

Rabbit tissue-specific stem cell engineering for controllable cartilage regeneration

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Disclosures: No conflicts of interest.

INTRODUCTION: Articular cartilage has a limited regenerative capacity and, once damaged, is often replaced by mechanically inferior fibrocartilage, which cannot meet the functional requirements of native cartilage. The transcription factor SRY-box 9 (SOX9) plays a central role in chondrogenesis by regulating the expression of type II collagen (COL2A1), preventing chondrocyte hypertrophy, and promoting the redifferentiation of osteoarthritic chondrocytes. Given that SOX9 expression strongly overlaps with COL2A1, it is widely considered to be a key regulator of cartilage-specific extracellular matrix synthesis [1]. However, direct overexpression of the SOX9 gene carries potential risks [2]. In this study, we employed gene transduction to insert SOX9 under the control of the type I collagen promoter, aiming to establish a more organized expression pattern within rabbit cartilage. As a common model for studying cartilage defects, rabbits offer a balance between logistical convenience and clinical relevance. However, one study noted that while rabbit mesenchymal stem cells (MSCs) differentiate into chondrocytes, they showed lower levels of differentiation potential compared to human MSCs [3]. Furthermore, compared with rabbit bone marrow-derived stem cells (BMSCs), rabbit subcutaneous adipose-derived stem cells (ScASCs) exhibited even lower potential for chondrogenic differentiation [4]. The above finding is in line with our previous report, in which rabbit ScASCs exhibited much lower chondrogenic potential compared with donor-matched infrapatellar fat pad-derived stem cells (IPFSCs) [5]. This study aims to investigate if SOX9 integration into the collagen I promoter can improve chondrogenic potential of rabbit ScASCs and donor-matched IPFSCs.

METHODS: ScASCs and IPFSCs were isolated from donor-matched rabbit tissues with Institutional Animal Care and Use Committee approval. ScASCs and IPFSCs were transduced with a custom lentivirus incorporating SOX9 into the type I collagen promoter (c1-SOX9), and green fluorescent protein (GFP) lentivirus-transduced (GFP) and non-transduced cells (CTR) served as controls. Both transduced and non-transduced cells were expanded for one passage on decellularized extracellular matrix (dECM) deposited by their corresponding cells. Expansion on plastic culture flasks (Plastic) served as a control. Cell proliferation was assessed by calculating population doubling time. Chondrogenic differentiation was assessed 21 days after induction by detecting sulfated glycosaminoglycans (GAGs) using Safranin O (SO)/Alcian blue (Ab) staining and detecting type II collagen using immunohistochemistry (IHC) and evaluating the expression of chondrogenic-related genes using real-time (RT)-quantitative PCR (qPCR). Differences were evaluated by Mann-Whitney U test ($p < 0.05$).

RESULTS: IPFSCs exhibited excellent chondrogenic potential compared to donor-matched ScASCs, which have superior adipogenic capacity. Lentiviral-transduced cells, particularly c1-SOX-transduced IPFSCs, exhibited reduced cell proliferation, which was reversed by expansion on dECM. After 21 days of chondrogenic induction, the c1-SOX9 group displayed the largest pellets and the most intense staining for sulfated GAGs and type II collagen compared to the control groups (Figure 1). Expansion on dECM significantly enhanced the chondrogenic potential of the c1-SOX9 group, particularly for IPFSCs. RT-qPCR results consistently showed increased expression of ACAN and COL2A1 mRNAs in the C1-SOX9 group compared to the corresponding control groups. In contrast, expression of COL1A1 and COL10A1 mRNAs decreased in the c1-SOX9 group compared to the control group.

DISCUSSION: This study confirmed that MSCs derived from different tissues have different chondrogenic potential [5]. After transduction of the c1-SOX9 gene and pretreatment with dECM, rabbit IPFSCs displayed superior chondrogenic capacity compared with donor-matched ASCs, suggesting that combining c1-SOX9 transduction with dECM expansion is a promising strategy for improving cartilage repair in rabbit models.

SIGNIFICANCE/CLINICAL RELEVANCE: This study established a rabbit model of controllable SOX9 overexpression, which enhanced hyaline cartilage formation, reduced fibrocartilage formation, and improved cartilage repair outcomes.

ACKNOWLEDGEMENTS: This project was supported by Research Grants from the National Institutes of Health (AR078846).

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