Big tasks for small RNAs- a new class of RNAs in the pathogenesis of osteoarthritis

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Purpose

Chondrocytes acquire a modified phenotype with ageing, resulting in increased risk of osteoarthritis (OA) due to alterations in the cartilage extracellular matrix (ECM). 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. This is interesting as in OA there is an imbalance between ECM protein anabolism and catabolism. To ensure continuous 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 human OA knee cartilage of young (n=6; mean age ±SD 22.7±4.1 years) normal and old n=6; (66.4±15.9 years) donors and hybridised onto Affymetrix miRNA 4.0 arrays. The probe set for *Homo sapiens* was used to determine differentially expressed snoRNAs. Relative ribosome number and chondrocyte marker gene expression was determined using qRT-PCR of 5.8, 18 and 28S rRNAs and of COL2A1, ACAN, SOX9, COL10A1, RUNX2, MMP13, ADAMTS5, COX-2, IL6, BAPX1 mRNAs. Total DNA content by SYBR Green detection and total protein was determined using BCA assay.

Results

Normal samples correlated closely together, however OA samples clustered into three groups. When PCA was integrated with the Kellgren and Lawrence scores of OA donors the sub-populations were separated on OA severity. Analysis of the three subgroups identified 26 snoRNAs reduced in OA and 11 snoRNAs increased. These include 25 box C/D and 11 box H/ACA snoRNAs. To address the potential impact of aberrant snoRNAs expression on rRNA maturation, we determined the relative ribosome content in healthy and OA human articular chondorcytes (HAC). 18S and 5.8S rRNA (not 28S) levels were decreased in OA, together with a typical OA chondrocyte gene expression profile. Pre-rRNA levels were higher in OA, indicating aberrant pre-rRNA processing. In concert array results indicated that expression of U3 and U13 snoRNAs is deregulated in OA. In contrast to the majority of the snoRNAs these direct site-specific endoribonucleolytic cleavage of pre-rRNA. To address a potential involvement of the inflammatory compound of OA, healthy HACs were exposed to IL1β. Similar to OA chondrocytes 18S and 5.8S rRNA decreased on exposure and pre-rRNA increased. To address the question whether the chondrocyte phenotype responds in an OA-like fashion as a result of alterations in the cell's translation capacity we inhibited rRNA transcription using actinomycin D using normal HACs. 18S and 5.8S rRNA levels/cell were significantly downregulated whereas 28S rRNA levels remain unaffected. Due to ribosome depletion a reduction in total protein content/cell was observed, confirming functionally decreased translation capacity. The expression of RUNX2 and COL10A1 was upregulated, whereas COL2A1 expression was downregulated. Findings indicate that as a result of decreased chondrocyte ribosome content and translation capacity, chondrocytes phenotypically respond in an OA-like fashion.

Conclusions

Since we found evidence for altered ribosome abundancy and auxiliary rRNA maturation machinery in ageing chondrocytes accompanied by differential OA cartilage-specific expression of snoRNAs, we believe that the translational capacity of the articular chondrocyte in OA is impaired, due to dysregulation of expression and function of specific snoRNAs, thereby contributing to the development of the OA chondrocyte phenotype.