Synovial fluid snoRNAs metanalysis in equine osteoarthritis

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Abstract

Background: Osteoarthritis (OA) is a complex, multifactorial degenerative joint disease affecting multiple tissues within the joint. There are currently no effective curative treatments available, and therapy is often limited to stagnating the disease and supplying analgesia. Novel approaches to understanding OA may lead to discovery of new therapeutic targets and disease biomarkers. Small nucleolar RNA-molecules (snoRNA) are 60-300 nt long, and modify the maturation and modification of other non-coding RNA molecules, primarily ribosomal RNAs (rRNA). Several snoRNAs have no identified target and are referred to as novel or non-canonical snoRNAs. SnoRNAs PTMs cluster around specific, important sites of the ribosome such as the peptidyl transferase center (PTC) on the large subunit (LSU) and decoding sites on the LSU and small subunit (SSU). These clusters are thought to stablise protein synthesis. We have previously identified differential expression of snoRNAs in cartilage, synovial fluid, and synovium in OA. A comparison of end-stage OA compared to healthy joint cartilage found a significant reduction in translational activity of the ribosome. We suggest that OA could, in part be due to ribosome dysfunction. The aim of this study was to interrogate two snoRNA datasets to determine whether snoRNA expression alters in both synovial fluids derived from horses with clinical OA and horses subject to an osteochondral fragment model of equine OA.

Method: A metanalysis was undertaken on two snoRNA Sequencing (snoRNA-seq) datasets. Study 1: Synovial fluid was collected from eight horses (mean age 4.8 years, 2 geldings, 6 mares, standardbreds). An equine carpal osteochondral fragment model of OA was performed. Synovial fluid was collected at day 0, 28 and 70. SnoRNA-seq was performed using NEBNext® Small RNA Library Prep Set for Illumina® using 150bp paired end reads. Study 2: Equine synovial fluid and serum was collected from 13 horses (9 OA: 6.5±3.5 years, 7 males, 2 mares; 4 control: 6.3±7.5 years). Horses with either clinical signs of OA (lameness, pain, positive flexion tests, radiographic signs, and positive response to intra-articular analgesia) or post-mortem alterations in the

metacarophalangeal joint based on macroscopic scoring (1-4) were included in the OA group. Horses with no signs of post-mortem macroscopic changes were assigned as the control group.

For snoRNA-seq samples were submitted for library preparation using RealSeq-Biofluids kit (Somagenics) with Illumina NovaSeq SP. In both studies sequencing reads were mapped against the equine genome on RNA central database using bowtie and allowing for one mismatch. Analysis of differential expression was undertaken with edgeR v3.28.SnoRNAs with a p-value <0,05 and logFC of <-1 or >1 were considered to be significantly altered. The data from both studies was analysed in RStudio (ggplot2), and each snoRNA with a p-value <0.05 was individually linked onto the associated maps of the human ribosomes. For validation, synovial fluid from 12 horses (age mean: 16.6 years) from independent cohort studies were used. The samples were collected based on post-mortem examinations (divided in 3 subgroups, control, mild and moderate). RNA-extraction (miRNEasy Serum/Plasma Advanced Kit, Qiagen) and qPCR (miRCURY LNA miRNA PCR Assey, Qiagen) was performed for SNORD58 and U3, as these snoRNAs were DE expressed in both studies.

Results: In study 1, 30 snoRNAs with a p-value of <0.05 were altered between day 0-28 (11 downregulated, 25 upregulated), 25 between day 28-70 (23 downregulated, 2 upregulated) and 25 between day 0-70 (18 downregulated, 7 upregulated). 12 snoRNAs were altered in synovial fluid in study 2 (3 upregulated, 9 downregulated in OA), and 14 snoRNAs were upregulated in serum from OA horses. The distribution of snoRNA PTM targets from both studies display signs of clustering on important areas of the ribosome. Five snoRNAs from study 1 are identified to target PTMs in the PTC. The PTC has catalytic properties and is responsible for peptide bond formation between amino acids and peptide release during mRNA decoding.

Discussion: This study provides evidence of altered snoRNA expression throughout the development of equine OA as evidenced by the identification of differentially expressed snoRNAs as both multiple time points in the in vitro model and at a single time point in clinical samples. Thus, snoRNAs could act as potential biomarkers for stage-specific OA or targets for novel treatment strategies. Furthermore, this work supports our hypothesis that OA is an acquired ribosomopathy as a potential explanation model for the development of the altered chondrocyte phenotype evident in OA. By mapping of individual snoRNA PTMs we provide evidence of clustering in the PTC, a central area of the ribosome. Interestingly this study also found one

snoRNA altering the 5.8S ribosome (SNORA71), which, to the authors' knowledge, has not been previously described in OA.