**Membrane channel gene expression in human costal and articular chondrocytes**

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**Abstract**

Chondrocytes are the uniquely resident cells found in all types of cartilage and key to their function is the ability to respond to mechanical loads with changes of metabolic activity. This mechanotransduction property is, in part, mediated through the activity of a range of expressed transmembrane channels; ion channels, gap junction proteins, and porins. Appropriate expression of ion channels has been shown essential for production of extracellular matrix and differential expression of transmembrane channels is correlated to musculoskeletal diseases such as osteoarthritis and Albers-Schönberg. In this study we analyzed the consistency of gene expression between channelomes of chondrocytes from human articular and costal (teenage and fetal origin) cartilages. Notably, we found 17 ion channel genes commonly expressed between articular and both types of costal cartilage chondrocytes. There were several other ion channel genes expressed only in articular (6 genes) or costal chondrocytes (5 genes). Significant differences in expression of *BEST1* and *KCNJ2* (Kir2.1) were observed between fetal and teenage costal cartilage. Interestingly, the large Ca2+ activated potassium channel (BKα, or *KCNMA1*) was very highly expressed in all chondrocytes examined. Expression of the gap junction genes for Panx1, *GJA1* (Cx43) and *GJC1* (Cx45) was also observed in chondrocytes from all cartilage samples. Together, this data highlights similarities between chondrocyte membrane channel gene expressions in cells derived from different anatomical sites, and may imply that common electrophysiological signaling pathways underlie cellular control. The high expression of a range of mechanically and metabolically sensitive membrane channels suggest that chondrocyte mechanotransduction may be more complex than previously thought.

**Introduction**

The biology of cartilage is complex and includes tissue-dependent remodeling in response to environmental stimuli (Mow and Guo, 2002). Mechanical signals cause changes in cartilage biosynthetic activity, including changes in matrix production (Liu et al., 2013) and gene expression (Amanatullah et al., 2014). The cartilage repair process is impacted by a lack of blood supply to the tissue, resulting in a relatively hypoxic and acidic environment for cells. The fundamental processes that underlie mechanotransduction in cartilage could potentially be harnessed to enhance tissue regeneration. A promising example of this approach recently used an agonist to the mechanosensitive TRPV4 channel to induce matrix synthesis (O'Conor et al., 2014). Appropriate expression of ion channels is essential for production of extracellular matrix in trachea (Lin et al., 2014) and in chondrocytes grown *in vitro* (Wu and Chen, 2000, Mouw et al., 2007). Differential expression of transmembrane channels is also correlated to musculoskeletal diseases such as osteoarthritis and Albers-Schönberg (Cleiren et al., 2001, O'Conor et al., 2013). Whilst the role of cartilage varies between anatomical sites, it seems likely that key fundamental tissue specific processes will be conserved between different sites.

 The biology of cartilage is driven by osmotic and ionic gradients created by the fixed charge density of proteoglycans which draw sodium and water into the tissue (Wilkins et al., 2000). This osmotic gradient is maintained and resisted by matrix proteins secreted by chondrocytes resulting in a hydrostatic pressure contributing to the ability of cartilage to withstand biomechanical stresses and strains (Mow and Guo, 2002). Largely understudied is the response of cells to ionic, osmotic, and pH gradients that are generated as cartilage is exposed to continually changing biomechanical forces generated during movement. Under these conditions, cells attempt to maintain homeostasis by moving ions back and forth across the cell membrane, effectively achieved by the expression of a range of transporters and ion channels. There is interest in the number and diversity of ion channels expressed by chondrocytes since many have unknown functions, but appear conserved between animal species (Barrett-Jolley et al., 2010).

Compromised cell-volume regulation has been associated with cartilage degeneration (Bush and Hall, 2003), and a number of ion channels, including TRPV4 (Clark et al., 2010a) and transporters (Sanchez and Wilkins, 2003) are clearly involved with the homeostatic control of chondrocyte volume through modulation of the cell membrane potential (Lewis et al., 2011a, Lewis et al., 2011b). The resting membrane potential of articular chondrocytes is more positive compared to many other cell types (Binggeli and Weinstein, 1986). This is intriguing because cells with a more positive resting membrane potential tend to have greater regenerative capabilities (Levin and Stevenson, 2012). The identity of ion channels present in chondrocytes is therefore of biological significance in organogenesis, tissue repair, and regeneration. Such knowledge will provide clues as to which ion channels are important for universal cartilage functions, while others may be involved with site-specific functions like cell proliferation, migration, and differentiation (Adams and Levin, 2013, Levin, 2014).

 In addition to ion channels, the membrane channel superfamily includes the so called gap junction proteins comprising both connexins and pannexins (Iovine et al., 2008). Gap junction proteins form intercellular pores that are permeable to small inorganic ions and larger organic molecules, such as ATP. Gap junction proteins also form “hemi-channels”, where the proteins form a transmembrane pore, like an ordinary, but very large ion channel (Stout et al., 2002). Unpaired hemichannels are capable of activation by pH, voltage, and intracellular calcium stimuli (Herve, 2013). Gap junctions have many roles, including mechanotransduction in cartilage (Knight et al., 2009), and can be activated through numerous mechanisms, including stretch (Salameh and Dhein, 2013) or voltage-dependent conformational changes (Bargiello et al., 2012). Gap junctions, through their ability to directly connect cells, are able to create networks of cells, an important component of successful tissue regeneration (Levin, 2013). Expression of gap junction genes is also tissue specific. This is significant in cartilage regeneration because recent reports (Mayan et al., 2013a, Mayan et al., 2013b) have described chondrocyte networks mediated by gap junctions. Overall, this suggests that transmembrane transport by membrane channel proteins may play an as yet unknown role in human cartilage tissue regeneration and we hypothesize that a diverse array of cell membrane ion transporters will be present in primary human chondrocytes.

In this paper, we define a complex and diverse array of ion channels present in a variety of chondrocytes, identify common gap junction genes expressed in human chondrocytes, and suggest a means of communication that is presently under exploited in the regeneration of cartilage.

**2. Materials and Methods**

**2.1 Cartilage samples**

Cartilage samples were obtained from 9 human subjects. Normal costal cartilage was obtained from a 15-year-old and two 17 year old males (CON8, CON9 and CON10 respectively) and processed within 24 hours. Femoral head articular chondrocytes from 54 and 57-year-old males and a 59-year-old female were purchased from PromoCell (Heidelberg, Germany). Cells were isolated from apparently normal cartilage of patients undergoing knee/hip replacement surgeries. All cells were cultured in Chondrocyte growth medium (PromoCell). Three fetal samples of costal cartilage were obtained; F1, a 37 weeks gestation female with chorioamnionitis, F2, a 21 weeks gestation female with intrauterine demise due to severe chorioamnionitis and congenital pneumonia, and F3, a 15 week gestational male with severe congenital cardiac disease. Fetal cells were grown in DMEM/F12 (HyClone, Logan, Utah, USA) (Cetinkaya et al., 2011) supplemented with 10% FBS (Atlanta Biologicals, Norcross, GA, USA), 2 mM L-glutamine (InVitrogen, Carlsbad, CA, USA), 50 IU/mL penicillin, and 50 mg/mL streptomycin (InVitrogen) at 37°C with 5% CO2 in humidified air. For experiments, chondrocytes were maintained in suspension culture (Bosnakovski et al., 2004, Wa et al., 2015) to maintain their differentiated phenotype, and briefly expanded in tissue culture flasks. All experiments used cells that had been passaged less than 4 times.

**2.2 Reverse Transcription and Real-Time PCR Analysis**

RNA was directly isolated from cells in tissue culture dishes, and genomic DNA eliminated using a Direct-zol™ RNA MiniPrep (Zymo Research, Irvine, CA, USA). Complimentary DNA (cDNA) was generated using an RT-First Strand Kit (Qiagen, Valencia, CA, USA). Polymerase chain reactions (PCRs) were performed using SYBR green detection (Qiagen) and customized ion channel array plates (Qiagen) in a BioRad CFX96 system (BioRad, Hercules, CA, USA). These customized plates provide gene expression data on 84 different ion channel-related genes. Manufacturer guidelines were used for PCR reaction volumes and cycle parameters. The cycling parameters were 95oC for 10 minutes, then 40 cycles of 95oC for 15 seconds and 60oC for 60 seconds. Reaction specificities were assessed with a melt curve of 65oC to 95oC in 0.2oC increments. The data was standardized to five (*ACTB, B2M, GAPDH, HPRT1* and *RPLP0*) reference gene expression values for all samples using the ∆Cq method. Gap junction gene expression was determined using a connexin and pannexin gene array (Qiagen CA, USA), with data standardized to the five reference genes described above. Aggrecan and COMP gene expression was by RT-PCR using commercially available primers (Qiagen) and data standardized against two reference genes (*GAPDH* and 18srRNA). Gene expression was calculated as $2^{-(Cq\_{GOI }-Cq\_{Ref})}$, where CqGOI is the Cq value of the gene of interest and CqRef, is the Cq value for the averaged reference genes. Although the assay range using the RT2 profiler array (Qiagen) is 6.8-35 Cq, in order to minimize the possibility of false-positives and account for variability, a constant concentration of total cDNA was used in all reactions between all samples and a raw Cq cutoff of 30 was used (Canales et al., 2006, Arikawa et al., 2008).

**2.3 Immunocytochemistry**

Confirmation of *KCNMA1*(KCa1.1α), *KCNA2* (KV1.2), *SCN9A*(NaV1.7), and *GJA1* (Cx43) gene expression were made by immunocytochemistry. Cells were grown on cover slips and fixed in 4% paraformaldehyde for 20 minutes then permeabilized with PBS + 0.5% TRITON-X100 for 10 minutes. Washes with PBS were performed after both steps and cells were then blocked in 10% boiled goat serum (BGS) for 1 hour. Incubation with primary antibodies was performed in 5% BGS at 4°C overnight then washed three times in PBS + 0.1% Tween-20 (PBS-T) for 5 minutes each. Rabbit polyclonal primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) for KCa1.1α (sc-25686), KV1.2 (sc-292447), NaV1.7 (sc-130096), and Cx43 (sc-9059). Negative controls were produced using normal rabbit IgG (Santa Cruz). Secondary staining was with goat anti-rabbit Alexa Fluor 488 (Life Technologies, Carlsbad, CA, USA) in 5% BGS for 45 minutes at room temperature then washed three times in PBS-T for 5 minutes each. The nuclei were counterstained using 1 µg/ml DAPI (4',6-diamidino-2-phenylindole; Sigma) in PBS-T for 5 minutes followed by three PBS-T washes for 5 minutes each. After a brief rinse in PBS, the coverslips were mounted on slides using VECTASHIELD antifade mounting medium (Vector Labs, Burlingame, CA, USA). Electronic fluorescent images were captured using an Olympus DP70 CCD camera through an Olympus BX51 microscope (Olympus America Inc., Center Valley, PA, USA).

**3. Results**

**3.1 Chondrocytes express ion channel and gap junction genes.**

*3.1a Ion channels*: Eighty four genes commonly expressed in excitable tissues were examined for gene expression in each of the 9 human chondrocyte samples. The ∆Cq was calculated for each sample using five different reference genes. A threshold for detection was conservatively set at a Cq of 30. Expression values of all genes examined can be found in Supplement TableS1. Table 1 summarizes expression of those genes that were considered to be uniformly expressed in the three types of cartilage examined. We classified channel genes ontologically under the following four groups:

*Calcium Permeable Ion Channels:*

 Of the twelve voltage-gated Ca2+ channel genes examined and involved in calcium release from intracellular stores, three (*CACNA1A* (Cav2.1), *CACNB1* (Cavβ1), *CACNB3* (Cavβ3) show expression in all samples tested (Table 1). Expression of *RYR3* was observed in all fetal samples. *CACNA1C* (CaV1.2) is the α-subunit of a voltage-dependent calcium channel mediating Ca2+ entry during depolarization. Here we show for the first time its consistent expression in human chondrocytes derived from three independent samples of articular cartilage. Expression of this gene was found in 2/3 fetal and 1/3 costal samples which suggest either age related or functional related changes in expression.

 *Sodium Selective Ion Channels.*

Expression of six sodium ion channels was detected. The voltage-gated sodium ion channel *SCN8A* (NaV1.6α) was expressed across all samples. Of the two β-subunits investigated, *SCN1B* (NaV1.1β) was also expressed across all samples, whereas *SCN2B* (NaV1.2β) is expressed in all three articular samples, as well as 2/3 costal and fetal samples (Table 1). The roles of the sodium channel β-subunits are not known, but they may have a role in connecting sodium channels to particular cellular membrane domains or modulation of the behavior of the ion permeable alpha subunit itself. *SCN2A* (NaV1.2α) was expressed in a single articular sample (ART2) and, along with *SCN3A* (NaV1.3α), were both expressed in two of the three control costal chondrocytes (CON8 and CON10) and one fetal sample each (F1 and F2 respectively). This is the first time that expression of these six genes has been shown in chondrocytes.

*Potassium Transport:*

Seven potassium transport genes are shown in Table 1. *KCNMA1* (KCa1.1 BKα subunit) is expressed at very high levels in all samples of chondrocytes, whereas *KCNN2* (KCa2.2α) was expressed only in costal cartilage (CON8, 9, and 10, and F1. Table S1). Due to the nature of samples collected, articular samples come from older individuals whereas costal cartilages are from teenagers. A larger age-matched population will help answer questions arising due to age or site related changes in expression. Both genes have previously been described in chondrocytes involved in mechanical stretch and cell volume regulation (Martina et al., 1997, Mobasheri et al., 2010, Lewis et al., 2013b). *KCNJ15* (Kir4.2)*, KCNAB2* (Kvβ2)*,* and *KCNK1* (TWIK1) are also expressed in all samples. Kir4.2 has not been described in chondrocytes but has a role in insulin secretion in diabetes. Kvβ2 is a β-subunit that associates with active α-subunits forming a voltage-gated channel and whose function in chondrocytes is not known. TWIK1 has a role in modulating chondrocyte membrane potential (Clark et al., 2011). *KCNJ2* (Kir2.1) is expressed in all samples except F3. This gene has not previously been described in chondrocytes and functions as an inwardly rectifying K+ channels in other tissue types with likely functions in membrane potential stabilization. The voltage-gated potassium channel gene, *KCNB1* (KV2.1) has previously shown to have a role in maintenance of membrane potential in mouse chondrocytes (Clark et al., 2010b). We observed expression in all articular samples as well as CON8, CON10, and F1. *KCNJ14* (Kir2.4)*,* expressed in all fetal samples as well as CON8, ART2 and ART3, has not been previously described in chondrocytes.

*Chloride Transport:*

*CLCN3* (CLC3) and *CLCN7* (CLC7) genes are both highly expressed in all types of chondrocytes examined (Table 1). Previous studies have shown that both *CLCN3* and *CLCN7* are expressed in the human chondrosarcoma cell line OUMS-27 (Kurita et al., 2015), and that it plays a role in cell volume regulation and cell death. *CLCN2* (CLC2) expression was observed in the two of the three articular samples (ART2 and ART3), in all fetal costal cartilage samples, and in one of the costal cartilage samples (CON8), indicative of a temporal or site-specific role in chondrocyte development.

*Non-selective cation transporters:*

Eight non-selective cation channels were detected. The gene *BEST1*, showed high expression in all three articular and fetal samples but was only expressed in 1/3 teenage costal cartilages (CON8). In other tissue types (cardiomyocytes) its product functions as a chloride ion channel. *TRPC1,* *TRPV2*,and *TRPV4* are expressed in all samples (Table 1) and known to all be expressed in chondrocytes. *TRPC1* is a non-selective calcium permeant cation channel (Gavenis et al., 2009), and *TRPV4* a Ca2+ permeable osmomechano-sensitive-TRP channel contributing to early stages of hypo-osmotic stress (Hdud et al., 2014, O'Conor et al., 2014). *TRPV2* is activated at high temperatures (>59oC), its role in chondrocyte biology is unknown. *HCN2* (BCNG2) is a hyperpolarization-activated channel whose activity is modulated by intracellular chloride ions and pH, where an acidic pH shifts the activation to more negative voltages. BCNG2 was expressed in 2 of the costal cartilage samples (CON8 and CON9), 2 of the articular cartilage samples (ART1 and ART3) and 1 fetal sample (F1). *ACCN2* (ASIC1) is an acid sensing channel, activated by a drop in extracellular pH and showed expression in all articular and fetal samples and 2/3 costal cartilage samples (CON8 and CON9). These are all conditions that chondrocytes are exposed to in cartilage and may be expected to have a role in their biology. *TRPV1* and *TRPV3* have been identified in chondrocytes. *TRPV1* show enhanced expression in freshly isolated chondrocytes from older patients with osteoarthritis (Gavenis et al., 2009). We show expression in ART2, and ART3 for both *TRPV1* and *TRPV3*.

Expression results summarized from Table 1 are shown in Figure 1 and demonstrate the number and diversity of ion channels expressed in chondrocytes and how they are distributed. Expression of 14 genes is shared between all samples. One gene is uniquely expressed in teenage costal cartilage and six in articular cartilage, with one gene (*KCNJ2/*Kir2.1) shared between them. Four genes are uniquely expressed in fetal costal cartilage, with none shared between only fetal and teenage costal cartilage. Expression of *ACCN2/*ASIC1 and *BEST1* are shared between fetal and articular chondrocytes. During tissue development changes in gene expression may be expected. Comparing gene expression between fetal and teenage costal cartilage, of note was the 4-fold higher expression of *BEST1* in fetal costal cartilage and 12-fold higher expression of Kir2.1 in teenage cartilage. Articular and costal chondrocytes share expression of many membrane channel genes; however there are a number that are specific to either costal or articular chondrocytes that may be reflective of different functional properties of the tissues they were derived from. Taken together, there is a surprising number and diversity of expression in human chondrocytes where, however, channel function is largely unknown.

*3.1b Gap junctions*:

RT-PCR from chondrocytes derived from two different human sources (normal teenage costal cartilage (CON8) and femoral head articular cartilage (ART1)), was performed on 17 connexin and 3 pannexin genes. All genes showed relative fold differences in expression lower than the mean of reference genes, however, three genes, *GJA1* (Cx43), *GJC1* (Cx45), and Panx1 showed much higher expression than all other genes (Table S2) in both samples suggestive of tissue specificity. Confirmation of only Cx43, Cx45, and Panx1 gene expression was determined in two additional costal cartilage and two additional articular cartilage samples (Table2).

**3.2 Ion channel and gap junction immunocytochemistry**

Although it was not possible to confirm the presence of all expressed ion channel genes, we confirmed the presence of KCa1.1 expressed from gene *KCNMA1* (Fig 1A) and Kv1.2 from gene *KCNA2* (Fig 1B), but with little presence of Nav1.7 from gene *SCN9A* (Fig 1C), albeit that it does show some expression in CON8. The gap junction protein Cx43 was also confirmed to be abundantly present (Fig 1D). All immunocytochemistry was performed on CON8.

**3.4 Determination of chondrocyte phenotype**

Chondrocyte phenotype was determined by expression of aggrecan (*ACAN*) and *COMP*, genes normally expressed by differentiated chondrocytes. Both genes were expressed in all samples with relative ΔCq values given in TableS3 relative to two reference genes.

**4. Discussion**

In this work we analyzed the expression of a panel of membrane channels from a range of cartilage phenotypes. We identify channels which are conservatively expressed across phenotypes and others which are differentially expressed. Several of the channels we identified have not previously been described/identified in cartilage. These channels belong to several different classes and families. Some groups of channels are expected based on electrophysiological studies, but the specific members have not been identified and characterized. One such group is the potassium transport channels. There was preexisting evidence that members of the voltage-gated Kv and inwardly rectifying potassium channels (Kir) subgroups exist at the RNA and protein level (Mobasheri et al., 2005, Clark et al., 2010b, Varga et al., 2011). However, our results show expression of 5 additional Kv and Kir channels (Kvβ2, Kv2.1, Kir2.1, Kir2.4, and Kir4.2). The function of these channels in chondrocytes is thought to be involved in differentiation, maintenance of the membrane potential, and regulation of calcium signaling. Previous reports (Wright et al., 1992, Wilson et al., 2004, Clark et al., 2010b, Lewis et al., 2011b, Varga et al., 2011) have shown that the resting membrane potential (RMP) of chondrocytes is significantly more depolarized than excitable tissues (-40mV to -10mV in chondrocytes compared to -60 to -95mV in excitable cells (Hodgkin and Huxley, 1945, Hodgkin and Horowicz, 1960). This membrane potential is significantly more positive than the equilibrium potential for K+ ions and therefore allows rapid efflux from the cells when channels open. The higher expression of Kir2.1 in teenage costal cartilage compared to fetal indicates an increasingly important role in membrane potential homeostasis in a tissue outside of its embryonic environment.

 When cartilage is compressed, water is exuded and osmolality increases within the tissue leaving chondrocytes susceptible to shrinkage. Cellular homeostasis is maintained by entry of cations and water into the cells (Lewis et al., 2011b). The two primary candidate proteins mediating this cation influx are the Na+, K+, 2Cl(-) (NKCC1) co-transporter (Qusous et al., 2011) (Kerrigan et al., 2006) and the epithelial sodium channel (Lewis et al., 2013a). Under relaxation, water reenters cartilage and the osmolality decreases. Hypo-osmolality cause’s chondrocytes to swell, however, the relatively positive RMP allows K+ to leave the cell along with excess water, reducing swelling and maintaining homeostasis. Associated with any loss of cations there must be an equivalent loss of anions, such as chloride ions. Chloride channels are known to be involved in RMP maintenance and cell volume regulation in chondrocytes. The ion channels CLC1, CLC2, and CLC3 were thought to be present in chondrocytes based on electrophysiological and pharmacological studies (Sugimoto et al., 1996, Isoya et al., 2009), with both CLC2 and CLC3, and additionally CLC4, CLC6, and CLC7, expression recently observed in human-derived chondrosarcoma cells (Kurita et al., 2015). Our own analysis confirms the expression of CLC3 and CLC7 in all cartilage types, while CLC2 and BEST1were expressed primarily in fetal costal and articular chondrocytes. When chloride channels are blocked using NPPB (5-nitro-2-(3-phenylpropyl-amino) benzoic acid) chicken mandibular mesenchymal stem cells (MSCs) undergoing chondrogenesis showed a decrease both in rate of proliferation and production of extracellular matrix proteins, including inhibition of terminal differentiation measured by collagen X expression (Tian et al., 2010). This suggests further functional roles for chloride channels in chondrocyte biology.

 Voltage-gated sodium channels comprise a family of membrane channel genes that are expressed in all of our chondrocyte samples. Previously, NaV1.4 was found to be expressed in chondrogenic MSCs and present at the protein level (Varga et al., 2011). However, due to the depolarized nature of chondrocytes, it is possible that they do not function as conventional sodium transporters. In contrast, the β-subunits of NaV have established non-sodium transport functions including mechanotransduction, and cell adhesion (Brackenbury and Isom, 2008) In our analysis, we have identified expression of several NaV α-subunits and two β-subunits including NaV1.6α and NaV1.1β that are expressed in all samples and have not previously been described in chondrocytes.

 Voltage-gated calcium channels (CaV) mediate calcium entry during depolarization in excitable cells. In mouse limb-bud chondrocytes, CaV1.4 is associated with β1-integrin suggesting involvement in mechanotransduction (Shakibael and Mobasheri, 2003). Our analysis showed expression of CaV1.2, CaV2.1, CaVβ1, and CaVβ3, though function of these genes remains unclear, we speculate they may have similar functions.

 Gap junction proteins are expressed in a very tissue specific manner, undoubtedly due to functional requirements of any one tissue, and impairment of their function contributes to the pathophysiology of many disorders. Typically, 2-3 gap junction genes are expressed in any one tissue, although compensatory mechanisms exist for functional loss of a gap junction. We observed expression of three gap junction genes, *GJA1* (Cx43)*, GJC1* (Cx45), and Panx1, in both human primary costal and articular cartilage. Cx43 has been previously observed in the primary cilium of bovine chondrocytes and involved in ATP release in response to mechanical stimulation (Knight et al., 2009). A study on ATP binding receptors in chondrocytes (Rosenthal et al., 2010) indicates that P2X receptors act as ATP-gated ion channels that quickly increase intracellular calcium. Other studies show interactions between P2X receptors and pannexins, and a considerable body of evidence shows P2X receptors in neurons are important mediators of pain (North and Jarvis, 2013). Cx43 and Cx45 genes identified in human articular cartilage have been suggested to be involved in intercellular signaling (Mayan et al., 2013c), but ours is the first report of Panx1 gene expression. Expression of Cx43 and Cx45 is of interest because of the rectifying properties of Cx43:Cx45 heterotypic channels whereby Cx45 is able to modulate the fast gating properties of Cx43, possibly through docking induced conformational changes in Cx43 (Elenes et al., 2001). In addition to Cx43 and Cx45, Mayan’s group (Mayan et al., 2013c) also noted expression of the genes for Cx32 and Cx46. Cx32 has been strongly associated with the neurophysiological disorder Charcot-Marie-Tooth disease (Bergoffen et al., 1993). Interestingly, Cx46 is expressed in lens tissue, and like cartilage, does not have a blood supply and survives under hypoxic conditions. The promoter region of Cx46 is strongly influenced by hypoxia (Molina and Takemoto, 2012), and there is a possibility that our observations did not include Cx46 as our cells were not grown under hypoxic conditions. Of the three pannexin genes, *Panx3* has been shown to regulate intracellular ATP/cAMP and promote chondrocyte differentiation in mice (Iwamoto et al., 2010). *Panx3* is also expressed in hypertrophic chondrocytes during mouse long bone development, but expression is very strongly linked to osteogenic differentiation (Bond et al., 2011). Pannexin 2 expression has been observed in, but not restricted to, the central nervous system (Penuela et al., 2013) and has not been observed in chondrocytes. Pannexin 1 has been reported in many tissues including brain, testis, skeletal muscle and skin (Baranova et al., 2004). The expression of Panx1here in cultured cells suggests a role for pannexin genes in chondrocyte differentiation. Pannexins, including Panx1, are mechanosensitive suggesting the presence of Panx1 related channel activity through biophysical stimuli (Sandilos and Bayliss, 2012). Their presence could act as a growth regulator (modulating cell cycle progression) with differentiation tied in to growth regulation and mechanical stress.

Overall these results show remarkable consistency in expression of subsets of ion channel genes. The consistency is all the more remarkable because of the different sources and ages of chondrocytes, suggesting that these genes are important in general chondrocyte biology. The roles of each gene briefly described are known in other cell types, and may not have the same function in chondrocytes. There is expression of certain genes (Table 1) where variation may be accounted for by site, disorder, age, or individual differences, however larger numbers of samples need to be analyzed from these sub-groups to confirm this.

Gap junction proteins are crucial mediators of cell communication during embryogenesis, tissue regeneration, and disease (Peiris and Oviedo, 2013). Additionally, an important role of ion channel-driven resting membrane potential is tissue patterning (Liebau et al., 2013). Mesenchymal stem cells express a variety of ion channels involved in numerous cellular processes, and their use for cell replacement therapy and tissue engineering has gained considerable interest (Zhang et al., 2014a, Zhang et al., 2014b) suggesting that multiple functional ion transporters may be necessary for regenerative pathways in cartilage that are presently understudied.

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