様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Identification and characterization of PLA2 enzyme required for sensory nervous system development

研究課題名（英文）Identification and characterization of PLA2 enzyme required for sensory nervous system development

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,300,000円

研究成果の概要（和文）：神経軸索誘導分子は、成長中の神経系内で正しい神経回路形成に必要とするシグナル伝達分子。私は以前、リゾホスファチジルグルコシドという誘導分子が脊髄の痛覚ニューロン回路構築を制御することを解明した。本研究では、神経系の幹細胞であるラジアルグリアがリゾホスファチジルグルコシドを作る分泌型ホスホリパーゼA2 V（sPLA2 V)という酵素を発現していることを発見しました。sPLA2 Vの遺伝子欠損マウスの脊髄には痛覚ニューロンが誤った位置に軸索を伸長する。したがってラジアルグリア細胞のsPLA2 V酵素は痛覚ニューロンの神経回路形成に必要であることを明らかにしました。

研究成果の学術的意義や社会的意義
I determined that a single isoform of secreted PLA2 enzyme, sPLA2 V, is required for normal embryonic development of nociceptive axon tracts in spinal cord. sPLA2/LysoPtdGlc-signaling is required for normal nociception, therefore this mechanism may play a role in disorders such as neuropathic pain.

研究成果の概要（英文）：Axon guidance cues are signaling molecules that instruct growing axons to form the correct connections within the developing nervous system. I previously have determined that lysophosphatidylglucoside is an axon guidance cue required for the development of nociceptive (pain-sensing) axon circuits in the spinal cord. In this project I have discovered that radial glia, the progenitor cells in the nervous system, possess an enzyme called secreted phospholipase A2 (sPLA2), specific isoform V, which produces lysophosphatidylglucoside (LysoPtdGlc). Spinal cords that lack the gene for sPLA2 V develop nociceptive axons in the wrong position. Therefore, radial glial sPLA2 V is required for correct nociceptive axon tract formation.

研究分野：Biology

キーワード：Lipid biology　Neuroscience　Developmental biology　Molecular biology　Cell biology

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

(1) To build a functioning brain and nervous system, neurons must extend axons and form the correct synaptic connections during embryonic development. One mechanism by which this is achieved is axon guidance, where signaling molecules guide extending axons to their correct ultimate position within the nervous system. In my previous research, I determined that the novel lysophospholipid lysophosphatidylglucoside (LysoPtdGlc) is a novel axon guidance cue. In this project I hypothesized that a phospholipase enzyme produces LysoPtdGlc that acts as a guidance cue for nociceptive axons in developing spinal cord.

(2) Secretory phospholipase A_2 (sPLA2) is a family of ten enzymes that specifically hydrolyze the ester-linked fatty acid chain at the sn-2 position of glycerol backbone in glycerophospholipids. It is the most likely candidate enzyme responsible for production of LysoPtdGlc. Many sPLA2 enzymes have been implicated in pathological conditions such as anaphylaxis, asthma and obesity. However, no role for sPLA2 enzymes has been found in nervous system during development or non-disease states.

２．研究の目的

The objective of this research project is to determine which isoform(s) of sPLA2 are required for the axon guidance of nociceptive sensory afferents in spinal cord development. To achieve this, I aim to identify specific isoforms of sPLA2 enzymes that are expressed by embryonic spinal cord radial glial cells at the appropriate developmental stage. Then, analyze the projection and layout of nociceptive axon tracts in knockout mice that lack specific sPLA2 enzymes identified in screening of spinal cord radial glia cultures to determine which are necessary for normal development.

３．研究の方法

This research is in collaboration with Prof. Makoto Murakami (Tokyo University) and Prof. Kei Yamamoto (Tokushima University). I utilized a loss-of-function approach to determine the isoform of sPLA2 required for normal development of nociceptive circuitry in spinal cord.

(1) Analyses of sPLA2 enzymes that are expressed in spinal cord at the developmental stage when axon guidance is taking place utilizing RT-PCR or where possible, by specific monoclonal antibody staining. The developmental stage when sensory afferents are entering the spinal cord and making synaptic connections with interneurons in the superficial dorsal horn is approximately around E12-E15.

(2) Analyses of knockout mice genetically lacking specific sPLA2 enzymes using immunofluorescence staining and laser scanning confocal microscopy. These knockout mice lines are already made and provided by my collaborators Prof. M. Murakami and Prof. K. Yamamoto. I imported three mouse lines to RIKEN animal facility and established breeding colonies for two. These are analyzed at the same developmental stages identified in (1).

４．研究成果

Discovery of radial glia-specific secreted phospholipase A2 (sPLA2) isoform V that modulates LysoPtdGlc/GPR55 signaling in development of nociceptive circuitry in developing spinal cord

(1) Radial glia specifically express sPLA2 V (gene name: *pla2g5*)

My earlier research found that spinal cord radial glia specifically express the membrane glycerophospholipid phosphatidylglucoside (PtdGlc) during embryonic development (Ref.1). In this current project I discovered that sPLA2 expressed by these radial glia hydrolyzes membrane PtdGlc to release the water-soluble lysolipid derivative lysophosphatidylglucoside (LysoPtdGlc) into the extracellular space. After dissecting, extracting and culturing radial glia cells to compare with whole spinal cord tissue, I utilized digital PCR to quantify mRNA expression levels of sPLA2 in embryonic spinal cord. I discovered that the isoform sPLA2 V (gene name: *pla2g5*) is specifically expressed by radial glia and not by other cells in the spinal cord. In contrast, sPLA2 X (gene name: *pla2g10*) is expressed by non-glial spinal cord cells, whilst *pla2g1b* and pla2g3 are detected at very low levels in both glial and non-glial cells. The sPLA2 with the highest level of expression in spinal cord was sPLA2 XII (*pla2g12a*) but this isoform is an atypical sPLA2 that lacks lipase activity and so was not pursued further in this project. *pla2g2d*, *pla2g2e* *pla2g2f*, *pla2g10* mRNA were not detected in radial glia at the stages examined.

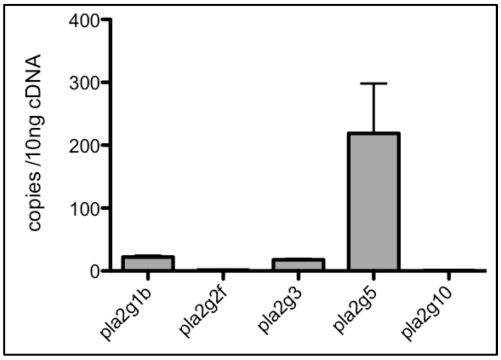

Figure 1: digital PCR quantification of sPLA2 messenger RNA in spinal cord radial glia

(2) Loss-of-function of sPLA2 *in vivo* induces axon guidance errors in nociceptive sensory circuitry

Since I had previously demonstrated that glial-derived LysoPtdGlc signaling at GPR55 is an in vivo axon guidance mechanism (Ref. 1), I hypothesized that spinal cord lacking glial sPLA2 V may have a phenotype similar to GPR55 loss-of-function. Using a knockout mouse line, I discovered that embryos with genetic deletion of sPLA2 V (*pla2g5*$^{-/-}$) enzyme have a defect in nociceptive axon (identified by TrkA expression) tract formation. Specifically, TrkA-expressing axons show an abnormal expansion into the medial spinal cord from

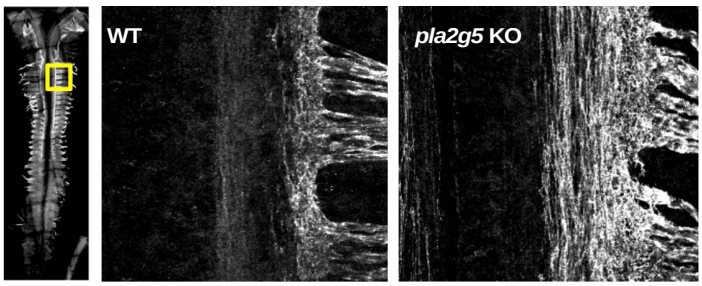

Figure 2: TrkA-expressing nociceptive axons form thickened abnormal tracts in spinal cord of sPLA2 V (*pla2g5*) knockout mice (right) compared to wildtype embryos of the same stage (center). Dorsal views of wholemount immunostain of TrkA in mouse E14 spinal cord.

which they are absent in wild-type (Fig.2) as usually proprioceptive axons predominate in this region. In conclusion, the presence of radial glial sPLA2 V activity modulates the LysoPtdGlc/GPR55 signaling mechanism that is required for spinal cord sensory axon tract development. I am currently carrying out behavioral analyses in adult mice lacking sPLA2 V to test if they possess persistent functional defects in nociception.

Literature cited:

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５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Guy Adam T.、Kano Koki、Ohyama Jyunpei、Kamiguchi Hiroyuki、Hirabayashi Yoshio、Ito Yukishige、Matsuo Ichiro、Greimel Peter	４．巻 10
２．論文標題 Preference for Glucose over Inositol Headgroup during Lysolipid Activation of G Protein-Coupled Receptor 55	５．発行年 2018年
３．雑誌名 ACS Chemical Neuroscience	６．最初と最後の頁 716～727
掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acschemneuro.8b00505	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計4件（うち招待講演　1件／うち国際学会　2件）

１．発表者名 Adam T. Guy
２．発表標題 Identification of phospholipase A2 enzymes required for LysoPtdGlc/GPR55-mediated nociceptive circuit development
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２．発表標題 Role of phospholipase A2 enzymes in LysoPtdGlc/GPR55-mediated axon guidance of nociceptive sensory afferents in developing spinal cord
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Guy AT, Inoue M, Greimel P, Yamamoto K, Hirabayashi Y, Murakami M, Kamiguchi H

２．発表標題
Role of phospholipase A2 enzymes in nociceptive sensory afferent projection in spinal cord development

３．学会等名
MRC Centre for Neurodevelopmental Disorders Symposium "The Developing Brain in Health and Disease"（国際学会）

４．発表年
2017年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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