氏名（本籍）	石井　柳太郎
学位の種類	博士（ 医学 ）
学位記番号	博甲第　　9919　　号
学位授与年月	令和　3　年　3　月　25　日
学位授与の要件	学位規則第４条第１項該当
審査研究科	人間総合科学研究科
学位論文題目	Defining compartmentalized stem and progenitor populations with distinct cell division dynamics in the ocular surface epithelium (眼表面上皮における幹細胞ダイナミクス解析)
主　　査	筑波大学教授　　加藤光保　医学博士
副　　査	筑波大学教授　　福田邦明　博士（医学）
副　　査	筑波大学准教授　　西村　健　博士（医学）
副　　査	理研遺伝子材料開発室室長　三輪佳宏　博士（理学）

論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Ryutaro Ishii describes the dynamic behavior of multiple stem cell populations in the ocular surface epithelium and territorial segregation of these epithelial stem cells. The summary is as follows:

（**目的　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, 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 transiently-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. The author’s 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.

（材料と方法　**Materials and methods**）

To evaluate the distribution of LRCs in the mouse ocular surface epithelium, the author injected EdU 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 $Slc1a3^{CreER}$, $Dlx1^{CreER}$ and $K14^{CreER}$ 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.

（結果　**Results**）

EdU pulse-chase experiments 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: $Slc1a3^{CreER}$, $Dlx1^{CreER}$ and $K14^{CreER}$, the author detected distinct dynamics of epithelial SCs in the cornea and conjunctiva. $Slc1a3^{CreER}$ labeled cells in the limbal LRC region as well as peripheral cornea, whereas $K14^{CreER}$ and $Dlx1^{CreER}$ preferentially labeled the central cornea. In conjunctiva, LRC-dense fornix region was preferentially marked by $Slc1a3^{CreER}$ and the bulbar and palpebral conjunctiva were marked by $K14^{CreER}$. 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, long-term lineage tracing was performed. In the limbus, long-lived SCs were labeled with $Slc1a3^{CreER}$ 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 $Dlx1^{CreER}$ and $K14^{CreER}$, 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 $Slc1a3^{CreER+}$ 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.

（考察　**Discussion**）

Genetic tools revealed dynamic behavior of multiple SC populations during tissue homeostasis and injury repair. The author considers that 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, the author thinks that 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.

（結語　**Conclusion**）

The ocular surface epithelium is composed of distinct compartments, which are characterized by the anatomical location, marker expression, and cell division dynamics. Genetic tools are used by the author to precisely mark and examine the dynamic behavior of multiple SC/progenitor populations in the ocular surface epithelium during homeostasis and injury repair, which will contribute in the future for clinical application to treat extensive ocular injuries, such as Stevens-Johnson syndrome or severe chemical burns.

審査の結果の要旨

Abstract of assessment result

（批評　**General Comments**）

This study demonstrated the dynamic behavior of multiple stem cell populations in the ocular surface epithelium and territorial segregation of these epithelial stem cells during homeostasis and injury repair. The study has high novelty and social value. Data are clear and convincing, and discussion is reasonable.

（最終試験の結果　**Assessment**）

The final examination committee conducted a meeting as a final examination on January 5, 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

（結論　**Conclusion**）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.