氏　　名　江川 孝彦

学位(専攻分野)　博士(理学)

学 位 記 番 号　総研大甲第 2509 号

学位授与の日付　2024 年 3 月 22 日

学位授与の要件　生命科学研究科 生理科学専攻
学位規則第6条第1項該当

学 位 論 文 題 目　Identification of physiological substrates for depalmitoylating enzyme ABHD17 in neurons

論 文 審 査 委 員　主　査　久保 義弘
生理科学コース 教授
古瀬 幹夫
生理科学コース 教授
深田 正紀
生理科学コース 教授
服部 光治
名古屋市立大学 大学院薬学研究科 教授

博士論文の要旨

氏　　名：Egawa, Takahiko

論文題目：Identification of physiological substrates for depalmitoylating enzyme ABHD17 in neurons

Protein palmitoylation is the most common post-translational lipid modification. By adding the 16-carbon saturated fatty acid to a protein, palmitoylation increases hydrophobicity of the protein and regulates trafficking, localization, and function of the protein. The unique reversibility of palmitoylation is thought to confer the palmitoylation-depalmitoylation cycle on the protein and thereby dynamically regulate the protein localization and function. The research on protein palmitoylation has been recently accelerated since palmitoyl acyltransferases (PATs) were identified and many palmitoyl-proteins, approximately 10% of all proteins, were isolated by proteomic analyses. On the other hand, a limited number of palmitoyl-proteins that undergo depalmitoylation have been reported, including PSD-95, H/N-Ras small GTPases, and G-protein alpha subunits (Gα), and the physiological significance of depalmitoylation is not yet fully understood. Recently, the laboratory I belong to reported a subset of α/β-hydrolase fold domain (ABHD) enzymes, ABHD17A, 17B, and 17C, as depalmitoylating enzymes for PSD-95. The ABHD17 family proteins (ABHD17s) are palmitoylated at the N-terminus and localized at the plasma membrane depending on their palmitoylation. However, the physiological functions of ABHD17s are largely unknown.

Here, I aimed to elucidate the physiological significance of depalmitoylation through the functional characterization of mouse ABHD17s. Due to the difficulty in generating

specific ABHD17B antibodies, I took advantage of knock-in (KI) mice in which ABHD17B is tagged with enhanced green fluorescent protein (EGFP) at the C-terminus (referred to as ABHD17B-EGFP KI mice). I confirmed that ABHD17B-EGFP is functional as the membrane-bound, depalmitoylating enzyme in heterologous cells. I attempted to identify substrate proteins for ABHD17s through the comprehensive identification of ABHD17B-interacting proteins in the mouse brain. I purified ABHD17B-EGFP from KI mouse brains by immunoprecipitation using the GFP-trap system and evaluated by silver staining that ABHD17B-EGFP and its interacting proteins were specifically obtained. By label-free quantitative proteomic analyses using liquid chromatography with tandem mass spectrometry, I identified 263 proteins that specifically and reproducibly interact with ABHD17B-EGFP. In combination with the palmitoyl-protein database analysis, I found that palmitoyl-proteins are enriched in the ABHD17B-interactome as compared to the mouse brain proteome (58% of ABHD17B-interactome vs. 18% of brain proteome), suggesting that the ABHD17B-interactome includes novel substrates for ABHD17s. I also classified the function of ABHD17B-interacting proteins by gene ontology (GO) enrichment analysis and found that sets of proteins relevant to the biological processes involved in the G protein-coupled signaling pathway and regulation of cAMP-mediated signaling are significantly enriched. Based on these results, I selected 11 proteins as substrate candidates for ABHD17B, including Gprin1 which is considered to regulate G protein signaling and Pde2a3 which catalyzes the hydrolysis of cAMP and cGMP. To examine if these candidates are actually palmitoylated in the mouse brain, I performed the acyl-biotin exchange (ABE) assay, which is a commonly used method to detect protein palmitoylation. I found that 7 out of 11 selected proteins, Gprin1, Pde2a3, Rap2a, Efr3b, M6a, Prrt2, and Atlastin-1, are palmitoylated in the mouse brain. To investigate if ABHD17B depalmitoylates these substrate candidates, I optimized and employed a metabolic labeling method coupled with the click reaction between the alkyne palmitic-acid derivative, 17-octadecynoic

acid (17-ODYA), and the fluorescent azide. I found that 17-ODYA incorporated into Gprin1, Pde2a3, Rap2a, and Efr3b is greatly reduced when ABHD17B is co-overexpressed in HEK293T cells. In addition, I found that HA-tagged Gprin1, Pde2a3, Rap2a, and Efr3b are localized at the plasma membrane in COS7 cells and co-expression of ABHD17B delocalizes these proteins from the plasma membrane to the cytoplasm. Next, I investigated if endogenous substrate candidates are depalmitoylated by overexpressed ABHD17B in cultured rat hippocampal neurons. The acyl-PEGyl exchange gel shift (APEGS) assay, a quantitative detection method of protein palmitoylation, revealed that all four substrate candidates, Gprin1, Pde2a3, Rap2a, and Efr3a, a homologue of Efr3b, are highly palmitoylated in the steady-state of neurons and depalmitoylated when ABHD17B is expressed using the adeno-associated virus (AAV) system. To further examine if these candidates physiologically undergo depalmitoylation, I treated neurons with 2-bromopalmitate (2-BP), an inhibitor of palmitoylation to extract the depalmitoylation process, and performed the APEGS assay. I found that endogenous Gprin1 and Pde2a3, but not Rap2a and Efr3a, are constitutively depalmitoylated in neurons. Importantly, when neurons were treated with ABD957, a specific inhibitor of ABHD17s, I found that 2-BP-induced depalmitoylation of Gprin1 and Pde2a3 is significantly inhibited. These overall results indicate that Gprin1 and Pde2a3 are novel substrates for ABHD17s in rat hippocampal neurons.

In summary, I comprehensively identified ABHD17B-interacting proteins in the brain and found that Gprin1 and Pde2a3 are physiological substrates for ABHD17s in neurons. I demonstrated evidence that Gprin1 and Pde2a3 undergo the dynamic palmitoylation-depalmitoylation cycle in neurons and that ABHD17s are responsible for the depalmitoylation process of Gprin1 and Pde2a3 and regulate their localization. My study will contribute to understanding of physiological significance of the ABHD17-mediated depalmitoylation and palmitoylation-depalmitoylation cycle.

（別紙１ Separate Sheet 1）

Results of the doctoral thesis defense

博士論文審査結果

Name in Full
氏　　名　　江川　孝彦

Title
論文題目　　Identification of physiological substrates for depalmitoylating enzyme ABHD17 in neurons

タンパク質のパルミトイル化は、タンパク質の輸送、局在、機能の調節に役割を果たす重要な脂質修飾であり、様々な翻訳後脂質修飾の中で唯一可逆的であることが知られている。パルミトイル化の研究は、パルミトイル化酵素 zDHHC の分子同定やプロテオミクス解析等により進展し、全タンパク質の 10%ものタンパク質がパルミトイル化を受けていることが明らかになった。一方で、脱パルミトイル化については、出願者の所属研究室で脱パルミトイル化酵素 ABHD17A,B,C が同定されたものの、脱パルミトイル化を受けることが確定したタンパク質は、PSD-95 等、限定的であり、その生理的意義は未だ明らかではない。

そこで、本研究において、出願者の江川孝彦氏は、ABHD17B の新規基質の同定を目指し、ABHD17B と結合するマウス脳のタンパク質の網羅的同定を行った。ABHD17B に緑色蛍光タンパク質(EGFP)のタグをつけた遺伝子を発現するノックインマウスを共同研究により CRISPR-Cas9 法を用いて作成し、タグ抗体により免疫共沈降するタンパク質を回収し、タンデム質量分析法により 263 個のタンパク質を同定した。そして、その多くが G タンパク質系のシグナリング、cAMP を介するシグナリングに関係する分子であることを見出した。それぞれの代表例として、Gprin1、Pde2a が同定された。また、アシルビオチン交換法により、解析した 11 個のタンパク質のうち、7 個がパルミトイル化されていることが観察された。このことから ABHD17B の基質が含まれていることが示唆された。

さらに、これらの候補タンパク質のうち、Gprin1, Pde2a, Rap2, Efr3 が、ABHD17B を強制発現した HEK293T 細胞において、実際に脱パルミトイル化を受けること、脱パルミトイル化により細胞膜から細胞質に移動することを見出した。

ラット海馬の培養神経細胞においても、アデノ随伴ウイルスを用いた ABHD17B の過剰発現により上記 4 タンパク質がいずれも脱パルミトイル化されることが、acyl-PEGyl 交換ゲルシフト解析法により観察された。さらに、ABHD17s の阻害剤を用いた同解析の結果から、Gprin1 と Pde2a が、神経細胞において実際に ABHD17s により脱パルミトイル化を受ける新規基質であることが確定した。

本研究により、江川氏は、タグ付き ABHD17B を発現するノックインマウスを用いた ABHD17B の結合タンパクの網羅的解析等を行うことにより ABHD17B の新規基質候補を同定し、さらに、Gprin1 および Pde2a がラット脳の神経細胞において確かに ABHD17s により脱パルミトイル化を受けていることを明らかにした。本研究は、洗練された生化学的実験手法等を駆使した実験を徹底的に遂行することにより確実な結論を導いたもので、パルミトイル修飾サイクルの生理学的役割の理解に向けて重要な分子細胞生理学的意義を有する。以上の理由から審査委員会は本論文が学位の授与に値すると全員一致で判断した。