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研究課題名（和文）腸におけるGPCRを介したアディポネクチン発現制御機構の解明と抗糖尿病効果の検討

研究課題名（英文）Elucidation of the mechanism of GPCR-mediated regulation of Adiponectin in intestinal cells to its anti-diabetic effects.

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研究成果の概要（和文）：GPCR61タンパク質は、腸細胞におけるアディポネクチンの発現を調節します。GPCR61のリガンドであるプラスマログンを摂取すると、アディポネクチンの発現が誘導され、Pls-GPCR61シグナリングによって肝臓などの組織における脂肪沈着が減少する可能性が示唆されます。GPCR61ノックアウト（KO）マウスでは、体重増加と脂肪肝が観察されました。RNAシーケンシングにより、KOマウスにおけるGPCR61の包括的なシグナルメカニズムが明らかになりました。

研究成果の学術的意義や社会的意義
This research provides a possible mechanism how we are susceptible to diabetes and other diseases during aging process when GPCR61 and plasmalogens are reduced. Therefore, this study could help in finding future therapeutics to cure various aging-related diseases including diabetes.

研究成果の概要（英文）：GPCR61 showed to regulate the expression of Adiponectin in intestinal cells and the oral ingestion of plasmalogens (Pls), which is the ligands of GPCR61 induced expression of Apiponectin, suggesting that Pls-GPCR61 signaling enhance adiponectin to reduce diabetic syndrome such as fat-deposition in the tissues including liver. WE successfully generated GPCR61 knockout (KO) mice which showed increased body weight marked with fatty liver. Next generation sequencing (RNASeq) data using the GPCR61 revealed a comprehensive mechanism of GPCR61 signaling in mice tissues including brain and immune cells. Our present research provided important clues in understanding the role of GPCR61 not only in the intestine but also in the brain tissues and immune cells.

研究分野：Molecular mechanism of diseases

キーワード：GPCR61　Plasmalogens　Adiponectin　Fatty liver　Intestinal cells　Central nervous system

１．研究開始当初の背景

Plasmalogen (Pls) levels in human blood correlate positively with high-density lipoprotein (HDL), suggesting potential anti-diabetic effects. Age-related reduction in Pls may increase diabetes risk. Reduced blood Adiponectin is linked to fatty liver, a common companion of diabetes. Pls ingestion enhances Adiponectin expression, hinting at a possible solution. GPCR61 appears to mediate Pls signaling, offering a pathway to activate Adiponectin in intestinal cells. Pls shows promise in addressing diabetes and related conditions.

２．研究の目的

The aim of this study was to investigate the mechanism by which plasmalogens (Pls), special phospholipids, reduce fatty liver. Previous research has suggested that the GPCR61 receptor may be involved in mediating the effects of Pls and that Pls could activate GPCR61 to exhibit anti-diabetic effects by increasing the expression of Adiponectin. To elucidate the mechanism by which Pls reduce fatty liver, we generated GPCR61 knockout mice and conducted in vitro studies to examine the role of Pls-GPCR61 signaling. Additionally, we employed ZDF rats, a diabetic animal model, to investigate the effects of Pls on reducing fatty liver and inducing Adiponectin.

３．研究の方法

（１）GPCR61 knockout mice were generated using the CRISPR-Cas9 method.

（２）Real-time PCR studies were conducted to analyze gene expression.

（３）Western blotting assays were performed to study protein expression.

（４）Chromatin immunoprecipitation (ChIP) assays were performed to study the recruitment of PPAR-gamma to the promoter region of target genes.

（５）Immunohistochemistry (IHC) assays were performed to examine protein expression in tissues such as the intestine, liver, and brain.

（６）Oil-red staining assays were conducted to examine the fat content of liver tissues.

（７）ZDF rats were used as a diabetic model to examine the anti-diabetic effects of orally ingested plasmalogens.

４．研究成果

（１）GPCR61 induced Adiponectin expression in intestinal cells: In vitro studies demonstrated that Pls treatment induced the expression of Adiponectin in rat intestinal cell line (IEC-6). Overexpression of GPCR61 also induced Adiponectin expression in IEC-6 cells and primary cultured mouse intestinal cells. Activation of the AMPK signaling pathway was identified as the critical upstream signaling pathway for Pls-GPCR61-mediated regulation of Adiponectin. Immunohistochemistry studies showed that oral ingestion of Pls increased GPCR61 expression in mouse intestinal cells, along with increased expression of Adiponectin.

（２）Pls treatment reduced fat in the liver of ZDF rats: The oral ingestion of Pls was found to reduce fatty liver in ZDF rats, as confirmed by Oil-red staining to detect fat content in tissues. This reduction was associated with increased expression of Adiponectin in the liver and intestine. Real-time PCR studies revealed that Pls treatment in ZDF rats induced the expression of key genes involved in fat metabolism in liver tissues. Chromatin immunoprecipitation assays showed that Pls treatment increased the recruitment of the PPAR-gamma transcription factor to target genes, suggesting that Pls-GPCR61 signaling could exhibit anti-diabetic effects associated with enhanced PPAR-gamma function. Pls treatment also enhanced the expression of beta-oxidation-related genes, suggesting a potential role of increased beta-oxidation in reducing liver fat content.

(3) Pls treatment reduced brain inflammation in ZDF rats: Brain inflammation has been associated with fatty liver and diabetes. IHC studies demonstrated that Pls treatment reduced the expression of activated microglia (Iba1-positive cells) and astrocytes (GFAP-positive cells) in the brains of ZDF rats. Analysis of NOS2 protein expression in the brain and intestinal tissues revealed that Pls treatment reduced NOS2 expression in ZDF rats. In vitro studies showed that GPCR61 overexpression reduced LPS-induced NOS2 expression in mouse microglial cells, suggesting that Pls-GPCR61 signaling could reduce brain inflammation.

(4) GPCR61 knockout mice exhibit fatty liver: GPCR61 knockout mice were generated successfully by deleting exon-2 using CRISPR-Cas9 techniques. Electroporation was utilized to introduce a mixture of guide RNA and Cas-9 into fertilized oocytes. These knockout mice displayed fatty liver accompanied by increased body weight. Additionally, these mice exhibited reproductive and memory issues. To investigate the cause of the diabetic-like syndrome and reproductive problems, high-throughput RNA-sequencing studies were conducted, resulting in the identification of several hundred differentially expressed genes compared to wild-type mice. Ongoing bioinformatics analyses are focused on understanding the defective signaling pathways in the brains of GPCR61 knockout mice. This study aims to uncover the mechanisms of GPCR61-mediated effects in the brain that regulate the diabetic-like syndrome and other issues, such as reproductive defects. The results of the RNA-seq study can also shed light on the mechanisms by which Pls-GPCR61 signaling reduces neuroinflammation.

(5) Conclusion and Importance of Our Study
Our study elucidates the mechanism of Pls-GPCR61 signaling in Adiponectin expression, which contributes to the reduction of fatty liver in the animal model. Activation of GPCR61 is associated with a decrease in inflammation in the intestine and brain. Further investigation, focusing on bioinformatics assays using the RNA-seq data, will provide detailed insights into the molecular events underlying the Pls-GPCR61 signaling pathways.
Furthermore, we observed a reduction of GPCR61 during aging in mice, suggesting that a decrease in GPCR61 in humans may be linked to diabetic-like syndrome and reproductive defects. Therefore, our study indicates a potential therapeutic alternative for treating fatty liver by employing plasmalogen treatments that activate GPCR61 in tissues.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関