# Abstract

Joint tissues release extracellular vesicles (EVs) that potentially sustain joint homeostasis and contribute to osteoarthritis (OA) pathogenesis. EVs are putative novel therapeutics for OA, and transport biologically active molecules (including small non-coding RNAs (SNCRNAs)) between cells. This study identified altering SNCRNA cargo in EVs in OA which may act as early diagnostic markers and treatment targets.

OA was surgically induced in four skeletally mature Standardbred horses using an osteochondral fragment model in the left middle carpal joint. The right joint underwent sham surgery. Synovial fluid (SF) and plasma were obtained weekly throughout the 70-day study. EVs were isolated using size exclusion chromatography and characterised using nanoparticle tracking (Nanosight), and exosome fluorescence detection and tetraspanin phenotyping (Exoview). RNA was extracted from EVs derived from SF (sham and OA joints) and plasma collected at days 10, 35, 42, 49, 56, 63, and subjected to small RNA sequencing on a NovaSeq SP100 flow cell (Illumina).

Nanosight-derived EV characteristics of size and concentration were not significantly different following disease induction. The diameter of the temporal population of plasma and SF-derived exosomes changed significantly for CD9 and CD81 following OA induction with significant temporal, and disease-related changes in CD63 and CD81 protein expressin in plasma and SF.

In SF and plasma-derived EVs snoRNAs, snRNAs, tRNAs, lncRNA, y-RNA, piRNAs and scRNA were found. Following pairwise analysis of all-time points we identified 27 miRs DE in plasma and 45 DE miRs in SF. Seven were DE in plasma and SF; miR-451, miR-25, miR-215, miR-92a, miR-let-7c, miR-486-5p, miR-23a. In plasma and SF 35 and 21 snoRNAs were DE with four DE in plasma and SF; U3, snord15, snord46, snord58.

This work has identified alterations to OA EV sncRNAs in plasma and SF providing a greater understanding of the role of EVs in early OA.

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