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学位論文題目	Elucidating the mechanism of epidermal stem cell maintenance by cellular glycosylation and extracellular matrix protein （糖鎖と細胞外マトリクスに着目した表皮幹細胞制御メカニズムの解明）

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, the author describes the mechanism of epidermal stem cell maintenance by cellular glycosylation and extracellular matrix protein. The summary is as follows:

（目的 Purpose）

Aging in the epidermis is marked by a gradual decline in barrier function, impaired wound healing, hair loss, and an increased risk of cancer. This could be due to age-related changes in the properties of epidermal stem cells or defective interactions with their microenvironment. Currently, no biochemical tools are available to detect and evaluate the aging of epidermal stem cells. The cellular glycosylation is involved in cell-cell communications and cell-matrix adhesions in various physiological and pathological conditions. In addition, it has been reported that the mouse epidermis contains two distinct stem cell populations that divide at a different rate and reside in the spatially segregated, distinct niches. However, it remains largely unknown which factors regulate the localization and function of heterogeneous populations of stem cells in the epidermis. Extracellular matrix (ECM) is one of the critical components of the stem cell niche and provides structural and biochemical support for stem cells. Fibulin-7 (Fbln7),

a fibulin family of ECM proteins, is known to play an essential role in the regulation of kidney calcification and odontoblasts differentiation by interacting with heparin and other ECM components. To identify aging biomarker and Fibulin-7 function for epidermal stem cells, the author explored the changes of glycans in epidermal stem cells as a potential biomarker of aging and analyzed epidermal phenotypes of Fibulin-7 knockout mouse.

（対象と方法　Materials and Methods）

The author isolated epidermal stem cells from young (2-month-old) and old (22-24-month-old) C57BL/6 mice by flow cytometry, then purified membrane protein from these isolated stem cells. To detect the change of glycosylation, lectin microarray and lectin blotting analyses were performed. The author also analyzed mRNA expressions of 84 genes regulating mouse glycosylation in young and old epidermal stem cells. After identifying candidate genes, the author overexpressed the candidate genes to check functions of these genes. For analyzing epidermal phenotypes of Fibulin-7 knockout mouse, Fbln7 wild-type (Fbln7+/+), Fbln7 heterozygous (Fbln7+/-) and Fbln7-/- mice were used in this study. The K5-tTA/pTRE-H2B-GFP/K14-CreER/Rosa26tdTomato (The Jackson Laboratory, no. 007905) quadruple transgenic mice were used for the isolation of LRCs and non-LRCs epidermal stem cells. For lineage-tracing experiment, Dlx1-CreER (C57BL6) (The Jackson Laboratory, no. 014551) or Slc1a3-CreER (C57BL6) (The Jackson Laboratory, no. 012586) mice were crossed with Fbln7+/- mice to generate Fbln7+/-;Dlx1-CreER and Fbln7-/-;Slc1a3-CreER, respectively. and further with Rosa-tdTomato (The Jackson Laboratory, no. 007905) reporter mice. For RNA-sequencing of basal epidermal stem cells, Fbln7+/+ and Fbln7-/- mice at the age of 2 months and 1 year were used.

（結果　Results）

The author identified that epidermal stem cells exhibited a significant difference in glycan profiles between young and old mice. In particular, the binding of a mannose-binder rHeltuba was decreased in old epidermal stem cells, whereas that of an α2-3 Sia-binder rGal8N increased. Gene expression analysis by quantitative PCR array further showed that these glycan changes were accompanied by the up-regulation of sialyltransferase and mannosidase genes in old epidermal stem cells. Overexpression of three glycogenes (*Man1a, St3gal2, St6gal1*) resulted in significantly less ability to proliferate as compared to the control keratinocytes. In addition, the author demonstrated that Fibulin-7 was highly expressed in fast-dividing stem cells in the epidermis and functionally crucial for these cells to control their proliferation and long-term maintenance. The genetic ablation of *Fbln7* leads to a transient increase in epidermal proliferation at a younger age, followed by a gradual decrease in proliferation over time.

（考察　Discussion）

The author demonstrated the age-related global alterations in cellular glycosylation patterns in epidermal stem cells. These glycan modifications detected by lectins may serve as molecular markers for aging, and further functional studies will provide a better understanding of the process of skin aging. In addition, upon the loss of fast dividing populations, the author showed that slow-cycling stem cells marked by Dlx-CreER made ectopic clones in the fast-dividing region, indicating a possible compensation between different stem cell populations. Given that Fibulin-7 could potentially bind with glycans, IGFBP2, and basement membrane components, Fibulin-7 might act as a hub to create a specialized micro-environment for fast-dividing stem cells and define the territorial segregation of different stem cell populations in the epidermis.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The author demonstrated that high mannose-type N-glycans are globally replaced by α2-3/6 sialylated complex type N-glycans with age and proposed a concept, “glycome shift” as a new molecular factor of epidermal stem cell aging. In addition, the author demonstrated that Fibulin-7 is highly expressed in fast-dividing stem cells in the epidermis and functionally crucial for these cells to control their proliferation and long-term maintenance. These findings are basic information to understand the mechanism of epidermal stem cell maintenance by cellular glycosylation and extracellular matrix protein and are highly evaluated by the reviewers.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on June 29, 2020. 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 Human Biology.