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学位論文題目	Comprehensive analysis of physiological functions of core 1-derived *O*-glycan using *Cosmc* deficient mouse (Cosmc 欠損マウスを用いた、コア 1 型 O 型糖鎖の生理機能解析)

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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Riku Suzuki describes roles of mucin type *O*-glycan using mice with tamoxifen-inducible, β cell-specific, and adipocyte-specific excision of the C1galt1 specific molecular chaperone (Cosmc) gene, and examines their phenotypes. The content is summarized as follows:

（目的　Purpose）

Mucin type *O*-glycan is one of the posttranslational modifications present on membrane bound and secretory proteins. Although the core 1-derived *O*-glycan is the most abundant core structure of mucin-type *O*-glycan expressed in a variety of tissues, its physiological role is not fully understood. Importantly, both core 1 β1,3-galactosyltransferase (C1galt1) and C1galt1 specific molecular chaperone (Cosmc) are known to be required for the biosynthesis of the core 1-derived *O*-glycan. In this study, the author generates mice with tamoxifen-inducible CAGCre-ER^{TM}/*Cosmc*-KO (iCAG-Cos) to elucidate the physiological function of core 1-derived *O*-glycans, especially in adult stages. The author finds that pancreatic β cells where insulin is secreted to lower blood glucose levels possess core 1-derived *O*-glycan. To evaluate the roles of the core 1-derived *O*-glycan in glucose homeostasis, the author also generates

pancreatic β cell-specific *Cosmc*-KO (RIP-Cos) mice.

（対象と方法　Materials and Methods）

Ten days after tamoxifen administration to achieve conditional excision of the *Cosmc* gene, iCAG-Cos mice are dissected to collect tissues and blood samples. The change of glycan structure is confirmed by Helix pomatia agglutinin (HPA) blot analysis and HPA staining. Hematoxylin and Eosin staining of each organ is performed to observe tissue morphology. Blood samples are used to conduct blood count and biochemical tests. Insulin content test, intraperitoneal glucose tolerance test, measurement of blood insulin levels after glucose or arginine administration are performed using RIP-Cos to evaluate glucose homeostasis. Immunohistochemical staining is performed to observe glucose transporter 2 (GLUT2) using pancreatic sections.

（結果　Results）

The author finds that the iCAG-Cos mice exhibit a global loss of core 1-derived *O*-glycans and high mortality and a drastic reduction in weights of the thymus, adipose tissue, and pancreas 10 days after tamoxifen administration. The author also finds that the iCAG-Cos mice show leukocytopenia, thrombocytopenia, severe acute pancreatitis, and atrophy of white and brown adipose tissue, as well as spontaneous gastric ulcers and severe renal dysfunction, which are considered the causes underlying high mortality of the iCAG-Cos mice. On the other hand, adipose tissue-specific (Adipoq-Cos) and pancreas-specific (Pdx1-Cos) *Cosmc*-KO mice do not show the atrophy of adipocytes and acute pancreatitis, respectively. Serological analysis indicates that the iCAG-Cos mice have lower blood glucose and total blood protein levels and higher triglyceride, high-density lipoprotein, and total cholesterol levels than those of the control. The author shows that blood glucose levels are higher in RIP-Cos mice than those of wild-type mice under glucose administration, that glucose tolerance of RIP-Cos is impaired, but that insulin production of RIP-Cos is normal. Moreover, the author indicates that insulin secretion levels after glucose and arginine administration are lower than and comparable to those of RIP-Cos compared to wild-type mice, respectively, and suggests that glucose-sensitive insulin secretion is impaired in RIP-Cos mice. Since blood glucose is incorporated into pancreatic β cells via GLUT2, its expression on cell surface is important for glucose-sensitive insulin secretion. Therefore, the author performs immunohistochemical staining of GLUT2, and demonstrates that the expression of GLUT2 is significantly reduced in RIP-Cos compared to wild-type mice, which is considered as one of the causes of impaired glucose tolerance observed in RIP-Cos.

（考察　Discussion）

Little information has been available regarding the long-term effect of core 1-derived *O*-glycan deficiency that could not be analyzed due to the early death of conventional *Cosmc*-KO mice. In this study, the author develops iCAG-Cos mice, in which atrophy of adipocytes, acute pancreatitis, spontaneous gastric ulcers, and kidney impairment, the last two of which are considered as the causes of the high mortality observed in the iCAG-Cos mice. In addition, kidney dysfunction and acute pancreatitis pose difficulties in analyzing the physiological functions of other tissues, because these disorders are known to seriously affect systemic homeostasis. In these circumstances, the author uses iCAG-Cos mice and suggests that loss of core 1-derived *O*-glycans is a possible cause of acute pancreatitis and adipose tissue atrophy, while the detailed mechanism is still unknown. The author illustrates that RIP-Cos mice show impaired

glucose tolerance, glucose-responsive insulin secretion and decreased expression of GLUT2, whereas arginine-induced insulin secretion is normal. The decrease in glucose uptake into pancreatic β cells due to reduced expression of GLUT2 is considered to be the cause of the impaired glucose-responsive insulin secretion leading to defective glucose tolerance. The author indicates that core 1-derived *O*-glycan in pancreatic β cells is important for maintaining expression levels of GLUT2 and contribute to glucose homeostasis. Further study focusing on the mechanism to regulate GLUT2 expression levels is expected to elucidate the function of core 1-derived *O*-glycan. Finally, the author identifies various cells that possess core 1-derived *O*-glycans in multiple organs by HPA staining, the findings in this study will be important to deepen the understanding of the function of core 1 derived *O*-glycans in a variety of tissues of adults as future studies.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

In the final examination, Riku Suzuki presented a number of systemic, tissue or organ phenotypes of mice with inducible or tissue-specific *Cosmc* excision. Unlike previous studies using mouse models that conventionally knock out the *Cosmc* gene with the phenotype of early embryonic lethality, the author successfully generates mice in which *Cosmc* could be inductively or tissue-specifically excised, and obtains new hints to consider the *Cosmc* functions in adult stages. The author has a deep understanding of the subjects, in particular Cosmc-related regulation of glycosylation, and fulfills necessities to graduate from the Human Biology Program.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on January 20, 2022. 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.