論文概要 (Thesis Abstract)

○ **Theme: Defining compartmentalized stem and progenitor populations with distinct cell division dynamics in the ocular surface epithelium**
（論文題目: 眼表面上皮における幹細胞ダイナミクス解析）

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Purpose: Adult tissues contain label-retaining cell (LRC)s, which are relatively slow-cycling and considered to represent a unique property of tissue stem cell (SC)s. These tissue SCs have the ability to self-renew and differentiate and play an important role in homeostasis and injury repair. Traditionally, the hierarchical stem/progenitor model, in which slow-cycling SCs give rise to short-lived, fast-dividing progenitor or so-called transit-amplifying cells, has been applied to various epithelial or non-epithelial tissues. Recent studies have challenged the generality of the stem/progenitor model and suggested that a relationship between LRCs and their SC potential can be tissue- or context-dependent. In the ocular surface epithelium, LRCs are detected in the limbus, a boundary between the cornea and conjunctiva, and the fornix region of the conjunctiva. These LRCs have been considered to be SCs; however, these characters remain unclear due to the lack of appropriate molecular markers. My research aims to understand the character of the mouse ocular surface epithelium and how its SC populations behave in homeostasis and respond during wound healing.

Materials and methods: To evaluate the distribution of LRCs in the mouse ocular surface epithelium, EdU was injected intraperitoneally twice a day for 1 week, followed by 5 weeks of chase without EdU before the animals were sacrificed. The LRC locations were analyzed by whole-mount staining of ocular epithelial sheets. Lineage tracing experiments were performed by using Slc1a3CreER, Dlx1CreER and K14CreER in order to investigate the SC/progenitor cell behavior. Mice were injected intraperitoneally with Tamoxifen at 2 weeks, 1 month, 3 months and 1 year after the last injection. Limbal physical removal or chemical injury by applying sodium hydroxide solution were used as injury models. Clone distribution and numbers were quantified using ImageJ (Fiji) software.

Results: EdU pulse-chase experiment showed LRCs were enriched in the limbus and fornix conjuctiva. By combining EdU pulse-chase analysis and lineage tracing with three CreER transgenic mouse lines: Slc1a3CreER, Dlx1CreER and K14CreER, I detected distinct dynamics of epithelial SCs in the cornea and conjunctiva. I found Slc1a3CreER labeled cells in the limbal LRC region as well as peripheral cornea, whereas K14CreER and Dlx1CreER preferentially labeled the central cornea. In conjunctiva, LRC-dense fornix region was preferentially marked by Slc1a3CreER and the bulbar and palpebral

conjunctiva were marked by K14CreER. These results suggest the possible heterogeneity of ocular surface epithelium regarding cell division dynamics and molecular characters. To analyze the behavior of LRC and non-LRC populations in each compartment, I performed long-term lineage tracing. In the limbus, long-lived SCs were labeled with Slc1a3CreER and they either migrate centripetally toward the central cornea or laterally expand their clones within the limbal region. In the central cornea, cells were mostly non-LRCs, labeled by Dlx1CreER and K14CreER, and the number of clones declined after a short period of time with rare long-lasting clones, suggesting their properties as short-lived progenitor cells. In the conjunctival epithelium, which consists of bulbar, fornix and palpebral conjunctiva, each territory was regenerated by compartmentalized, distinct SC populations without migrating one region to another. The limbal injury altered limbal SC dynamics toward the limbal-expansion mode and induced rapid expansion of the Slc1a3CreER+ population within the limbus. On the other hand, chemical burn induced disruption of SC compartments and invasion of all three conjunctival SC populations into the corneal region.

Discussion: My genetic tools revealed dynamic behavior of multiple SC populations during tissue homeostasis and injury repair. The territorial segregation of epithelial SCs is determined by yet unidentified mechanism and possibly involves stromal architecture, extracellular matrix and secreted factors. Since epithelial SC heterogeneity is associated with differential tumorigenic ability, regenerative capacity and interaction with non-epithelial cell types, it will be interesting to further unravel the biological significance of multiple SC/progenitor populations in the ocular surface epithelium and their specific roles in different physiological and pathological conditions.

Conclusion: I define distinct compartments in the ocular surface epithelium, which are characterized by the anatomical location, marker expression, and cell division dynamics. My work provides genetic tools to precisely mark and examine the dynamic behavior of multiple SC/progenitor populations in the ocular surface epithelium during homeostasis and injury repair, and to molecularly characterize each population. It will contribute in the future for clinical application to treat extensive ocular injuries, such as Stevens-Johnson syndrome or severe chemical burns.