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A ROLE FOR THE SNORNA U3 IN THE ALTERED TRANSLATIONAL CAPACITY OF AGEING AND OSTEOARTHRITIC CHONDROCYTES

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Purpose

Cartilage is severely affected by the ageing process, being particularly susceptible to the age-related disease, osteoarthritis (OA). OA is characterised by uncontrolled synthesis of extra cellular matrix degrading enzymes together with reduced anabolic capacity. Small nucleolar RNAs (snoRNAs) are non-coding small guide RNAs, some of which are implicated in the endoribonucleolytic cleavage of the pre-ribosomal RNA (pre-rRNA), such as U3 snoRNA. We investigated the role of U3 snoRNA in cartilage ageing and OA, postulating that its dysregulation alters ribosome biogenesis and consequently changes the chondrocyte’s protein translational capacity.

Methods

Cartilage for microarray analysis was collected at total knee arthroplasty surgery from the lateral (old normal; n=10) and medial (old OA; n=10) femoral condyles from donors who were 62.6 ± 7.3 (mean ±standard deviation) years and the medial side of the lateral femoral condyle following anterior cruciate ligament repair surgery from young donors; 23.7 ± 3.8 years. 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. OA severity was based on Kellgren and Lawrence scoring and confirmed histologically using a modified Mankin scoring system. Ribosomal RNA expression in ageing equine and OA human articular chondrocytes (HAC) was measured using qRT-PCR. Translational capacity of OA chondrocytes was assessed using puromycilation assays. Changes in expression of U3 in OA-like conditions were studied in human chondrocytes using interleukin-1β (IL-1β), TNFα and OA synovial fluid. U3 knockdown for 24 and 48 hours in SW1353 cells was undertaken using antisense oligonucleotides and confirmed using northern blotting and qRT-PCR. Northern blotting was undertaken on rRNA intermediates following U3 knockdown. P53 protein expression was undertaken using western blotting.

Results

Microarray analysis showed reduced expression of U3 snoRNA in knee articular cartilage as a consequence of age and OA. *In vitro* HAC cultures demonstrated a decrease in U3 levels accompanied by a reduction in rRNA and an increase in precursor-rRNA in OA. In accordance with decreased rRNA levels, puromycilation assays revealed an overall reduced ribosome translational capacity in OA chondrocytes. Anti-sense oligonucleotide-mediated knockdown of U3 snoRNA expression in SW1353 chondrocytic cells revealed a reduction in 5.8S, 18S, 28S rRNAs. Accumulation of early (A0 cleavage) 47S pre-rRNA intermediates (5’ ETS) was evident following U3 snoRNA knockdown and late pre-18S rRNA intermediates accumulated later after U3 knockdown. Following U3 snoRNA depletion, chondrocyte phenotype marker gene expression demonstrated a shift towards a more predominant hypertrophic/catabolic chondrocyte phenotype, associated with activation of ER stress and alterations in SMAD signalling routes. In accordance with disturbed ribosome synthesis, U3 knockdown caused stabilization of p53 protein levels and decreased overall ribosome translational capacity. Finally, we discovered that U3 snoRNA expression in HAC responds to environmental conditions: exposure to OA synovial fluid or healthy synovial fluid decreased or increased U3 snoRNA expression, respectively. Similarly, catabolic provocation of chondrocytes by IL-1β or TNFα or anabolic stimulation by BMP-7 suppressed or induced U3 snoRNA expression, respectively.

Conclusions

U3 directs pivotal processing of pre-rRNA enabling the assembly of ribosomes. The *in vitro* model of OA indicates that reduced U3 in ageing and OA may lead to functional phenotypic consequences related to rRNA processing. In concert, our data illustrates that ageing and OA chondrocytes suffer from an overall impaired protein translational capacity compared to normal chondrocytes.