggesting a possible involvement with progression of the disease.

There are several possible roles for K_{Ca} channels in chondrocytes. The ionic composition of cartilage is rather different to that of plasma, in particular there is a chronic hypertonicity with sodium ions being elevated by some 100 mM.[77] This unusual ionic composition of cartilage will change upon loading,[2] as does the ionic composition of chondrocytes themselves.[78] Since intracellular calcium changes under these loading conditions, calcium-activated channels such as K_{Ca} are ideally positioned to mediate scellular responses. Whether they play a direct role in maintenance of chondrocyte volume is yet to be proven. However, there is circumstantial evidence that K_{Ca} is involved with the volume reduction mechanism. For example, K_{Ca} can be activated by membrane stretch and pressure[69,79] and parathyroid related peptide, which suppresses the hypertrophy of chondrogenesis,[80] increases chondrocyte K_{Ca} activity via a PKA-dependent mechanism.[72] The implication is that downregulation of K_{Ca} would be necessary to allow volume increases in chondrocyte hypertrophy.

In other tissues (for example, glomus cells of the carotid body), BK channels often demonstrate oxygen sensitivity in cell-free membrane patches suggesting that a significant component of the oxygen-sensing machinery must be closely associated with the channel protein complex.[81] Recent proteomic studies have identified the constitutively expressed form of hemoxygenase, hemoxygenase-2 (HO-2), as a BK α-subunit protein partner. This enzyme-ion channel complex has been suggested to be directly involved in hypoxic inhibition of BK channel activity.[82,83] It is therefore possible that the chondrocyte BK channel may also be involved in oxygen sensing. The presence of the low conductance, Ca^{2+}-activated potassium channel transcripts subtypes SK1 (KCNN1, K_{Ca}2.1), SK3 (KCNN3, K_{Ca}2.3) and the intermediate Ca^{2+}-activated potassium channel transcript (IK, KCNN4, SK4, K_{Ca}3.1) have also been demonstrated in OUMS-27 cells (a chondrosarcoma cell line),[75] albeit at relatively low abundance compared with KCNMA1. The other SK channel subtype, SK2 was not detected.[75] SK channels (SK1, SK2 and SK3) have a distinct pharmacological profile including a notably low sensitivity to TEA, but high sensitivity to apamin, consistent with the pressure-activated channel first observed by Wright et al.[84] Both SK and BK have been proposed to be involved with response to osmotic challenge in chondrocytes[74,84] and this hypothesis is discussed in more detail below. Another recent discovery is that histamine, an important mediator of inflammation, activates BK channels and significantly hyperpolarizes OUMS-27 cells.[75] The proposed mechanism is itself quite interesting; the relatively high input resistance chondrocyte is hyperpolarized from a RMP of -20 mV by some 30 mV. The authors propose that this allows increase in intracellular Ca^{2+} by increasing the driving force for passive entry of Ca^{2+} via some as-yet-unknown constitutive pathway. This is exciting, since it provides a direct link between inflammation and chondrocyte function. A similar hyperpolarization/Ca^{2+} entry mechanism has also been proposed to occur when chondrocytes are placed under hydrostatic pressure.[79] It should be noted that for any cell type, a hyperpolarization-driven passive entry of Ca^{2+} ions will lead to some degree of positive feedback, since the newly elevated intracellular Ca^{2+} will, in turn, activate further K_{Ca} (discussed in detail in Nilius and Droogman's review of endothelial cell Ca^{2+} handling[85]). Since cartilage has considerably greater extracellular Ca^{2+} than plasma, the potential for this positive feedback scheme could be even greater for chondrocytes than for endothelial cells.[77]

Inward Rectifier Potassium Channels

Inward rectifiers (Kir) allow potassium ions to move easily into the cell at membrane potentials negative to the potassium equilibrium potential (E_K), but restrict potassium outflow at potentials positive to E_K. The asymmetry in the current-voltage relationship

of strong inward rectifiers results from either the channel's molecular characteristics and/or its susceptibility to voltage-dependent block by Mg^{2+} and/or intracellular polyamines.[86,87] In many cell types, this results in an ion channel which stabilizes the membrane potential by actively resisting membrane depolarisation. With the exception of Kir 6.x, study of inwardly rectifying channels is severely hampered by a lack of selective pharmacological inhibitors. Although Kir channels are blocked by polyamines[86] and many inorganic ions such as Ba^{2+}, Cs^+, Ag^+, etc.,[88,89] pimozide[90] and CEC[91] are the only pharmacological inhibitors of other Kir channels known to the authors. Interestingly, pimozide does inhibit chondrocyte K^+ efflux in response to hypotonic challenge[92] and Kir2.2 (KCNJ12) was identified by Clark et al. in a very thorough examination of cultured human chondrocytes,[60] but with the exception of Kir 6.x, a systematic analysis of chondrocyte inwardly rectifying potassium channels has yet to be conducted.

Kir 6.x are unique members of the greater inwardly rectifying potassium channel subgroup, which, when combined with a sulphonylurea receptor (SUR), form the ATP-sensitive potassium K_{ATP} channels. They are one of the more weakly rectifying Kir channels and, to date, these are the only inwardly rectifying potassium channels categorically identified in chondrocytes.[93] K_{ATP} channels are closed by the binding of intracellular ATP and, thus, couple changes in cellular metabolism to membrane excitability.[94] They are expressed in pancreatic β-cells, certain types of neurons, cardiac, skeletal and smooth muscle and are important in regulating secretory processes, cardioprotection and muscle tone.[54,95,96] Their properties vary considerably from tissue to tissue, reflecting heterogeneity in channel structure. K_{ATP} channels form as 4+4 octamers of Kir 6.x pore-forming subunits and proteins.[97] Two Kir6 subunits, Kir6.1 and 6.2, have been identified, and two SUR genes are known, SUR1 and SUR2, the latter giving rise to SUR2A and SUR2B by alternative splicing. β-cell and cardiac K_{ATP} channels comprise Kir6.2/SUR1 and Kir6.2/SUR2A respectively, and it is likely that the dominant channel in most vascular smooth muscle comprises Kir6.1/SUR2B.[98,99] In our own experiments, we demonstrated the presence of single channel activity, which was inhibited by both intracellular ATP and by the sulphonylurea compound glibenclamide. This is strongly suggestive of the presence of the full K_{ATP} complex, the SUR protein together with either Kir6.1 or Kir6.2. In subsequent immunohistochemical studies we have located both Kir 6.1 and Kir6.2, SUR2A, SUR2B (unpublished observations). Moreover, western blot analysis showed that Kir 6.1 does not change with age or through the progression of OA (unpublished observations). Since we were able to exclude the presence of SUR1 by immunohistochemistry (unpublished observations) and by comparisons of glibenclamide sensitivities in other tissues, SUR2 is most likely the SUR subunit making up K_{ATP} channels in human chondrocytes.[98-100]

The discovery of K_{ATP} channels in chondrocytes has quite striking implications. Cartilage is an avascular connective tissue in which the availability of oxygen and glucose is significantly lower than synovial fluid and plasma, particularly in deeper layers. Chondrocytes are capable of existing under hypoxic conditions.

In fact, chondrocytes need such conditions for survival, chondrogenesis and matrix synthesis.[101-103] Therefore, the chondrocyte requires sophisticated mechanisms to sense the quantities of available oxygen, glucose and ATP levels as well as the concentrations of other important metabolites. Presumably, chondrocyte K_{ATP} channels are involved in coupling metabolic and electrical activities through the sensing of extracellular glucose and resultant intracellular ATP levels in a scheme analogous to that seen in pancreatic β-cells.[54] This raises the distinct possibility that nutritional state of joints and synovial fluids may influence the functioning of chondrocytes and, thus, the health of cartilage. Our work also highlights the possibility that altered K_{ATP} channel function in OA chondrocytes may result in impaired intracellular ATP sensing and sub-optimal metabolic regulation; if this turns out to be the case, it provides a possible novel therapeutic target.

Tandem Pore Potassium Channels (K2P)

Tandem, or two-pore potassium channels, are the most recently discovered family of potassium channels. The key defining feature of these channels is that each subunit has two domains (P-domains), which contribute to the ion channel pore,[104,105] whereas other potassium channels have only one.[106] They can be thought of as being structurally analogous to two inwardly rectifying α-subunits joined together and, thus, form dimers in the membrane rather than the more common tetramer.[107] These channels are most commonly thought of as contributing the elusive "leak" conductance[108] seen in neurons and muscle.[106] However, they have now been identified somewhat ubiquitously and serve as both stabilizers of the RMP and sensors for pH, stretch and several other physiological signals.[105] Members of this family were discovered recently in human chondrocytes.[60] The presence of TASK-2 was shown by immunocytochemistry (*ibid*) and three K2P gene transcripts [KCNK1 (TWIK-1), KCNK5 (TASK-2) and KCNK6 (TWIK-2)] were detected with quantitative RT-PCR.[60] TASK-2 gene transcription changes during OA suggest that TASK-2 loss could be involved with the progression of OA (see below, "Potassium channel involvement in maturation, proliferation and viability").

Biomechanical Signaling and Potassium Channels in Chondrocytes

Chondrocytes are exposed to biomechanical signals occurring from at least two sources. First, as pressure is applied to joints, water is squeezed out and there is an increase in osmolarity. Conversely, as the pressure is released, water returns to the cartilage and osmolarity decreases. Since intracellular osmolarity must match extracellular osmolarity, mechanisms must clearly be in place to allow appropriate influx and efflux ions and/or water. Second, membrane compression naturally involves instantaneous membrane deformation. Many of these changes occur in the context of volume regulation, discussed below. However, the biomechanical signal of membrane deformation appears to be far more central to chondrocyte function than this. A consensus is emerging that static compression decreases chondrocyte

production of ECM,[109] whereas dynamic compression increases it.[110-113] The sequence of events which lead to proteoglycan secretion in response to mechanical stimulation is not known, but it is thought to involve ion channels, since various ion channel blockers themselves reduce both proteoglycan secretion and calcium waves.[24,114] Whether the involvement of ion channels in the control of ECM secretion is direct [via $(Ca^{2+})_i$ etc] or indirect, via the RMP is not known. In the majority of mammalian cells, the RMP is largely dependent upon potassium ion distribution and the activity of potassium ion channels. This appears to be partly the case with chondrocytes too (see below).[60] Furthermore, physical or osmotic pressure changes activate potassium channels and also hyperpolarize the chondrocyte membrane.[74,79,115] These changes in RMP are accompanied by changes of intracellular calcium.[116] The secretion of ECM is reduced by a range of potassium ion channel inhibitors.[116,117] Whether the activation of these potassium channels by stretch is direct, or secondary, to activity of some other species such as TRPV,[10,118] ENaC and/or integrins[21] is not yet proven.

Chondrocyte volume homeostasis was reviewed recently.[34] Essentially, following compression or exposure to hypo-osmotic challenge, chondrocytes exhibit (condition dependent) regulatory volume decrease and potassium channels have been implicated in this process. We have hypothesized that the relatively depolarized state of the chondrocyte membrane may facilitate volume decrease, since it increases the driving force for K^+ to leave the cell (**Fig. 2**). The identity of the specific potassium channels has not yet been established, but we have discussed above a number of examples of channels that could be involved. In particular, Ca^{2+}-activated potassium channels may open following elevation of intracellular Ca^{2+}. Hyperosmotic challenge can also activate BK channels,[74] and this may have significant consequences to chondrocyte biosynthetic function. It seems unlikely that this mechanism is directly involved in regulatory volume increases, since the potassium gradient of the cells leads to passive potassium efflux rather than influx.

Potassium and the Resting Membrane Potential (RMP)

The RMP of chondrocytes is likely to be important for a number of functions, such as matrix biosynthesis[114] and volume regulation.[119] While the RMP of large cells and muscle fibers can be readily determined by sharp electrodes, the RMP of rather small cells, such as chondrocytes, is impossible to determine categorically. A few authors have used sharp electrodes to record the RMP from chondrocytes.[79,84,119] While this allows one to record from cells deep in slices of cartilage, the high input resistance to leak resistance ratio (input resistance: 2 GΩ,[75] 11 GΩ,[60] 3 GΩ,[58] estimated leak resistance: 200 MΩ[120]) of the system allows for significant underestimation of RMP.[58,121] Most authors have
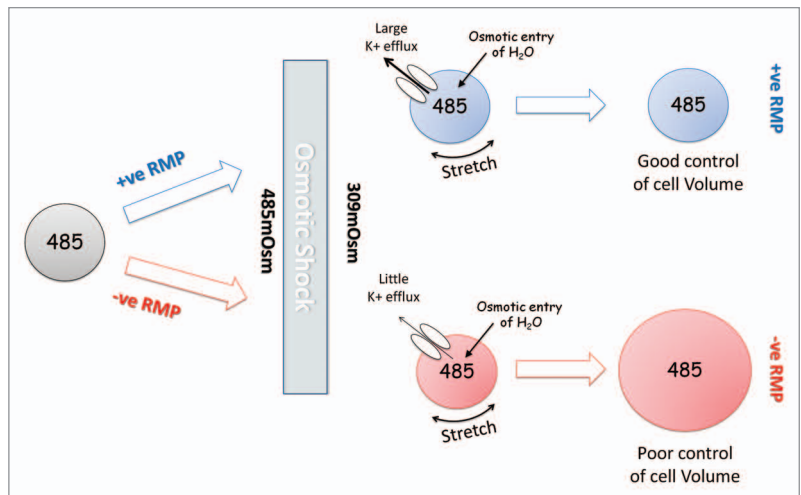

Figure 2. Role of potassium channels and membrane potential in volume control: if extracellular osmolarity decreases (in the example, this is from 485 mOsm to 309 mOsm) there would be an osmotic influx of water into the cell through aquaporin channels. In our hypothesis this activates potassium channels and an efflux of potassium reduces the intracellular osmotic pressure and thus the drive for water to enter. At positive membrane potentials, there is a large driving force for potassium efflux; however, at very negative membrane potentials there is little driving force for potassium to leave the cell. Thus, volume would increase unchecked until either osmotic balance is reached or the cell lyses.

therefore used whole-cell patch-clamp recording as an alternative.[26,56,58,60,122] Whole-cell patch-clamp measurement of RMP, however, also potentially (to a lesser degree) allows underestimation of RMP,[121] again due to the membrane resistance to leak resistance ratio. Chondrocytes form very good membrane "Giga" seals prior to "break-in" (reported as 30 GΩ,[75] 28 GΩ,[56] 17 GΩ,[58] 42 GΩ[60]) and while it is impossible to calculate what this resistance is post-"break-in," it is clearly much greater than that achieved with sharp electrode. Unfortunately, patch-clamp measurement of RMP suffers from more fundamental limitations: the membrane potential is highly dependent upon the intracellular ionic composition, but this is an unknown parameter and has to be artificially set in whole-cell patch-clamp experiments. Furthermore, while frequently not stated in the methods, it is common practice for electrophysiologists to use the RMP itself as an indication of cell viability. If the RMP is positive to a "threshold" value, the cell is excluded without further consideration (see for example, refs. 123–126). Therefore, it is perhaps unsurprising that a wide range of RMP have been reported for chondrocytes since the original report of -10.6 mV.[127] The most common observation is that the chondrocyte RMP is in the region of -40 mV or less (note, this excludes the underestimation phenomenon also described by Wilson et al. 2011).[26,56,58,60,122] At such depolarized levels, and assuming a significant membrane permeability to K^+ ions and E_{K^+} of -85 mV, one would predict a heavy contribution of the Na^+-K^+-ATPase to the RMP and, thus, sensitivity of RMP to ouabain. To the authors' knowledge, this has not been investigated. The very first experiments investigating the ionic basis of the chondrocyte RMP used an optical dye approach and found the SITS (Cl^- channel blocker),

Table 1. Potassium ion channel gene transcripts significantly altered in osteoarthritis

Gene symbol	Encoded Ion channel	Abundance ratio	p value
KCNK5	K2P5.1 (Task-2)	-4.7	4.8E-16
KCNMA1	K_{Ca} 1.1 (BK)	3.1	5.0E-10
KCNN4	K_{Ca} 3.1 (SK4 or IK)	10.2	2.0E-17
KCNT2	K_{Ca} 4.2 (BK)	-2.2	2.0E-07

Data analyzed from.[142] Similar data was also obtained by Dehne and coworkers.[143] A negative abundance ratio indicates an x-fold decrease and a positive value indicates an x-fold increase in transcript abundance calculated from Affymetrix microarray data.

4-AP (K⁺ channel blocker) and verapamil (voltage-gated Ca^{2+} channel blocker) to all decrease the RMP, but TEA to slightly increase it.[128] Electrophysiological experiments then confirmed the importance of the "maxi" chloride channel,[122] but several potassium channel studies then also suggested a role for Kv channels in maintenance of the chondrocyte RMP.[56,58,59] More recent experiments suggest that a TASK-2 conductance is also a major contributor to the RMP.[60] This is particularly interesting since the chondrocyte environment is believed to be somewhat acidic in comparison to plasma[129] and is likely to acidify further during joint inflammation.[130] This positions the acid-sensing TASK-2[60,105] in an important location to alter chondrocyte function in health and disease. TASK-2 inhibition by extracellular acidification itself would alter membrane potential and, thus, indirectly mediate pH effects on other cellular systems such as volume regulation,[34] intracellular Ca^{2+} or biosynthetic activity. Furthermore, TASK-2 gene transcription appears to be decreased in OA and TASK-2-mediated cellular control could therefore be lost as the disease progresses, (see below).

Potassium channel involvement in maturation, proliferation and viability. Potentially, mesenchymal stem cell chondrogenesis could be used to supplement eroded cartilage in the treatment of OA.[131] A few studies have investigated the role of ion channels in this process. Somewhat surprisingly, exposure of chondrocytes to lidocaine, a potassium channel blocker, increases the expression of a marker for chondrocyte maturation CD44.[132] Furthermore, a recent study by Varga et al.[133] investigated the role of different isoforms of Kv in maturation. In this chondrogenesis model, cells appear to subtly switch expression from a predominantly Kv1.1 phenotype toward one expressing first Kv4.1, and then Kv1.3; this then correlates with a decrease in the frequency and amplitude of (Ca^{2+}) sparks. If sufficiently selective pharmacological tools become available, it may become possible to steer differentiation toward a chondrocyte-like phenotype.

Kv channels have also been linked to the cell proliferation of a number of different cancers.[42,43] The mechanism of this is not known, although modulation of RMP is the strongest candidate.[43] This has been investigated in chondrocytes using a range of ion channel-inhibitors including classical potassium ion channel blockers TEA, 4-aminopyridine (4-AP).[117,128,134] These flow cytometry and thymidine incorporation studies showed that both TEA and 4AP decreased proliferation and cell viability.[117,128] Again, the suggestion was that these effects may be

mediated by changes in RMP. Of particular significance in these studies, and others,[135-138] is the observation that the local anesthetic lidocaine decreased cell viability, since local anesthetics are routinely injected into joints to control pain in arthroscopy. This effect may be linked to Kv ion channel block,[139] although interestingly, the Kv1.4 channel-blocker curcumin[140] actually exerts anti-apoptotic effects on IL-β stimulated chondrocytes. Cytotoxicity is less severe with mepivacaine;[137] however, clearly further studies are required to confirm the mechanism of action and source alternative local anesthetics with reduced cytotoxicity.
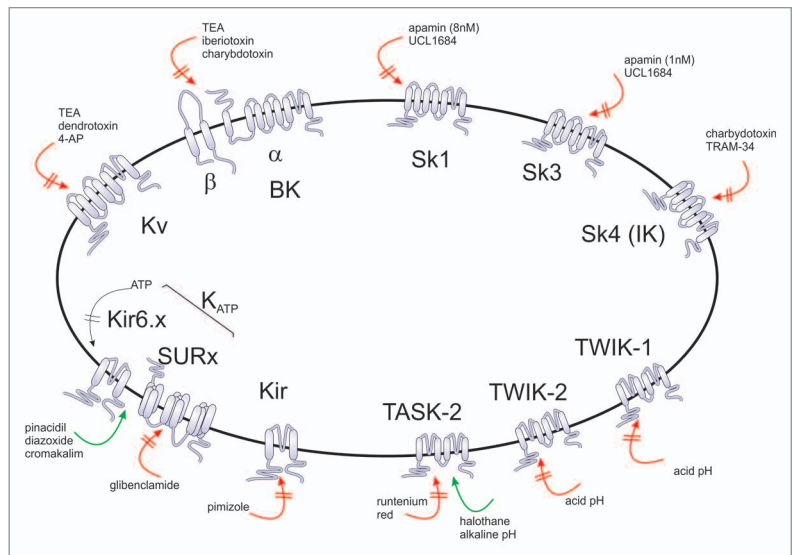
A key question is whether there are alterations in potassium channel expression in chondrocytes from OA cartilage. Although few studies have investigated this at a functional level, transcriptomic data does suggest that there are major changes to many channels involved with volume regulation and apoptosis,[34] these include the genes for several potassium channels (**Table 1**). Interestingly, these data show a maintained or increased transcription of K_{Ca} expression in general, but a switch from K_{Ca} 4.2 to K_{Ca} 1.1 and K_{Ca} 3.1 (IK). Furthermore, there is a large decrease in TASK-2 transcription. Since TASK-2 appears to be an important contributor to the chondrocyte RMP, the prediction from this data are that the OA chondrocyte would be depolarized. This is supported by a recent study where OA chondrocytes are reported to be approximately 15 mV depolarized relative to controls.[141]

Whether these ion channel transcript abundances correlate to changes in protein expression and whether the changes are resultant, coincident or causal to OA remains to be determined.

Concluding Remarks

The past 10 years have seen enormous advances in our understanding of chondrocyte physiology. Functional (electrophysiological studies), quantitative immunohistochemical and transcriptomic techniques have increased the rate of identification of chondrocyte potassium channels (summarized in **Fig. 3**). Most strikingly of all have been the developments in our knowledge of ion channel function in chondrocytes. When ion channels, especially voltage-gated ion channels, were first identified in chondrocytes, their function was somewhat of a mystery since these cells are non-excitatory. Now it is clear that ion channels have multiple roles, including involvement in apoptosis, volume homeostasis, maturation and chondrogenesis. The big question now is can this information be used constructively to target conditions such as OA? For example, could potassium channel modulators be used to aid chondrogenesis? If not, is potassium channel expression changed in OA, and/or can pharmacological modulation of potassium ion channels be used in treatment? Transcriptomic analysis of chondrocytes from OA models reveal several potassium channel transcripts levels to be changed in OA.[34,142,143] This makes chondrocyte potassium channels potentially useful biomarkers of the altered chondrocyte phenotype in OA. Whether expression changes are resultant or causal to OA is unknown, but pharmacological intervention with these channels is clearly an avenue worthy of future research.

Figure 3. Summary of the potassium channels discovered in chondrocytes and the pharmacological agents known to act on them. These channels include the calcium-activated potassium group of channels, consisting of SK1, SK3, IK (SK4) and BK,[69,75] voltage-gated potassium channel (Kv),[56,58,62] the K$_{ATP}$ channel consisting of a Kir6.x and SUR subunit[93] and the potassium two-pore channels TWIK-1 and 2 and TASK-2.[60] Note that apamin and UCL1684 also block SK2,[146,147] but this has not yet been reported in chondrocytes. The diagram illustrates just one subunit and primary accessory protein of each ion channel species.

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