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Modeling vascular diseases using human induced pluripotent stem cells

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APPENDIX

Summary

Samenvatting

Curriculum Vitae

List of publications

Acknowledgments

SUMMARY

Endothelial cells (ECs) and myeloid cells derived from human induced pluripotent stem cells (hiPSCs) provided a novel and powerful tool for the study of various vascular and inflammatory diseases which are caused or correlated to dysfunctions of these cell types. This thesis mainly focuses on the derivation and characterization of ECs and myeloid cells from hiPSCs, and their application in modeling two vascular diseases Hereditary Hemorrhagic Telangiectasia type 1 (HHT1) and Pseudomyogenic hemangioendothelioma (PHE).

Chapter 1 provides general introduction of hiPSC-derived ECs and macrophages. This chapter summarized the current developed differentiation method for these cell types and their phenotype and functional activities compared to their primary counterparts *in vivo*. As we are mainly interested in two vascular diseases HHT1 and PHE, this chapter also describes known underlying disease mechanism of these diseases, with an emphasize ECs and myeloid cells.

In **chapter 2 and 3**, an efficient protocol was developed to derive monocytes and macrophages from hiPSCs and performed detailed functional characterization and comparison with peripheral blood (PB)-derived monocytes and macrophages. We showed that hiPSC-derived monocytes were functional after cryopreservation and could be used to model the inflammation response *in vitro* in a microfluidic chip. Both pro-inflammatory and anti-inflammatory M1 and M2 subtypes of macrophages could be polarized from hiPSC-monocytes, which were similar to PB-derived macrophages in terms of their gene expression profiles and functional activities. Interestingly, we observed a higher endocytosis and efferocytosis activities with hiPSC-derived macrophages than blood-derived macrophages, although both showed similar bacterial and tumour cell phagocytosis capacities. In summary, hiPSC-monocytes and macrophages could be generated robustly from independent hiPSC-lines, which were fully functional and comparable with PB-derived myeloid cells.

In **chapter 4**, the development process of EC and cardiomyocytes from hiPSCs in a co-differentiation system was investigated utilizing an ETV2^{mCherry} reporter line and bulk and single cell RNA sequencing (RNAseq) approaches. This chapter showed that EC and cardiomyocytes had a common development origin from cardiac mesoderm precursors in this co-differentiation system. A critical role of transient expression of ETV2 was revealed during endothelial fate specification. Besides, this chapter indicated that functional cardiomyocytes could also differentiate from ETV2+ progenitors.

In **chapter 5**, a 3D vasculature model was established in a microfluidic chip using ECs derived from isogenic HHT1 hiPSC lines, which were generated from a mosaic HHT1 patient. Disease related phenotypes were observed using this 3D vascular model with mutant hiPSC-ECs, including increased inflammatory responses and defected vascular

organization. TGFß, BMP and WNT signaling pathways were revealed being dysregulated in mutant ECs based on their whole transcriptomic profile. Work of this chapter further revealed the underlying disease mechanism of HHT1 which is crucial for developing potential therapeutic strategies in the future.

In **chapter 6 and appendix**, a PHE tumor specific hiPSC line was established by introducing a chromosomal translocation into WT hiPSCs using CRISPR/Cas9. Then ECs were differentiated from the disease line in order to model PHE *in vitro*. Mutated hiPSC-ECs showed a number of disease related phenotypes *in vitro* and *in vivo* after transplantation into mice, which correlated to disease symptoms observed in PHE patients. RNAseq of WT and mutated hiPSC-ECs revealed transcriptome alterations causing hiPSC-EC dysfunction, which reflected the PHE phenotypes. Signaling pathways dysregulated in PHE hiPSC-ECs were also identified, which can be potentially be targeted for the treatment of PHE in the future.

Finally, **chapter 7** is a general discussion about the research conducted in this thesis, which summarized both the importance of our findings and limitations of the work. This chapter also includes future perspectives of the research in each chapter of this thesis, which are either already under investigation in our lab or could be potentially conducted in the future.