SMALL NUCLEOLAR RNAS AS MEDIATORS OF OXIDATIVE STRESS IN CROSS SPECIES CARTILAGE AND OSTEOARTHRITIS

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PURPOSE

Small nucleolar RNAs (snoRNAs) are non-coding guide molecules that classically play an important role in guiding the chemical modifications of other RNAs to accommodate for changing cellular requirements. SNORD32A, -33 and -35A (oxi-snoRNAs) are a unique class of snoRNAs which themselves are regulated by reactive oxygen species (ROS) and oxidative stress. This is though guiding Nm modification of mRNA (evidenced for peroxidasin), in addition to their roles modifying rRNA. Furthermore others have demonstrated that they act in concert in stress response pathways. They are intronically encoded in the ribosomal protein host gene RLP13A. Oxidative stress, as a result of overwhelming ROS accumulation, significantly contributes to the ageing process. There is an age-related increase in ROS resulting in oxidative stress, a predominantly catabolic process evident in osteoarthritis (OA), one of the most common age-related chronic diseases affecting a multitude of species.

Gaining an awareness of, and elucidating the mechanisms by which abnormal tissue homeostasis contributes to cartilage ageing and OA-related regulatory dysfunctions, is crucial to our understanding of the disease. We hypothesise a role for these oxidative stress snoRNAs in cartilage ageing and OA.

METHODS

RNA was extracted from cartilage collected at total knee arthroplasty surgery from the medial (old normal; n=10; damaged) and lateral (old OA; n=10; intact), femoral condyles from donors aged 62.6 ± 7.3 (mean ±standard deviation) years and the lateral femoral condyle following anterior cruciate ligament surgery from young donors (young normal); 23.7 ± 3.8 years. Microarray analysis was undertaken using Affymetrix miRNA 4.0 arrays and differentially expressed (DE) snoRNAs were defined as FDR<0.05. Oxi-snoRNA expression was validated using qRT-PCR in independent human samples and equine young (2.33±1.2), old (17.67±2.5) and OA (18.67±1.2) chondrocytes. RLP13A expression was measured in the independent cohort samples. Changes in expression of snoRNAs were studied in OA-like conditions in human articular chondrocytes (HAC) and equine chondrocytes using interleukin-1 (IL1β) and HAC using OA synovial fluid. HAC and equine chondrocytes were treated with hydrogen peroxide (50uM), or tert-butyl hydroperoxide (TBHP) (25uM) to induce oxidative stress and fibronectin fragments (FN -f)(1uM), to generate physiological levels of ROS. QRT-PCR was used to determine induction of oxidative stress (CAT, GPx5 and SOD1) and oxi-snoRNAs expression. Phenotypic changes following SNORD-32A and-33 knockdown in SW1353 cells was undertaken following antisense oligonucleotides (ASO) using qRT-PCR. Phenotypic changes were assessed by measuring gene expression of rRNA, chondrogenic and hypertrophic genes.

RESULTS

Microarray analysis identified an increase in OA cartilage of SNORD33 and -35A (4.6 and 3.1. log2 fold change). The expression of SNORD32A, -33, -35A were significantly reduced in ageing human cartilage (3.9, 7.2, 4.5 log2 fold change). Using an independent cohort, the expression of SNORD32A ($p<0.05$), SNORD33 ($P<0.001$), SNORD35 ($p<0.07$) was higher in OA human cartilage (damaged) compared to old (intact) human cartilage. RPL13A was expressed higher in old versus OA ($p<0.03$) and young versus OA ($p<0.001$). In equine chondrocytes expression of SNORD32A ($p<0.01$), SNORD33 ($P<0.05$), SNORD35 ($p<0.05$) was higher in OA. In ageing HAC, we confirmed a reduction in SNORD33 ($p<0.02$) and SNORD35 ($p<0.05$) and in equine chondrocytes in snord32A ($p<0.05$) and snord35 ($p<0.01$) between young and old.

We discovered that oxistress snoRNA expression in HAC and equine chondrocytes responds to catabolic and environmental conditions. The addition of IL1β or OA synovial fluid for 24h to HAC increased the expression of SNORD33 ($p<0.05$, $P<0.001$). In healthy HAC there was a significant increase in expression of SNORD32A ($p<0.001$) and SNORD33 ($p<0.001$) following hydrogen peroxide treatment for 24h.

In young equine chondrocytes whilst there was an increase in snord32A and snord33 following hydrogen peroxide treatment for 1h, this was only significant for snord33 ($p<0.01$). Following TBHP treatment for 48h there was an increase in snord32A in young ($p<0.05$) and old ($p<0.05$) equine chondrocytes, an increase in snord33 in old ($p<0.05$) and a non-significant increase in snord35.

Following successful SNORD33 knockdown ($p<0.008$), and D32A ($p<0.02$) there was a reduction in 5.8S rRNA ($p<0.008$), 18S rRNA ($p<0.03$) and 28S rRNA $p<0.02$), along with a reduction in SOX9 ($p<0.03$) and MMP13 ($p<0.01$) and an increase in COL2A1 ($p<0.01$) (all SNORD33).

CONCLUSIONS

We identified changes in oxi-snoRNAs in ageing and OA, and this was a cross species phenomenon. This is interesting as not all snoRNA effects are the same between species. We have previously identified RLP13A as being an age-related gene using the Human Ageing Genomics Resource. However in agreement with other studies oxi-snoRNA expression did not mirror host gene expression. Our *in vitro* experiments demonstrated that oxi-snoRNAs respond to their environment (synovial fluid) and oxidative stress stimuli (hydrogen peroxide and TBHP). Physiological levels of ROS generated using FN –f increased the expression of oxi-snoRNAs but this did not reach statistical significance. The effects of a loss of D32A and D33 snoRNAs on cartilage homeostasis and OA phenotypic gene expression raises the possibility that a variation in the expression of these snoRNAs may contribute to the OA disease phenotype. SnoRNAs can be targeted by antisense knockdown, thus our findings suggest that molecular therapies could be designed to target these noncoding RNAs as possible future treatments in human and equine OA. We are investigating our findings further using induction of oxidative stress in parallel with ASO knockdown.