Characteristics of the extracellular vesicles from human intervertebral disc cells

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INTRODUCTION: Extracellular vesicles (EV) are membrane-bound structures produced by all cells. They carry a variety of cargoes, including nucleic acids, proteins, and lipids. EVs play important roles in cell communication and regulation. Human mesenchymal stem cell (MSC) secretome has been shown to be affected differentially by the presence of tissue with varying disease states, and we have also shown that conditioned media from human intervertebral disc (IVD) cells of different degeneration grades alter gene expression in MSCs. However, limited knowledge exists regarding IVD cell-derived EVs. The **objective** of this study is to characterize EVs of non-degenerate, degenerate organ donor tissue, and degenerate tissue from patients undergoing surgery for low back pain (LBP) and to determine the cell source generating the most regenerative EVs.

METHODS: This study was approved by McGill University ethical review board (#2019-4896). IVD tissue was collected from organ donors without LBP and surgical patients with LBP with informed consent. IVD cells were isolated from nucleus pulposus (NP), inner annulus fibrosis (iAF), and outer AF tissue of organ donors with varying degrees of degeneration. NP and iAF cells were cultured together for EV collection. EVs were enriched using Amicon® Ultra-15 Centrifugal Filter Units and then purified by size-exclusion chromatography. Nanoparticle tracking analysis was conducted to measure EV size and concentration. Transmission electron microscopy (TEM) and Western blot were performed to examine EV structure and markers. Tandem mass tag-mass spectrometry (TMT-MS) and microRNA sequencing were conducted to determine protein and microRNA cargoes. Unpaired t-tests with Welch's correction and Welch's ANOVA tests were conducted. $p \leq 0.05$ was considered statistically significant.

RESULTS: Western blot data (N=3) confirmed the presence of EV markers ANXA5, FLOT1, and CD81, and the absence of the mitochondrial protein Tom20 and the Golgi protein 58K Golgi. TMT-MS data (N=3-6) further confirmed the relative expression of ANXA5, FLOT1, and CD81. On top of this, the relative expression of other EV markers CD9, CD44, CD47, CD55, CD59, CD82, TSG101, and ALIX were also detected. ANXA5, CD44, and CD47 exhibited decreased and increased trends in mildly-degenerate and degenerate states, respectively. TEM (N=3) revealed round and single-lobed flat structures in EVs. A significant increase in EV size and concentration (N=3-6) was associated with disease progression from non-degenerate to mildly-degenerate in exosome (p=0.0137) and intermediate microvesicle (p=0.0032) subgroups. Intermediate microvesicles showed an increasing trend at the degenerate state (p=0.0348). TMT-MS detected 993 proteins across non-degenerate, mildly-degenerate, and degenerate groups (**Fig. 1A**), covering protein clusters such as proteasomes, extracellular matrix (ECM) proteins, and proteoglycans. Gene ontology analysis ranked ECM-receptor interaction the strongest association of biological process with the lowest false discovery rate (FDR) (FDR=4.28E-36) (**Fig. 1B**). KEGG pathway analysis ranked cell adhesion (FDR=1.42E-17), wound healing (FDR=3.47E-16), and cell migration (FDR=8.16E-15) as the top three pathways with the lowest FDRs (**Fig. 1C**). In addition, TMT-MS detected 115 proteins shared by non-degenerate and mildly-degenerate groups and 11 proteins only in mildly-degenerate group.

DISCUSSION: Our findings indicate that IVD cells from mildly-degenerate IVDs display increased EV production containing ECM components for regeneration and proteasomes for anabolic and catabolic processes with the potential to counteract IVD degeneration. Further studies are needed with additional samples from degenerating tissues, and the anticipation of forthcoming microRNA sequencing data that may reveal more regulatory insights.

SIGNIFICANCE/CLINICAL RELEVANCE: This study characterizes IVD cell-derived EVs to evaluate their potential to reduce IVD-related LBP. By identifying the conditions leading to the most regenerative EVs and recognizing distinct EV markers across different disease states, the findings may pave the way for potential therapeutic applications and non-invasive diagnostic approaches for IVD degeneration.

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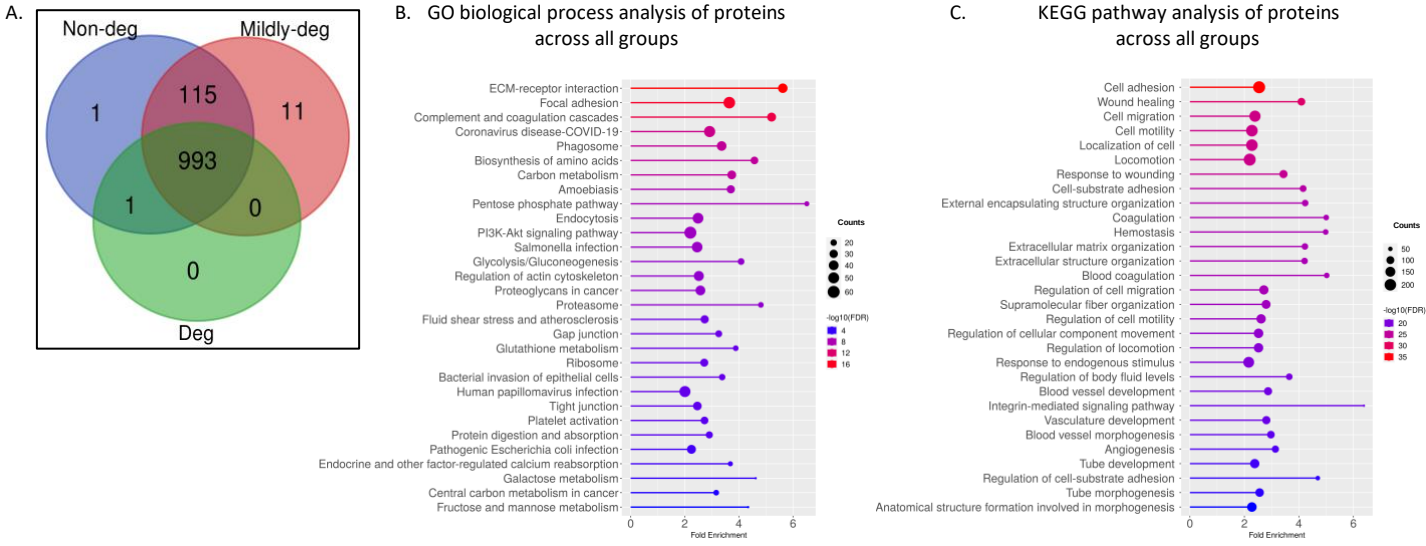

Fig. 1. A. Venn diagram shows the protein distribution of EV cargoes across non-degenerate, mildly-degenerate, and degenerate groups. **B.** Gene ontology-biological process analysis of proteins across all groups. Ranked by FDR. **C.** KEGG pathway analysis of proteins across all groups. Ranked by FDR.