A new method to engineer stem cells to automatically convert fibrocartilage into articular cartilage

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INTRODUCTION: Once damaged, articular cartilage has limited self-healing capacity. If the damage extends deep into the subchondral bone, it often results in fibrocartilage formation. Cell therapies in animal studies and clinical trials have ultimately led to fibrocartilage formation. Given that SOX9 is a key transcription factor for chondrogenesis, many studies have focused on overexpressing SOX9 in implanted cells to promote the expression of cartilage matrix components, such as type II collagen and proteoglycans. Unfortunately, overexpression of SOX9 can contribute to various diseases, such as osteoarthritis and cancer risk. In this study, human annulus fibrosus cells (AFCs) were selected as a cell source for fibrocartilage formation; SOX9 was integrated into the type I collagen promoter to control SOX9 expression, which is triggered only during fibrocartilage formation. We hypothesized that engineering of stem cells by integrating SOX9 into the type I collagen promoter would allow stem cell-based tissue constructs to differentiate into articular cartilage rather than fibrocartilage, as upregulated type I collagen triggers SOX9 expression.

METHODS: Human AFC cells, purchased from ScienCell, were transduced with lentiviruses expressing green fluorescent protein (GFP), SOX9, and SOX9 integrated into the type I collagen promoter (c1-SOX9). Transduced cells were selected using puromycin. Both lentivirus-transduced and non-transduced AFC cells were expanded on decellularized extracellular matrix (dECM) deposited by AFC cells. Cell proliferation was assessed by calculating population doubling time, quantifying EdU incorporation by flow cytometry, and measuring transcript levels of the cell cycle regulators CDKN1A, E2F1, and MKI67 by real-time quantitative PCR (RT-qPCR). MSC surface markers, including CD73, CD90, CD105, CD146, and SSEA4, were assessed by flow cytometry. After 21 days of in vitro induction, trilineage differentiation, including chondrogenesis, adipogenesis, and osteogenesis, was evaluated. Chondrogenic differentiation was assessed by RT-qPCRdetecting the expression of SOX9, ACAN, COL2A1, PRG4, COL1A1, and COL10A1, Alcian blue (Ab) staining for sulfated glycosaminoglycans (GAGs), and immunohistochemistry (IHC) for types I, II, and X collagen. Lineage-specific differentiation genes (adipo: CEBPA, FABP4, PPARG, LPL; osteo: BGLAP, RUNX2, ALPL, SP7) quantified by RT-qPCR and lineage-specific staining (adipo: Oil Red O; osteo: Alizarin Red S) were performed to assess adipogenic and osteogenic differentiation. Differences were evaluated by Mann–Whitney U test ($p < 0.05$). Data are presented as mean ± SD for n=3. Groups not connected by the same letter are significantly different ($p < 0.05$) in each comparison.

RESULTS: Lentiviral transduction of SOX9 and c1-SOX9 into human AFC cells effectively silenced the COL1A1 gene and significantly upregulated the expression of SOX9, COL2A1, and ACAN during chondrogenic induction, exceeding that of the non-transduced and GFP-transduced control groups (Figure 1). Notably, compared with the non-transduced control group, SOX9 overexpression significantly inhibited AFC proliferation, while c1-SOX9 transduction maintained a relatively high proliferation rate. dECM-expanded c1-SOX9 and SOX9-transduced AFCs produced significantly larger pellets under chondrogenic induction, with marked upregulation of SOX9 and ACAN and downregulation of COL1A1, whereas non-transduced cells showed minimal pellet enlargement and modest gene changes (Figure 1). dECM-expanded c1-SOX9 and SOX9-transduced AFCs exhibited reduced expression of COL1A1, RUNX2, and ALPL mRNAs upon osteogenic induction.

DISCUSSION: Although AFC cells transduced with SOX9 combined with dECM expansion produced the highest chondrogenic transcript levels after chondrogenic induction, c1-SOX9 transduction paired with dECM expansion provided superior proliferation kinetics without compromising chondrogenic output, making it a more feasible clinical strategy for hyaline cartilage regeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: The use of c1-SOX9 transduction combined with dECM expansion for pretreatment is a promising strategy for future stem cell–based cartilage regeneration.

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Figure 1