The emerging chondrocyte channelome

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INTRODUCTION

Chondrocytes are metabolically active cells found in mature articular cartilage (Iannotti, 1990; Archer and Francis-West, 2003). The extracellular matrix (ECM) of cartilage is composed of elastic and collagen fibers (mainly type II collagen), which provide tensile strength with embedded proteoglycans forming a gel-like ground substance that provides elasticity and the ability to resist compressive forces (Buckwalter and Mankin, 1998). Chondrocytes occur singularly or in groups or clusters of three or more cells within spaces called lacunae in the ECM (Stockwell, 1975). Articular cartilage has a high matrix to cell ratio, with chondrocytes occupying only 10% of the total tissue in mammals (Carney and Muir, 1988). Articular cartilage is a type of hyaline cartilage that covers the surfaces of bones which meet at a synovial joint (Mankin, 1982). Synovial joints include a cavity between the bones within the articular capsule in order to allow free movement (Edwards et al., 1994). The synovial cavity contains synovial fluid, which acts as a lubricant to decrease friction between the bones meeting at the synovial joint and absorbs shock. Friction would be undesirable because it would damage the joint and also generate heat, thereby causing pain (Tatari, 2007). Articular cartilage is avascular without a perichondrium connective tissue surround. In human articular cartilage, chondrocytes may be as far away as 3 mm from the nearest artery. Therefore, synovial fluid supplies chondrocytes in adult articular cartilage with oxygen and nutrients, and removes carbon dioxide and metabolic waste products, by diffusion (Lee and Urban, 1997; Allan, 1998). Synovial fluid is periodically washed over the surface of the articular cartilage by the movement of the joint (Lee and Urban, 1997). Oxygen and substrate concentrations within cartilage reduce near to the cartilage-bone margin to almost zero (Otte, 1991). Therefore, chondrocytes generate ATP by substrate-level phosphorylation during anaerobic respiration, leading to the accumulation of lactate and lowering of the pH through the production of H+ ions, which can continue in anoxic conditions (Lee and Urban, 1997). The extracellular pH affects the chondrocyte metabolism and its ability to synthesize matrix. Low pH reduces lactate production, but also slows down the synthesis of glycosaminoglycans. However, the rate of collagen synthesis appears to be independent of pH (Wu et al., 2007). Chondrocytes embedded within the ECM have an unusual ionic environment because they are surrounded by negatively charged proteoglycans, which attract large numbers of cations, such as Na+ ions, creating a high extracellular osmolarity and contributing to the low pH (Urban et al., 1993).

Chondrocyte primary function is to synthesize and secrete proteoglycans, collagen and non-collagenous proteins to maintain the cartilage ECM (Fassbender, 1987). Chondrocytes maintain cartilage by establishing a balance between replacing degraded
macromolecules and increasing synthesis in response to injury (Martin and Buckwalter, 2000). Proteoglycans contribute to cartilage rigidity, stability and durability during compression (Redini, 2001). Types II, IX, and XI collagen form the tensile fibril networks within cartilage. Type VI collagens form adjacent to chondrocytes and may be involved in attachment of the chondrocyte to the ECM (Bruckner and van der Rest, 1994). Non-collagenous proteins, such as anchorin CII, are also involved in chondrocyte anchorage (Fernandez et al., 1990). The cartilage matrix protects chondrocytes from mechanical stress placed on the joint (Buckwalter and Mankin, 1998; Martin and Buckwalter, 2000). Chondrocyte metabolic activity is directly correlated with the weight of mechanical stress placed on the cartilage; increased activity when the cartilage is heavily loaded provides maximum proteoglycan content (Urban, 1994). The ability of articular cartilage to withstand and respond to pressure and shearing forces is vital for it to fulfill its function. Accumulating evidence suggests that the resting membrane potential (RMP) is vital for fulfilling this function. The RMP has been shown to be central to the secretion and synthesis of substances in a variety of other cell types (Breittmayer et al., 1996; McCarty, 1999; Penyige et al., 2002). It therefore seems likely that if the RMP of chondrocytes is changed by ion channel manipulation, their ability to produce ECM will be compromised. This conjecture is indeed supported by experiments where RMP modifying ion channel blockers reduced the production of matrix mRNAs (Wu and Chen, 2000), proteins and sulfated glycosaminoglycans (Mouw et al., 2007). Chondrocyte proliferation is also inhibited by channel blockers lidocaine and verapamil (Wohlrab et al., 2001, 2005) and apoptosis increased (Grishko et al., 2010). As with other cells, the chondrocyte RMP is determined by the balance of positive and negative ion permeabilities in the cell membrane (Hodgkin and Huxley, 1952a). These permeabilities are, in turn, controlled by the chondrocyte channelome (the complement of expressed ion channels and porins).

Ion channels are the essential components that control ion movement in and out of the cell (Hodgkin and Huxley, 1952a). They are embedded within the plasma membrane and usually consist of one or more proteins with a central aqueous pore, which opens by conformational change (Neher and Sakmann, 1992). The stimulus for opening (gating) is specific to each ion channel, and may be voltage, chemically or mechanically induced (Hille, 2001). A number of studies have now shown the presence of an ever-expanding list of ion channels in chondrocytes (Figure 1), and this review will summarize the data to date, both on the variety of expression and the proposed roles of these channels.

**K<sub>C</sub> Channels**

One of the first discovered ion conductances in biology was the potassium delayed rectifier (Hodgkin and Huxley, 1952b; Ramage et al., 2008). The ion channels underlying this are now known to be

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**FIGURE 1** | Summary of the chondrocyte channelome. Many studies have now identified ion channels and porins in chondrocytes. Frequently the function of these channels is either unknown or controversial. This figure illustrates some of the major channel proteins identified to date, either by electrophysiological, immunological or molecular biological techniques. Note in this figure, K<sub>Ca</sub> is taken to be equivalent to any calcium activated potassium channel including BK and SK. AQP, aquaporin channel; BK, calcium-activated potassium channel, high conductance; CIC, chloride channel; ENaC, epithelial sodium channels; K<sub>ATP</sub>, ATP dependent potassium channel; Kv, voltage-gated potassium channel; NMDA, N-methyl D-aspartate; SK, calcium-activated potassium channel, low conductance; TRP, transient receptor potential channel; VGCC, voltage-gated calcium channels; VGSC, voltage-gated sodium channel; This data is summarized more fully in Table 1. For references please see text and or Table 1.
members of the K\textsubscript{v} potassium channel family. This family is one of the largest ion channel families with at least 40 members (Gutman et al., 2005) of six transmembrane domains. Interestingly these were also one of the earliest ion channels discovered in chondrocytes (Walsh et al., 1992; Sugimoto et al., 1996). K\textsubscript{v} channels have now been reported in chondrocytes by a number of authors and have been shown to be archetypal slowly inactivating ion channels (Walsh et al., 1992; Wilson et al., 2004; Mobasher et al., 2005a; Ponce, 2006). In essence, these channels are very similar to those channels found in skeletal muscle (Pallotta and Wagener, 1992) and in neurons (Barrett-Jolley et al., 2000) where, in those cell types, they are critical for repolarization of the membrane following an action potential. The role of a delayed rectifier channel in the chondrocyte plasma membrane is far less clear. Since the chondrocyte exists at far more depolarized levels than neurons or skeletal muscle (Wright et al., 1992; Wilson et al., 2004), logic would suggest that these channels would be constantly inactivated. Close study of the mathematical relationship between voltage, time and fractional inactivation (Hodgkin and Huxley, 1952b) reveals that a certain, albeit small, proportion of these channels will remain active even at the relatively depolarized RMP of a chondrocyte. This is supported by the observation by Wilson et al. (2004) and Clark et al. (2010b) that TEA inhibition of the potassium channels does have a significantly depolarizing effect on chondrocyte RMP, as it does with other non-excitable cells such as those of smooth muscle (Telezhkin et al., 2001; Park et al., 2007).

Relatively few studies have attempted to establish the molecular identity of the delayed rectifier in chondrocytes. However, reports suggest that these channels are similar between species (chicken, canine, equine, and elephant) in terms of their steady-state half-activation voltage and slope (Wilson et al., 2004; Mobasher et al., 2005a; Ponce, 2006). Half activation parameters range from 12 to 25 mV; typical of K\textsubscript{v} 1.x or K\textsubscript{v} 4.x potassium channels (Coetzee et al., 1999). Activation time constants are, however, quite fast compared with many K\textsubscript{v} channels (Mobasher et al., 2005a). Such rapid kinetics have been reported for members of the K\textsubscript{v} 1.x family and also homomeric K\textsubscript{v} 3.4 (Coetzee et al., 1999). The inactivation time constant in the order of seconds (Mobasher et al., 2005a) is typical of K\textsubscript{v} 1.x, K\textsubscript{v} 2.x and K\textsubscript{v} 3.x channels (Coetzee et al., 1999). Together these data suggested that the potassium channel of chondrocytes is likely to be a member of the K\textsubscript{v} 1.x. Pharmacological data are discussed in (Mobasher et al., 2005a) and are not entirely consistent for K\textsubscript{v} 1.x channels or one particular K\textsubscript{v} channel. We therefore feel that the key, published data identifying the subunit identity of the chondrocyte K\textsubscript{v} channels are the immunohistochemical and RT-PCR data. Such data have unequivocally revealed the presence of K\textsubscript{v} 1.4 subunits in equine chondrocytes (Mobasher et al., 2005a) and K\textsubscript{v} 1.6 in the mouse (Clark et al., 2010b). Since K\textsubscript{v} channels are known to exist as functional heteromultimers (Villalonga et al., 2010) we would tentatively suggest that articular chondrocytes may express K\textsubscript{v} 1.x, probably as a heteromultimer including the K\textsubscript{v} 1.4 or K\textsubscript{v} 1.6 subunit(s) and probably some other, as yet unidentified, K\textsubscript{v} subunit(s).

**INWARDLY RECTIFYING POTASSIUM CHANNELS**

Study of inwardly rectifying potassium channels is greatly hampered by a lack of selective inhibitors. Barium and chloroethylclonidine are inhibitors of the strong inward rectifiers (Standen and Stanfield, 1978; Barrett-Jolley et al., 1999) and glibenclamide is an inhibitor of ATP dependent potassium channels (Tomai et al., 1994). So far only K\textsubscript{ATP} channels have been observed in chondrocytes (Mobasher et al., 2007). K\textsubscript{ATP} channels are a widely expressed subfamily of inwardly rectifying potassium channels. These channels are closed by intracellular ATP and thus serve to couple metabolism to membrane excitability (Quayle et al., 1997; Ashcroft and Gribble, 1998; Minami et al., 2004). Structurally these channels exist as heteromultimers. Each functioning protein consists of four ATP binding cassette proteins (SUR) surrounding four inwardly rectifying potassium channel subunits (Kir 6.x) (Babenko et al., 1998). Of particular interest to investigators of chondrocyte function is the fact that, in addition to being opened by decreasing intracellular ATP (Figure 2), K\textsubscript{ATP} channels are also frequently observed to be opened by low oxygen tension and hypoxia (Dart and Staden, 1994). This suggests that these channels are important in hypoxia-mediated cell signaling (Phillis, 2004). We showed recently that K\textsubscript{ATP} channels were expressed in articular chondrocytes (Mobasher et al., 2007). We used polyclonal antibodies raised against the K\textsubscript{ATP} channel to show expression in both human and equine chondrocytes. Expression was largely restricted to the superficial and middle zones of normal cartilage and the superficial zone of fibrillated osteoarthritic cartilage in clusters (Mobasher et al., 2007). In patch-clamp studies we found the biophysical properties of K\textsubscript{ATP} channels to be broadly similar to K\textsubscript{ATP} channels expressed elsewhere (Babenko et al., 1998; Mobasher et al., 2007). Several K\textsubscript{ATP} subtypes (i.e., Kir 6.1 and Kir 6.2) are each potentially coupled with one of the SUR subtypes; SUR1, 2A or 2B (Babenko et al., 1998). Pharmacological properties of K\textsubscript{ATP} channels are thus very different between tissues. Glibenclamide is sometimes used as a functional discriminator between K\textsubscript{ATP} subtypes. It is highly active in pancreatic β-cells (IC50 of <10 nM, Krause et al., 1995), but rather less potent in muscle (IC50 25–100 nM, Beech et al., 1993; Barrett-Jolley and Davies, 1997; Barrett-Jolley and McPherson, 1998). In pharmacological studies of chondrocytes, the K\textsubscript{ATP} channel's IC50 is within the range seen in muscle (Mobasher et al., 2007). It therefore seems highly likely that chondrocytes express at least one subtype of K\textsubscript{ATP} channel and that these may be important for regulation of cartilage metabolism and sensing ATP levels within the cell (Mobasher et al., 2005b).

**LARGE CALCIUM-ACTIVATED POTASSIUM CHANNELS**

Several studies have putatively identified BK channels in chondrocytes (Grandolfo et al., 1990, 1992; Long and Walsh, 1994; Martina et al., 1997; Mozrzymas et al., 1997; Mobasher et al., 2010). In our own study (Mobasher et al., 2010), the principal stretch-activated channel we identified had a slope conductance, reversal potential, and pharmacology consistent with it being a large calcium-activated potassium channel (BK) (Latorre et al., 1989; Cui et al., 2009). We found the sensitivity to iberiotoxin to be statistically significant but weak (Mobasher et al., 2010). This is interesting because whilst the BK channel can exist as a standalone six trans-membrane α-subunit, complete with potassium conducting pore and Ca\textsuperscript{2+} sensor (Wang and Sigworth, 2009), the presence or absence of a β-subunit determines many of the channel's functional properties (Salkoff et al., 2006; Torres et al., 2007). In particular, low sensitivity to iberiotoxin is highly characteristic of the expression of...
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small calcium-activated Potassium channels

In addition to the body of work showing the presence of BK channels, there have also been a few reports of SK activity in chondrocytes (Wright et al., 1996; Lee et al., 2000; Ramage et al., 2008; Funabashi et al., 2010b). In the study by Wright et al. (1996), osmotic shock led to a hyperpolarization, which was largely insensitive to iberiotoxin, but highly sensitive to the SK channel inhibitor, apamin. Interestingly, in our own study of stretch activated potassium channels in chondrocytes (Mobasheri et al., 2010), whilst single channel studies clearly identified BK channels, the hypo-osmotic hyperpolarization was resistant to the low concentrations of TEA which would be expected to block BK channels. The hyperpolarization was, however, inhibited by symmetrical 10 mM TEA. This was an observation consistent with the original observations of an SK component to the hyperpolarization shown by Wright et al. (1996), since both SK and BK are rather resistant to extracellular TEA (Latorre et al., 1989).

**Transient receptor potential channels**

Transient receptor potential (TRP) channels are a family of loosely related ion channels that show relatively little selectivity between permeable cations such as sodium, calcium, and magnesium. They were initially proposed to couple hypo-osmotic shock to intracellular Ca²⁺ mobilization in chondrocytes on the basis of gadolinium sensitivity (Sanchez et al., 2003), but since then several

**Figure 2 | K<sub>ATP</sub> channels in chondrocytes.** Chondrocytes have been shown to express K<sub>ATP</sub> channels. The function of these is generally accepted to be coupling metabolic status with membrane potential and thus cell activity. In other cell types, endogenous triggers for activation of K<sub>ATP</sub> include decrease of intracellular ATP (Babenko et al., 1998), increase in ADP (Dunne and Petersen, 1988), extracellular hypoxia (Dart and Standen, 1994) or other chemical signals such as adenosine (Dart and Standen, 1993; Barrett-Jolley et al., 1996), angiotensin (Sampson et al., 2007) etc.; K<sub>ATP</sub>, ATP dependent potassium channel.

1 Transient receptor potential channels. Authors: David E. Clapham, Bernd Nilius, Grzegorz Owsianik. Last modified on 2010-04-07. Accessed on 2010-06-24. IUPHAR database (IUPHAR-DB), http://www.iuphar-db.org/DATABASE/FamilyMenuForward?familyId=78.
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and cytoskeletal complexes in close proximity. The presence of L-type (and T-type) calcium channels in chondrocytes was recently supported by Mancilla et al. (2007), however, (Sanchez and Wilkins, 2004) found that osmotically induced changes in intracellular calcium ions were not influenced by more selective L-type calcium channel blockers (including verapamil). In contrast aggrecan and collagen synthesis induced by electrical stimulation of cartilage is dependent upon the activity of VGCCs (Xu et al., 2009). Clearly, further evidence for the presence of this channel is needed to clarify these data.

voltage-gated sodium channels (vgsc)
Voltage-gated sodium channels (VGSC) are integral membrane proteins that are activated in response to voltage-changes across the plasma membrane (Catterall, 1991, 1992, 1995, 2002). The presence of tetrodotoxin sensitive VGSC in rabbit chondrocytes has been reported by Sugimoto et al. (1996) and in chondrocytes from osteoarthritic cartilage by Ramage et al. (2008). It would be interesting to see how the expression of this channel fits into the control of the chondrocyte membrane potential, since current studies have failed to observe sufficient hyperpolarization of chondrocytes for a typical VGSC to be substantially reactivated. Under conditions of constant depolarization, for example, these channels would be permanently inactivated.

TRP channels have been identified in osteoarthritic cartilage by PCR (Gavenis et al., 2009). TRPV4 has also been identified in both porcine and canine chondrocytes (Phan et al., 2009; Lewis et al., 2010) by PCR. TRPV4 is an established stretch activated channel and is widely regarded to be a conduit for stretch-activated entry of calcium ions (Nilius et al., 2004). TRPV4 has been shown to be a regulator of the chondrogenic SOX9 pathway (Muramatsu et al., 2007) and the deficiency of TRPV4 in knockout mice leads to a loss of Ca2+ response to hyp-osmotic challenge and the onset of osteoarthritic changes (Clark et al., 2010a). Thus, it may be that by linking chondrocyte membrane stretch to calcium mobilization TRPV4 is key to regulation of chondrogenesis, activation of calcium activated potassium channels and volume regulation.

VOLTAGE-GATED CALCIUM CHANNELS
Voltage-gated calcium channels (VGCC) are a group of calcium permeable voltage-gated ion channels found in excitable cells (e.g., muscle, glial cells, neurones, etc., Goldin, 2001; Dolphin, 2009). The presence of L-type VGCCs was suggested by Wright et al. (1996) on the basis of pharmacological inhibition of calcium dependent hyperpolarization by somatostatin and cadmium. It should be noted that whilst this is a plausible hypothesis, both somatostatin and cadmium affect a range of other ion channels including transient receptor potential channels (Carlton et al., 2004; Alexander et al., 2008), which may be present in chondrocytes. Ultrastructural studies have confirmed the presence of L-type VGCCs in mouse limb bud chondrocytes (Shakibaei and Mobasheri, 2003). These channels appear to be organized around β-1 integrin receptors with kinases and cytoskeletal complexes in close proximity. The presence of L-type (and T-type) calcium channels in chondrocytes was recently supported by Mancilla et al. (2007), however, (Sanchez and Wilkins, 2004) found that osmotically induced changes in intracellular calcium ions were not influenced by more selective L-type calcium channel blockers (including verapamil). In contrast aggrecan and collagen synthesis induced by electrical stimulation of cartilage is dependent upon the activity of VGCCs (Xu et al., 2009). Clearly, further evidence for the presence of this channel is needed to clarify these data.

VOLTAGE-GATED SODIUM CHANNELS (VGSC)
Voltage-gated sodium channels (VGSC) are integral membrane proteins that are activated in response to voltage-changes across the plasma membrane (Catterall, 1991, 1992, 1995, 2002). The presence of tetrodotoxin sensitive VGSC in rabbit chondrocytes has been reported by Sugimoto et al. (1996) and in chondrocytes from osteoarthritic cartilage by Ramage et al. (2008). It would be interesting to see how the expression of this channel fits into the control of the chondrocyte membrane potential, since current studies have failed to observe sufficient hyperpolarization of chondrocytes for a typical VGSC to be substantially reactivated. Under conditions of constant depolarization, for example, these channels would be permanently inactivated.

EPITHELIAL SODIUM CHANNELS
Epithelial sodium channels (ENaC) have been identified in chondrocytes both immunohistochemically (Trujillo et al., 1999) and functionally (Lewis et al., 2008). They are members of the
degenerin (DEG) and ENaC superfamily (Mano et al., 2009). ENaC is a heteromeric channel, formed of up to four subunits; α, β, δ, and γ (Canessa et al., 1994). Using immunohistochemistry, the ε and β subunits have been shown to be present in chondrocytes (Trujillo et al., 1999). ENaCs are significantly more permeable to sodium than potassium (Eaton et al., 1995) and are sensitive to the channel inhibitor amiloride (IC50 100–200 nM; Alexander et al., 2008). The ENaC main function in the kidney, bladder, and colon is control of sodium reabsorption (Rossier et al., 2002). They are found in lung tissue (Mall et al., 1998) and the taste buds (Lindemann, 2001) and are known to regulate blood volume and pressure through sodium balance in the cardiac system (Canessa et al., 1993). ENaC is known to have roles in various disease states, including cystic fibrosis and Liddle’s Syndrome (Snyder et al., 1995; Stutts et al., 1995). Differential expression and up-regulation of the subunits between normal and disease states is thought to contribute to cellular changes in disease (Burch et al., 1995; Greig et al., 2004). In chondrocytes the role of ENaC is less clear; however, it is thought to be one of mechanotransduction, possibly where the channel contributes to the maintenance of the RMP. This, in turn, may regulate signaling pathways that allow chondrocytes to maintain their ECM and prevent chondrocyte apoptosis (Wright et al., 1996; Shakibaei et al., 2001; Shakibaei and Mobasher, 2003). It is thought that the mechanotransduction pathways involving ENaC become progressively defective during osteoarthritis, leading to a loss of chondroprotective mechanisms (Salter et al., 2004). It is possible that ENaC subunits are differentially expressed in chondrocytes, potentially to cope with different mechanical stresses throughout the zones of articular cartilage, and changes in chondrocytic properties during disease (Trujillo et al., 1999; Shakibaei et al., 2001).

**CHLORIDE CHANNELS**

The chloride channel family (CIC) is widely expressed in many tissue types. It was first discovered by Jentsch et al. (1990) using *Xenopus* oocytes, who isolated and sequenced the channel primary structure using cDNA. Using the same cDNA, CIC-1 was identified in rat skeletal muscle. In skeletal muscle, CIC-1 is involved in stabilization of the RMP (Gronemeier et al., 1994). CICs have been identified in rabbit articular cartilage (Sugimoto et al., 1996; Tsuga et al., 2002; Isoya et al., 2009) and in OUMS-27, the human chondrocyte-derived cell line (Funabashi et al., 2010). A commonly used pharmacological inhibitor of CICs is 4-acetamido-4’-isothiocyanatostilbene-2,2-disulfonic acid (SITS) (Pesente and Signorile, 1979; Lefevre et al., 1996; Vaca, 1999; Alexander et al., 2008). This and other CIC inhibitors were used by Sugimoto et al. (1996) and Tsuga et al. (2002) to show that CICs are important for control of the RMP. Furthermore, exposure of chondrocytes to high concentrations of SITS leads to signs of necrotic damage (Wohlrab et al., 2004) suggesting that activity of CICs may be critical to the survival of chondrocytes. Chondrocytes may express a number of CICs. So far the only one successfully identified in molecular terms is the cystic fibrosis transmembrane conductance regulator (CFTR) (Liang et al., 2010). This is particularly interesting since CFTR is known to function both as a channel in its own right, and as a regulator of other ion channels known to be expressed in chondrocytes (Mall et al., 1998; Nilius and Droogmans, 2003; Arniges et al., 2004). As yet no studies have successfully identified other CICs expressed by chondrocytes. Such identification will be a tricky task since the CIC family is large and the available pharmacological inhibitors are rather non-selective between each of the family members (Alexander et al., 2008). In our own laboratories we have attempted to locate CIC-1 mRNA using the primers based on the sequence already identified for canine skeletal muscle (Rhodes et al., 1999). These studies show a lack of CIC-1, but as yet there have been no positive studies on chondrocytes. The identity was suggested to be the maxi-CIC by Yabu and colleagues (Sugimoto et al., 1996; Tsuga et al., 2002), but further studies will be needed to clarify this. The CIC identified in rabbit articular cartilage by Isoya et al. (2009) proved to be swelling activated, but whilst its molecular identity is unknown, CIC-3 was suggested as a possibility on the basis of its biophysical and pharmacological properties. In terms of the function of CICs in chondrocytes, at least two clear possibilities exist; the first would be that they are required for setting of the membrane potential as implied above, but the second would be that they could be important as anionic osmolyte channels. The latter hypothesis arises from the fact that any osmotic loss of K+ ions as a part of volume regulation would need to be matched by an effective anion loss. CICs would be an obvious candidate to fulfill such a role.

**AQUAPORIN CHANNELS**

Aquaporins (AQP) are a family of small integral membrane proteins related to the major intrinsic protein (MIP), sometimes called AQP0 (Agre et al., 1993). The first AQP discovered, AQP1, was identified during experiments investigating the identity of the rhesus blood group antigens (Agre et al., 1987; Denker et al., 1988; Smith and Agre, 1991). Oocytes from *Xenopus laevis* microinjected with *in vitro*-transcribed mRNA of AQP1 (previously known as CHIP28) exhibited increased osmotic water permeability compared to uninjected controls. This observation, combined with the reversible inhibition induced by mercuric chloride, provided the first molecular evidence for water channels (Preston et al., 1992). Since the identification of AQP1 the field has expanded to now include study of AQP in all types of organisms. In mammals, over a dozen AQP have been identified. The classical AQP transport water exclusively. However, a second class of AQP has now been identified (Rojek et al., 2008); these so-called aquaglyceroporins also transport small, uncharged molecules such as glycerol and urea; examples include AQP3, AQP7, and AQP9 (Carbrey and Agre, 2009). Many models of chondrocyte function involve changes in volume (Hall et al., 1996). For this to occur there must be pathways for the movement of water into and out of the cell. The discovery of AQP channels in chondrocytes would appear to provide an appropriate mechanism (Mobasher and Marples, 2004; Mobasher et al., 2004a,b; Trujillo et al., 2004; May et al., 2007). Whilst studies have already shown a loss of volume regulation with inhibition of AQP channels (May et al., 2007) and reductions in migration and adhesion (Liang et al., 2008), it would be interesting to investigate whether cell survival or progression of osteoarthritis are also affected by AQP block or by AQP knockouts.

**NMDA CHANNELS**

There have been a few reports of expression of excitatory neurotransmitter receptor (NMDA) channels in chondrocytes (Millward-Sadler et al., 2001; Salter et al., 2004; Ramage et al., 2008). These are
Table 1 | Ion channels that have been identified in the chondrocyte channelome.

| Channel family | Identified in chondrocytes | Species | General phenotype | Proposed role in chondrocytes | References |
|----------------|-----------------------------|---------|-------------------|-------------------------------|------------|
| **POTASSIUM CHANNELS** | | | | | |
| $K_v$ | $K_{v,1.4}$ | Canine, chicken, elephant, equine, rat | Voltage activated | Regulation of membrane potential | Walsh et al. (1992), Wilson et al. (2004), Mobasheri et al. (2005a), Ponce (2006) |
| $K_{ATP}$ | Kir6.x/SURx | Equine, human | Closed with normal intracellular [ATP] | Coupling cellular activity to metabolic state | Mobasheri et al. (2007) |
| BK | KCNMA1, KCNMNB1 | Equine | Large Ca$^{2+}$ activated | Coupling of membrane stretch to RVD | Long and Walsh (1994), Mobasheri et al. (2010) |
| SK | – | Human | Small Ca$^{2+}$ activated | Intracellular Ca$^{2+}$ regulation | Wright et al. (1996) |
| **NON-SELECTIVE CATION CHANNELS** | | | | | |
| TRPV4 | – | Porcine, canine, equine | Mono and divalent cation conductor | Coupling membrane stretch and BK channel function; osmoregulation | Phan et al. (2009), Lewis et al. (2010), Lewis et al. (2010), Gavenis et al. (2009) |
| TRPV5 | – | Canine, equine | Highly calcium selective channel ($P_{Ca}/P_{Na} > 100$) | Not known | |
| TRPV6 | – | Canine, equine | Highly calcium selective channel ($P_{Ca}/P_{Na} > 100$) | Not known | |
| TRPC1/2/6, TRPM5/7, TRPV1/2 | – | Human osteoarthritic cartilage | Cation channels | Not known | |
| **SODIUM CHANNELS** | | | | | |
| VGSC | – | Rabbit | TTX sensitive | Mechanotransduction | Sugimoto et al. (1996) |
| ENaC $\alpha$- and $\beta$-subunits | | Canine, human | Low conductance, amiloride sensitive | Mechanotransduction | Trujillo et al. (1999), Shakibaei and Mobasheri (2003), Lewis et al. (2008) |
| **OTHER CHANNELS** | | | | | |
| Chloride | Maxi-Cl | Rabbit | Large conductance (~280 pS) | Control of membrane potential | Sugimoto et al. (1997), Tsuga et al. (2002) |
| VGCC | – | Rabbit, mouse | Voltage-activated | Calcium signaling | Poiraudet et al. (1997), Shakibaei and Mobasheri (2003) |
| Aquaporins | AQP-1, AQP-3 | Equine, porcine, bovine, cell lines, ovine | Water and small solute transport | Regulation of cell volume | Mobasheri and Marples (2004), Mobasheri et al. (2004a, b), Trujillo et al. (2004), May et al. (2007) |
| ASIC | – | Bovine | Voltage activated | Intracellular pH regulation | Sanchez et al. (2006) |
| NMDA | – | Human | Ligand-gated ion channel | Response to mechanical stimulation | Millward-Sadler et al. (2001), Salter et al. (2004), Ramage et al. (2008) |
| Connexins | Connexin 43 | Bovine | Gap junction hemichannels | Mechanocoupling | Knight et al. (2009) |

Ion channels either identified or proposed to exist in chondrocytes. AQP, aquaporin channel; ASIC, acid sensing ion channel; BK, calcium-activated potassium channel; high conductance; ENaC, epithelial sodium channels; $K_{ATP}$, ATP-dependent potassium channel; Kir, inwardly rectifying potassium channel; $K_v$, voltage-gated potassium channel; MIP, major intrinsic protein; NMDA, N-methyl-D-aspartate; SK, calcium-activated potassium channel; low conductance; SUR, sulfonylurea receptor; TRP, transient receptor potential channel; TTX, tetrodotoxin; VGCC, voltage-gated calcium channels; VGSC, voltage-gated sodium channel.
interesting observations however the role of these ligand-gated ion channels in chondrocyte function is not yet understood. It does not seem likely that they are involved with neurotransmission because, despite some similarities between neurone and chondrocyte phenotype, no “pre-synaptic” neurones project to the immediate vicinity of the chondrocytes. It again appears likely that NMDA channels are in someway involved in the mechatransduction pathway, since mechanically induced hyperpolarizations are reduced by NMDA antagonists (Salter et al., 2004). Furthermore, glycine induces a number of changes on chondrocytes in cartilage explants (including accumulation of calcium) and these effects can be reduced with an NMDA antagonist as, presumably, glycine acts via the glycine binding site of the NMDA receptor (Takahata et al., 2008).

OTHER ION CHANNELS

Two further ion channels recently identified in chondrocytes are the acid sensing channel, ASIC1a and ASIC3 (Kolker et al., 2010; Yuan et al., 2010) and the connexin 43 hemichannel (Knight et al., 2009). ASIC are very small cation selective channels closely related to ENaC (reviewed by Wemmie et al., 2006). As their name implies, they are opened by extracellular protons. This is particularly relevant to chondrocyte biology since chondrocytes are routinely exposed to relatively acidic conditions, as low as pH 6.6 for example (Wilkins et al., 2000). In vitro studies show that these channels mediate an increase in intracellular calcium upon exposure of chondrocytes to acidic conditions. This intracellular Ca\textsuperscript{2+} is likely to be a signal for production of enzymes and for proliferation. Potentially, inappropriate increases in calcium could result in cell death from either necrosis or apoptosis (Kolker et al., 2010; Yuan et al., 2010). The role of the connexin 43 is possibly more complex. Knight et al. (2009) found it to be constitutively active in about 40% of chondrocytes, and as such it might be expected to profoundly depolarize the membrane. In summary, the suggested scheme of connexin 43 involvement was that mechanical stimulation of chondrocyte cilia activates the hemichannel which then acts as a conduit for ATP release. This released ATP then acts on chondrocyte membranes (via P2 purinoceptors) to increase intracellular Ca\textsuperscript{2+} (Knight et al., 2009).

CONCLUSIONS

There is growing interest in the expression and function of ion channels in chondrocytes. Part of this interest stems from the realization that many ion channels are involved in mechatransduction, chemotransduction and osmoregulation. It is important to bear in mind that ion channels are also important drug targets because of their localization in the chondrocyte plasma membrane. A number of research groups, including ours, have used electrophysiology, molecular biology and immunohistochemistry to study ion channels in articular chondrocytes. Table 1 contains a summary of the ion channels studied in the chondrocyte channelome so far. It is likely that some ion channels in chondrocytes are multifunctional, serving a number of different physiological purposes. The processes of mechanical and chemical sensing and metabolic regulation may well be intricately linked and make use of a number of ion channels as common denominators. In summary, ion channels are important for chondrocyte function and further investigations are required to explore the full complement of channels present in the chondrocyte channelome. This knowledge will help us understand the unique biology of chondrocytes and may lead to the development and formulation of therapeutic strategies to treat arthritis.

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