Differentiating Proteomic Changes due to Healthy Aging and Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a widespread, costly, age-related chronic disorder that accounts for the majority of knee disability. In the knee joint, OA is characterized by articular cartilage and meniscal degradation, where some studies have suggested that the meniscus degenerates earlier than articular cartilage and may be a potential indicator of OA development.[1] In a recent study, Folkesson et al. provided insight on the pathophysiology of meniscal degeneration by identifying a large number of meniscal protein changes between healthy and OA knees. However, this study did not consider the confounding effect of changes that naturally occur during healthy aging. The objective of this study was to quantify the proteomic changes in the human knee meniscus that occur during healthy aging, and to compare these results to proteomic changes that occur in OA knees.[2] This will help us better understand the age-related pathophysiology of meniscal degeneration and OA.

METHODS: A total of 40 meniscus specimens were excised from the lateral menisci of 10 unpaired fresh frozen human cadaveric knee joints, with five knees from donors under the age of 40 (age = 33 ± 5 years), and five knees from donors over the age of 65 (age = 72 ± 7 years). No medical history of injury and no visual signs of meniscal damage or degeneration was associated with donor knees. Meniscal tissue (10.3 ± 0.5 mg, n=40) was minced, homogenized, agitated, incubated, and centrifuged to remove the supernatant. This supernatant was vortexed to ensure protein solution homogeneity before quantifying the protein concentration and quality with a BCA assay kit and SDS-PAGE electrophoresis. Fifty µg of protein from each sample was analyzed for ECM protein makeup using a 20-sample data independent acquisition quantitative proteomic platform at the University of Arkansas for Medical Sciences, and $\log_2$ fold changes of protein quantity were compared between age groups. Proteomics comparisons (young vs. older) were made by fitting a repeated-measures one-way ANOVA to the $\log_2$-fold protein amounts of each sample. Raw *p*-values were adjusted using a false discovery rate. This data was compared to Folkesson et al., who reported that 131 proteins had $\log_2$ fold changes greater than one between 17 menisci without OA (51 ± 18 years) and 16 menisci with OA (62 ± 8 years).

RESULTS: In total, 1582 proteins were detected in our analysis, of which 503 showed significant changes with aging (Fig. 1A). Of these 503 proteins, 15 had absolute $\log_2$ fold changes greater than two (Fig. 1B). When comparing proteins with $\log_2$ fold changes greater than one for both aging and OA, 12 common proteins were identified (Fig. 1C), and no common proteins were found to decrease in both aging and OA.

DISCUSSION: Healthy aging and OA appear to be two distinct processes with different protein changes occurring in each. Only 12 common proteins were found to significantly increase in both aging and OA over a 1 $\log_2$ fold change (Fig. 1C). Five of these proteins had nearly identical magnitudes of change, and with the exception of PA2GA and APOA4, all other protein changes were similar in magnitude as well. This suggests a potential common mechanism for the increased expression of these proteins in aged and OA meniscus, particularly between the 5th and 6th decades of life. Since these 12 proteins occur in healthy aging, they would not be suitable meniscal biomarkers for OA development. For example, increased MGP has been associated with OA[3], but our study would indicate that this increase may simply be due to normal changes with aging. Our study also supports previous findings, as SMOC2 has been shown to accumulate in skeletal muscle with aging[4], and this appears to also be the case for meniscus. The 15 proteins we identified to have the largest magnitudes of change due to aging could be used as biomarkers of healthy aging (Fig. 1B). Their roles are not yet fully understood, but some are ECM regulators (CHRD, MA2B2, TIMP3), others are involved in homeostasis (TMED2, BZW2) or immune regulation (LAG3, APOA4), and one is a structural protein (COL2A1). Previous studies have shown that plasma LAG3 increases in aging[5], but the other proteins have not previously been classified as aging biomarkers. Only three of these proteins were also reported in Folkesson's OA analysis (COL2A1, APOA4, and TIMP3)(Fig. 1B). COL2A1 and TIMP3 both showed lesser magnitudes of change in OA. COL2A1 is of particular interest as it is a structural protein that interacts with proteoglycans to improve compressive strength via osmotic loading. The six time increase in COL2A1 in healthy aging compared to OA is unexpected, since increased COL2A1 synthesis by chondrocytes under compressive loading[7] has been linked to age-related loss of meniscal vascularity[6] and subsequent hypoxia, which are factors that impede healing and would therefore be associated with a higher risk of degeneration. In conclusion, this study has identified biomarkers for healthy aging in meniscus, and has highlighted a group of proteins that were previously associated with OA development, but are likely only increasing due to the normal aging process.

SIGNIFICANCE/CLINICAL RELEVANCE: Understanding the protein changes that occur in aging and differentiating them from those that occur in OA will assist in the pathophysiological understanding of OA development and provide biomarkers to differentiate healthy aging from OA.

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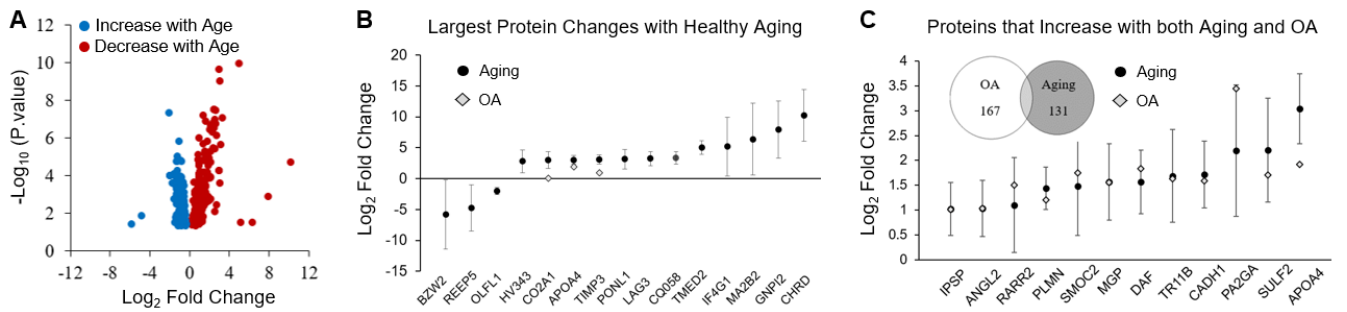

Fig.1: Proteomics data from human meniscus. A)Proteins that significantly changed with healthy aging, B) with the 15 largest protein changes due to aging shown with OA proteins where detected. C) Common proteins between healthy aging and OA that increased over 1 $\log_2$ fold change. Venn diagram shows total proteins that increased in Aging (grey) and OA (white), as well as overlapping proteins (light grey), which are displayed in the graph below.