Regenerative therapy, Drug development

Generation of 3D lacrimal gland organoids from human pluripotent stem cells

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Abstract

The surface of the eye (ocular surface) consists of the cornea and conjunctiva, and the presence of a lacrimal fluid layer is essential for the homeostasis of the ocular surface. The lacrimal fluid layer is mainly maintained by the secretion of tear fluid and mucin from the lacrimal gland, but when the lacrimal gland is damaged by autoimmune diseases such as Sjögren's syndrome, the ocular surface becomes dry, resulting in severe dry eye. Our research group had previously succeeded in producing the cornea and conjunctiva from human iPS cells, but the generation of the lacrimal gland had not yet been reported. Noting that the cornea, conjunctiva and lacrimal gland have the same developmental origin, we applied the two-dimensional ocular organoids (named SEAM) previously used to induce the cornea and conjunctiva and newly attempted to induce lacrimal gland primordia. First, we found that lacrimal gland-like cell clusters appeared in the iPS cell-derived SEAM, and successfully produced 3D lacrimal gland organoids by conducting 3D culture in Matrigel.

Background & Results

We found that in the region of the ocular surface ectoderm (common developmental primordium of the lacrimal gland and cornea) within the induced SEAM (zone-3), there were widely distributed as cell clusters with PAX6/SOX9-positive gland-like structures. When these gland-like cell clusters were isolated and cultured in Matrigel in 3D culture, budding and elongation of ducts were observed, and further branching occurred, resulting in a lacrimal gland-like structure. Furthermore, lacrimal gland progenitor cells were successfully isolated using a cell sorter. Immunostaining of the generated lacrimal gland-like organoids confirmed the expression of lacrimal gland markers such as PAX6, AQP5 and SOX9. In addition, a swelling assay was performed to verify the lacrimal gland organoid lacrimal fluid secretion capacity, and significant swelling of the terminal end of the organoid was observed upon forskolin stimulation.

Next, transplantation experiments were performed using nude rats to investigate the viability and functional maturation of the lacrimal gland organoids in vivo. When lacrimal gland organoids were examined 4 weeks after transplantation, lumen formation and cell polarity, which were insufficient in vitro, became remarkable. Furthermore, the expression of lactoferrin and lysozyme, defence proteins of the lacrimal fluid, which were hardly expressed in vitro, was observed and significantly increased after transplantation evaluated by ELISA. These results indicate that human iPS cell-derived lacrimal gland organoids are functional in vivo.

Significance of the research and Future perspective

The results of this research have made it possible to produce 3D lacrimal gland tissues from human iPS cells and will enable the development of regenerative Therapy for severe dry eye. In addition, the ability to produce large quantities of human lacrimal gland organoids from human iPS cells is expected to make significant progress in drug discovery and research for the pathology of dry eye and other ocular diseases.

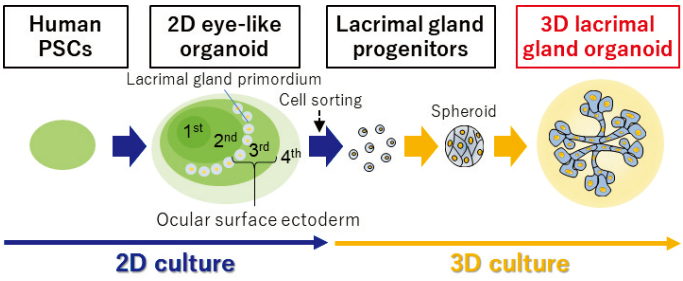

Fig.1 Summary of the study
Ocular surface ectoderm, which is thought to be the common primordium of the lacrimal gland and cornea, is induced in zone-3 of eye-like organoids derived from human iPS cells. After further differentiation culture, we induced and isolated lacrimal gland progenitor cells by cell sorting and successfully made 3D lacrimal gland organoids by 3D culture in Matrigel.

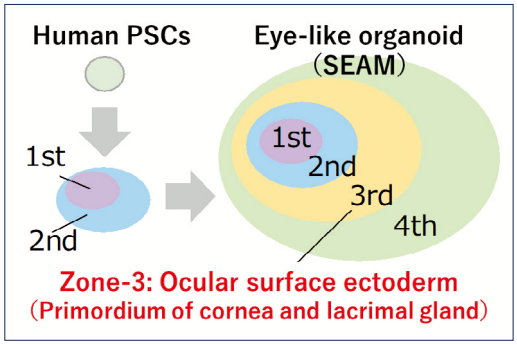

Fig.2 Schematic illustration of SEAM
SEAM typically forms a concentric four zones, and the ocular surface ectoderm, which is the primordium of the lacrimal gland and corneal epithelium, develops in zone 3.

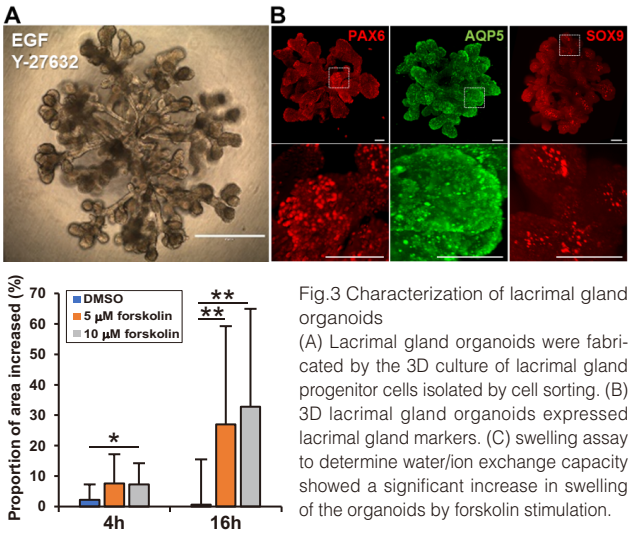

Fig.3 Characterization of lacrimal gland organoids
(A) Lacrimal gland organoids were fabricated by the 3D culture of lacrimal gland progenitor cells isolated by cell sorting. (B) 3D lacrimal gland organoids expressed lacrimal gland markers. (C) swelling assay to determine water/ion exchange capacity showed a significant increase in swelling of the organoids by forskolin stimulation.

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URL

Keyword pluripotent stem cells, lacrimal gland, iPS cells, ocular surface, regenerative medicine