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

<u>Lisanne T. Laagland</u>¹, Deepani W. L. Poramba Liyanage¹, Paul Bensadoun², Mathis Soubeyrand³, Romain Desprat⁴, Anne Camus⁵, Jean Marc Lemaitre⁶, Benjamin Gantenbein⁷, Marianna A. Tryfonidou¹

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

²IRMB, Univ Montpellier, INSERM, Montpellier, France

³ESTeam Paris Sud, INGESTEM National IPSC Infrastructure, UPSud, Paris-Saclay University, Villejuif 94800, France; UMR-S 935, INSERM, University Paris Sud, Villejuif 94800, France.

⁴SAFE-iPSC Facility, CHU Montpellier, Montpellier, France

⁵Nantes Université, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-4400 Nantes, FR

⁶IRMB, Univ Montpellier, INSERM, Montpellier, France; SAFE-iPSC Facility, CHU Montpellier, Montpellier, France

⁷Bone and Joint Program, Department for BioMedical Research (DBMR), Medical Faculty, University of Bern, Bern, CH-3008, Switzerland; Department for Orthopaedic Surgery and Traumatology, Insel University Hospital, Medical Faculty, University of Bern, Bern, CH-3010, Switzerland

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, donormatched 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 + 3 μ M CHIR99021)¹. Thereafter, the cells were further driven towards the NClineage by transfection with synthetic *NOTO* mRNA¹ and further matured using a 3D pellet culture in discogenic medium containing 10ng/mL TGF- β 1. 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.



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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: ¹Colombier *et al.*, 2020

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