SMALL NON-CODING RNA SIGNATURES IN CARTILAGE AGEING

Author Block: M. J. Peffers1, P. Balaskas2, Y. Fang2, J. A. Green3, T. M. Haqqi3, T. J. Welting4; 1Univ. of Liverpool, Neston, United Kingdom, 2Univ. of Liverpool, Liverpool, United Kingdom, 3Northeast Ohio Med. Univ., Rootstown, OH, 4Maastricht Univ. Med. Ctr., Maastricht, Netherlands

Abstract:

Purpose: Osteoarthritis (OA) is one of the leading welfare issues in horses in the UK, and results in substantial morbidity and mortality. In this whole joint disease cartilage is particularly susceptible to age-related changes, although it is not an inevitable consequence of ageing but due to a complex relationship between age and other predisposing factors. Age-related changes affect both chondrocyte physiology as well as extracellular matrix properties. Aged chondrocytes show increased senescence and higher expression of catabolic markers; features also seen in OA chondrocytes. The exact mechanisms through which age can affect cartilage health remain elusive, though it is believed to be a combination of different molecular pathways. Recent advances in the field have recognised epigenetics in ageing and diseased articular cartilage as an area of growing interest. One class of epigenetic modifiers are small non-coding RNAs (sncRNAs). They are short, typically <200bp, RNA species, which are not translated into protein but have other structural or regulatory roles. We hypothesised that cartilage sncRNA signatures will change with age and present a novel source of epigenetic effectors involved in cartilage ageing.

Methods: RNA was extracted from chondrocytes isolated from macroscopically normal cartilage of the metacarpophalangeal joints of ten horses; five young donors (4 years old) and five old donors (>15 years old) and subjected to small RNA-Seq on Illumina MiSeq. Size selection of libraries at 120-400bp (including 120bp adapters) generating 150bp paired end reads. tRNA fragment (tRF) analysis was undertaken following alignment of trimmed reads to NCBI horse genome reference sequences. Candidate tRNA reads were extracted according to whether they overlapped the ranges covered by tRNA features with read pairs then stitched into RNA fragments using PEAR. Only the perfectly mapped fragments were extracted and taken as tRNA fragments for further explorations. Statistical analyses focused on the fragment length and the mapping start location. Putative snoRNAs were derived from our experimental small RNA sequencing data using ShortStack and SnoReport. Validation of selected sncRNAs was undertaken using qRT-PCR. Differentially expressed (DE) genes were identified with DESeq2 ($P < 0.05$). Ingenuity Pathway Analysis (IPA) was used to identify targets for the DE microRNAs by uploading DE mRNAs identified from our previous ageing equine cartilage RNASeq study. Additionally, RNA was extracted from smooth, unaffected human knee OA cartilage with a Mankin score of 2 or less and from human knee OA damaged cartilage with a Mankin score of 4 or greater. We then used a qRT-PCR based approach to profile the expression of tRNAs and tRFs.

Results: In equine cartilage our study identified the expression of miRNAs, snoRNAs, snRNAs, lncRNAs and tRNAs. There were six snoRNAs, 11 microRNAs and 24 tRNAs that were DE with age. Additionally, we identified 86 age-related tRFs; 41 were derived from the 5' end and 45 from the 3' end. Our age-specific miRNA interactome identified 67 miRNA-mRNA pairings with the 352 DE age-related mRNAs from our previous ageing equine cartilage RNASeq study. IPA identified their top

canonical pathways as GP6 signalling, osteoarthritis and hepatic fibrosis. Top molecular functions as cell morphology, movement and development; top physiological systems as tissue morphology and top regulatory effect networks in response to chondrocytes age were cell viability and cell survival. When comparing equine and human OA data sets, nine tRNAs, three tiRNAs and a specific tRF-5 5003c fragment, induced with age in equine samples were also induced in damaged human OA cartilage. Two tRNAs and two tiRNA 5'halves were reduced with age in equine samples and in damaged human OA cartilage.

Conclusions: We have identified a set of sncRNAs including microRNAs which, together with DE age-related mRNAs enabled pathways to be elucidated for cartilage ageing, an important factor in OA development. For the first time we identified tRNA fragments that may also be targets for future therapeutic interventions in OA. These tRFs under stress conditions can inhibit protein synthesis. Given our previous findings that in ageing chondrocytes there is a reduction in protein translational capacity, that oxidative stress has a role in ageing and a previous in vitro study which identified reduced protein synthesis through tRF formation, our findings point to a role of tRNAs and tRFs in cartilage ageing and therefore potentially OA. This is an exciting future area of research.

Category (Complete): Omics Investigations of OA

Keyword (Complete): Chondrocyte ; Aging ; Horse ; Knee Osteoarthritis

Presentation Preference (Complete): Either Podium or Poster

Additional (Complete):

Type of Abstract (Required): Basic Science

Are you age 40 or under and within 5 years of your research degree? (Required): No

Do you want to be considered for a Need Based Travel Assistance Award? (Required): No