

# Regulation of pathological chondrocyte activity by the primary cilium

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**Purpose:** OA is defined by increased activity of catabolic enzymes that target the ECM, principally ADAMTS5 and MMP13. This enzymatic activity is regulated at the point of gene transcription, induced by inflammatory signalling pathways such as NFκB, and controlled by post-translational turnover, for example by LRP1-mediated endocytosis. The primary cilium is a singular organelle assembled by almost all cell types. The ciliary-associated proteome includes core cilia proteins such as intraflagellar transport proteins (IFT). The cilium modulates cell behaviour, acting as a hub for cellular signalling. The cilium is influential during musculoskeletal development; many ciliopathies have a musculoskeletal phenotype. In cells of the joint, such as mesenchymal stem cells, osteocytes and chondrocytes, genetic disruption of core cilia machinery alters the response to a variety of physiologically important stimuli including mechanics, inflammatory cytokines, changes to osmolarity and oxygen tension, growth factors, and Indian hedgehog. Moreover, such stimuli alter cilia trafficking and ciliary architecture and function. Recently, we showed the cilium modifies the response to interleukin-1 (IL-1) by tuning NFκB signalling. Here we investigate the influence of the cilium to the catabolic activity of chondrocytes in order to test for roles of the cilium in disease pathogenesis.

**Methods:** The study used primary human and porcine chondrocytes and murine chondrocytes with a hypomorphic mutation to IFT (IFT88ORPK). Chondrocytes were cultured with/without cytokines IL-1β or TNFα. Aggrecan cleavage was assessed by western blotting (WB) for ARGS and AGE3 epitopes, using a cell-aggrecan co-culture system. LRP1 expression in chondrocytes was assessed by immunofluorescent (I.F) staining. Gene expression analyses were conducted by qPCR. Primary cilia architecture and p65 dynamics was assessed by I.F. with Image J analysis.

**Results:** IFT targeting altered aggrecan catabolism. ARGS and AGE3 epitopes were increased in non-stimulated chondrocytes to levels comparable to that seen with cytokine treatment. Microscopy revealed LRP1 expression to be associated spatially with the microtubule organising centre/basal body and thus highly concentrated at the ciliary base. This organisation was lost when ciliary trafficking was inhibited. Unstimulated IFT88ORPK cells showed increased ADAMTS4 and 5 and TIMP3 gene expression. In contrast, ADAMTS1, MMP3 and MMP13 mRNA were decreased. Inflammatory gene regulation, in response to IL-1, was almost abolished in IFT88ORPK. However, inductions of MMP3 and 13 were still observed in IFT88ORPK. ADAMTS4 and 5 showed no cytokine-regulation in IFT88ORPK cells. The transcription factor NFκB p65 accumulated at the base of the primary cilium during cytokine treatment in human chondrocytes. IFT88 mutation abolished TNFα-induced nuclear-trafficking of NFκB p65 (100% inhibition 30mins post treatment). A 25% inhibition of IL-1β-induced nuclear-trafficking of p65 was observed in IFT88 mutant chondrocytes.

**Conclusions:** Our data indicate that the cilium influences catabolic activity of chondrocytes in vitro. The alteration of mRNA levels, inflammatory signalling and the association of the primary cilium with the spatial organisation of LRP1 suggest the cilium exerts influence at potentially multiple levels to control of catabolic activity in chondrocytes. Which ciliary mechanism is most relevant in disease pathogenesis remains unclear but on-going work is dissecting how the ciliome components interact with both inflammatory signalling and endocytosis in regulating matrix turnover.