Background: Osteoarthritis (OA) is the most common joint disease in elderly and is a major cause of disability. Currently no disease modifying agents exist to treat OA. ZCCHC6 (zinc-finger CCHC-domain containing protein 6) is a member of family of terminal uridylyltransferases (TUTs) and is known to carry out template independent addition of Uridine at the 3’ ends of miRNAs. ZCCHC6 has been implicated in miRNA-mediated regulation of cytokine gene expression; however, the role of ZCCHC6 in the pathogenesis of OA is unknown.

Objectives: The aim of this study was to investigate the effect of ZCCHC6 depletion on proinflammatory cytokine, IL-6, expression in human and mouse chondrocytes and to study the role of ZCCHC6 in OA pathogenesis in vivo using Zcchc6−/− mice.

Methods: Human or mouse chondrocytes were prepared by sequential enzymatic digestion and treated with recombinant human or mouse IL-1β to mimic OA pathogenic conditions. Cytokine profiling was done using qPCR array and multiplex assay. IL-6 expression in chondrocytes was analysed by qPCR and immunoblotting/ELISA. IL-6 mRNA stability was determined by Actinomycin-D chase experiments. The 3’-uridylation of miRNAs was analysed by deep sequencing of small RNAs using Illumina MiSeq. ZCCHC6 and IL-6 expression in OA cartilage was analysed by immunohistochemistry. For in vivo studies, OA was induced by surgical destabilisation of medial meniscus (DMM) in the knee joints of mice. The severity of OA was assessed by safranin O/fast green staining followed by OARSI scoring.

Results: ZCCHC6 expression was upregulated in OA cartilage. Cytokine expression profiling in Zcchc6 depleted human and Zcchc6−/− mouse chondrocytes revealed IL-6 as a major target of ZCCHC6. Depletion of ZCCHC6 expression in human chondrocytes resulted in decreased expression of IL-6 at mRNA and protein levels. ZCCHC6 depletion suppressed IL-1β induced expression of IL-6. Overexpression of ZCCHC6 in human chondrocytes resulted in increased expression of IL-6 in the presence or absence of IL-1β. Chondrocytes from Zcchc6−/− mice also showed decreased levels of IL-6 expression compared to Zcchc6+/+. Furthermore, overexpression of mouse Zcchc6 in Zcchc6−/− chondrocytes rescued IL-6 expression. IL-6 mRNA half-life was significantly reduced in Zcchc6−/− mouse chondrocytes and ZCCHC6 depleted human chondrocytes. Deep sequencing of small RNAs in ZCCHC6 depleted human chondrocytes showed reduced 3’-uridylation of IL-6 targeting miRNAs miR-26b/26a/26b. Human chondrocytes transfected with miR-26a/26b mimic suppressed IL-6 expression, however, miR-26b mimic with additional ‘UU’ failed to suppress IL-6 expression. Zcchc6−/− mice expressed low levels of MMP13 and showed lesser matrix degradation in KO mice also showing decreased levels of IL-6 expression compared to Zcchc6+/+. Furthermore, overexpression of mouse Zcchc6 in Zcchc6−/− chondrocytes rescued IL-6 expression. IL-6 mRNA half-life was significantly reduced in Zcchc6−/− mouse chondrocytes and ZCCHC6 depleted human chondrocytes. Deep sequencing of small RNAs in ZCCHC6 depleted human chondrocytes showed reduced 3’-uridylation of IL-6 targeting miRNAs miR-26b/26a/26b. Human chondrocytes transfected with miR-26a/26b mimic suppressed IL-6 expression, however, miR-26b mimic with additional ‘UU’ failed to suppress IL-6 expression. Zcchc6−/− mice expressed low levels of MMP13 and showed lesser matrix degradation in the joints with DMM surgery. Zcchc6−/− mice developed less severe OA as determined by safranin O/fast green staining followed by OARSI scoring. Synovitis was also decreased in the Zcchc6−/− mice DMM joints in comparison to Zcchc6+/+DMM joints.

Conclusions: Our data demonstrate that ZCCHC6 is upregulated in OA cartilage and regulates IL-6 expression via miR-26-3’-uridylation. These data identify a previously unknown function of ZCCHC6 in OA pathogenesis and identifies a potential therapeutic target for the management of OA.

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