TEMPORAL PROTEOMIC LANDSCAPE OF PLASMA AND SYNOVIAL FLUID DERIVED EXTRACELLULAR VESICLES USING AN EXPERIMENTAL MODEL OF EQUINE OSTEOARTHRITIS

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Purpose (the aim of the study)

Joint tissues release extracellular vesicles (EVs) that potentially sustain joint homeostasis and contribute to osteoarthritis (OA) pathogenesis. We have shown that EVs are putative novel biomarkers and therapeutics for OA, transporting biologically active molecules between cells. This study identified altering protein cargo in EVs in OA in addition to demonstrating that these EVs can affect cell signalling. EVs comprise an as yet inadequately investigated intercellular communication pathway in the field of OA.

Methods

OA was surgically induced in Standardbred horses (n=4) using an osteochondral fragment carpal fragment model. The opposite carpus underwent sham surgery. Synovial fluid (SF)and plasma were obtained weekly throughout the 70-day study. EVs were isolated using size exclusion chromatography from plasma and SF collected at days 0, 10, 35, 42, 49, 56, 63 and characterised using nanoparticle tracking, exosome fluorescence detection and tetraspanin phenotyping. Protein extracted from plasma and SF EVs was subjected to SWATH proteomics using a Triple TOF 6600 mass spectrometer following production of spectral libraries. Retention time alignment and peptide/protein quantification were performed by Data-Independent Acquisition by Neural Networks. Multi-level PCA was carried out using the MixOmics R package to account for intra-class correlation between the horses. All fold-changes and p-values were calculated relative to time zero. Bioinformatics on differentially expressed proteins was undertaken using Ingenuity Pathway Analysis (IPA). The activity of SF-derived EVs from the same samples in 13 pathways was determined in stably expressing response element (NFκB-RE, SBE, NFAT5-RE, TCFLEF-RE, CRE, ARE, AP1, SRE, SRF-RE, SIE, ISRE, GRE, NBE) driven Nano luciferase SW1353 reporter cells upon stimulation with either 10% PBS vehicle or 10% EVs.

Results

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 both CD9 and CD81 following OA induction with significant temporal, and disease-related changes in CD63 and CD81 tetraspanin protein expression in plasma and SF.

The plasma spectral libraries identified 437 and 2271 proteins in plasma and SF respectively. 111 and 70 proteins were differentially expressed at various time points compared to time 0 in SF and plasma derived EVs respectively (Figure 1). IPA identified significant common canonical pathways for both included activation of acute phase protein signaling ($p<6.5E-04$), complement/coagulation system ($p<5.4E-03$) and prothrombin activation ($p<3E-04$).

Fitting a linear model there was a time and OA dependant response on intracellular signalling responses in chondrocytic cells to EV treatment (Figure 2).

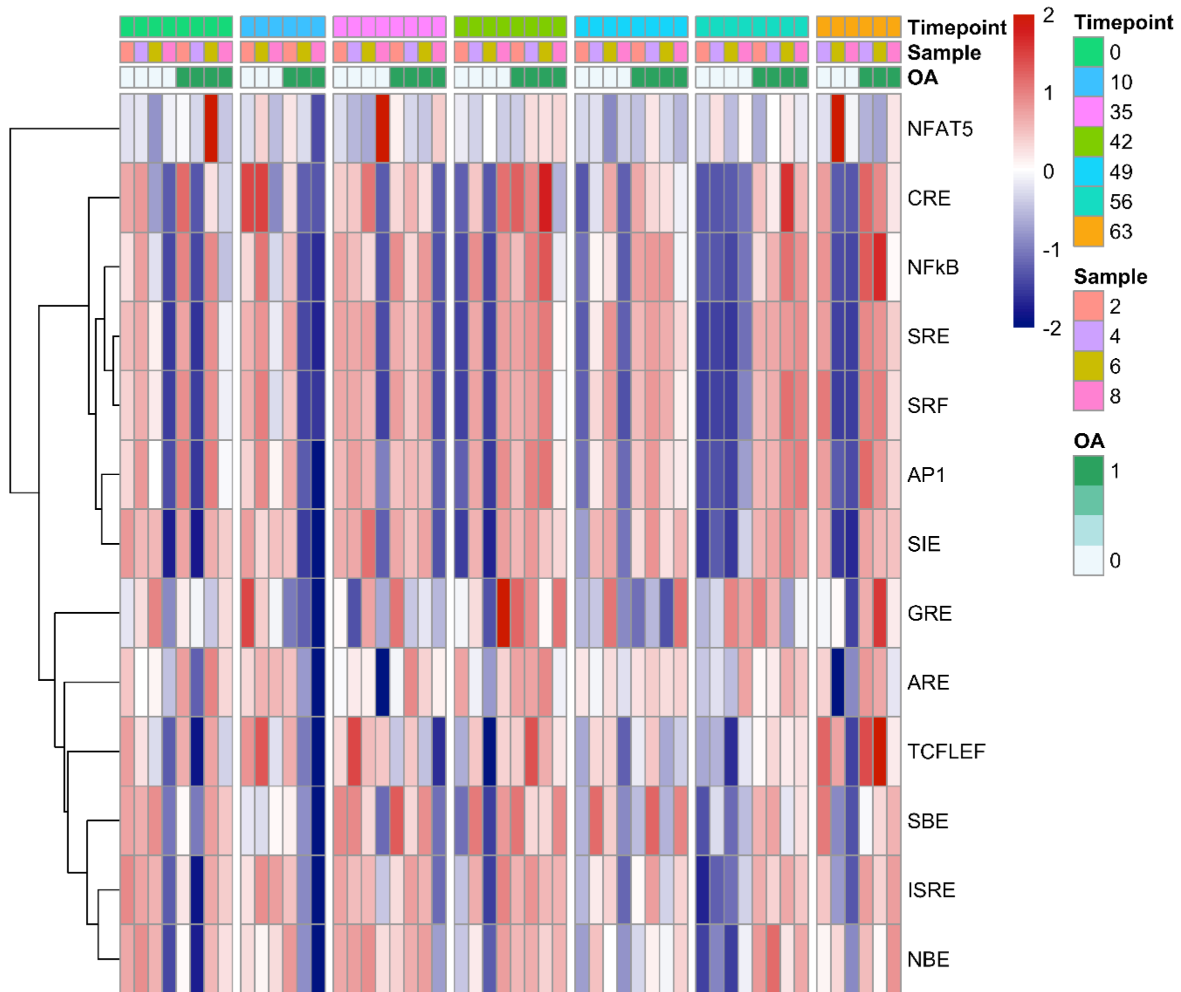

Figure 2: Heatmap of signalling transcription reporters demonstrate functionality of EVs.

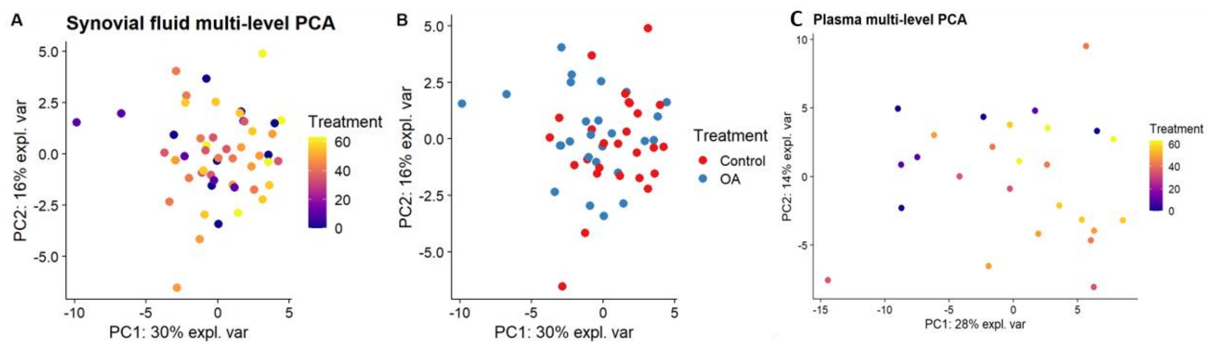

Figure 1: Multi level PCA of A SF with time, B. SF with disease and C. plasma with time

d. Conclusions

This study aimed to elucidate the role of EVs in early processes in OA which have the greatest potential to be modified by therapeutic interventions. Differences in tetraspanin expression indicate that the EVs present in the SF and plasma could have different functional activities, as tetraspanins are important for EV functionality as their function is dependent on their ability to interact with target cells.

Dynamic temporal EV protein changes in OA at the local and systemic level point to a changing role of EVs in disease pathogenesis. These proteins mapped to previously identified pathways were implicated in OA were activated.

EVs from OASF induced MAPK, AKT, RhoGTPase and NFκB signaling with potential effects on OA-related processes, including chondrocyte dedifferentiation, fibrosis, inflammation and ECM-degradation. There was a trend for OASF EVs at later time points to have a more pronounced effect on transcription factor reporter assays.

OA-associated changes in EV protein profiles in this OA model provided a unique opportunity to understand the role of EVs in OA propagation and disease progression.