Dynamic ribosomes in osteoarthritis

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Aim: Osteoarthritis (OA) is characterized by progressive loss and destruction of articular cartilage. Articular chondrocytes produce and maintain the cartilage extracellular matrix (ECM), which requires continuous translation of mRNAs by ribosomes. Recent studies demonstrate that ribosomes can be dynamic and heterogeneous. Post-transcriptional modifications (PTMs) of ribosomal RNAs (rRNAs), such as 2'-*O*-ribose methylation (2'-*O*-Me), are a major source of ribosome heterogeneity that fine-tune translational activity. These modifications are accurately guided by specific small nucleolar RNAs (snoRNAs). Here we hypothesized that chondrocyte rRNA 2'-*O*-Me profiles are altered in osteoarthritis and contributes to OA progression.

Methods: Non-OA human primary articular chondrocytes (HACs, 5 donors) were treated with human end-stage OA synovial fluid (OA-SF) for 14 days. COL1A1 protein expression was evaluated by immunoblotting. 2'-*O*-Me levels on rRNA were measured and quantified by RiboMethSeq. CRISPR/Cas9 was used to knockdown snoRNAs in SW1353 cells. CRISPR/Cas9-treated cells were used as control. The efficiency of genomic modification was determined by gDNA analysis (surveyor nuclease) and mRNA analysis (RT-qPCR). Cells were transfected with bicistronic IRES (Internal Ribosome Entry Site) reporters (CrPV IGR, P53, or HCV IRES) to assess ribosome modus, or with UAA in 0 frame, HIV-1 -1 PRF (programmed ribosomal frameshifting) reporters to determine ribosome fidelity. Gene expression was measured by RT-qPCR. Proteomic analysis was performed by LC-MS/MS.

Results: We set up an OA-mimicking *in vitro* model using non-OA HACs treated for a prolonged time with end-stage OA-SF. In the model we found decreased expression of *COL2A1* and increased expression of *COL1A1*. Immunoblotting confirmed the increase in type I collagen protein expression after OA-SF treatment (Figure 1A). To evaluate altered rRNA PTMs in this model, we performed 2'-*O*-Me profiling of rRNAs. Five out of 109 known rRNA 2'-*O*-Me sites were significantly altered, including 2'-*O*-Me of U14 in 5.8S rRNA (Figure 1B). To assess the relevance of 2'-*O*-Me of U14, we targeted the responsible SNORD71 snoRNA gene by CRISPR/Cas9. As expected, this led to decreased expression levels of *SNORD71* (Figure 1C) as well as decreased 2'-*O*-Me of U14 (Figure 1D). Measurements of ribosome function uncovered significant changes in translation modus (Figure 1E) and fidelity (data not shown) in *SNORD71* KD cells when compared to CRISPR controls. Moreover, *SNORD71* KD cells showed higher expression of type I collagen protein (Figure 1F), which is in agreement with our results obtained in primary HACs treated with OA-SF. Simultaneously, *COL1A1* gene expression was unchanged (Figure 1G).
Conclusions: Exposure of human non-OA articular chondrocytes to synovial fluid from patients suffering from end-stage knee OA led to a fibrotic chondrocyte phenotype, characterized by collagen type I deposition. This was accompanied by specific changes in the epitranscriptome of ribosomal RNAs, including decreased 2'-*O*-Me of U14 in 5.8S rRNA.

In addition, we showed that functional impairment of *SNORD71,* which mediates this modification, affected ribosome modus and fidelity and led to increased collagen type I protein expression. The fact that *COL1A1* gene expression was not changed hints at preferential protein translation. We demonstrated that chondrocytes respond to OA-associated changes in their environment by adjusting rRNA PTMs and altering their translation characteristics. This impacts the translation of important components of the ECM. Thereby we linked the rRNA epitranscriptome to OA pathophysiology.

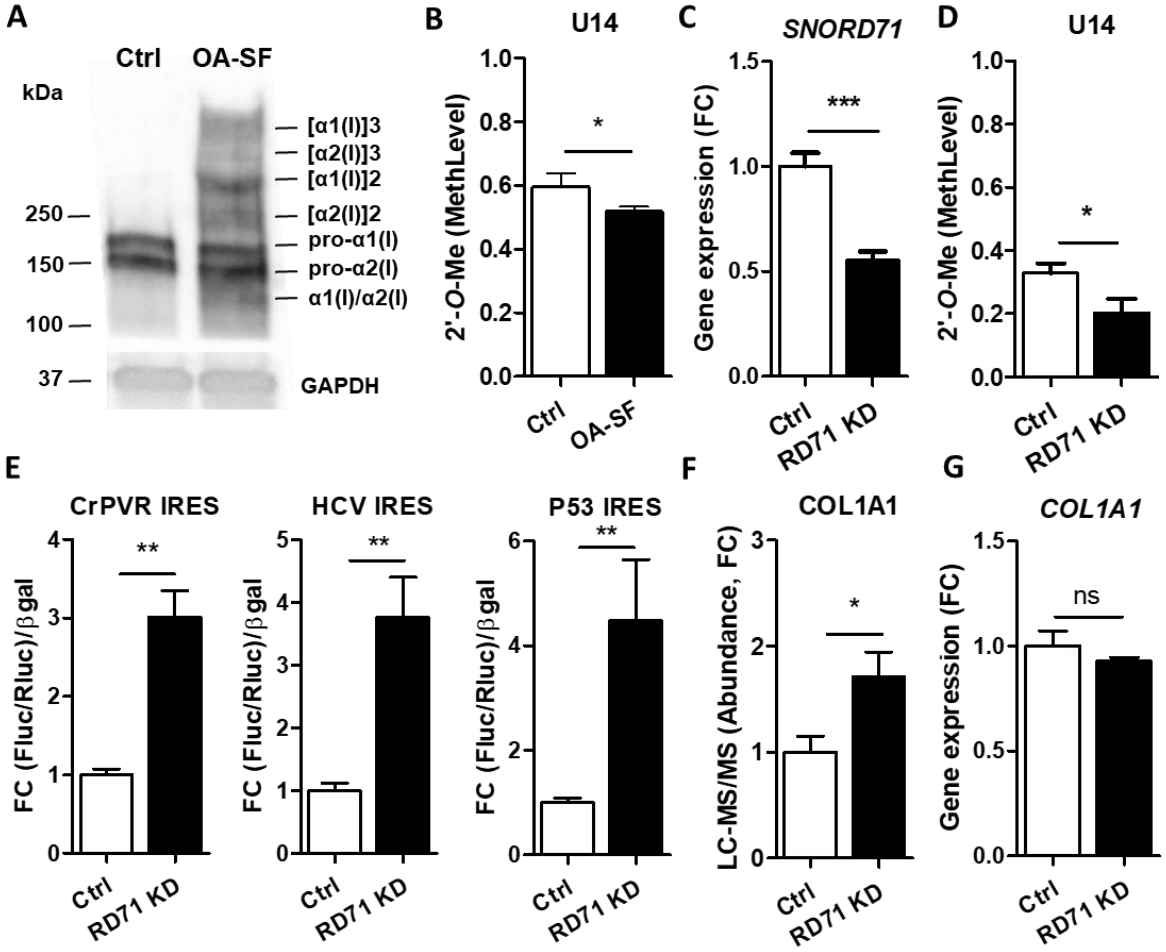

Figure 1: Exposure of human primary chondrocytes to end-stage OA-SF led to an increase in COL1A1 deposition (A), and impacted rRNA 2'-*O*-Me profiles in a site-specific manner, including 2'-*O*-Me of U14 on 5.8S (B). CRISPR/Cas9-mediated knockdown of *SNORD71*, snoRNA mediating modification at this site, led to a significant decrease in *SNORD71* expression levels (C) and U14 2'-*O*-Me (D). KD of SNORD71 affected the intrinsic capacity of ribosomes to initiate translation from several IRES elements (E). COL1A1 protein (F) expression was increased in SNORD71 cells, while COL1A1 gene expression (G) was stable.