Exosomes secreted by LRP1$^+$ ligament-derived stem cells promote tendon-bone healing after ACL reconstruction via miR-708-5p/Bambi axis

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INTRODUCTION: ACL reconstruction is a common surgical intervention for ACL injuries, yet optimal postoperative graft-bone tunnel healing remains challenging, often leading to poor clinical outcomes. This study investigates the role of exosomes derived from LRP1-positive ligament-derived stem cells (LRP1$^+$LDSCs-Exos) in promoting tendon-bone healing in an ACL reconstruction rat model.

METHODS: Single-cell RNA sequencing compared the characteristics of LRP1$^+$LDSCs and LRP1$^-$LDSCs. LRP1$^+$LDSCs were isolated from rat ACLs via flow cytometry, and exosomes were extracted. In vitro, these exosomes were co-cultured with rat bone marrow mesenchymal stem cells (BMSCs) to evaluate their effects on BMSC migration (scratch/Transwell assays) and chondrogenic differentiation (Alcian Blue, WB, RT-qPCR). In vivo, LRP1$^+$LDSCs-Exos were loaded into gelatin methacryloyl (GelMA) hydrogel and injected into bone tunnels of ACL-reconstructed rats. Healing was evaluated at 4/8 weeks by micro-CT, biomechanical testing, and histology. miRNA sequencing identified miR-708-5p as a key candidate, its role was validated via gain/loss-of-function experiments, and its target Bambi was confirmed by dual-luciferase assay.

RESULTS SECTION: scRNA-seq of human ACLs revealed an LRP1$^+$LDSC subpopulation with enhanced stemness (CytoTRACE score) and chondrogenic potential (GO/GSEA enrichment) compared to LRP1$^-$LDSCs. In vitro, LRP1$^+$LDSCs-Exos (100 μg/mL optimal dose) significantly enhanced BMSCs migration and chondrogenic differentiation (increased SOX9, Aggrecan, COLII expression; $p<0.01$ vs. controls and LDSCs-Exos). In vivo, LRP1$^+$LDSCs-Exos-GelMA treatment yielded superior outcomes: reduced bone tunnel expansion ($p<0.001$), increased bone volume fraction (BV/TV, $p<0.0001$), higher failure load and stiffness ($p<0.01$), and enhanced histological scores with more fibrocartilage and Sharpey-like fibers at the TBI. Mechanistically, miR-708-5p was highly enriched in LRP1$^+$LDSCs-Exos. It directly targeted and suppressed Bambi, a negative regulator of BMP signaling. Inhibiting miR-708-5p abolished the pro-chondrogenic effects in vitro and the therapeutic benefits in vivo, while overexpressing Bambi similarly counteracted the effects.

DISCUSSION: This study identifies LRP1 as a novel marker for a chondrogenic LDSC subpopulation. We demonstrate that LRP1$^+$LDSCs-Exos delivered via GelMA hydrogel significantly promote TBI healing post-ACLR by transferring miR-708-5p to BMSCs, which alleviates Bambi-mediated inhibition of BMP signaling, thereby driving chondrogenic differentiation. Limitations include the use of a rodent model, which may not fully recapitulate human healing, and a focus on a single miRNA within the complex exosomal cargo, suggesting other components may contribute synergistically.

SIGNIFICANCE/CLINICAL RELEVANCE: This work provides a novel, targeted cell-free therapeutic strategy using exosomes from a defined progenitor cell subpopulation to enhance graft-bone integration. By elucidating the specific miR-708-5p/Bambi mechanism, it identifies a potential therapeutic target to accelerate healing and improve outcomes after ACL reconstruction, potentially reducing failure rates and enabling a faster return to activity.