**SnoRNA signatures in cartilage ageing and osteoarthritis**

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Purpose

Osteoarthritis (OA) presents as a change in the chondrocyte phenotype and an imbalance between anabolic to catabolic processes. Factors such as age affect its onset and progression, and the origin of the phenotype is poorly understood. SnoRNAs direct chemical modification of RNA substrates and are involved in endoribonucleolytic pre-rRNA processing. The post-transcriptional 2’O-ribose methylation and pseudouridylation carried out by snoRNAs fine-tunes spliceosome and ribosome function, accommodating changing requirements for protein synthesis during health and disease. Control of snoRNA levels may be pivotal in regulating the transcriptional and translational capacity of high protein producing chondrocytes. To ensure continuous extracellular matrix (ECM) deposition it is essential for a chondrocyte to control the number and quality of its ribosomes. We tested the hypothesis that the ribosome’s translational capacity alters with age and disease due to dysregulation of expression and function of specific snoRNAs; contributing to the development of the OA chondrocyte phenotype.

Methods

Total RNA was extracted from cartilage collected at total knee arthroplasty surgery from the medial (old normal; protected (P); n=10) and lateral (old OA; unprotected (U);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. Microarray analysis was undertaken uisng Affymetrix miRNA 4.0 arrays. Differentially expressed (DE) snoRNAs were defined with a FDR<0.05. OA severity was based on Kellgren and Lawrence scoring and confirmed histologically using a modified Mankin scoring system. Results were validated with an independant cohort. Ribosomal RNA expression in ageing and OA was measured using qRT-PCR. Translational capacity of ageing and OA chondrocytes was assessed using puromycilation assays. Changes in expression of snoRNAs in OA-like conditions were studied in chondrocytes using interleukin-1 (IL-1) and OA synovial fluid. SnoRNA knockdown was undertaken using antisense oligonuleotides.

Results

The translational capacity of chondrocytes was reduced in OA. There was a reduction in expression of 5.8 and 18S rRNA in cartilage ageing and OA. In cartilage we identified panels of snoRNAs differentially expressed due to ageing and OA. 297, 378 and 368 snoRNAs were detected above background in young, P and U respectively. Using principle componeent analysis P were split into two groups due to a ten year age gap. Between two and 126 snoRNAs were DE between contrats. A panel of eight snoRNAs (SNORD116, 96A, 26, 33, 44, 95, 98) were validated in an independent cohort. Knockdown of SNORD26 resulted in increased expression of rRNA, an increase in SOX9, RUNX2, COL10 and a reduction in aggrecan. *In vitro* experiments using OA-like conditions effected snoRNA expression with an increase in SNORD26 following IL-1 and OA synovial fluid treatment.

Conclusions

A dysregulation of protein synthesis in the chondrocyte is evident in OA. We demonstrate that snoRNA expression changes in cartilage ageing and OA and in OA-like conditions and when the espression of these snoRNAs is altered this affects rRNA and chondrogenic gene expression. Thus we propose an additional dimension in the molecular mechanisms underlying cartilage ageing and OA through the dysregulation of snoRNAs, whose consequences include alterations to the post translational modification landscape of rRNA. SnoRNAs may be novel molecules to target in the treatment of OA.