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主　論　文　の　要　旨

論文題目　Reproducible production and image-based quality evaluation of retinal pigment epithelium sheets from human induced pluripotent stem cells
（細胞医薬品としてのヒトiPS細胞由来網膜色素上皮シートの作製法と品質評価に関する研究）

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論　文　内　容　の　要　旨

Our vision is initiated in the retina that collects the light from the surrounding environment. The primary light-sensing neurons are p]hotoreceptor cells of the retina. The retinal pigment epithelium (RPE) is the monolayer cells adjacent to photoreceptor cells that support the survival and function of photoreceptor cells. Dysfunction of RPE causes photoreceptor cell death, leading to the loss of vision. Retinal degeneration diseases, such as age-related macular degeneration (AMD), occur by the early loss of RPE in the macular region. AMD patients will lose their central vision and high-acuity vision. Until now, no effective treatments have been available for these patients. The transplantation of RPE cells holds a great promise in rescuing the vision. However, the limited source of donor RPE cells has hindered the clinical application of the RPE transplantation. Pluripotent stem cells can overcome this limitation. Both embryonic stem cells and induced pluripotent stem cells (iPSC) have the potential to self-renew and develop into any cell types of the body. In addition, human iPSC (hiPSC) can circumvent ethical problems because hiPSC can be generated from human somatic cells without embryo destruction.

For the development of RPE sheet transplantation, however, two major issues need to be addressed. (1) RPE sheets in high purity are necessary to guarantee the safety of transplantation, such as avoiding tumorigenesis risks. Although the efficiency of RPE differentiation from hiPSC has been improved, manual selection is necessary to obtain pure RPE sheets. However, manual selection is time-consuming and labor-intensive and needs trained experts. (2) For clinical application purposes, any RPE sheets with abnormal function need to be detected and eliminated before transplantation. However, technology

for evaluating the quality of RPE sheets has been poorly developed. Conventional evaluation methods that need the extraction of DNA, RNA, and proteins are destructive and not suitable for cell products for regenerative medicine. To solve the issues of purification and quality evaluation, I conducted the following sets of experiments.

(1) Generation of RPE Cells from hiPSC for RPE Sheets

Stepwise treatment of dissociated hiPSC with six signaling pathway inhibitors along with nicotinamide (RPE6iN) induced MITF+ cells, polygonal pigmented cells, and ZO-1+ cells, suggesting that RPE6iN induced RPE cells in high efficiency. RPE6iN-induced cells were seeded on transwell to produce RPE sheets. The RPE sheets expressed markers of mature and polarized RPE cells. However, the barrier function of the RPE sheets varied between lots, consistent with general issues reported in previous studies. RPE sheets with low TER values showed the mislocalization of tight junctions and loss of adheres junctions. These results suggest that the cellular morphology of RPE sheets with low TER values is different from those with high TER values. I conclude that functional RPE sheets in high purity were generated after further culture of RPE6iN-induced cells on the transwell and that the quality of the RPE sheets varied between lots.

(2) Morphology-based Prediction Model for RPE Sheet Function

To support the robust RPE sheet production ability of the RPE6iN method by evaluating the quality of RPE sheet products, I developed machine learning models to predict barrier function based on morphological information of microscopic images. Cellular morphological feature rules from the F-actin-stained images were extracted and used for model construction. The models were effective at identifying low-quality RPE sheets for elimination, even when using label-free images. Using these models, I also identified failure samples only from the non-labeled images of RPE sheets across different hiPSC lines and different manufacturing facilities.

The RPE6iN-based RPE sheet generation combined with the non-destructive image-based prediction offers a comprehensive new solution for the large-scale production of pure RPE sheets with lot-to-lot variations. These findings will not only add to our understanding of retinal differentiation and RPE cell biology, but also contribute to *in vitro* disease modelings, such as pathogenesis analysis and drug screening. The studies described in this dissertation will facilitate the manufacturing and quality control of RPE sheet products and provide a foundation for the development of hiPSC-based cell products for regenerative medicine.