*Keio University*

# ABSTRACT

No.

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Thesis Title

Human Induced Pluripotent Stem Cell-Derived Ectodermal Precursor Cells Contribute to Hair Follicle Morphogenesis *In Vivo*
(ヒト iPS 細胞由来外胚葉前駆細胞は in vivo での毛包再構成に寄与する)

Thesis Summary

Currently, attempts to regenerate hair follicles (HF) centre on combining epithelial and mesenchymal HF components and grafting them into an *in vivo* environment. However, currently available isolation techniques are not efficient enough to collect the necessary number of human epithelial bulge stem cells for *in vivo* assays. In addition, previous investigators have reported that neonatal human keratinocytes (KCs) more efficiently formed HF like structures than adult human KCs at the same passage. Therefore, human induced pluripotent stem cells (hiPSCs) could be a more easily accessible source of epithelial component for HF bioengineering.

In this study, ectodermal precursor cells (EPCs) with the capacity to crosstalk with hair inductive dermal cells were generated from hiPSCs and assessed for HF forming ability *in vivo*. EPCs derived from three hiPSC lines generated with 4 or 3 factors (POU5F1, SOX2, KLF4 +/- MYC) were converted into embryoid bodies (EBs), exposed to retinoic acid to promote ectodermal lineages and bone-morphogenetic protein-4 (BMP4) to suppress neural lineages. Flowcytometric analyses demonstrated that generated cells mostly expressed KRT18, a marker of epithelial progenitors, and a small proportion of them expressed KC markers. Interestingly, when each hiPSC-EPC population was exposed to high calcium conditions, upregulation of KC terminal differentiation markers was observed, especially in 201B7 EPC lines. These findings implied that hiPSC-EPCs contained the cells with KC differentiation potential.

When co-cultured with human dermal papilla (DP) cells, a 4 factor 201B7 hiPSC-EPC line upregulated follicular KC markers more significantly than normal human adult KCs and other hiPSC-EPC lines. DP cells preferentially increased DP biomarker expression in response to this line. Interestingly, 201B7 hiPSCs were shown to be ectodermal/epithelial prone, and the derived EPCs were putatively in a WNT-activated state. Importantly, co-transplantation of 201B7 hiPSC-EPCs, but not normal human KCs, with trichogenic mice dermal cells into immunodeficient mice resulted in HF formation. Human HF stem cell markers were detected in reconstituted HFs; however, a low frequency of human-derived cells implied that hiPSC-EPCs contributed to HF morphogenesis via direct repopulation and non-cell autonomous activities. The current study suggests a previously unrecognized advantage of using hiPSCs to enhance epithelial-mesenchymal interactions in HF bioengineering.