Current Topics

Ion Channels as Therapeutic Targets for the Immune, Inflammatory, and Metabolic Disorders

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Articular chondrocytes are embedded in the cartilage of diarthrodial joints and responsible for the synthesis and secretion of extracellular matrix. The extracellular matrix mainly contains collagens and proteoglycans, and covers the articular cartilage to protect from mechanical and biochemical stresses. In mammalian chondrocytes, various types of ion channels have been identified: e.g., voltage-dependent K+ channels, Ca2+-activated K+ channels, ATP-sensitive K+ channels, two-pore domain K+ channels, voltage-dependent Ca2+ channels, store-operated Ca2+ channels, epithelial Na+ channels, acid-sensing ion channels, transient receptor potential channels, and mechanosensitive channels. These channels play important roles for the regulation of resting membrane potential, Ca2+ signaling, pH sensing, mechanotransduction, and cell proliferation in articular chondrocytes. In addition to these cation channels, Cl− channels are known to be expressed in mammalian chondrocytes: e.g., voltage-dependent Cl− channels, cystic fibrosis transmembrane conductance regulator channels, swelling-activated Cl− channels, and Ca2+-activated Cl− channels. Although these chondrocyte Cl− channels are thought to contribute to the regulation of resting membrane potential, Ca2+ signaling, cell volume, cell survival, and endochondral bone formation, the physiological functions have not been fully clarified. Osteoarthritis (OA) is caused by the degradation of articular cartilage, resulting in inflammation and pain in the joints. Therefore the pathophysiological roles of Cl− channels in OA chondrocytes are of considerable interest. Elucidating the physiological and pathological functions of chondrocyte Cl− channels will provide us a more comprehensive understanding of chondrocyte functions and may suggest novel molecular targets of drug development for OA.

Key words  chloride channel; cartilage; chondrocyte; osteoarthritis; voltage-dependent Cl− channel; TMEM16

1. INTRODUCTION

The surface of bones in diarthrodial joints is covered by articular cartilage, which contains a relatively low density of chondrocytes (1–10% of total tissue volume).1–3 Chondrocytes are the only cells found in articular cartilage. During joint movement, these chondrocytes are continuously exposed to a variety of stresses such as mechanical loads to the joints, osmolarity fluctuations in the synovial fluid, and biochemical stimuli by endogenous substances. In response to these stresses, articular chondrocytes synthesize and secrete the extracellular matrix, which is essential for normal joint movement. The extracellular matrix of cartilage is formed by cross-linked meshwork of collagen fibrils (mainly type II).4–6 Other minor collagens (type VI, IX, and XI) regulate the fibril assembly, organization, and degradation, and thus support the fibrillary collagen network in articular cartilage. Non-collagenous proteins, such as annexin V (also known as anchorin CII), are involved in the anchorage of chondrocytes. This matrix is packed with large aggregating proteoglycans (mainly aggrecan) containing glycosaminoglycans (chondroitin sulfate, keratan sulfate, and hyaluronic acid), which contribute to the stability of cartilage against physical shocks and biochemical stresses on the diarthrodial joints. In the process of responding to a variety of stimuli, ion channels are thought to regulate chondrocyte functions via changes in membrane potential and intracellular Ca2+ concentration ([Ca2+]i).

2. CATION CHANNELS IN CHONDROCYTES

Extensive electrophysiological and pharmacological studies have revealed the functional expression of various types of cation channels in mammalian chondrocytes (Table 1). Voltage-dependent K+ (Kv) channels substantially contribute to maintenance of resting membrane potential in articular chondrocytes.7 Whereas, voltage-dependent Ca2+ (CaV) channels and store-operated Ca2+ (SOC) channels are involved in Ca2+ signaling required for chondrogenesis and chondrocyte functions including extracellular matrix production and cell proliferation.4 Large-conductance Ca2+-activated K+ (BKCa) channels, epithelial Na+ channels (ENaCs), transient receptor potential vanilloid subfamily 4 (TRPV4) channels, and mechanosensitive Piezo channels are potentially responsible for sensing the membrane stretch and osmolarity.8,9 ATP-sensitive K+ (KATP) channels sense ATP levels within the cells and modulate the cartilage metabolism.10,11 Two-pore domain K+ (K2P) channels and acid-sensing ion channels (ASICs) are modulated by the endogenous stimuli such as acidic pH.12,13

3. PHYSIOLOGICAL FUNCTIONS OF Cl− CHANNELS

The Cl− channel superfamily includes voltage-dependent...
Table 1. Functional Expression of Cation Channels in Chondrocytes

| Channels                             | Subtypes          | Species            | Functions                                | Refs. |
|-------------------------------------|-------------------|--------------------|------------------------------------------|-------|
| Voltage-dependent K⁺ channels      | Kᵦ,1.4            | Equine, elephant   | Regulation of membrane potential         | 8     |
|                                     | Kᵦ,1.6            | Mouse              | Regulation of membrane potential         | 9     |
| Ca²⁺-activated K⁺ channels          | Kᵦ,1.1 (BKCaα)    | Human, OUMS-27     | Regulation of membrane potential         | 10–12 |
| ATP-sensitive K⁺ channels           | Kir6.1            | Human, Equine      | Metabolic regulation                      | 14    |
| Two-pore domain K⁺ channels         | TASK2             | Human              | Regulation of membrane potential, pH sensing | 11    |
| Voltage-dependent Ca²⁺ channels    | Ca₁,2 (α₁c)       | Rat                | Proliferation, differentiation           | 15    |
| Store-operated Ca²⁺ channels       | Orai1, 2, STIM1    | OUMS-27            | Store-operated Ca²⁺ entry                | 16    |
| Epithelial Na⁺ channels             | ENaCα, β, γ       | Canine             | Volume regulation                        | 17    |
| Acid-sensing ion channels           | ASIC1a            | Rat                | Acid pH sensing, apoptosis                | 18, 19|
|                                     | ASIC3             | Mouse              | Acid pH sensing                          | 20    |
| Transient receptor potential channels| TRPV4             | Porcine, bovine    | Osmotic sensing                          | 21, 22|
|                                     | TRPV5             | Mouse              | Mechanotransduction                      | 23    |
|                                     | TRPV6             | Human, mouse, rat  | Extracellular matrix secretion, proliferation, apoptosis | 25    |
|                                     | TRPA1             | T/C28a2, mouse     | Activation of inflammatory signals        | 26    |
| Mechanosensitive channels           | Piezo1, 2         | Human, mouse, porcine | Mechanotransduction                       | 23, 27|

Abbreviations: Kir, inward rectifier K⁺ channel; TASK, TWIK-related acid-sensitive K⁺ channel; TRPA, transient receptor potential ankyrin subfamily channel.

Cl⁻ (CIC) channels, cystic fibrosis transmembrane conductance regulator (CFTR) channels, swelling-activated Cl⁻ channels, Ca²⁺-activated Cl⁻ (ClC) channels, and ligand-gated Cl⁻ channels (γ-aminobutyric acid type A (GABAₐ) and glycine receptors). These Cl⁻ channels are ubiquitously expressed in many types of cells. Their Cl⁻ conductance plays pivotal roles for controlling the membrane potential and cell volume. Besides, Cl⁻ efflux counterbalances K⁺ and results in maintaining electroneutrality. Therefore Cl⁻ channels are thought to be involved in diverse physiological mechanisms regulating ion homeostasis, cell excitability, muscle tonus, neurotransmission, transepithelial transport, cell volume, and cell survival.

In addition to cation channel currents, Cl⁻ currents have been recorded in articular chondrocytes. In articular chondrocytes, the resting membrane potential is regulated by Cl⁻ conductance in addition to K⁺ conductance. The dependence of resting membrane conductance to Cl⁻ is stronger than to K⁺, although there is still a balance between them in chondrocytes. Pharmacological blockade and genetic knockdown of Cl⁻ channels cause membrane hyperpolarization of chondrocytes. In non-excitatory cells such as chondrocytes, membrane hyperpolarization facilitates Ca²⁺ entry through non-selective cation channels. An increase in [Ca²⁺]ᵦ promotes the synthesis and secretion of extracellular matrix of cartilage. However, the physiological significance of chondrocyte Cl⁻ channels has not been completely elucidated.

4. CIC CHANNELS IN CHONDROCYTES

Voltage-dependent Cl⁻ channels are widely expressed in many tissues and involved in several physiological functions including membrane excitability, transepithelial transport, volume regulation, acidification of intracellular organelles, and cell survival. This family of channels is coded by nine CIC genes (CIC-1–7, K₁₇, and K₈) and distributed in the plasma and intercellular membranes as Cl⁻ channels or Cl⁻/H⁺ transporters. Each CIC channel/transporter has a distinct electrophysiological and pharmacological profile. For instance, CIC-2 currents show an inward rectification, whereas CIC-4, CIC-5, and CIC-7 currents exhibit strong outward rectification. CIC-3 currents are activated by membrane hyperpolarization but inactivated in a time-dependent manner at strongly depolarizing voltages. CIC-2 and CIC-7 activities are enhanced by acidic pH, whereas CIC-4 and CIC-5 activities are reduced by acidic pH. CIC-2 and CIC-3 currents are activated by hyposmotic stress. In addition, CIC-3, CIC-4, and CIC-7 currents are sensitive to 4,4′-disothiocyanostilbene-2,2′-disulfonic acid (DIDS), whereas CIC-2 and CIC-5 currents are resistant to DIDS.

Voltage-dependent Cl⁻ currents have been recorded in rabbit articular chondrocytes and are sensitive to a Cl⁻ channel blocker, 4-acetamide-4′-isothiocyanato-2,2′-stilbenedisulfonic acid (SITS). Also, voltage-dependent Cl⁻ currents sensitive to classical Cl⁻ channel blockers, niflumic acid, DIDS, and anthracene-9-carboxylic acid (9-AC) as well as SITS, have been identified in a cell line of human chondrocytes, OUMS-27. DNA microarray analyses revealed that two human (CIC-3 and CIC-7) and three rodent (CIC-3, CIC-4, and CIC-6) CIC transcripts are detectable in chondrocytes. In addition, mRNA expression of CIC-3 has been observed in rabbit articular chondrocytes, although it is potentially responsible for swelling-activated Cl⁻ channels. Recently, we have found that acid- and DIDS-sensitive CIC-7 channels are predominantly and functionally expressed in OUMS-27 cells and contribute to regulation of resting membrane potential and [Ca²⁺]ᵦ. Osteopetrosis associated transmembrane protein 1 (Ostm1) has been recently identified as an auxiliary subunit of CIC-7; however, expression analysis of Ostm1 in articular chondrocytes has not been examined to date.

5. CFTR CHANNELS IN CHONDROCYTES

Cystic fibrosis is a life-threatening disease caused by mutation in the CFTR gene. The deletion mutation ΔF508 is the most frequent cause of cystic fibrosis and disrupts the functional expression of CFTR channels. CFTR channels are predominantly distributed in epithelial tissues including the lung, pancreas, small intestine, sweat duct, and salivary glands, and
are responsible for Cl− conductance associated with transepithelial transport. Therefore dysfunction of CFTR channels fatally affects endothelial functions by sticky mucus and secretions. CFTR currents are voltage-independent and activated by cAMP and protein kinase A (PKA). CFTR channels are blocked less potently by traditional Cl− channels blockers. So far, there is one successful publication showing the functional expression of CFTR channels in mouse chondrocytes. The Cl− currents are activated by cAMP and inhibited by a specific CFTR channel blocker, CFTRinh-172. CFTR channels may contribute to endochondral bone formation. CFTR is also recognized as a regulator of other ion channels, such as ENaCs, Kir6.1, and TRPV4, which are known to be expressed in chondrocytes. Therefore CFTR channels may be functionally coupled with these channels for chondrocyte functions.

6. SWELLING-ACTIVATED Cl− CHANNELS IN CHONDROCYTES

Regulating cell volume during osmotic stresses is necessary for maintaining cellular homeostasis in many cell types. When a cell is exposed to extracellular hypotonic environment, it triggers passive uptake of water and thus swells. In turn, cell swelling facilitates ion efflux through K+ and Cl− channels and subsequent extrusion of increased volume of water in the process of regulatory volume decrease (RVD). Finally, the cell volume recovers to normal level. On the other hand, hypertonic stress causes osmotic cell shrinkage. Uptake of osmolytes and water is rapidly activated in the process of regulatory volume increase (RVI) for recovery to normal cell volume. The Cl− channel conductance activated by cell swelling plays a key role in cell volume regulation. Swelling-activated Cl− channels are ubiquitously distributed in mammalian cells and involved in many physiological and pathological functions such as cell proliferation, differentiation, migration, and apoptosis, in addition to cell volume regulation. Swelling-activated Cl− currents show outwardly rectification, time-independent activity at negative potentials (−50 mV), and rapid inactivation at positive potentials (>50 mV). The activity is blocked by DIDS, SITS, and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), whereas the inhibitory effects of niflumic acid and 9-AC are less than these on other Cl− channels.

Articular chondrocytes are exposed to a dynamic extracellular osmotic environment and thus require volume regulatory mechanisms. When articular chondrocytes of the cartilage in synovial joints are exposed to extracellular hyposmotic environment, they exhibit rapid RVD following hyposmotic cell swelling and subsequent activation of swelling-activated Cl− channels. Swelling-activated Cl− channels have been identified in bovine, rabbit, and rat articular chondrocytes. Swelling-activated Cl− currents in chondrocytes are moderately outwardly rectifying, and exhibit a time-independent activity at potentials negative to +50 mV and a rapid inactivation at more depolarizing potentials. The hyposmotic-induced currents are blocked by non-specific Cl− channel blockers, DIDS, SITS, and NPPB. The RVD response following activation of swelling-activated Cl− channels may be related to expression of ClC-3 and TMEM16A in rat articular chondrocytes. However, some biophysical characteristics of these currents are different from those of swelling-activated Cl− channels in chondrocytes. Although leucine-rich repeats containing 8A (LRRC8A) has been recently identified as a volume-regulated anion channel or swell-activated Cl− channels, there are currently no reports suggesting the expression of LRRC8A in articular chondrocytes.

7. ClCa CHANNELS IN CHONDROCYTES

The ClCa channels are ubiquitously expressed in many excitable cells, such as smooth muscles cells, neurons, and cardiac myocytes. They are also found in non-excitable cells, such as epithelia cells, endothelial cells, and interstitial cells of Cajal. In these cells, ClCa channels play important roles in many physiological processes including smooth muscle contraction, neuronal signaling, sensory transduction, cardiac excitability, and epithelial secretion. In many types of cells, the intracellular Cl− concentration ([Cl−]i) is supposed to be 30–50 mM, suggesting that its equilibrium potential of Cl− (ECl) is estimated between −40 and −25 mV. The resting membrane potential of human chondrocytes has been reported between −50 and −40 mV under conditions of 30 mM [Cl−] in the pipette solution. Therefore blockade of ClCa channels shifts the
resting membrane potential in the hyperpolarizing direction and facilitates Ca\(^{2+}\) influx through non-selective cation channels in non-excitable cells such as chondrocytes. Classical Cl\(^{-}\) channel blockers, such as niflumic acid, DIDS, SITS, NPPB, and 9-AC, are potent but not specific for Cl\(_{\text{Ca}}\) currents.

Currently, TMEM16A and TMEM16B proteins in the TMEM16 family (TMEM16A–H, J, K) are the preferred candidates for Cl\(_{\text{Ca}}\) channel conductances in native tissues. TMEM16A is functionally expressed in a large variety of tissues, including airway epithelial cells, vascular smooth muscle cells, interstitial cells of Cajal in gastrointestinal tract, and nociceptive neurons. On the other hand, localization of TMEM16B expression seems limited to sensory nervous systems (olfactory neurons and retinal photoreceptors), hippocampus neurons, and pinealocytes. The biophysical characteristics of TMEM16A channels show significant differences from those of TMEM16B channels. First, the single-channel conductance of TMEM16A channel (8 pS) has been reported to be greater than that of TMEM16B channel (1 pS). However, a recent report suggests that there is no significant difference between TMEM16A (3.5 pS) and TMEM16B (3.9 pS) channels. Second, the half maximal [Ca\(^{2+}\)] level required for activation of TMEM16A channel (0.4–0.6 \(\mu M\)) is lower than that of TMEM16B channel (1–3 \(\mu M\)). Third, the kinetics of activation and deactivation of TMEM16A current are much slower than those of TMEM16B current, with time constants differing by more than 10-fold.

It has been reported that TMEM16A, TMEM16C, TMEM16E, and TMEM16F transcripts are expressed in mouse chondrocytes, suggesting that these gene products are required for endochondral ossification and bone development. TMEM16A mRNA is also expressed in rat articular chondrocytes, although it is recognized as a swelling-activated Cl\(^{-}\) channel component. In contrast to TMEM16A and TMEM16B, other members of TMEM16 family are considered not ion channels responsible for Cl\(_{\text{Ca}}\) channels.

Histamine is an early pharmacological mediator to initiate a local inflammatory response. Histamine released from mast cells binds to histamine receptor type 1 (H\(_1\)R) and activates G\(_{\text{q}}\)-phospholipase C (PLC)-IP\(_3\) signal pathway. Subsequent Ca\(^{2+}\) release from IP\(_3\) receptors (IP\(_3\)Rs) on the endoplasmic reticulum (ER) enhances the activity of BK\(_{\text{Ca}}\) channels in a cell line of human chondrocytes, OUMS-27 cells (Fig. 1). When Ca\(^{2+}\) in the ER is depleted by Ca\(^{2+}\) release, Ca\(^{2+}\) influx through SOC channels, mainly consisting of Orai1/2-STIM1 complex in OUMS-27 cells, is evoked. This SOC entry is enhanced by membrane hyperpolarization following BK\(_{\text{Ca}}\) channel activation. Ca\(^{2+}\) influx is expected to activate BK\(_{\text{Ca}}\) channel progressively, hyperpolarize chondrocytes, and finally induce more [Ca\(^{2+}\)] elevation, which is recognized as a positive feedback mechanism.

Pharmacological blockade and genetic knockdown of Cl\(^{-}\) channels also cause membrane hyperpolarization followed by [Ca\(^{2+}\)] increase in OUMS-27 cells. Sustained [Ca\(^{2+}\)] increase is likely to induce extracellular matrix synthesis, cytokine production, and cell proliferation in chondrocytes. In contrast, acidification at inflammatory region activates CIC-7 channels, causing membrane depolarization in OUMS-27 cells. Depolarizing stimulation reduces the activity of SOC channels followed by a decrease in [Ca\(^{2+}\)]. Taken together, these ion channels may be considered potential targets of drug development for arthritis.

Fig. 1. Functional Coupling among SOC, BK\(_{\text{Ca}}\), and CIC-7 Channels Following Histamine Stimulation in Chondrocytes

Mast cells release histamine, an inflammatory mediator. Histamine induces [Ca\(^{2+}\)] increase through activation of H\(_1\)R-G\(_{\text{q}}\)-PLC-IP\(_3\) signaling pathway in a cell line of human chondrocytes, OUMS-27 cells. Ca\(^{2+}\) release from IP\(_3\)Rs on the ER activates BK\(_{\text{Ca}}\) channels, resulting in membrane hyperpolarization. Ca\(^{2+}\) influx in the ER elicits Ca\(^{2+}\) influx through SOC channels, mainly consisting of Orai1/2-STIM1 complex. Ca\(^{2+}\) entry via SOC channels is enhanced by membrane hyperpolarization due to BK\(_{\text{Ca}}\) channel activation (positive feedback mechanism). Sustained [Ca\(^{2+}\)] rise may facilitate chondrocyte functions such as extracellular matrix synthesis, cytokine production, and cell proliferation. In addition, inflammatory acidification activates CIC-7 channels, resulting in membrane depolarization. Depolarizing stimulation reduces the activity of SOC channels followed by a decrease in [Ca\(^{2+}\)], and attenuates chondrocyte functions. (Color figure can be accessed in the online version.)
9. PATHOPHYSIOLOGICAL FUNCTIONS OF CL− CHANNELS IN CHONDROCYTES

Osteoarthritis (OA) is an inflammatory and degenerative joint disease characterized by degradation and loss of articular cartilage. Although the pathogenic process in OA remains unclear, abnormal osmolality of synovial fluid is likely to be associated with cause and development of OA. Because the osmolality of synovial fluid in OA patients is lower than in normal subjects (295–340 mOsm), articular chondrocytes in OA patients are thereby exposed to hypotonic environment. Hypotonic stress can cause Cl− efflux and associated RVD. We have found that CIC-7 is downregulated by hypotonic stress (270 mOsm) that mimics an external OA environment, resulting in enhanced death of OUMS-27 cells. On the other hand, expression levels of BKCa and TRPV4 channels are increased by hypotonic stress (280 mOsm) in equine articular chondrocytes.

A crucial role of chondrocyte volume control in apoptosis has been proposed. Activation of swelling-activated Cl− channels mediates cellular volume decrease leading to apoptosis in several cell types. Apoptosis is accompanied by nonmotonic shrinkage of cells, apoptotic volume decrease (AVD), which precedes cytochrome c release, caspase-3 activation, and DNA laddering. Apoptosis of chondrocytes in articular cartilage has been implicated in initiating the progression of OA. Therefore, it can also be induced by several proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α). Moreover, cartilage degradation in OA patients is facilitated by upregulation of matrix metalloproteinases (MMPs).

Recent microarray analyses demonstrate that TMEM16A CLCa channels are upregulated at the transcript levels in chondrocytes from OA patients. In addition, intermediate-conductance Ca2+-activated K+ (IKCa) also known as KCa4.2 (Na+-activated K+ channels), ENaC, TRPV4, and transient receptor potential melastatin subfamily 7 (TRPM7) channels are downregulated in OA chondrocytes. Contradictory results have been obtained regarding the expression changes of BKCa (Kca1.1) channels in OA chondrocytes. Also, TRPV5 channels are upregulated in rat OA models, whereas TRPV6 channels are functionally attenuated in chondrocytes from OA patients and rat OA model. Recently, voltage-dependent Na+ (Na1.7) and TRPV4 channels have been identified as potential biomarkers of OA in genetic association studies.

10. CONCLUSION

OA is the most common joint disease worldwide. OA is caused by mechanical damage to the articular cartilage and associated with chronic inflammation of joints. Therefore OA adversely affects QOL by loss of joint function following inflammation, pain, activity limitation, and disability. Currently, there are no effective treatment options against OA. OA chondrocytes may be characterized by altered membrane potential and intracellular Ca++ homeostasis, which are regulated by the activity of ion channels including Cl− channels. Therefore Cl− channels may be considered novel targets of therapeutic intervention(s) and drug development for OA.

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