IMPAIRED CHONDROCYTE U3 SNORNA EXPRESSION IN OSTEOARTHRITIS AND ITS IMPACT ON THE CHONDROCYTE'S PROTEIN TRANSLATION APPARATUS

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INTRODUCTION: Osteoarthritis (OA) is the most prevalent degenerative joint disease with no disease-modifying treatment available. During OA progression, articular chondrocyte homeostasis is disturbed and presents with a catabolic signature. Pathways controlling ribosome activity have previously been described in the regulation of chondrocyte homeostasis. However, ribosome biogenesis has not been implicated in OA. We hypothesized that U3 small nucleolar RNA (snoRNA), a key factor in the endoribonucleolytic ribosomal RNA (rRNA) maturation, is critical for chondrocyte homeostasis via regulating the availability of mature

METHODS: Old OA cartilage for microarray analysis was collected following total knee arthroplasty surgery (n=6) and young non-OA cartilage was collected following anterior cruciate ligament repair (n=6) (both with ethical permission). Total RNA was extracted and hybridised onto Affymetrix miRNA 4.0 arrays. The probe set for *Homo sapiens* was used to determine differentially expressed snoRNAs, with additional validation using RT-qPCR. U3 snoRNA expression during knee OA-progression was determined *in vivo* in an experimental mouse model for traumatic OA (destabilization of medial meniscus (DMM) model (n=3)) using a U3 snoRNA-specific probe for *in situ* hybridization (ISH). Ribosomal RNA expression, and chondrocyte phenotypic markers were measured using RT-qPCR. U3 snoRNA knockdown and increased ectopic expression in non-OA chondrocytes was undertaken following transfection of antisense oligonucleotides (ASO) or a U3 snoRNA mini-gene, respectively. Overall protein translational capacity was assessed using puromycylation assays. Differential proteomes after U3 snoRNA knockdown in SW1353 cells were established by LC/MS-MS, followed by label-free quantitation and ingenuity pathway analysis (IPA). Transcriptional regulation of U3 snoRNA gene expression was determined via a human U3 snoRNA promoter-luciferase reporter assay.

RESULTS: Microarray and RT-qPCR analyses demonstrated reduced expression of U3 snoRNA in old OA articular cartilage, compared to young non-OA cartilage. Additionally, U3 snoRNA levels were reduced in isolated OA chondrocytes compared to non-OA chondrocytes, as well as in the load-bearing area of traumatic murine OA joints. OA synovial fluid furthermore reduced U3 expression via inhibition of U3 gene promoter activity. Altering U3 snoRNA expression using an anti-sense approach or a U3 snoRNA mini-gene changed the expression of chondrocyte phenotypical genes relevant for OA. Expression of rRNAs was reduced after U3 snoRNA knockdown and increased following ectopic induction of U3 snoRNA expression. This is in line with reduced expression of rRNAs observed in OA chondrocytes compared to non-OA chondrocytes, which is accompanied with reduced expression of U3 snoRNA. OA chondrocytes showed reduced protein translation capacity compared to non-OA chondrocytes, and this was recapitulated following U3 snoRNA knockdown in articular chondrocytes. Reciprocally, ectopic expression of U3 snoRNA led to an increase in chondrocyte protein translation capacity. Analysis of the full impact of reduced U3 snoRNA levels on the SW1353 proteome uncovered a global deregulation of cellular protein synthesis pathways, confirming the central role of U3 snoRNA in chondrocyte protein translation. Finally, cellular U3 snoRNA levels could be induced by BMP-7 treatment via upregulation of U3 promoter activity, leading to higher rRNA expression levels and improve protein translation capacity.

DISCUSSION & CONCLUSION: We demonstrate that U3 snoRNA expression, a critical non-coding RNA needed for ribosome biogenesis, is reduced in OA chondrocytes. This has consequences for chondrocyte rRNA levels and protein translational capacity. U3 snoRNA expression might be a therapeutic target for OA treatment

and our data indeed show that BMP-7, a chondrocyte anabolic morphogen, is capable of inducing chondrocyte U3 levels, and improving chondrocyte protein translation capacity.

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