Regenerative medicine, Drug screening, Developmental biology

Generation of functional conjunctival epithelium, including goblet cells, from human iPSCs

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Abstract

We have established a new method for generating functional mucin-producing conjunctival epithelial cell sheets from human iPSCs (hiPSCs). The conjunctiva, which sits on the sclera (the white of the eye) and lines the inside of the eyelids, is essential for mucin secretion and the establishment of a healthy tear film.

We demonstrated that conjunctival epithelial lineage cells developed in epidermal growth factor (EGF)-treated hiPSC-derived eye-like organoids and that they could be isolated by using a cell sorter. We then succeeded in reconstituting the conjunctival epithelium, including goblet cells, from the isolated cells under the influence of keratinocyte growth factor (KGF).

This study enables us to obtain a large amount of human conjunctival cells, which has been difficult until now, and great progress is expected in drug discovery research and regenerative therapy for dry eye disease.

Background & Results

The conjunctiva, which sits on the sclera and lines the inside of the eyelids, is the source of mucins, which are vital for the stability of the tear film. Tear film instabilities due to conjunctival disease result in dry eye disease, which is manifested by physical discomfort and visual impairment

We have previously established a method for inducing "Self-formed Ectoderm Autonomous Multi-zone" (SEAM, Fig. 1), which is an organoid containing various ocular cells using human iPS cells. And we have succeeded in producing corneal epithelial tissue from SEAMs. In the present study, we aimed to differentiate, isolate, and mature conjunctival cells from SEAMs by properly using growth factors under appropriate conditions.

Firstly, we showed that ocular surface epithelial stem/progenitor cells, which express p63 and PAX6, are present in the EGF or KGF-treated SEAM zone 3, but that differentiation into corneal epithelial cells does not take place in the EGF-treated SEAM (Fig. 2A–B). Secondly, EGF-treated SEAMs were stained with three cell surface markers (CD200, SSEA-4, and ITGB4), divided into six fractions, and analyzed in detail (Fig. 3A). As a result, it was clarified that the cells of the P2 fraction (CD200-, SSEA-4low, ITGB4+ were differentiated into mature conjunctival epithelium including conjunctival goblet cells by stratified culture. Unexpectedly, expression of the goblet cell marker MUC5AC was upregulated when KGF, but not EGF, was added to the maturation medium (Fig. 3B–C). The iPSC-derived conjunctival epithelium contained MUC5AC producing goblet cells (Fig. 3D) and showed characteristic of native conjunctival epithelium (Fig. 3E– F). These results suggest that key complementary roles of EGF and KGF in directing the differentiation and maturation, respectively, of the human conjunctival epithelium.

Significance of the research and Future perspective

We succeeded in generating human conjunctival cells, which were difficult to obtain previously, from iPS cells. This finding has a range of potential applications to help us better understand human conjunctival development. Furthermore, this study could help with identifying novel drugs for dry eye syndrome and could further open new avenues for regenerative therapies.

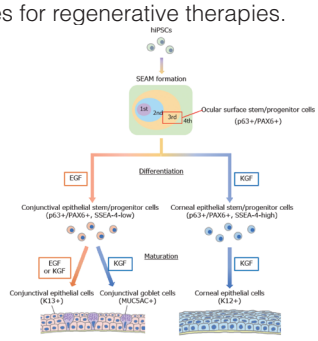

Figure 1. Schematic of ocular surface epithelial cell generation

Ocular surface epithelial stem/progenitor cells are present in the zone-3 of multizonal colony (SEAM), which is an organoid containing various ocular cells derived from human iPSCs. In differentiation culture, conjunctival epithelial lineage cells were predominantly induced in the EGF-treated SEAM derivatives, whereas corneal epithelial lineage cells predominated in the KGF-treated SEAM derivatives. KGF was necessary for maturation of the hiPSC/SEAM-derived conjunctival epithelium, and goblet cells.

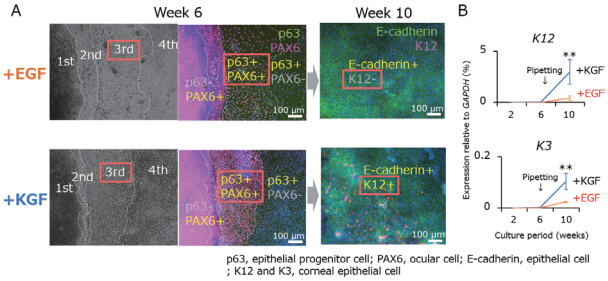

Figure 2. EGF inhibits the differentiation of hiPSC-derived SEAMs into corneal epithelial cells

(A) Immunostaining for EGF and KGF-treated SEAMs (+EGF and +KGF). K12 (corneal epithelial cell marker) expression was not detected in the EGF-treated SEAM derivatives. (B) Gene expression analysis for EGF and KGF-treated SEAMs (+EGF and +KGF). K12, and K3 (corneal epithelial differentiation cell markers) were significantly downregulated in the EGF-treated SEAM ($p < 0.01$).

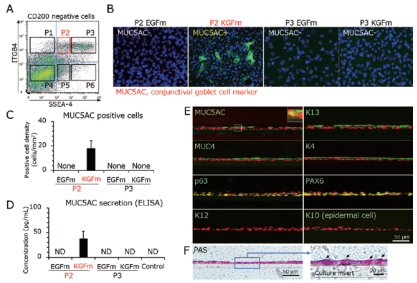

Figure 3. Isolation of Conjunctival epithelial progenitors from EGF-treated SEAM derivatives and generation of conjunctival epithelial tissue by maturation culture in KGF containing medium.

(A) FACS analysis of the EGF-treated SEAM derivatives (10–12 weeks) and isolation of each cell fraction. (B) MUC5AC immunostaining for P2 and P3 cells after cultivation. (C) The density of MUC5AC+ cells from the P2 and P3 fraction cells after maturation culture. (D) ELISA for MUC5AC in culture supernatants of the P2 and P3 fraction cells after maturation culture. (E) Immunostaining for conjunctival functional proteins and ocular surface epithelium-related markers (green) in the hiPSC/SEAM-derived conjunctival epithelium. (F) PAS-staining of the hiPSC/SEAM-derived conjunctival epithelium.

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Keyword conjunctival epithelium, goblet cells, EGF, KGF, SEAM