**STRATIFYING OSTEOARTHRITIS USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY PROTEOMIC ANALYSIS OF SYNOVIAL FLUID**

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Purpose

Osteoarthritis (OA) is characterised by a loss of articular cartilage, abnormal bone proliferation, synovial membrane dysfunction and subchondral sclerosis. Approximately 60% of equine lameness can be attributed to OA and is considered a leading welfare issue, resulting in substantial morbidity and mortality. Currently the pathogenesis of OA is unknown and there are no disease-modifying treatments available. Synovial fluid (SF) is an integral articular component, closely associated with other articular tissues which are primarily altered during joint pathologies. However, to date few studies have used a global approach to investigate and stratify the equine SF proteome according to OA severity. The identification of differentially expressed proteins according to OA severity will aid our understanding of underlying disease pathogenesis, identify markers of early OA to allow timely management interventions, as well as help in identifying potential therapeutic targets.

Methods

SF was collected from 47 metacarpophalangeal and metatarsophalangeal joints of 36 racing thoroughbred horses following euthanasia.Condylar subchondral bone/cartilage sections were decalcified and stained with haematoxylin/Eosin and safranin O. Osteoarthritic pathology was histologically assessed using the OARSI modified Mankin scoring system for horses and categorised as mild (n=16), moderate (n=19) or severe (n=12). SF was hyaluronidase treated and 2 mg of protein loaded onto ProteoMiner™ equalisation columns to deplete high abundant proteins with reduction, alkylation and trypsin digestion undertaken directly on the beads in order to quantify less abundant proteins. 100 µg of protein from native SF of each donor was reduced alkylated and trypsin digestion undertaken in-solution, quantifying the higher abundant proteins. All samples were individually run using liquid chromatography-tandem mass spectrometry on an UltiMate 3000 Nano LC System coupled to a Q Exactive Quadrupole-Orbitrap instrument. Progenesis™ QI 2.0 was used for label-free quantification with protein identifications carried out using PEAKS 8.5 in the Unihorse database. ANOVA values of p<0.05 and altered regulation of >2-fold were regarded as significant.

Results

In total, during this study 431 proteins were identified within equine SF with the ProteoMiner™ equalisation columns providing a 69% increase in the number of protein identifications compared to native SF. Twenty six proteins were identified as being differentially expressed between differing severities of OA. These differentially expressed proteins have been found to be involved within various biological pathways, including GTPase activity/binding (tubulin beta chain and guanylate binding protein family number 6), the complement system (complement C1s), cobalamin binding (transcobalamin 2) and cell matrix adhesion (fibrinogen beta and gamma chains).

Conclusions

By using a global proteomic analysis approach and reducing the protein concentration dynamic range, we have identified a panel of proteins which change in accordance with OA severity, including novel proteins not previously identified in OA SF. These potential biomarkers hold promise in the stratification of equine OA including as indicators of early OA, which may allow for timely interventional management as well as providing further information to greater understand the complex pathogenesis behind this condition and help to identify further therapeutic targets.