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Differentiation of human induced pluripotent stem cells towards notochordal-like cells: the role of tissue source

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INTRODUCTION: Notochordal cells (NCs) are linked to a healthy intervertebral disc (IVD), and they are considered an exciting target for cell-based therapy. However, NCs are scarcely available as they are lost early in life, and attempts at *in vivo*expansion have failed because NCs lose their specific phenotype. The production of Notochordal-like cells (NLCs) from human induced pluripotent stem cells (iPSCs) is a viable alternative. However, current attempts have been challenged by the low differentiation efficiency into the NC lineage. Therefore, the aim of this study was to build on the tissue-specific epigenetic memory of hiPSCs derived from IVD progenitor cells (TIE2+-cells) to improve hiPSC differentiation towards mature, matrix-producing NLCs.

METHODS: hiPSCs were generated from TIE2$^+$ cells of three adult donors. As a comparison, donor-matched minimally invasive peripheral blood mononuclear (PBM) cell-derived iPSCs were used. Firstly, the iPSCs were differentiated into mesendodermal progenitors by Wnt pathway activation (N2B27 medium + 3µM CHIR99021)[1]. Thereafter, the cells were further driven towards the NC-lineage by transfection with synthetic *NOTO* mRNA[1] and further matured using a 3D pellet culture in discogenic medium containing 10ng/mL TGF-β1. Read-out parameters included cell morphology, gene and protein expression and matrix deposition.

RESULTS: Both TIE2$^+$ and PBM cell-derived hiPSC showed successful differentiation towards mesendodermal progenitor cells following Wnt activation on day 2, indicated by the cells moving out of the colonies after CHIR stimulation. Accordingly, a decreased gene expression of pluripotency markers (*OCT4, SOX2, NANOG*), and upregulation of Wnt-target genes (*LEF1, NODAL*) and mesendodermal markers (*TBXT, FOXA2, TBX6*) was observed compared to mTESR1 controls. This was confirmed by immuno-stains for FOXA2 and TBXT. At day 3, we confirmed a 9-fold increase in *NOTO* mRNA levels after transfection in all donor lines. At day 28, the appearance of vacuolated NLCs was observed in both TIE2$^+$ and PBM cell-derived pellet cultures confirming successful commitment towards the NC-lineage. Interestingly, while DMMB-assay detected GAG deposition in both lines, a significant increase in GAG content was seen in the TIE2$^+$ cell-derived pellets.

DISCUSSION & CONCLUSIONS: Tissue-specific TIE2^{+} cell-derived iPSCs may allow for an improved iPS-NLC differentiation efficiency, indicated by the increased potency for deposition of GAG-rich matrix. Detailed analysis of the phenotypic markers and matrix deposited at the end of the 28 day maturation is ongoing to further document the phenotype of these iPS-NLCs. Delineating which epigenetic features are retained after reprogramming of these two cell lines, could shed light on the differences in their differentiation capacity.

REFERENCES: [1]Colombier *et al.*, 2020

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