The Catabolic Effects of Endothelial Cell-Derived Microparticles on Intervertebral Disc Cells

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Disclosures:

Introduction: Spine pathologies arisen from intervertebral disc degeneration (IDD) represent a worldwide public health problem due to the associated physical and psychosocial disability as well as substantial economic losses \cite{1, 2}. IDD is often associated with annulus fibrosus (AF) fissures, followed by a neovascularization process. The vessel ingrowth is considered pathological, since normal IVDs are mostly avascular. For example, increased vascularization is observed in herniated disc tissues. Endothelial cells (ECs) are the primary cell type involved in neovascularization. It is well established that cells can communicate using several different mechanisms including direct cell-cell contact and cell fusion, and paracrine signaling through cytokines, growth factors, prostaglandins, and other factors. However other pathways were recently identified, including a new mode of cellular communication mediated by microparticles (MPs). This represents one of the most active and exciting areas of cellular communication research.

Microparticles are cell-secreted microvesicles or exosomes, consist of particles smaller than 1 \textmu m which contain subcellular components (membrane, cytoplasm, mRNAs, proteins) of their parental cells. MPs can be released by multiple cell types in the human body, but endothelial cells are one of the primary producers of MPs \cite{3}. Endothelial microparticles (EMPs) have been studied widely \cite{4-7}, in part because of their easy isolation from peripheral blood and ECs cultures. EMPs have been linked to different diseases such as acute coronary syndrome, severe hypertension, metabolic syndrome, renal disease, diabetes, oncologic processes and extracellular matrix metabolism regulation.

Although neoangiogenesis has been documented in degenerated and injured discs, there are currently no literature reports that evaluate the effects of ECs or EMPs on IVD cells. Since ECs play an important role in vascular invasion of disc annulus fibrosis (AF) tissue during neoangiogenesis, the objective of this study is to investigate how secreted products of ECs, including endothelial microparticles and secreted proteins, influence the catabolic activities of AF cells in a cell culture model system.

Methods: Microvascular endothelial cell line, HEMC-I, was cultivated in monolayer culture until 95\% confluence for microparticles production. Clarified medium was subjected to ultracentrifugation at 100000g resulting in two fractions, the pellet fraction containing EMPs and the ultracentrifugation supernatant (SUP) containing secreted proteins and other factors as previously described \cite{8}. EMPs were confirmed through electron microscopy images of pellet resuspended in PBS (Fig. 1). ECs were also labeled with a membrane dye DiO (Invitrogen) to produce fluorescence-labeled EMPs for subsequent measurement of uptake of EMPs by annulus fibrosus (AF) cells, which was done by imaging living cells using the inverted Nikon TiE fluorescent microscope (Nikon Inc., Melville, NY). Human AF cells isolated from 12 surgical specimens. Passage 1 AF cell cultures were treated with 250\mu g of EMPs or 250\mu g SUP proteins for 72 hrs. Gene (qRT-PCR) and protein expression (Western blot) of key matrix metalloproteinases (MMPs) were determined.

Results: Live cell imaging assay revealed that AF cells uptake EMPs (Fig 3). RT-PCR analysis demonstrated increased mRNA expression of MMP-1 (36 fold), MMP-3 (5 fold) and MMP-13 (6 fold) in AF cell cultures treated with EMPs as compared to control cultures (Fig 4). Moreover, AF cells treated with SUP resulted in even larger increases in mRNAs of MMP-1 (218 fold), MMP-3 (34 fold) and MMP-13 (5 fold) compared to control. The protein products of these MMPs were also found in dramatically elevated levels in EMP-treated and SUP-treated AF cells as compared to untreated AF cells, consistent with the mRNA findings. MMP-2 gene expression did not show significant difference between the different treatments.

Discussion: Many reported neovascularization in degenerated and herniated discs, but the effects of the anticipated interaction between ECs and the resident disc cells have not been explored. In this study, we found drastic increases in gene expression of key MMPs in AF cells following exposure to protein products and microparticles made by ECs. This upregulation of catabolic activities in AF cells by EMPs might be explained in part by the uptake of EMPs by AF cells. However, additional studies are needed to identify the key factors in the SUP and EMP as well as the signaling pathways responsible for promoting the catabolic phenotype in AF cells.

Significance: The findings in this study suggest a potentially a new mechanism by which disc extracellular matrix is degraded during degenerative and neo-vascularization processes. If confirmed, such mechanism will provide specific molecular targets for therapeutic interventions to prevent or slow pathologic matrix degradation in injured and degenerated discs.

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