The anti-inflammatory effects of preconditioned bone marrow mesenchymal stromal cell- derived secretome on degenerated human nucleus pulposus cells in vitro

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INTRODUCTION: Due to their capacity to improve intervertebral disc metabolism and ameliorate low back pain (LBP), intradiscal delivery of mesenchymal stromal cells (MSC) for intervertebral disc degeneration (IDD) has been drawing increasing interest over the last decades[1]. Recent studies have shown that the majority of MSC anabolic effects are mediated by their secretome, which is constituted by extracellular vesicles, cytokines, and growth factors[2]. As the secretome composition strictly depends on cell state and microenvironment, several priming strategies have been tested to promote the release of regenerative and anabolic factors from stimulated cells[3]. The aim of this study was to investigate the effects of the secretome derived from bone marrow-MSCs (BM-MSCs) preconditioned with interleukin (IL)-1β on degenerative human nucleus pulposus cells (hNPCs) in a 3D in vitro model.

METHODS: The secretome was collected from BM-MSCs (BM-MSC-sec) after preconditioning with 10 ng/mL IL-1β. hNPCs were isolated from disc surgical specimens (n=5), culture-expanded in vitro, encapsulated in alginate beads and treated with either standard medium (CTRL), BM-MSC-sec (CM), 10 ng/mL IL-1β, or 10 ng/mL IL-1β for 24 hours and then BMSC-sec (IL-1β+CM). Cell proliferation was assessed by flow cytometry. Cell viability was evaluated with the LIVE/DEAD assay. Nitrite production was measured with the Griess assay. Reactive oxygen species (ROS) were quantified via immunofluorescence. Glycosaminoglycan (GAG) content was evaluated with the 1,9-dimethylmethylene blue assay. Gene expression levels of extracellular matrix (ECM) markers and inflammatory mediators were assessed with qPCR. The normality of data distribution was confirmed by the Wilk-Shapiro test. The analysis of the results was performed using one-way ANOVA.

RESULTS: BM-MSC-sec significantly increased hNPC proliferation ($p<0.05$) compared to the IL-1β group. After 24 hours of treatment, the percentage of dead cells was higher in IL-1β-treated hNPCs compared to control group and significantly lower in hNPCs treated with BM-MSC-sec, both alone and combined with IL-1β ($p<0.01$, Fig. 1). Nitrite and ROS production were significantly decreased ($p<0.05$, Fig. 2), and GAG content was increased in cells treated with BM-MSCs-sec and IL-1β preconditioning ($p<0.05$. Fig. 3). Furthermore, ECM gene expression levels were modulated by BM-MSC-sec treatment compared to controls.

DISCUSSION: Our results support the potential use of BM-MSC-sec as a cell-free strategy to treat IDD, possibly overcoming the drawbacks of cell therapy. Moreover, the secretome derived from IL-1β-preconditioned BM-MSCs was able to reduce hNPC death and attenuate ECM degradation and oxidative stress, thus counteracting IDD progression.

SIGNIFICANCE/CLINICAL RELEVANCE: MSC-derived secretome is biocompatible and may deliver incorporated therapeutic agents able to reprogram cell behaviour. Moreover, the specific profile of MSC-derived secretome may be a molecular "fingerprint" of the physiological state of the donor cell.

REFERENCES: 1. Vadalà G et al. Stem Cells Int. 2019;2019:2376172. 2. Tilotta V et al. Front Bioeng Biotechnol. 2023;11:1152207. 3.Maffioli et al. J Proteomics. 2017;166:115-126.

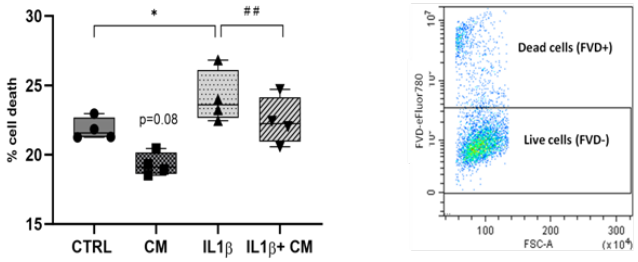

Fig. 1. BM-MSC-sec improved hNPC viability compared to the controls and cells treated with IL-1β only. **p<0.01 compared to control. ##p<0.01 compared to cells treated with IL-1β only.

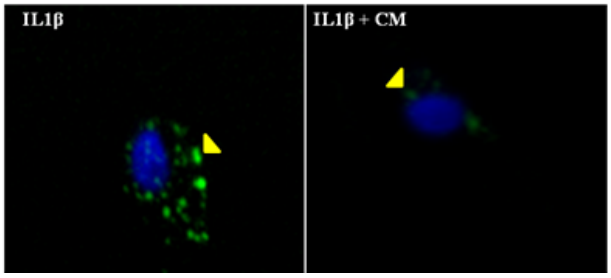

Fig. 2. A representative image showing intracellular ROS levels by molecular probe H2DCFDA staining in hNPCs treated with IL-1β and IL-1β + BM-MSC-sec.

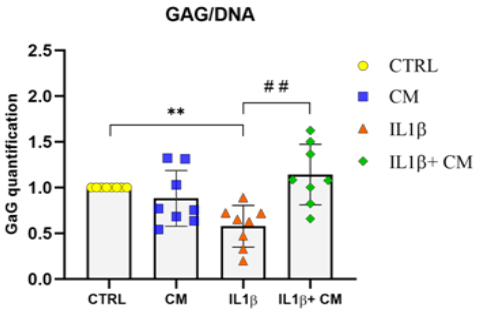

Fig. 3. Three-dimensional hNPC cultures treated with BM-MSC-sec and IL-1β showed significantly higher levels of GAG compared to cells treated with IL-1β only. **p<0.01 compared to control. ##p<0.01 compared to cells treated with IL-1β only.