THE CHONDROCYTE PRIMARY CILIUM – A PURINERGIC MECHANORECEPTOR?

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Purpose: Mechanical loading is essential for the health and homeostasis of articular cartilage and may be utilised within a tissue engineering context to promote the production of a functional repair tissue. Consequently there is much interest in elucidating the fundamental mechanotransduction pathways in chondrocytes. Previous studies have demonstrated the presence of primary cilia in articular chondrocytes and their interaction with integrins and the extracellular matrix. Furthermore, we have recently shown that in Tg737orpk mice that lack polaris, a protein involved in cilia assembly, chondrocytes have extremely short or missing cilia. These mice have defects in growth plate and developing articular cartilage as well as skeletal patterning abnormalities and stunted growth. This suggests that primary cilia are critical for the development and health of articular cartilage although the precise function of this fascinating organelle in cartilage has not been established. In various other cell types, primary cilia act as mechanoreceptors, such that cilia deflection initiates intracellular Ca²⁺ signaling as part of a mechanotransduction cascade. We have demonstrated that compression of chondrocytes also activates a Ca²⁺ signalling pathway, mediated by the release of ATP, which triggers an up-regulation of proteoglycan synthesis. However, the mechanism of ATP release and the involvement of the primary cilium are as yet unclear.

Methods: The present study used immunofluorescence and confocal microscopy of bovine and human articular cartilage to examine the expression and co-localisation of primary cilia and connexin 43 hemichannels, which are known to act as mechanosensitive ATP release channels in other cell types. In addition, we also examined the expression of purine receptors through which extracellular ATP may trigger Ca²⁺ signalling.

Results: Within bovine articular cartilage, approximately 40-60% of chondrocytes exhibited primary cilia with a higher percentage of ciliated cells in the deep zone. All cells were found to express connexin 43 hemichannels which was confirmed by immunolabelling of both the intracellular and extracellular domains. Furthermore, at least 50% of the primary cilia were decorated with connexin 43 (Fig. 1).

![Figure 1](image1)

Studies using human articular cartilage revealed that only chondrocytes within the superficial zone expressed connexin 43. Chondrocytes throughout all zones of the tissue expressed purine receptors P2X2, P2X4, P2X7 and P2Y1, whereas P2Y2 was expressed only by superficial zone cells.

Conclusions: These studies suggest that the chondrocyte primary cilium may serve as a mechanoreceptor or strain amplifier such that its distortion activates ATP release via connexin 43 hemichannels, initiating a purinergic Ca²⁺ signalling pathway.

NMDA RECEPTORS ON CHONDROCYTES MODULATE MECHANOTRANSDUCTION

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Purpose: The mechanisms by which mechanical forces regulate chondrocyte function are beginning to be understood. A number of different mechanoreceptors appear capable of recognising mechanical loads applied to chondrocytes and stimulation of these receptors results in generation of complex signal cascades involving activation of intracellular signalling molecules and production of autocrine and paracrine signalling molecules that, by regulating transcription factor activation influence chondrocyte gene expression and protein/proteoglycan production. We have previously identified involvement of substance P, a neuropeptide, in human articular chondrocyte mechanotransduction. As Substance P and glutamate are closely associated in the central and peripheral nervous system, being co-expressed by some neuronal cells we have extended our studies to investigate whether other classical neuronal signalling molecules including NMDA receptors are similarly involved in regulation of chondrocyte responses to mechanical loads.

Methods: Human knee joint articular chondrocytes were obtained from arthroplasty or above knee amputations. Expression of NMDAR was identified by immunohistochemistry, western blotting and RT-PCR. Function of NMDAR was studied by assessing calcium uptake, changes to cell membrane potential and activation of intracellular signalling molecules following addition of NMDA or mechanical stimuli in the presence or absence of specific receptor antagonists and blockade of activity of mechanotransduction associated molecules.

Results: NMDAR subunits NR1, 2A and 2B were detected at the molecular and protein level whereas NR2C, 2D and NR3 were not identified. 50µM NMDA with glycine induced calcium uptake by chondrocytes and this was associated with cell membrane hyperpolarisation of normal chondrocytes and depolarisation of OA chondrocytes. These electrophysiological responses were blocked by NMDAR antagonists (MK801 and 2-Amino-5-phosphonovaleric acid), PSD-95 dissociation (TAT-NR2B9c) and nNOS inhibition (L-NAME-Nitroarginine-2,4-L-diamino-butryc amide). Downstream effects of NMDAR stimulation included activation of both tetrodotoxin sensitive sodium channels and apamin sensitive SK ion channels. NMDAR blockade inhibits the electrophysiological response of both normal and OA chondrocytes to mechanical stimulation. In the mechanotransduction pathway function blocking antibodies to b1 integrin, CD47 and IL4R but not α1, α2, α3, α5 or IL1 receptor inhibited NMDA induced membrane responses.

Conclusions: We have demonstrated expression and functional activity of NMDAR, a subset of the glutamate receptor family, by normal and OA chondrocytes. Results obtained to date suggest roles for these molecules in chondrocyte responses to mechanical stimuli involving regulation of cell signalling through j1 integrin/CD47 and IL4R. Expression by chondrocytes of classically neuronal-associated molecules is increasingly being demonstrated. Roles for these molecules are likely to be diverse, complex and important. In neurons physiological levels of NMDA activation promote cell survival whereas intense or chronic activation of the receptors results in both activation of proapoptotic pathways and, in certain circumstances necrotic cell death. Whether similar functions for these molecules exist in cartilage, promoting chondrocyte survival or death in face of a wide range of mechanical and other insults that lead to development and progression of osteoarthritis remains to be seen.