Identification and evaluation of serum non-coding RNAs as biomarkers for musculoskeletal ageing and disease

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Purpose

The development of effective treatments for age-related musculoskeletal diseases and the ability to predict disease progression has been hampered by the lack of substantive biomarkers able to demonstrate tissue pathology preceding identifiable tissue alterations. We have previously identified age-related dysregulation of a defined set of small nucleolar RNAs (snoRNAs) in cartilage and tendon ageing as well as the age-related disease osteoarthritis (OA). SnoRNAs are a class of evolutionary conserved non-coding small guide RNAs of which the majority directs the chemical modification of other RNA substrates, including ribosomal RNAs and spliceosomal RNAs. Profiling the expression patterns of snoRNAs in disease may help comprehend their functional significance in the development and progression of age-related musculoskeletal diseases, and provide diagnostic biomarkers and therapeutic targets.

Methods

C57BL6/J male mice were used for the study. RNA was isolated from joints and serum of old (n=6; 18 months old), young (n=6; 8 months old) and old OA (n=6 destabilisation of medial meniscus (DMM), n=3 sham; 24 months old) mice. Samples were submitted for library preparation using NEB small RNA library kit. To reduce bias in our workflow we used tobacco acid pyrophosphatase to remove 5'CAP found on some snoRNAs. Samples were amplified for 15 cycles, mixed into 3 pools, and size selected using a range 120-300bp. The size selected material was purified with Ampure beads. Small RNA sequencing was undertaken on the Illumina HiSeq 2000 platform using 100 base paired-end reads. The reference genome used for alignment was the mouse reference genome assembly GRCh38. The snoRNA count data were used to perform a data variation assessment. Differential expression analysis was conducted using edgeR. Significantly differentially expressed (DE) snoRNAs were defined with a FDR-adjusted P-values<5%. Counts per million (CPM) were used to present expression values.

Results

Reads mapping percentages for joint libraries were between 12 ~ 29%, and for serum libraries 0.03 ~ 0.9%. Between 42 and 53% of the 1555 mouse snoRNA reference sequences were aligned with at least one read for joints and 16-26% for serum samples.

The types and expression levels of snoRNAs were identified in ageing and OA joints and serum for the first time (Table 1). Table 2 demonstrates the number of DE snoRNA in the different contrasts.

Table 1

Tissue parameters	No. snoRNAs expressed
Young normal joint	1171
Old normal joint	1286
DMM joint	1218
Sham joint	1068
Young serum	597
Old serum	646
DMM serum	591
Sham serum	498

Table 2

	Young vs old joint	Young vs old serum	DMM vs sham joint	DMM vs sham serum	Old joint vs DMM joint	Old serum vs DMM serum
Number DE	10	39	5	28	21	79
↑	7	8	0	17	1	39
↓	3	31	5	11	20	40

Conclusions

The number of DE snoRNAs were generally small but greatest in ageing serum samples. A number of DE snoRNAs have previously been identified as having roles in oxidative stress, and cellular senescence and organismal ageing. Furthermore interesting snoRNA host genes were identified including a heparan sulphate sulphotransferase, transforming growth factor β and sorting nexin (all with roles in cartilage homeostasis) and Gas-5. Previously the snoRNA host gene GAS-5 has been suggested as a mechanism by which ageing and stress effect hippocampal function. A recent study identified that a single altered post translational modifications in rRNA due to a lack of a specific snoRNA can cause abnormal development and lethality in zebra fish. Snord 23 and snora 68 were DE in old vs old DMM joints and previously in our human OA snoRNA array studies and represent candidates for further work. Further studies will be undertaken to

determine the roles for these snoRNAs in ageing and musculoskeletal disease and identify possible biomarker candidates.