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rAAV sox9 gene transfer stimulates the chondrogenic differentiation activities in human peripheral blood aspirates

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Introduction: Implantation of genetically modified peripheral blood aspirates may be a promising approach to treat focal cartilage lesions. Here, we explored the effects of an rAAV sox9 vector in human peripheral blood aspirates on the chondrogenic differentiation activities in the samples.

Methods: rAAV-lacZ carrying the E. coli beta-galactosidase (lacZ) gene and rAAV-FLAG-hsox9 a human FLAG-tagged sox9 sequence were produced using standard protocols. Peripheral blood collected in the presence of hirudin from donors was transduced with rAAV (40 µl) or let untreated using chondrogenic medium for up to 21 days. Histological and immunohistochemical analyses were performed on paraffin-embedded sections of the constructs (5 µm). The proteoglycan contents in the aspirates were monitored by binding to dimethylmethylene blue dye and the DNA contents by Hoechst 33258 assay. Total RNA was extracted, and reverse transcription was carried out for cDNA amplification via real-time RT-PCR using GAPDH as control for normalization. A t-test was employed with $p \leq 0.05$ considered statistically significant.

Results: Transgene (sox9) expression was observed in rAAV sox9-treated aspirates relative to control conditions. Enhanced chondrogenic differentiation was achieved in aspirates transduced with rAAV sox9 after 21 days as noted by stronger toluidine blue staining and type-II collagen immunostaining. Real-time RT-PCR analyses showed enhanced chondrogenic differentiation with sox9 relative to the controls (up to 2- and 1.7- fold higher COL2A and ACN expression, respectively; $p \leq 0.001$) and reduced hypertrophic differentiation (up to 2.7-fold lower COL1A1 and COL10A1 expression, respectively; $p \leq 0.001$), probably resulting from increased levels of SOX9 expression (up to 5-fold difference; $p \leq 0.001$).

Conclusion: rAAV sox9 gene transfer is a potent approach to modify peripheral blood aspirates as a novel, implantable system to treat cartilage defects.