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研究課題名（和文）The nature and roles of cellular metabolism and energy in the regulation of neuronal chain migration

研究課題名（英文）The nature and roles of cellular metabolism and energy in the regulation of neuronal chain migration

研究代表者

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研究成果の概要（和文）：この研究の目的は、鎖状に移動するニューロンのエネルギー消費と代謝源を理解することです。マウスの前小脳ニューロンをモデルとし、RNA-seq実験から、代謝経路の切り替えがニューロンの移行に影響する可能性を仮定しました。この仮説を検証するため、エストロゲン関連受容体ガンマ（Esrrg）遺伝子をノックアウトしたマウスを作製しました。その結果、多くの前小脳ニューロンが過度に移動し、仮説を支持しました。また、蛍光ATPセンサーを使用してエネルギー状態を測定しましたが、技術的な問題により最近センサーの種類を変更し、現在テスト中です。

研究成果の学術的意義や社会的意義
神経細胞の移動と適切な終止は神経回路の形成に重要です。従来の研究は、ガイダンス、接着、細胞骨格の観点から神経細胞の移動を理解することに焦点を当てていましたが、本研究では神経細胞の移動と代謝経路の関係に注目しました。この側面はほとんど研究されていません。私たちの結果は有望であり、将来の詳細な研究への道を開きます。また、細胞代謝は環境と密接に関連しているため、この研究は代謝障害が神経細胞の移動にどのように影響するかを理解するための基礎も提供します。

研究成果の概要（英文）：The objective of this research is to understand the energy expenditure and metabolic sources of chain migrating neurons, using the migrating precerebellar neurons in mouse hindbrain as a model. Based on our previous RNA-seq experiment, we hypothesized that a switch of metabolic pathways might affect neurons' transition from migratory to stationary phases. To test this hypothesis, we generated a mouse line knocked out of the estrogen related receptor gamma (Esrrg) gene. Esrrg had previously been suggested to mediate the switch from glycolysis to OXPHOS. We found that a significant number of precerebellar neurons appeared to over-migrate, failing to terminate in ventral hindbrains, which supports our hypothesis. A second line of research was to measure the energy status of migrating neurons using a fluorescence ATP sensor. However, progress has been hampered by technical difficulties causing us to change the type of ATP sensor recently.

研究分野：Developmental Neurobiology

キーワード：Metabolic pathway　Chain migration　Esrrg　termination　ATP sensor

１．研究開始当初の背景

Very little is known about the nature and roles of cellular metabolism during neural development, particularly in neuronal migration. In recent years, it has been shown that cellular metabolism and energy adapt to cellular demands at high spatial-temporal resolutions and metabolic pathways could play instructive roles in cellular processes. Our previous RNA-seq experiment comparing transcriptomes of chain migrating versus migration-terminated mouse precerebellar neurons revealed an up-regulation of oxidative phosphorylation (OXPHOS) related genes in the latter population. This finding promoted us to hypothesize that a switch of metabolic pathway might regulate the termination phase of neuronal migration.

２．研究の目的

The objective of this research is to reveal the roles and nature of cellular energy and metabolic pathways in the transition of neurons from migration to termination.

３．研究の方法

Two approaches were proposed. (1) To visualize the energy status of chain migrating neurons, a fluorescent energy sensor (ATP sensory) will be introduced into mouse precerebellar neurons. The chain migration of these neurons will be replicated in an in vitro 3D culture system with which the energy expenditure of migrating neurons in a chain will be imaged. (2) To test if switch of metabolic pathways has a role in the process of termination of precerebellar neuronal migration, genes that are upregulated during the termination and are known to regulate metabolic switch will be chosen and mouse mutants deficient in these genes will be generated for functional analysis of a role of metabolic switch in migration termination.

４．研究成果

With respect to approach (1) described above, we have successfully established an in vitro 3D culture system in which chain migration of precerebellar neurons can be successfully followed over at least 24-48 hours. We tested a FRET based ATP sensor, ATeam1.03 by introducing the DNA construct encoding this ATP sensor into the precerebellar neurons by in utero electroporation. While the sensor was expressed in the cytoplasms of the migrating preecerebellar neurons and FRET signals could be detected on fixed cells, we encountered technical difficulties in detecting reliable FRET

signals on living migrating cells in 3D-matrix. Due to this technical obstacle, we decided to switch to a newly developed single wavelength ATP sensor iATPSnFR1.0 which is being currently tested.

With respect to approach (2) described above, we first carefully examined the differentially expressed genes identified from our previous RNA-seq experiment. We found that the estrogen related receptor gamma gene (Esrrg) is up-regulated in precerebellar neurons that have undergone migration-termination. Esrrg has been shown in previous studies to control the transition from glycolysis to OXPHOS metabolic pathway during heart development (Alaynick et al., 2007, Cell Metabolism), serving as a transcription factor that directly or indirectly regulate a nuclear-encoded mitochondrial genetic network. We chose to focus on Esrrg and successfully generated an Esrrg knockout mouse line. We then analyzed the development of precerebellar neurons in this mutant mouse line. While the tangential migration of two precerebellar neuronal types, namely the lateral reticular neurons (LRN) and external cuneate neurons (ECN), appear normal, their termination into their final destinations appear disrupted. Both neuronal groups appear to fail to stop migration in the superficial destination locations. Instead, they continued migration dorsally into deeper hindbrain parenchyma (see Figure below). Furthermore, they do not form aggregated clusters after migration as in the wild type but take on a dispersed existence. Our results lend support to our hypothesis that switch of metabolic pathways may regulate the precise termination of neuronal migration.

Figure 1

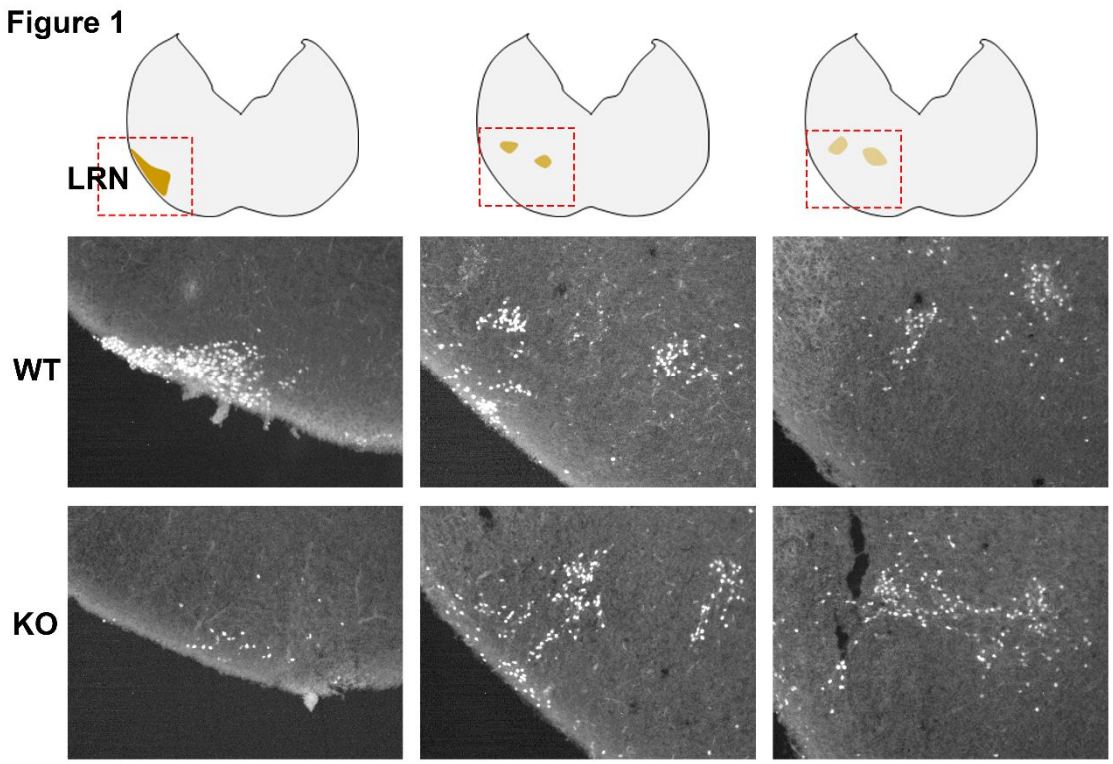

Figure 1: The Schematics on the top panel show the hindbrain sections from the caudal to rostral direction containing the LRN neurons (yellow) as in the wild type scenario. The LRN neurons originate from the dorsal tip of a hindbrain and migrate tangentially across the midline to settle in these contralateral final destinations. The mid- and bottom- panels show hindbrain sections stained with a marker for LRN, from the WT (wild type) and the KO (knock out) samples, respectively.

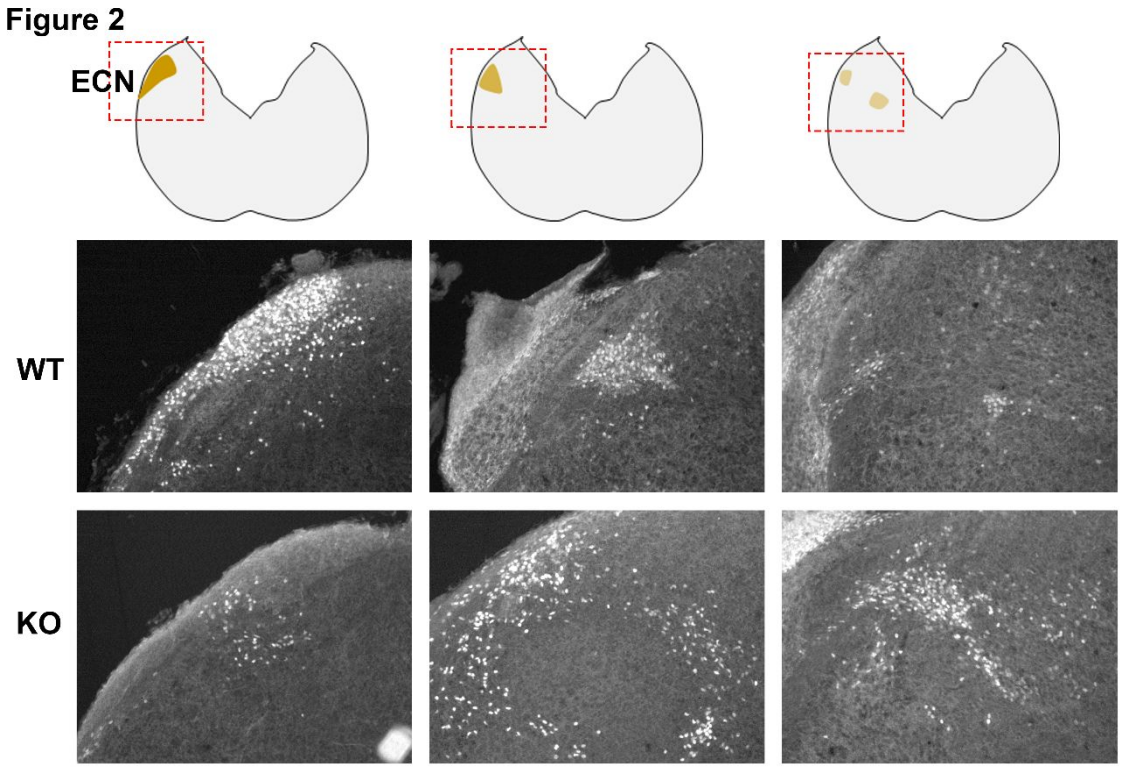

Figure 2: The Schematics on the top panel show the hindbrain sections from the caudal to rostral direction containing the ECN neurons (yellow) as in the wild type scenario. The ECN neurons originate from the dorsal tip of a hindbrain and migrate tangentially across the midline to settle in these contralateral final destinations. The mid- and bottom- panels show hindbrain sections stained with a marker for ECN, from the WT (wild type) and the KO (knock out) samples, respectively.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　2件／うち国際共著　0件／うちオープンアクセス　0件）

１．著者名 Zhu Yan、Hirata Tatsumi、Mackay Fabienne、Murakami Fujio	４．巻 10
２．論文標題 Chemokine receptor CXCR7 non-cell-autonomously controls pontine neuronal migration and nucleus formation	５．発行年 2020年
３．雑誌名 Scientific Reports	６．最初と最後の頁 11830
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41598-020-68852-z	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 －

１．著者名 Aki Masuda, Kazuhiko Nishida, Rieko Ajima, Yumiko Saga, Marah Bakhtan, Avihu Klar, Tatsumi Hirata, Yan Zhu	４．巻 10
２．論文標題 A global gene regulatory program and its region-specific regulator partition neurons into commissural and ipsilateral projection types	５．発行年 2024年
３．雑誌名 Science Advances	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/Sciadv.adk2149	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 －

〔学会発表〕　計3件（うち招待講演　1件／うち国際学会　0件）

１．発表者名 Yan Zhu
２．発表標題 A global transcriptional program that specifies axon laterality in commissural neurons
３．学会等名 International Symposium: Development and Plasticity of the Brain（招待講演）
４．発表年 2022年

１．発表者名 Yan Zhu
２．発表標題 NHLH-1 & -2, a pair of highly related bHLH transcriptional factors, synergistically control the expression of Robo3 in mouse precerebellar neurons
３．学会等名 Annual Meeting of the Japan Neuroscience Society
４．発表年 2021年

１．発表者名 Yan Zhu
２．発表標題 Uncovering a novel global transcriptional program and its interaction with local gene regulatory network for the specification of commissural neurons
３．学会等名 Annual Meeting of the Japan Neuroscience Society
４．発表年 2023年

〔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