Regenerative Potential of Mesenchymal Stem Cell-Derived Extracellular Vesicles Loaded with PTEN Small Interfering RNA for Facial Nerve Injury

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INTRODUCTION

Facial nerve injuries often lead to long-term functional deficits, with current treatments providing limited recovery. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) offer a promising regenerative strategy by supporting axonal growth, reducing inflammation, and promoting Schwann cell activity. We investigated bone marrow MSC-derived EVs loaded with glucose-conjugated PTEN siRNA (siPTEN) to enhance delivery and promote nerve regeneration in a rat model of facial nerve injury.

METHODS

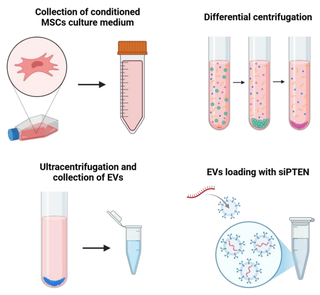

Figure 1: EVs were isolated from MSCs and loaded with siPTEN.

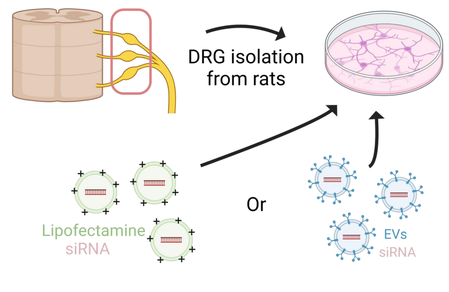

Figure 2: In vitro primary neurons were used to study the effect of siRNAs and EVs.

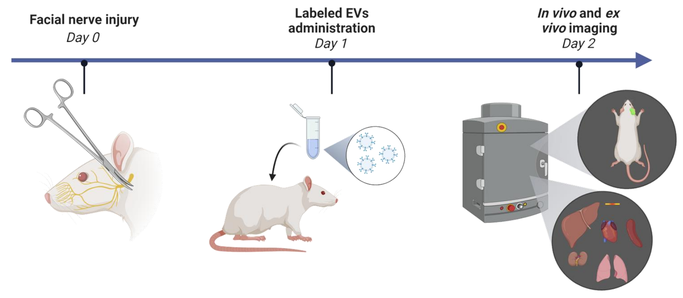

Figure 3: Labeled EVs were tracked post-injury via IVIS imaging to determine the optimal delivery route.

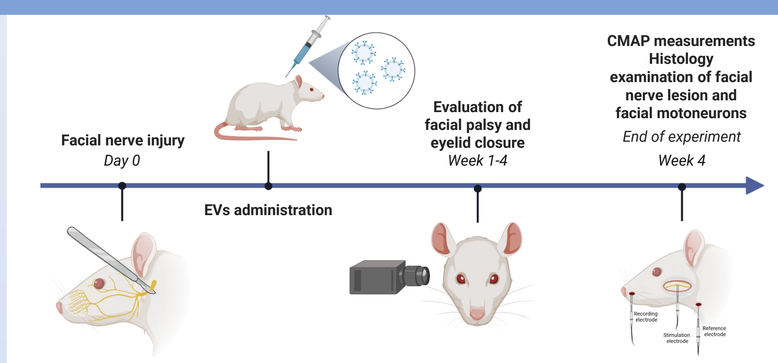

Figure 4: EV+siPTEN treatment was tested in vivo to evaluate its efficacy.

RESULTS

Characterization of Human bm-MSC-Derived EVs

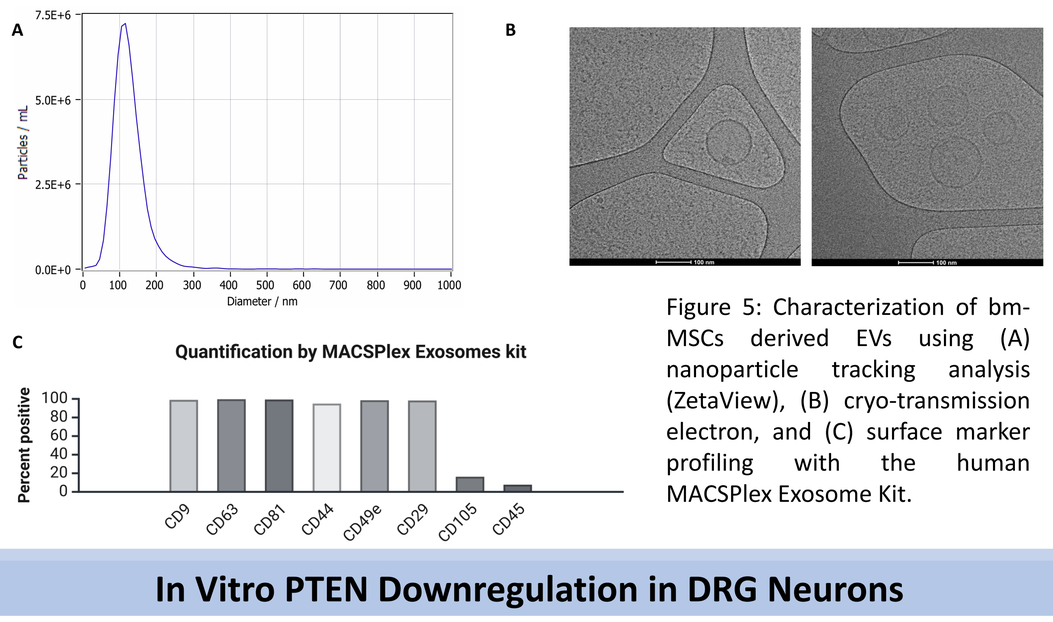

Figure 5: Characterization of bm-MSCs derived EVs using (A) nanoparticle tracking analysis (ZetaView), (B) cryo-transmission electron, and (C) surface marker profiling with the human MACSPlex Exosome Kit.

In Vitro PTEN Downregulation in DRG Neurons

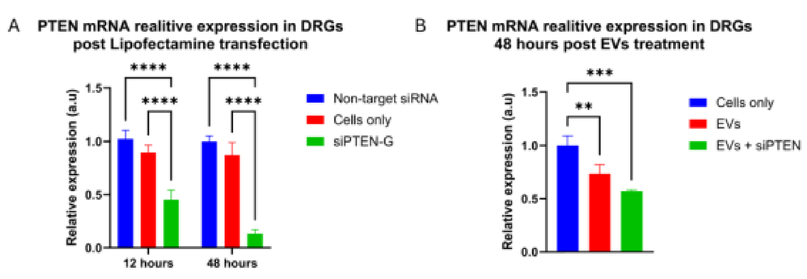

Figure 6: PTEN downregulation in vitro (A) post Lipofectamine transfection and (B) post loaded EVs treatment.

Targeted Delivery via Intralesional Injection

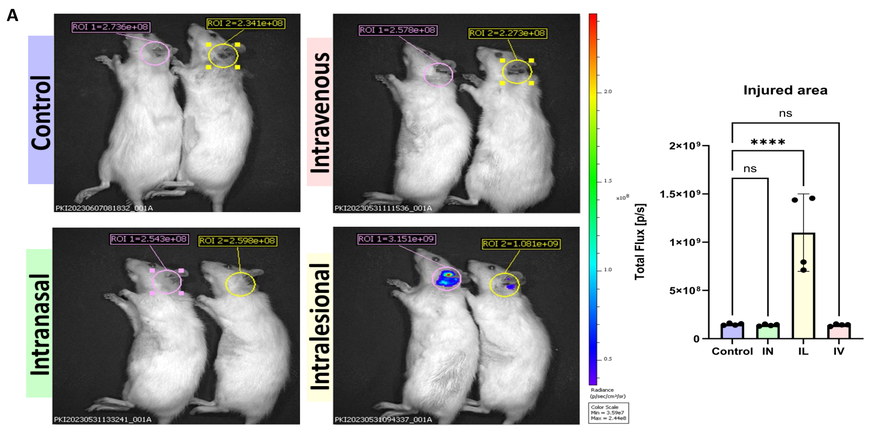

Figure 7: (A) Representative whole-body IVIS images and quantification of fluorescence intensity at the injury area after intravenous, intranasal, and intralesional administration of DiR-labeled MSC-EVs. (B) Quantification of fluorescence intensity in the isolated injured nerve and major organs.

Altered PTEN Expression Suggests a Role in Facial Nerve Injury

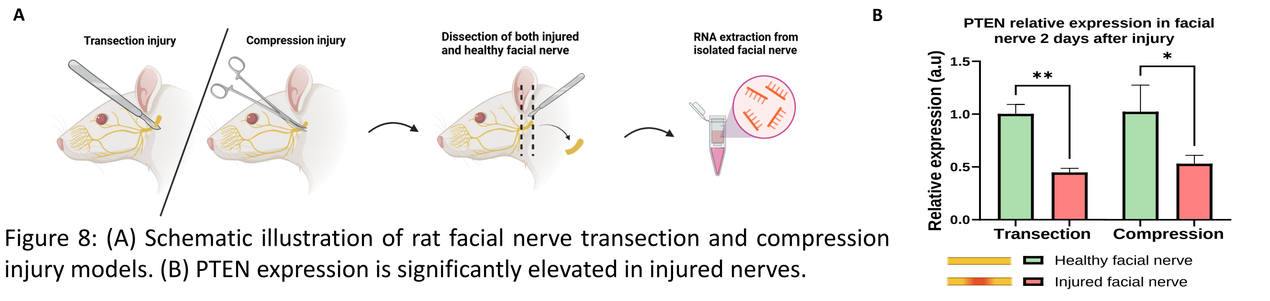

Figure 8: (A) Schematic illustration of rat facial nerve transection and compression injury models. (B) PTEN expression is significantly elevated in injured nerves.

EVs + siPTEN Therapy Improves Facial Nerve Regeneration

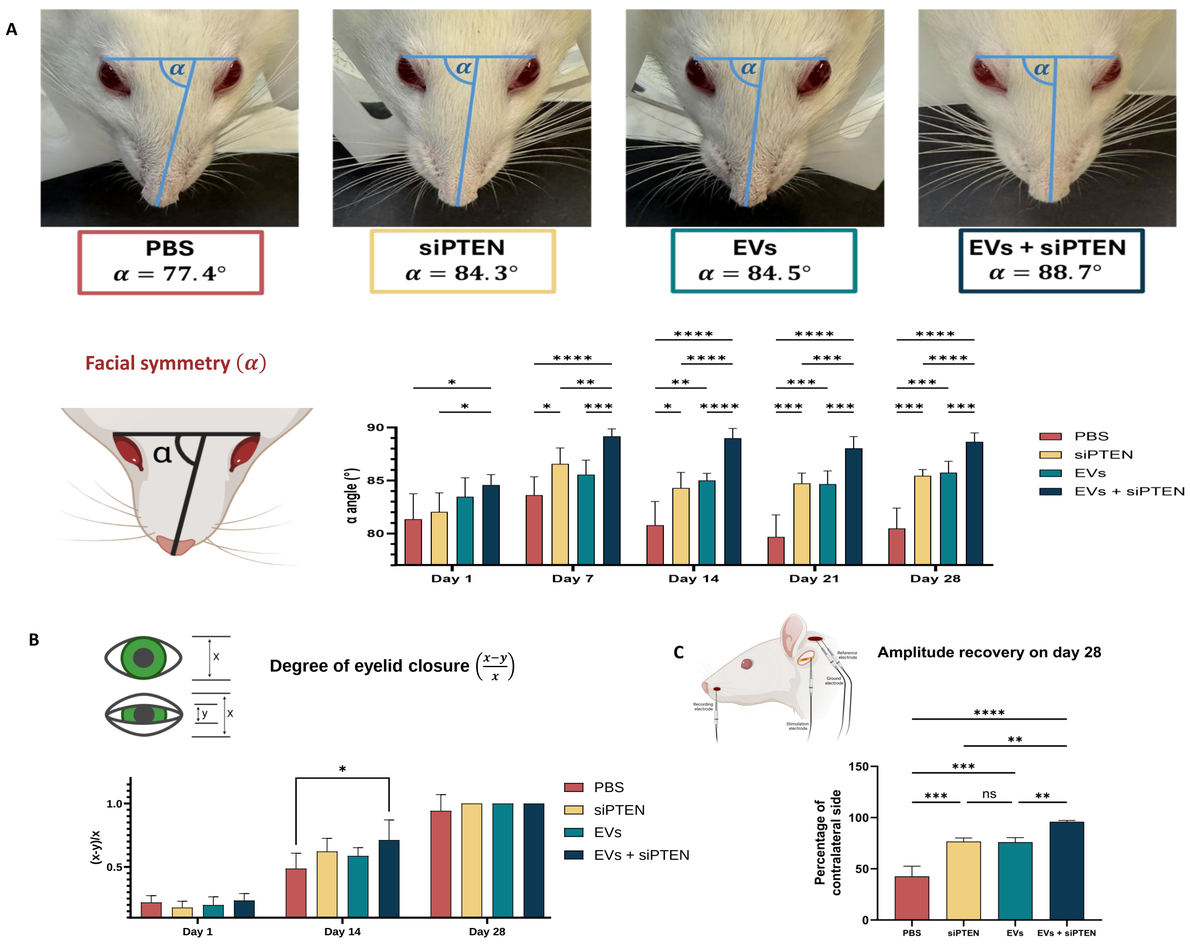

Figure 9: Facial symmetry (A), eyelid closure (B), and CMAP amplitude (C) were evaluated across four groups: PBS, siPTEN, EVs, and EVs + siPTEN. EVs loaded with siPTEN significantly improved all functional outcomes compared to controls, indicating enhanced facial nerve regeneration.

EVs + siPTEN Treatment Upregulates Regenerative Gene Markers

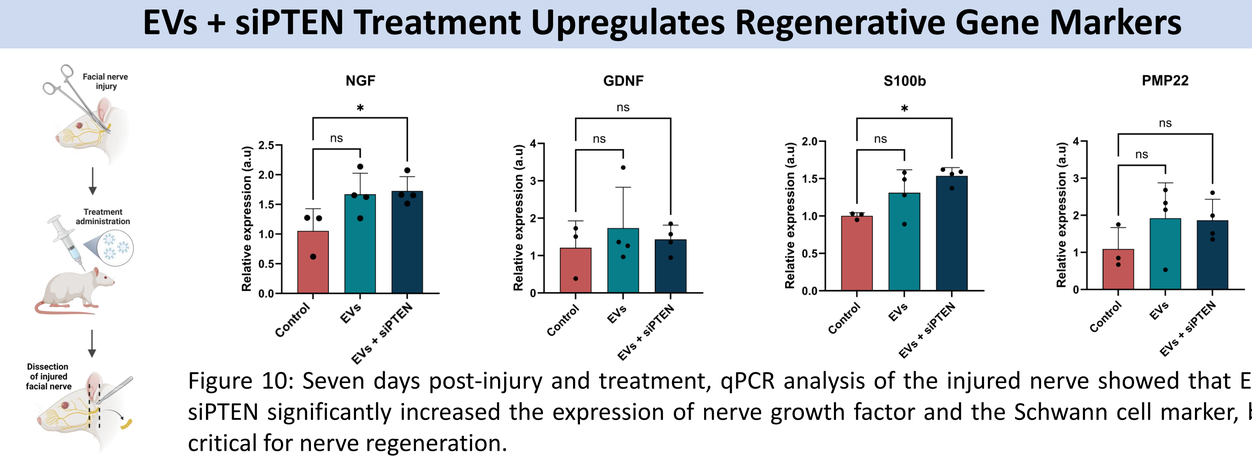

Figure 10: Seven days post-injury and treatment, qPCR analysis of the injured nerve showed that EVs + siPTEN significantly increased the expression of nerve growth factor and the Schwann cell marker, both critical for nerve regeneration.

SUMMARY

PTEN downregulation is a powerful strategy to promote axonal regrowth and facial nerve regeneration.

MSC-derived extracellular vesicles (EVs) are natural, biocompatible carriers that can deliver therapeutic siRNAs effectively.

Optimizing this approach holds strong potential to improve recovery and long-term outcomes after facial nerve injury.