Employing epigenetic memory and native instructive stimuli to stimulate iPS-NLC differentiation

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Notochordal cells (NCs) are linked to a healthy intervertebral disc (IVD), and are considered a promising candidate for cell-based therapies. However, NCs are scarcely available as they are lost early in life, and attempts at *in vitro* expansion have failed because NCs lose their specific phenotype. The production of notochordal-like cells (NLCs) from human induced pluripotent stem cells (hiPSCs) is a viable alternative. Therefore, this study aimed to build on the tissue-specific epigenetic memory of hiPSCs derived from IVD-progenitor cells ($TIE2^+$-cells) and the instructive capacity of decellularized notochordal cell-derived matrix (dNCM)[2] to improve hiPSC differentiation towards mature, healthy matrix-producing NLCs.

hiPSCs were generated from $TIE2^+$-IVD cells of three adult donors. As a comparison donor-matched minimally invasive peripheral blood mononuclear (PBM)-derived iPSCs were used. Firstly, hiPSCs were differentiated into mesendodermal progenitors by Wnt pathway activation (N2B27 medium + 3µM CHIR99021)[1] for 2 days. Thereafter, the cells were further driven towards the NC-lineage by transfection with synthetic *NOTO* mRNA[1] and matured by switching to a 3D-cell pellet culture in discogenic medium containing 10ng/mL TGF-β_1 or 3mg/mL dNCM until day 28. Read-outs included cell morphology, gene and protein expression and matrix deposition.

Both $TIE2^+$- and PBM-cell derived hiPSC showed successful differentiation towards mesendodermal progenitors 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* and *Nodal* signaling (*LEF1, NODAL*) and mesendodermal markers (*FOXA2, TBXT*) was detected, compared to mTeSR1 controls. This was confirmed by immuno-stains for FOXA2 and TBXT. On day 3, we detected a significant increase in *NOTO* mRNA levels in all donor lines after transfection compared to untransfected cell pellets. 3D-pellets of all donor lines showed glycosaminoglycan (GAG)- and collagen type II-rich areas after dNCM- but not TGF-β_1-treatment on day 28. This was confirmed with the DMMB-assay, showing a significantly increased GAG content in the 3D-pellets treated with dNCM compared to TGF-β_1. Next to that, $TIE2^+$-cell derived iPSC pellets contained a significantly higher GAG content after dNCM-treatment compared to the PBM-cell derived hiPSC pellets. Immunohistochemical evaluation showed a heterogeneous cell population including cells positive for chondrogenic- (ACAN, SOX9), NPC/NC- (panKRT, T), and IVD progenitor- markers (CD24, TIE2).

In conclusion, using tissue-specific $TIE2^+$-cell derived hiPSCs combined with dNCM-treatment may allow for an improved differentiation capacity indicated by the increased deposition of GAG and collagen type II-rich matrix. However, the obtained cell population is still very heterogeneous and further transcriptome analysis could unravel whether the 3D-pellets contain cells which were successfully driven towards the notochordal-lineage and how these can be enriched based on unique NC-specific markers. Next to that, delineating which epigenetic features are retained after reprogramming of these two cell lines, could shed light on the observed differences in their

differentiation capacity. These insights could be used for further optimization of iPS-NLC differentiation and allow for a more purified population of mature, healthy matrix-producing NLCs.

This work was funded by Horizon 2020 (no. 825925) and the Dutch Arthritis Society (LLP22).

References

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