Extracellular vesicles derived from Tie2-enhanced nucleus pulposus cells attenuate intervertebral disc degeneration and ameliorate pain: an in vivo study

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INTRODUCTION: Despite promising results in attempting intervertebral disc regeneration, intradiscal cell transplantation is affected by several drawbacks, including poor viability in the harsh disc environment, low cost-effectiveness, and immunogenic/tumorigenic concerns[1]. Recently, the development of cell-free approaches is gaining momentum in the field, with a particular regard towards extracellular vesicles (EVs)[2]. Nucleus pulposus cell (NPC) progenitors characterized by Tie2 expression have shown a higher chondrogenic differentiation potential compared to bone marrow-derived mesenchymal stromal cells (BM-MSCs)[3]. The aim of this study was to investigate the putative regenerative effects of EVs isolated from Tie2-overexpressing NPC progenitors on degenerative NPCs in vitro and in an in vivo rat model of intervertebral disc degeneration (IDD). We hypothesized that such EVs would exert a higher anabolic and anticatabolic effect compared to EVs derived from BM-MSCs and differentiated NPCs characterized by a low Tie2 expression.

METHODS: The current study was approved by the IRB of Tokai University School of Medicine. NPCs were isolated from young donors (n=6) and underwent an optimized culture protocol to maximize Tie2 expression (NPC^{Tie2+})[4] or a standard culture protocol (NPC^{STD}; DMEM high glucose + 10% FBS + 50 µg/ml ascorbic acid). EVs were extracted from NPC^{Tie2+} and NPC^{STD} and characterized according to the International Society of Extracellular Vesicles guidelines[5]. NPCs isolated from surgical specimens of patients with IDD (n=6) were treated with either NPC^{Tie2+}-EVs or NPC^{STD}-EVs, with or without 10 ng/mL interleukin (IL)-1β. Cell proliferation and viability were assessed with the CCK-8 assay. Cell apoptosis was evaluated with the Annexin V/PI assay. Cell senescence was investigated with β-galactosidase staining. EV uptake was assessed with PKH26 staining of EVs under confocal microscopy. IDD was induced by annular puncture of 3 caudal discs in 16 Sprague Dawley rats aged between 10 and 12 weeks. Animals were randomly assigned to sham injection, NPC^{Tie2+}-derived EV, NPC^{STD}-derived EV, or BM-MSC-derived EV intradiscal injection (n=4 per group) and sacrificed at 12 weeks. The Von Frey test was performed to assess mechanical tissue hypersensitivity at the tail base and surgical site every two weeks. Radiographic assessment of the disc height index (DHI) was performed once per month. After sacrifice, macroscopic evaluation of disc morphology was carried out using the Thompson score. The normality of data distribution was confirmed by the Wilk-Shapiro test. The analysis of the results was performed using one-way or two-way ANOVA.

RESULTS: EVs isolated from young donors significantly increased degenerative NPC viability at days 3 and 7 (n=4, Fig. 1A), especially in samples treated with $NPCs^{Tie2+}$-EVs ($p<0.001$). Likewise, $NPCs^{Tie2+}$-EVs significantly reduced cell senescence (n=3, $p<0.05$) and did not show to exert necrotic (n=3) nor apoptotic effects (n=3) on recipient cells compared to controls. Furthermore, EV uptake was successfully observed in all treated cells at 180 min following incubation (Fig. 1). According to the Von Frey test, the amount of force needed to elicit a nocifensive response at the tail base and surgical site was consistently lower in rats treated with NPC^{Tie2+}-derived EVs and NPC^{STD}-derived EVs at all timepoints ($p<0.05$, Fig. 2). Discs treated with NPC-derived EVs showed significantly lower Thompson scores compared to the sham group ($p<0.01$) and samples treated with BM-MSC-derived EVs ($p<0.01$). Similarly, treatment with both NPC^{Tie2+}-derived EV and NPC^{STD}-derived EV resulted in the preservation of DHI, whereas it progressively decreased with time in the other two groups.

DISCUSSION: NPC^{Tie2+}-EVs were demonstrated to enhance degenerative NPC viability, senescence, and apoptosis. The use of committed progenitors naturally residing within the nucleus pulposus may optimize EV regenerative properties and constitute the basis for a new therapy for IDD. Further in vitro characterization and applications in larger animals are warranted to confirm and expand the preliminary knowledge base provided by this study. Upcoming histological analyses may partially contribute to clarifying EV effects at the tissue level.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provided a preclinical proof of concept that may potentially identify a novel acellular therapeutic tool for IDD characterized by tissue specificity, cost-effectiveness, high tunability, and suitability for mass production.

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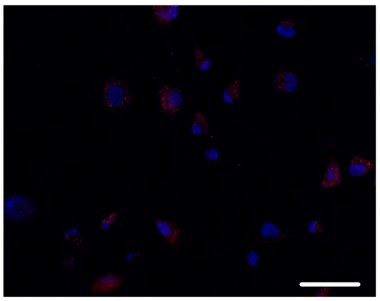

Fig. 1. PKH26 staining of EVs shows uptake in recipient NPCs at 180 min after incubation. Red = EVs; blue = nuclei (Hoechst 33342). Scale bar = 50 µm. Abbreviations: EVs = extracellular vesicles; NPCs = nucleus pulposus cells.

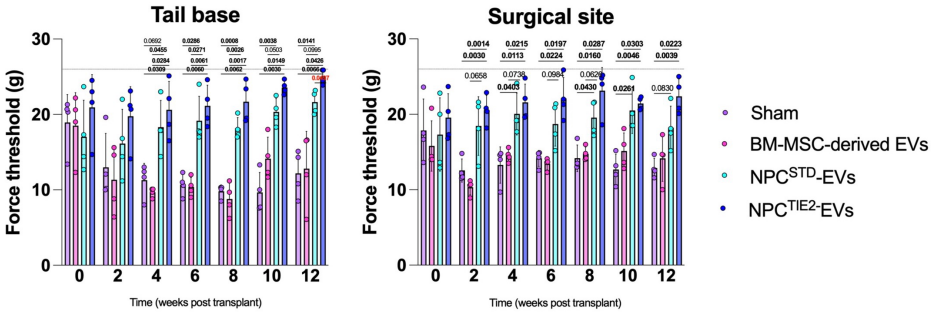

Fig. 2. The animals treated with NPC^{STD}-EVs and NPC^{Tie2}-EVs showed a higher force threshold to elicit a nocifensive response following mechanical stimulation of the tail base and surgical site at all timepoints. Abbreviations: BM-MSCs = bone marrow-derived mesenchymal stromal cells; EVs = extracellular vesicles; NPCs = nucleus pulposus cells.