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研究課題名（和文）Unravelling Mechanisms Underlying Termination of Neuronal Migration

研究課題名（英文）Unravelling Mechanisms Underlying Termination of Neuronal Migration

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研究成果の概要（和文）：正常に機能する脳をつくるには、個性の異なる神経細胞を正しい位置に配置することが不可欠である。この過程を担うのが、神経細胞の移動の「停止」であるが、その機構はよくわかっていない。本研究では、マウス後脳をモデルに用いて、神経細胞の移動停止に関わる機構について解析した。具体的には、RNA-seqを用いたアンバイアスな転写産物解析と、転写後調節を受ける候補分子に着目した解析の２種類のやり方で、この問題にアプローチした。その結果、1）神経細胞移動と停止に関与する転写因子群、2）神経細胞の最終位置を調節するリガンド－受容体系、3）細胞移動から停止に移行する際に分解を受ける接着分子、を見出した。

研究成果の学術的意義や社会的意義
本研究の網羅的RNA-seqで得られた情報は、本研究の深化のみならず、将来的に様々な発見につながる波及効果をもたらすと期待される。神経細胞の位置異常は、神経発達障害における主要な原因である。実際、本研究で解析したいくつかの分子については、神経発達障害との関連が示唆されている。したがって、本研究はこれらの病気や病態の理解にも貢献すると考えられる。さらに、神経細胞移動の方向づけや、目的地で移動を停止する機構を理解することは、将来的に神経発達障害や神経障害の治療法を考える上でも有益な知見となるはずである。

研究成果の概要（英文）：One of the challenges to generate a functional brain is to correctly position different types of neurons into their proper final destinations. This process takes place at the termination phase of neuronal migration, but its underlying mechanisms are still poorly understood. This research aims to uncover these mechanisms using the mouse hindbrain as a model system. We have taken two different approaches, an unbiased transcriptome profiling approach using the recently developed genomic technology RNA-seq, and a candidate gene approach focusing on molecules that are regulated at post-transcriptional level. From these approaches, we have successfully identified a pair of transcription factors that are involved in migration and termination of migration, a receptor/ligand system that fine-tune the final location of neurons, and a post-translational regulation of a cell adhesion molecule by proteolysis during transition from migration to termination.

研究分野：神経発生

キーワード：neuronal migration　termination　RNA-seq　bHLH　contactin-2　chain migration　protein processing　Robo3

１．研究開始当初の背景 （Background at the start of this research）

To build a structure that is architecturally as sophisticated as a mammalian brain is a daunting task coordinated by multiple strategies. Tangential migration of neurons over long distances is a strategy deployed by mammals to construct complex neural network efficiently. It enables neurons to be positioned optimally for wiring, as well as for intermingling with other neurons from distant origins. Clearly, the regulated termination of these migratory events is as important as their pathfinding, for mistakes in termination could lead to mis-positioning of neurons which would undermine the subsequent neural network formation. However, how neuronal migration is properly terminated and neurons correctly positioned in their final destinations is an issue that remains poorly understood, hence the focus of this research.

In this research, I propose to use the mossy fiber-projecting precerebellar neuronal system (PCN) in the mouse hindbrain to interrogate mechanisms underlying termination of tangential migration. PCN, born from the dorsal edge of the hindbrain, diverge into an anterior and a posterior tangential migratory pathway, namely, AES and PES, respectively. The two pathways display confined, yet different termination sites. Most AES terminate migration before crossing the midline to form, ipsilaterally, a medial Pontine Nucleus (PN), while PES cross the midline to form, contralaterally, an intermediate Lateral Reticular Nucleus (LRN) and a dorsal External Cuneate Nucleus (ECN). The stereotypic and confined termination sites make PCN an excellent model to investigate the cellular logic and environmental cues that underlie the termination of migration.

２．研究の目的（Objectives of this research）

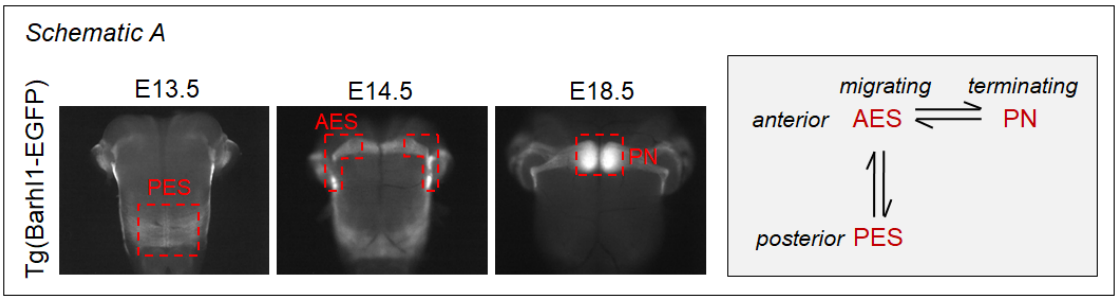
(1) Using transcriptome profiling, we aim to extract molecular differences at genome level between during-migration and migration-terminated, and between AES and PES, subpopulations of PCN. From this information, we hope to identify key molecular players and the mechanisms that they carry out.

(2) By using a candidate gene approach, we aim to identify molecules that are regulated post-transcriptionally at the transition from migration to termination and investigate their function and regulation.

３．研究の方法（Research plan and methodology）

RNA-Seq on neuronal subsets enriched by fluorescence cell sorting is employed to profile transcriptomes of three populations (see schematic A). The transcriptomes are subjected to bioinformatic analyses to extract functional molecular pathways and transcriptional networks. From these analyses, potential key players that may regulate

different aspects of migration termination are chosen for gain- and loss-of function



analyses. Functional analyses are performed by in utero electroporation mediated gene transfection, siRNA gene silencing, and generation of knockout mice by CRISPR/Cas9.

4．研究成果（Results of this research）

(1) RNA-Seq transcriptome profiling revealed molecular features associated with migration and migration-termination processes

We performed RNA-Seq on the three subpopulations of PCN (see Schematic A), and carried out a range of bioinformatic analyses on the transcriptomes and differentially expressed genes. From Gene Ontology, Reactome pathway, and Gene set enrichment analyses, we found distinct molecular features that are associated with neurons undergoing migration, neurons that are terminating migration, and neurons having different termination sites (Fig. 1 shows an example of GO analysis on AES versus PN). From these bioinformatic analyses, we selected a short list of candidate genes. The preliminary functions of these candidates in the development of PCN neurons were tested by gain-of-function experiments, the results of which serve the bases for future in-depth analyses.

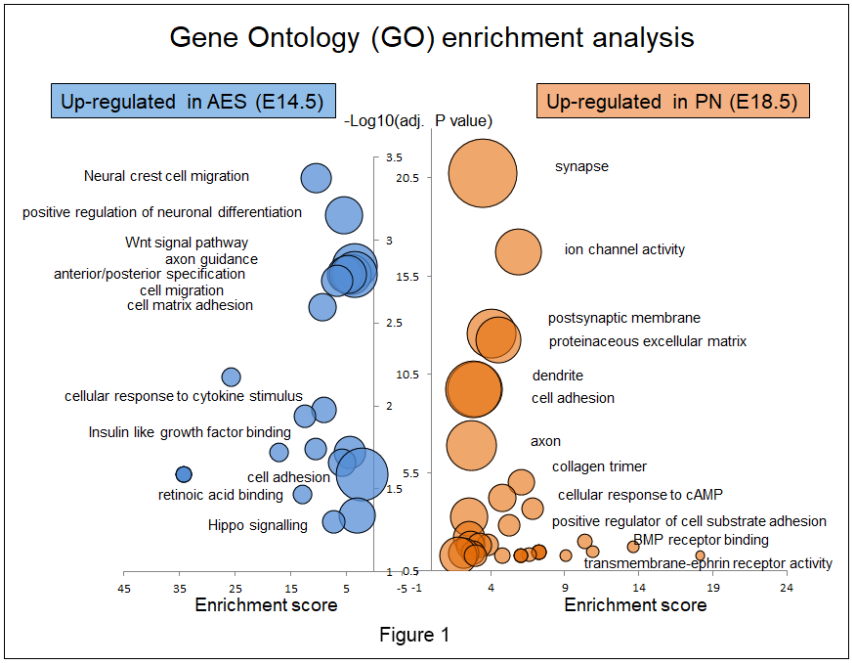


Figure 1

(2) NHLH1/2 are key transcription factors that synergistically guide commissural axons across midline via transactivation of Robo3

From the candidate list extracted from RNA-seq, we chose to focus on a pair of highly

related bHLH family transcription factors, namely NHLH1 and NHLH2. Expression of NHLH1/2 is highly enriched in migrating neurons at E14.5 and become drastically downregulated in terminated neurons. In silico search of NLHL1/2 DNA recognition site across the differentially expressed genes uncovered Robo3 as the top potential downstream target of NHLH1/2. We then showed by in utero electroporation of NHLH1/2 genes into developing neuroepithlium that NHLH1/2 are capable of inducing Robo3 expression in the midbrain, the cerebellum, the hindbrain and the spinal cord. By using a range of variants of NHLH1/2, and their VP16 or EnR fusions, we further showed that NHLH1/2 act as transcriptional activators of Robo3 and this function requires the bHLH domain.

To investigate the in vivo function of NHLH1/2, we then generated NHLH1 and NHLH2 deletion mice using CRISPR/CAS9 technology. Analysis of the central nervous system of the NHLH1/2 double deletion mice revealed a stunning failure of the commissural system across the neuroaxis from the spinal cord to the midbrain. Almost all commissural neurons failed to project their axons across the ventral midline. The hindbrain PN neurons, the model system we based this research on, instead of terminating their migration next to the midline, stopped migration before entering their presumptive nuclear region. Such detrimental phenotypes are absent in NHLH1 and NHLH2 single deletion mice, suggesting that NHLH1 &2 act synergistically. The failure of commissural system in NHLH1/2 deletion mice is highly reminiscent of the Robo3 mutant phenotype. Indeed, we found that both Robo3 protein and mRNA levels are drastically downregulated to close to background level in the NHLH1/2 double deletion mice.

In summary, we have identified NHLH1 and NHLH2 as the chief upstream transcriptional factors that specify commissural neuronal fate by transactivating the expression of Robo3 guidance receptor.

(3) Cell adhesion molecule Contactin-2 (CNTN2) regulates chain formation during tangential migration and is subjected to post-translational processing by β-secretase Bace1

Based on our previous expression studies, the literature and public databases, we have identified a number of candidate molecules whose down-regulation appears to coincide with the transition from tangential migration to termination. Among these candidates, CNTN2, which is highly expressed in the migrating AES neurons, is particularly interesting to us because its down-regulation appears to be regulated at post-translational level. We first analyzed the function of CNTN2 for the normal migration of AES neurons. Phenotype analysis of CNTN2 knockout mice showed that depletion of CNTN2 results in a significant number of neurons leaving the migratory stream prematurely without reaching their final destinations. In addition, migration appears to be slower. By using in vitro culture

systems, we found that while wild type neurons migrate in tightly bundled chains, CNTN2 depleted neurons are much less capable of forming chains. This defect may account for both the premature departure from migratory stream, as well as slower migrating speed (Fig. 2).

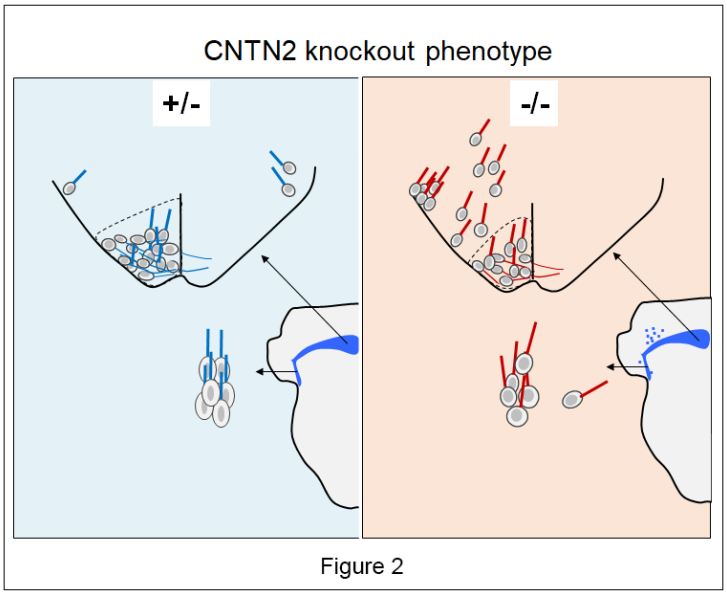


Figure 2

During termination of migration, neurons disseminate from tangential migratory chains and switch to radial migration before cessation of their motility. This dynamic cellular behavior suggests the necessity to dissemble migratory chains by down-regulating factors that facilitate chain formation. Referencing to our RNA-seq data as described above, we found that Bace1, an β-secretase, is expressed in the migrating neurons and showed an increased level during the termination of migration. We found that Bace1, indeed, can process CNTN2 when force-expressed, both in vitro and in vivo. Encouraged by this finding, we generated Bace1 knockout mice by CRISPR/CAS9 and demonstrated that indeed CNTN2 level is increased in Bace1 KO mice. We also found that the formation of Pontine nucleus is affected in Bace1 mutant. In summary, this line of research has uncovered a regulatory mechanism involving post-translational protein processing in the control of the termination of neuronal migration.

(4) Semaphorin6A/PlxnA2/PlxnA4 in the final positioning of hindbrain precerebellar neurons

The bioinformatic analyses on RNA-seq data revealed that a Semaphorin/Plexin signaling pathway is significantly upregulated in the terminating population of PCN. We found that PlxnA2 and PlxnA4 are upregulated in PCN undergoing termination, and these cells also express the PlxnA2/A4 ligand Sema6A. We therefore analyzed the PN, LRN and ECN formation in Sema6A mutant and PlxnA2/A4 double mutant mice. Interestingly, all the three mossy-fibre projecting precerebellar nuclei showed a common defect in both mutants. The ability of PCN to move into the deeper laminae of the nuclei is compromised, resulting in much more superficially aggregated nuclei. This result suggests that the Sema6A-PlxnA2/A4 signaling between the PCN neurons are crucial for the correct neuronal positioning during the termination phase of neuronal migration.

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　2件／うちオープンアクセス　0件）

１．著者名 Yumiko Hatanaka, Yan Zhu, Makio Torigoe, Yoshiaki Kita, Fujio Murakami	４．巻 92
２．論文標題 From Migration to settlement: the pathways, migration modes and dynamics of neurons in the developing brain	５．発行年 2016年
３．雑誌名 Proceedings of the Japan Academy Series B	６．最初と最後の頁 1-19
掲載論文のDOI（デジタルオブジェクト識別子） 10.2183/pjab.92.1	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

１．著者名 Yan Zhu, Tatsumi Hirata, Fabienne Mackay, Fujio Murakami	４．巻 10 (1)
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 該当する

〔学会発表〕　計5件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Masuda A, Nakaoka H, Toyoda A, Hirata T, Zhu Y
２．発表標題 Mechanisms underlying termination of tangential neuronal migration investigated by a RNA-seq based approach
３．学会等名 Biennial Meeting of the International Society for Developmental Neuroscience, Nara（国際学会）
４．発表年 2018年

１．発表者名 Masuda A, Nakaoka H, Toyoda A, Hirata T, Zhu Y
２．発表標題 Mechanisms underlying termination of tangential neuronal migration investigated by a RNA-seq based approach
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４．発表年 2018年

１．発表者名
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３．学会等名
International Symposium on Neural Development, Regeneration and Diseases

４．発表年
2020年

１．発表者名
Zhu Y, Masuda A, Hirata T

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 Japanese Society of Neuroscience, Kobe

４．発表年
2021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