Effect of heparan sulfate in perlecan on stem cell-based chondrogenesis via dECM expansion

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Disclosures: No conflicts of interest

INTRODUCTION: Mesenchymal stem cells (MSCs) are highly promising stem cells for tissue regeneration. Given that they inevitably lose their proliferative capacity and multi-differentiation potential during long-term in vitro expansion, preserving or enhancing their lineage commitment in vitro is a prerequisite for clinical translation. Perlecan, a heparan sulfate (HS)-rich basement membrane proteoglycan, is gaining increasing attention as an epigenetic cue regulating MSC fate within the stromal microenvironment [1,2]. However, there is a lack of research on the role of HS chains in perlecan-mediated MSC mesenchymal regeneration. This study aimed to evaluate the effects of perlecan HS chains on stem cells within the stromal microenvironment using heparinase I&III (HPA) digestion.

METHODS: Decellularized extracellular matrix (dECM) (s-dE) was generated from human adult infrapatellar fat pad-derived stem cells (IFPSCs) transduced with lentivirus carrying the SV40 large antigen (SV40LT). dECM generated from non-transduced IFPSCs (dE) served as a control. The effects of HS chains in HPA-digested dECM (dE and s-dE) on IPFSC proliferation and mesenchymal differentiation were evaluated. The proliferation capacity of the expanded cells was measured using population doubling time and relative EdU incorporation. Flow cytometry was used to assess the expression of MSC surface markers, including CD73, CD90, CD105, CD146, and SSEA4, in the expanded cells. Real-time quantitative PCR (qPCR) was also used to assess the expression of stemness genes, including BMI, KLF4, POU5F1, NOV, NANOG, SOX2, MYC, and NES, in the expanded cells. Chondrogenic differentiation was assessed by culturing the pellets for 21 days in chondrogenic induction medium. The expression of perlecan (HSPG2) and chondrogenesis-related genes (SOX9, ACAN, COL2A1, PRG4, COL1A1, and COL10A1) was analyzed by qPCR; sulfated GAGs were analyzed by Alcian blue staining; and collagen types I, II, and X were detected by immunohistochemistry (IHC). After 21 days of lineage-specific induction, adipogenic and osteogenic potentials were assessed. Adipogenesis-related genes (CEBPA, FABP4, PPARG, and LPL) were quantified by qPCR, combined with Oil Red O staining and quantitative analysis. Osteogenic potential was assessed by qPCR quantification of osteogenic genes (BGLAP, RUNX2, ALPL, and SP7), combined with Alizarin Red S staining and quantitative analysis. Statistical analysis was performed using the Mann–Whitney U test.

RESULTS: Immunofluorescence staining of dECM using the anti-HS antibodies 10E4 (N-sulfated epitope) and 3G10 (heparinase-generated neoepitope) confirmed successful HPA removal of HS chains. Enzymatic digestion of HS chains bound to perlecan with HPA confirmed their crucial role in dECM-mediated IFPSC lineage differentiation. Compared to s-dE, removal of HS from perlecan-enriched s-dE significantly enhanced chondrogenic capacity while inhibiting adipogenesis. Conversely, loss of HS from dE reduced chondrogenic potential while enhancing adipogenic capacity. Furthermore, osteogenic potential appeared to remain unchanged under both conditions.

DISCUSSION: Given that dE from high-passage non-transduced cells loses its ability to induce chondrogenesis, whereas passage-matched s-dE retains this capacity [3], this study demonstrated that HPA-mediated HS removal correspondingly enhanced or diminished cartilage commitment. This matrix-specific response makes perlecan a tunable cue for the construction of novel biomaterials that can selectively enhance cartilage regeneration based on intrinsic dECM composition.

SIGNIFICANCE/CLINICAL RELEVANCE: HPA-mediated HS editing of perlecan-enriched s-dE deposited by high-passage SV40LT MSCs appears to provide a dECM-based strategy to enhance stem cell-based cartilage regeneration.

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

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