Title: HETEROGENEITY OF XENO-FREE CULTURED HUMAN PLURIPOTENT STEM CELLS (HPSCS) INDUCES VARIATION IN THE DIFFERENTIATION EFFICIENCY OF HPSC-DERIVED CORNEAL LIMBAL STEM CELLS

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Abstract
Differentiation of corneal limbal stem cells (LSCs) from hPSCs presents a promising therapeutic approach to treat severe bilateral limbal stem cell deficiency. However, hPSC variation is known to challenge the robustness of the protocols and increase unwanted heterogeneity. Here, hPSCs were differentiated towards corneal epithelium, and the process was serially analyzed with real-time qPCR (RT-qPCR) and immunofluorescence (IF), to reassess the cell line-specific sensitivity of an established differentiation protocol. The results from five hiPSC lines were compared with a hESC line showing a good historical performance in this protocol.

Partial success in the stepwise differentiation towards LSCs was achieved, but it was indeed diminished by varying lineage commitment efficiency between the hiPSC lines and even between differentiation batches within individual cell lines. RT-qPCR results indicated high initial variation from line-to-line as well as from batch-to-batch within the undifferentiated hiPSCs. Interestingly, the variations of BMP4, LEF1, PAX6, and TGFB1 gene expressions, which represent some of the key factors in corneal epithelial induction, were notably high also in the control hESC line, suggesting that a standardization of the starting material is needed for this protocol. Some cell batches failed to upregulate PAX6 and developed a fibroblastic, mesenchymal morphology in association with elevated TGFB1 gene expression, which was linked to the decreased number of cells expressing LSC markers p63 and CK14 in the later phases of the differentiation.

These results emphasize the need for additional development and quality control to ensure reliable production of homogenous starting material for the LSC differentiations.