**A ROLE FOR SMALL NUCLEOLAR RNA HOST GENES IN CARTILAGE AGEING AND OSTEOARTHRITIS**

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**Purpose (the aim of the study)**

Cartilage is severely affected by ageing, being particularly susceptible to the age-related disease osteoarthritis (OA). Small nucleolar RNAs (snoRNAs) are small non-coding guide RNAs, whose roles include the post-transcriptional modification of other RNAs. Most snoRNAs are ‘hosted’ within introns of protein-coding and non-coding genes. It is increasingly apparent that these host genes contribute to the aetiology of disease. This study identifies potential host genes of snoRNAs with roles in cartilage ageing and OA.

**Methods**

Microarrays were used to analyse snoRNA expression in knee cartilage from ten young normal (YN) (23.7±3.8 years), ten old normal (ON) and ten OA donors (OOA) (62.6±7.3 years). Differentially expressed (DE) snoRNAs had an FDR<0.05. All samples were histologically scored. Expression changes were validated for snoRNAs using qRT-PCR on an independent cohort (n=7). DE of a subset of 12 host genes was measured using qRT-PCR. The Human Ageing Genomics Resource (HAGR) identified age-related host genes in non-diseased samples. Pathways and networks of DE host genes were interrogated using Ingenuity Pathway Analysis.

**Results**

Principal component analysis revealed YN clustering together. However ON clustered into two groups (ON1 and ON2), likely due to a ten-year age difference. The number of DE snoRNAs was; YN versus ON1; 126, YN versus ON2; 39, ON1 versus OOA; 39, ON2 versus OA; 2 and ON1 versus ON2; 52. There was good correlation between microarray results and qRT-PCR for the snoRNAs validated. Host gene expression was similar between the dependant and an independent cohort (SNHG5, SNHG1, EIF4G1, RPS12, LLR7C7, RPL13A, CCAR, RACK1, NOP56, TAF1D and GAS5).

GAS5 is a host gene to ten snoRNAs and whose expression increases in OA chondrocytes and regulates cell survival through microRNA 21. In this study we found an age-related reduction in five of its intronic snoRNAs in ageing; SNORD79, SNORD44, SNORD73, SNORD74, SNORD75 (Y vs ON) and an increase in two in ON versus OOA; SNORD74 and SNORD77. The latter was accompanied by an increase in GAS5 expression in OA.

P53 signalling has been implicated in cartilage ageing and OA. The expression of SNHG1, which along with its intronic snoRNAs have previously been identified as being repressed by p53 activation was reduced in ageing and increased in OA in parallel with one of its intronic snoRNAs SNORD26.

HAGR identified eight age-related host genes, including RLP13A, RPS12 in Y versus ON contrast. Six host genes were identified as age-related in the ON1 versus ON2 contrast, including RPL13A and RPS12.

The top canonical pathways identified from Y versus ON host genes were EIF2 (p=1.7E-25), mTOR signalling (p=2.1E-09) and regulation of EIF4 and p70S6K signalling (p=7.1E-09) and for ON versus OOA were EIF2 signalling (p=3.1E-12), regulation of EIF4 and p70S6K signalling (p=3.6E-04) and mTOR signalling (p=7.2E-04). The top networks identified in Y versus ON was ‘cell death and survival and organismal injury’, and for ON versus OOA was ‘immunological disease’.

**Conclusions**

Previously it was thought that as many host genes contained short, poorly conserved, open reading frames that they had no function other than carry the snoRNAs sequence within their introns. However work in cancer has identified that some host genes have roles in tumorigenesis and cell fate. Many of the protein coding snoRNA host genes are involved in protein synthesis enabling co-regulation of snoRNAs and proteins implicated in translation. mTOR signalling, is implicated in cartilage homeostasis and cartilage degeneration associated with OA. This was one of the principal pathways that host genes contributed to and host genes were within multiple levels of the signalling pathway.

Our work points to additional, as yet undefined roles for some of these snoRNA host genes in cartilage ageing and OA, both through the production of the snoRNAs which they express, and through functions of the genes themselves.