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学位の種類	博士（　人間生物学　）
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審査組織	グローバル教育院
学位論文題目	Intestinal CREBH prevents lithogenic diet-induced hypercholesterolemia by decreasing Npc1l1 expression (小腸の CREBH は Npc1l1 の発現を抑制し、 高コレステロール食による高コレステロール血症を抑制する)

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論文の要旨
Abstract of thesis

The transcription factor cyclic AMP-responsive element-binding protein H (CREBH, encoded by Creb3l3) is highly expressed in the liver and small intestine. Hepatic CREBH contributes to glucose and triglyceride metabolism by regulating Fgf21 expression. However, the intestinal CREBH function remains unknown. Thus, the applicant aimed to elucidate the role of intestinal CREBH in energy metabolism.

To investigate the influence of intestinal CREBH on cholesterol metabolism, the applicant compared plasma, bile, fecal, and tissue cholesterol levels between wild-type (WT) mice and mice overexpressing active human CREBH mainly in the small intestine (CREBH Tg mice) under different dietary conditions. Tg mice were a fed lithogenic diet (LD) for 2 weeks. Plasma, hepatic, gallbladder, and fecal lipid levels were measured. Images of the gallbladders were analyzed by the polarized light microscopy for the presence of cholesterol monohydrate crystals and sandy stones. mRNA expressions of the genes related to lipid metabolism in the liver and intestine were examined. In addition, the applicant performed luciferase assay using the Caco-2 colon cells and EMSA to determine the CREBH target gene.

The applicant revealed that endogenous CREBH was broadly expressed in the small intestine, but the

expression was higher in jejunum than other sub-regions. CREBH-Tg mice overexpressed the active form of human CREBH in small intestine, colon, adipose tissues, and muscle. To explore the effects of CREBH on cholesterol metabolism, the applicant examined numerous metabolic indices using WT and CREBH-Tg mice fed either a normal chow diet or a LD for 2 weeks. There were no differences in body weight, liver weight, white adipose tissue (WAT) weight, and food intake between WT and CREBH-Tg mice fed either normal chow or LD. There was also no difference in the level of plasma cholesterol between genotypes when fed a normal chow diet. However, it is found that LD-fed CREBH-Tg mice show 33% lower plasma cholesterol levels compared to LD-fed WT mice. Consistent with this result, the applicant found that the livers of CREBH-Tg mice appeared healthier than those of WT mice following 2 weeks of LD, and histological analysis of liver sections showed that CREBH overexpression decreased lipid droplet accumulation compared to WT mice. It is found that CREBH-Tg mice have significantly lower intestinal cholesterol levels than WT mice as well. Conversely, CREBH-Tg mice exhibited significantly higher fecal cholesterol output than WT mice, while fecal bile acid output did not differ between genotypes. These results indicate that CREBH overexpression reduces dietary cholesterol absorption in the intestine, resulting in lower liver and plasma cholesterol levels and higher fecal excretion.

Based on these findings that CREBH overexpression prevents LD-induced hypercholesterolemia and cholelithiasis, the applicant examined the mRNA expression levels of lipid metabolism-related genes in intestine to identify a gene(s) regulated by CREBH. The applicant found that intestinal cholesterol transporters expression including *Npc1l1*, *Abca1*, *Srb1*, *Abcg5/8* decreased in LD-fed CREBH Tg mice compared to that in LD-fed WT mice. It is known that NPC1L1 is a rate-limiting transporter mediating intestinal cholesterol absorption. Thus, to reveal the molecular mechanism how CREBH prevents systemic hypercholesterolemia, the applicant examined the effects of CREBH on *Npc1l1* promoter activity in Caco-2 colon cancer cell line using a series of mouse *Npc1l1* promoter-luciferase constructs. It is found that the *Npc1l11* promoter activity is reduced by overexpression of CREBH in a dose-dependent manner. EMSA assay indicated that HA-CREBH binds to *Npc1l1* promoter region -32 bp to +5 bp. These data indicate that CREBH directly regulates *Npc1l1* expression.

Taken together, it is likely that CREBH controls the cholesterol uptake in intestine through the transcriptional regulation of *Npc1l1* gene.

審査の要旨
Abstract of assessment result

【批評 Review】

This study found that intestinal CREBH functions as a metabolic regulator to attenuate diet-induced hypercholesterolemia and cholelithiasis by decreasing expression of the transporter gene *Npc1l1*. It is known that hepatic CREBH has the potential to ameliorate hypertriglyceridelemia. This study identifies intestinal CREBH as another possible therapeutic target for the treatment of hypercholesterolemia and related metabolic diseases. Thus, it is highly expected that drugs controlling CREBH activity throughout the intestinal−hepatic system could be efficacious treatments for metabolic diseases and surely have impact in this field.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 19th Jan, 2017. 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】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.