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研究課題名（和文）Pathologic hematopoietic stem cell determines co-morbid symptoms in autism

研究課題名（英文）Pathologic hematopoietic stem cell determines co-morbid symptoms in autism

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研究成果の概要（和文）：自閉症の病因における免疫調節異常のメカニズムを明らかにするために発表されました。特定の細胞型の胎児期まで遡って追跡することにより、HDAC1の変化が自閉症モデル系統の卵黄嚢およびAGMにおける最終的な造血に影響を及ぼし、それによってミクログリアと造血幹の発達に影響を与えることを発見した。その後脳炎症や免疫細胞プロファイルの偏りを引き起こします。活化ERVが発生中の転写プロファイルも操作することを示す別の論文を発表しました。ERV活化とウイルス感染の間の類似性は、MIA誘発自閉症モデルなどの環境危険因子の自閉症モデルにおける病因を再度反映します。ERVはゲノム内での CNV 形成も促進します。

研究成果の学術的意義や社会的意義
The mechanism of immune dysregulation discovered by study can provide the basis to develop biomarker to access the change in peripheral immune system for early autism diagnosis and intervention. This will be important for the increased MIA cases owing to COVID infection during the 2-year pandemic.

研究成果の概要（英文）：The research results were published to reveal the mechanism of immune dysregulation on autism etiology. By tracking the origin of immune dysregulation back to embryonic stage in specific cell types, we found an altered HDAC1 activity affects the definitive hematopoiesis in yolk sac and AGM in an autism model strain (BTBR), which therefore affects the development of microglia and hematopoietic stem cells and subsequently leads to brain inflammation and skewed immune cell profiles. We published another paper to show active ERV also manipulate the transcriptional profiles of BTBR during development. The analogy between ERV reactivation and viral infection again echoes the etiology of in the autism models of environmental risk factor, such as MIA- and VPA- induced models of autism. Active ERV also accelerate CNV formation in the genome. This study unravels the idiopathic mechanism of autism but also provde new insights to how the ancient viral infection affects autism susceptibility.

研究分野：neurodevelopmental disorders

キーワード：autism　epigenetics　immune dysregulation　gut dysbiosis　sc-RNA seq　endogenous retrovirus　genome susceptibility　copy number variation

１．研究開始当初の背景

Two key themes that have emerged across the last two decades of autism research: the established role of immune dysregulation on autism etiology and the prevalence of gut dysbiosis in autistic patients. Transcriptomic studies by using postmortem brains have provided fundamental evidence for the involvement of immune system in autism spectrum disorder (ASD). However, a lack of genetic component makes the molecular mechanism behind these immune changes remaining elusive, therefore, stagnating the development of precision therapy for ASD. In parallel, the emergence of gut-microbiota-brain axis starts another boom to pursue the role of dysbiosis in the pathogenesis of autism. However, the findings in autistic patients are often heterogenous and contradictory between studies. Knowing the causality of host immunity to gut microbiota, solving the etiology of immune dysregulation in autism will be the first step to open the correct door for targeting microbiome as a diagnosis or therapeutic tool.

Meanwhile, at the first place of autistic mouse model screening, we unexpectedly found the BTBR mice in Japan, BTBR TF/ArtRbrc (hereafter referred to BTBR/R), have an intact corpus callosum. However, for the *BTBR* $T^{+}Itpr3^{tf}$/J provided by The Jackson Laboratory (hereafter referred to as BTBR/J), which is the most frequently used BTBR strain for autism research, corpus callosum agenesis (AgCC) is one of its prominent phenotype. In fact, the two BTBR strains are of the same origins, developed by L. C. Dunn. The original stock was continuously maintained by Karen Artzt, who later sent it to the McArdle Laboratory at the University of Wisconsin in 1982 and RIKEN BioResource Research Center in 1987. Thereafter, RIKEN BRC maintains this BTBR/R strain by crossing T^{+} heterozygotes. In 1994, Alexandra Shedlovsky and Bill Dove at the McArdle Laboratory sent their BTBR stock to The Jackson Laboratory, but the T locus dropped before the deposition. This strain then becomes the current BTBR/J mice. The striking difference in AgCC phenotype urged us to explore that to what

extend the two BTBR strains are different.

２．研究の目的

Considering the critical developmental windows for immune insult of autism, we suggest that tracking the origin of immune dysregulation back to embryonic stage in specific cell types should provide a rational direction to explore the underlying mechanism. Meanwhile, we aimed to verify whether the altered immunity in BTBR mice can determine specific microbiota profiles to extend the knowledge of immune dysregulation with an eye towards clinical applications.

There are substantial variations, including brain anatomy, behaviors, and immune phenotype, have accumulated between the two strains after ~30-year separation, a relatively short time in terms of strain evolution. The accelerated strain segregation suggests an unknown mechanism leading to genome instability between BTBR/R and BTBR/J. To identify the motivating force behind this, we compared the CNV composition between the two BTBR strains, which is the most “efficient” genetic variation to affect gene expression.

３．研究の方法

Autism models of environmental risk factor, such as maternal immune activation (MIA), are associated with immune dysregulation but lacks for microglia phenotype. After a screening, we chose an idiopathic autism mouse model (BTBR strain) with face validity of systemic immune dysregulation from brain to the comorbid dysbiosis phenotype. By single-cell RNA sequencing (sc-RNA seq), we trace the developmental origins of immune dysregulation in a mouse model of idiopathic autism. For the microbiota study, two experiments are desinged as the following. First, to demonstrate the genetically pre-determined microbiome in BTBR mice, B6 and BTBR embryos were co-transplanted into a recipient mouse of a third strain, which provided an identical initial microbiota to the pups of both strains during delivery. Second, to further demonstrate the role of host immunity in microbiota composition, we analyzed the microbiome in B6 mice receiving BTBR bone marrow transplantation (BMT) ($B6^{BTBR}$).

On the other hand, the striking difference in AgCC phenotype urged us to explore that to what extend the two BTBR strains are different. We found substantial variations at multiple

levels, including brain anatomy, behaviors, and immune phenotype, have been accumulated between the two strains after a separation of ~30-year. Next, we compared the CNV composition between the two BTBR strains, which is the most “efficient” genetic variation to affect gene expression.

４．研究成果

It is found that both in aorta-gonad-mesonephros (AGM) and yolk sac (YS) progenitors, the dysregulation of HDAC1-mediated epigenetic machinery alters definitive hematopoiesis during embryogenesis and downregulates the expression of the AP-1 complex for microglia development. Subsequently, these changes result in the dysregulation of the immune system, leading to gut dysbiosis and hyperactive microglia in the brain. This explains why the dysregulation can occur in a systemic way. Immune dysregulation originating from YS and AGM can be restored by HDAC inhibitor at specific time window of embryonic development. We further confirm that dysregulated immune profiles are associated with specific microbiota composition. The autistic immune profiles create a gut environment, which determines the pattern of gut dysbiosis in the autistic mice.Therefore, gut microbiome may serve as a biomarker to identify autism of immune-dysregulated subtypes.

For the story of two BTBR strains, BTBR/R has more prominent phenotypes related to the core symptoms of autism but moderate changes in ultrasonic vocalization/normal hippocampus-dependent memory. Most importantly, we identified the genetic factor, *Draxin* mutation, which determines the AgCC phenotype and hippocampus shrinkage in BTBR/J, possible leading to the phenotype not directly related to autism. The insult of *Draxin* mutation likely compromise the validity of BTBR/J as an autism mode.

Intriguingly, by analyzing the repeat sequences in the identified CNV, we found the potential involvement of endogenous retrovirus (ERV) in speeding up CNV formation in both BTBR strains. Furthermore, by single-cell RNA sequencing (sc-RNA seq), the trace evidence of ERV activation during embryonic development was identified. These results suggest ongoing events of viral evasion centering on the host integrated stress response (ISR), which leads to a global alteration in the transcriptome of BTBR mice. These results unravel the idiopathic etiology of

the BTBR strain by suggesting it as a superimposed model of autism genetic susceptibility and endogenous virus infection. The ancient viral infection and reactivation affect host genome instability in the long term and have a continuing effect on embryonic development. With the new advance in this old model, our study provides insights into how ASD susceptibility evolves in the genome and suggests BTBR/R as a precise model to investigate the core etiology of autism.

The research results of this grant were published to reveal the mechanism of immune dysregulation on autism etiology. By tracking the origin of immune dysregulation back to embryonic stage in specific cell types, we found an altered HDAC1 activity affects the definitive hematopoiesis in yolk sac and AGM in an autism model strain (BTBR), which therefore affects the development of microglia and hematopoietic stem cells and subsequently leads to brain inflammation and skewed immune cell profiles. We published another paper to show active ERV also manipulate the transcriptional profiles of BTBR during development. Active ERV also accelerate CNV formation in the genome, therefore, providning provde new insights to how the ancient viral infection affects autism susceptibility

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　1件／うちオープンアクセス　3件）

１．著者名 Chia-Wen Lin, Jacob Ellegood, Kota Tamada, Ikuo Miura, Mikiko Konda, Kozue Takeshita, Koji Atarashi, Jason P Lerch, Shigeharu Wakana, Thomas J McHugh, Toru Takumi	４．巻 -
２．論文標題 An old model with new insights: endogenous retroviruses drive the evolvement toward ASD susceptibility and hijack transcription machinery during development	５．発行年 2023年
３．雑誌名 Mol Psychiatry	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） 10.1038/s41380-023-01999-z	査読の有無 有
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１．著者名 Chia-Wen Lin, Dian E Septyaningtrias, Hsu-Wen Chao, Mikiko Konda, Koji Atarashi , Kozue Takeshita , Kota Tamada, Jun Nomura, Yohei Sasagawa, Kaori Tanaka, Itoshi Nikaido, Kenya Honda, Thomas J McHugh, Toru Takumi	４．巻 27
２．論文標題 A common epigenetic mechanism across different cellular origins underlies systemic immune dysregulation in an idiopathic autism mouse model	５．発行年 2022年
３．雑誌名 Mol Psychiatry	６．最初と最後の頁 3343-3354
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41380-022-01566-y	査読の有無 有
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１．著者名 Dian Eurike Septyaningtrias, Chia-Wen Lin, Rika Ouchida, Nobuhiro Nakai, Wataru Suda, Masahira Hattori, Hidetoshi Morita, Kenya Honda, Kota Tamada, Toru Takumi	４．巻 161
２．論文標題 Altered microbiota composition reflects enhanced communication in 15q11-13 CNV mice	５．発行年 2020年
３．雑誌名 Neuroscience research	６．最初と最後の頁 59-67
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〔学会発表〕　計1件（うち招待講演　1件／うち国際学会　0件）

１．発表者名 Chiawen Lin
２．発表標題 A prenatal epigenetic fluctuation underlies systemic immune dysregulation in autism
３．学会等名 The Physiological Society of Japan（招待講演）
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