News Release

Title

Spatiotemporal regulation of immune cell distribution in fetal cerebral wall and its significance

–Microglia assist neuronal proper differentiation by staying away from the cortical plate–

Summary

In this study, Assistant Professor Yuki Hattori and Professor Takaki Miyata in Graduate School of Medicine, Nagoya University (dean: Kenji Kadomatsu, M.D., Ph.D.), demonstrated how microglia, the resident immune cells in the central nervous system, contribute to brain development in the embryonic stage.

While the function of microglia in the adult brain has been extensively studied, their roles in the fetal brain still remain unclear. In the embryonic brain, microglia show interesting behaviors in their distribution, i.e., they are transiently absent from the specific region called the cortical plate (CP), where the neural lineage cells that have differentiated into a mature state (neurons) accumulate, for only a brief period during the mid-embryonic stage. However, the molecular mechanism underlying microglial temporal disappearance and the physiological significance remain to be elucidated. The research group directly monitored microglial real-time movement by three-dimensional slice culture system of brain tissues and *in vivo* observation using two-photon microscopy, and demonstrated the molecular mechanism of microglial migration that leads to their disappearance from the mid-embryonic CP.

On the other hand, the research group investigated the significance of microglial temporary absence from the mid-embryonic CP based on the hypothesis that microglia need to transiently exit to avoid disturbing neuronal functional differentiation that occurs in the CP. Through the various experiments to artificially expose neurons in the CP to excessive microglia, the research group found that microglia disturbed the expression pattern of molecules which are essential for neuronal proper differentiation and maturation. These results suggest that microglia smartly change their distribution to fine-tune the expression of molecules needed for neuronal proper differentiation, thus securing the establishment of functional cortical circuit in the postnatal and adult brain.

This achievement is expected to lead to a breakthrough in the development of the extensive research of the influences of maternal inflammation (such as infectious and autoimmune diseases) on fetal brain development, and will contribute to the establishment of the novel preventive and therapeutic platform against fetal brain dysplasia in the future.

This study was performed with the cooperation of Professor Hiroaki Wake in Graduate School of Medicine, Nagoya University, Professor Takashi Nagasawa in Graduate School of Medicine, Osaka University, Associate Professor Shigenori Nonaka in National Institute for

Basic Biology, and Dr. Yoji Tsugawa in National Center for Geriatrics and Gerontology.

This work was published in *Nature Communications* on 2 April, 2020.

This study was supported by JSPS KAKENHI Grant numbers JP16H02457 [T. Miyata], JP16K15169 [T. Miyata], JP16J06207 [Y. Hattori] and JP18K15003 [Y. Hattori], and by a grant from the Uehara Memorial Foundation (Grant number 201910147) [Y. Hattori].

Key Points

- **The research group addressed the question why microglia transiently disappear from the cortical plate in the mid-embryonic stage and demonstrated the underlying molecular mechanism and its significance for the first time.**
- **This achievement is expected to lead to a breakthrough in the development of the extensive research of the influences of maternal inflammation on fetal brain development, and will contribute to the establishment of the novel preventive and therapeutic platform against fetal brain dysplasia in the future.**

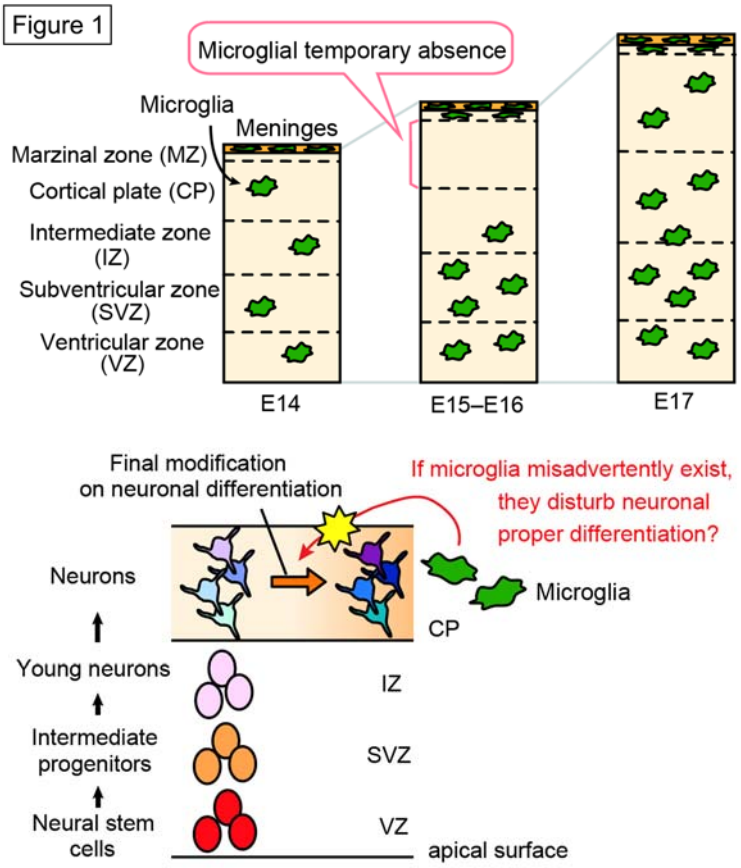
1. Research Background

Our brains are composed of not only neural lineage cells but also the immune cells called microglia. Microglia collaboratively work for brain proper function. There is mounting evidence of microglial function in the adult brain. For example, these cells contribute to the maintenance of homeostasis by removing dying neurons or cellular debris and monitor neuronal circuits for successful synaptic connections. In pathological contexts, such as neuropsychiatric disorders, neurodegeneration, and infectious diseases, microglia play critical roles as immediate responders with the potential to promote both central nervous system damage and repair. Despite recent progress in elucidating the function of microglia in the adult brain, the roles of microglia in the embryonic stage still remain unclear.

Embryonic neocortical development proceeds through the production and migration of neural lineage cells, and their positioning is well organized based on their differentiation status. On the other hand, microglia exist from the embryonic stage, despite being only a minor population of the cells that constitute the cerebral wall. Microglia exhibit interesting behavior in their distribution, i.e., they initially distribute in a homogenous pattern in the mouse cerebral wall until embryonic day (E) 14, but these cells temporarily disappear from the CP, where neurons that have migrated from the inner region accumulate, from E15 to E16 and show preferences for colonizing the ventricular zone (VZ), subventricular zone (SVZ) and intermediate zone (IZ), where undifferentiated neural progenitor cells exist (Figure 1). Of note, microglia reenter the CP at E17. However, the mechanism of microglial temporary absence from CP and its physiological significance have not been well understood.

To elucidate the molecular mechanism of microglial mysterious distribution in the cerebral wall during the embryonic period, the research group directly monitored microglial behavior by three-dimensional slice culture system of brain tissues and *in vivo* observation using two-photon microscopy, and demonstrated the molecular mechanism of microglial migration and disappearance from the mid-embryonic CP. Furthermore, the research group addressed the significance of microglial temporary absence from the cortical plate by investigating the hypothesis that microglia transiently disappear from the mid-embryonic CP to avoid disturbing neuronal functional differentiation through various experiments.

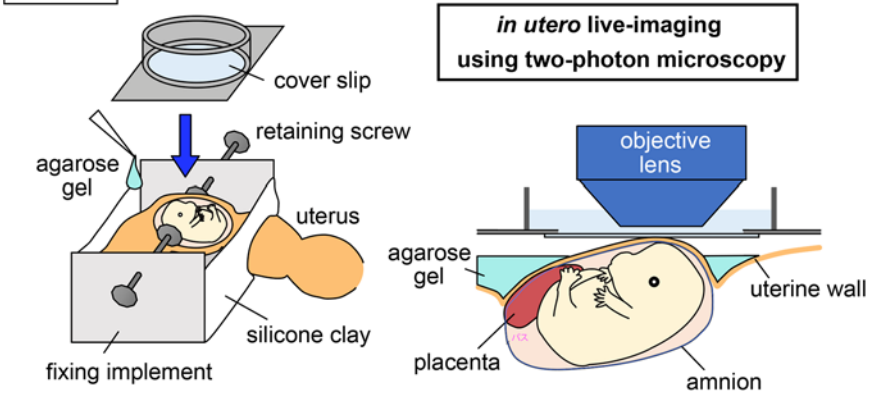
Figure 1



2. Research Results

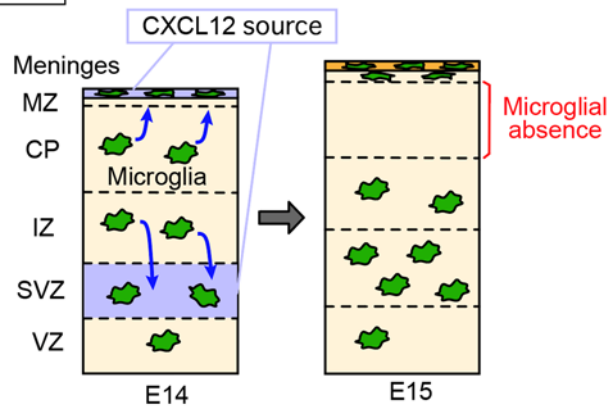
First, to monitor three-dimensionally microglial movement and their disappearance from the CP in E15–E16, the research group performed live-imaging of microglia in the cerebral wall using CX3CR1-GFP transgenic mice, in which microglia are labeled with GFP, by slice-cultured based system and *in vivo* observation using two-photon microscopy (Figure 2). Live-imaging demonstrated that microglia move throughout the cerebral wall most extensively at E14. Notably, these cells migrate in a bidirectional pattern in dependent on their original positioning, i.e., those initially positioned in the CP moved toward the meninges (outward) and accumulated in the marginal zone, whereas those existed in the IZ migrated toward the SVZ (inward). These results from live observation suggested that microglial bidirectional migration that occurs at E14 may result in their absence from the CP at E15.

Figure 2



To address the molecular mechanism underlying the bidirectional attraction of microglia in the developing cortex, the research group focused on CXCL12 and its receptor CXCR4, because CXCL12 is specifically produced in the meninges and SVZ whereas CXCR4 is expressed on microglia. Importantly, live observation of microglia using CXCR4 knockout (*Cxcr4*$^{-/-}$) mice, genetically modified mice lacking CXCR4, demonstrated that microglial bidirectional migration was strikingly inhibited and there were more stationary microglia. Further investigation of the microglial distribution pattern at E15 showed that the relative proportion of microglia in the CP was significantly greater in *Cxcr4*$^{-/-}$ mice. Together, these results indicate that the CXCL12-CXCR4 system plays a pivotal role in the microglial bidirectional migration and their proper positioning in the mid-embryonic cerebral wall. (Figure 3).

Figure 3

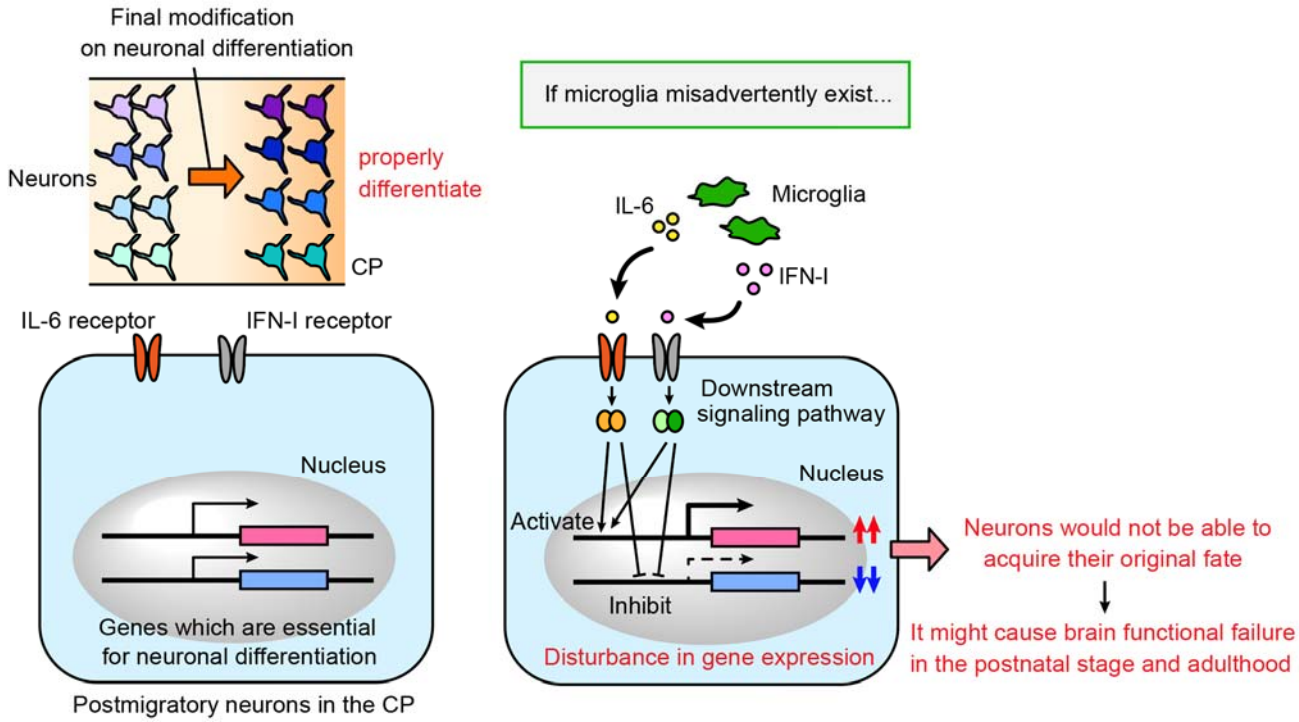


Next, the research group addressed the significance of microglial temporary exit from the CP. To test the hypothesis that the developing cortex expels microglia that would otherwise disturb neuronal differentiation which occurs in a limited developmental time window, they sought to expose CP-composing neurons to excessive microglia through various approaches, i.e., *in vivo* experiments to artificially deliver microglia into the CP using neuron-specific CXCL12 overexpression system and microglial attraction or transplantation into the CP in the cultured cortical slices. They found that microglial excessive exposure to neurons in the CP disturbed the expression pattern of molecules which are essential for neuronal proper differentiation and maturation. Although birthdate-dependent neuronal specification occurs in the VZ/SVZ, it is known that postmigratory neurons still undergo subsequent differentiation programs that confer their final fate in the CP. In light of this, these results strongly suggest that microglia may influence postmigratory cortical neurons in the expression of neuronal subtype-associated transcription factors, if these cells are inadvertently positioned in the CP.

The research group further explored the molecules derived/released from microglia which were involved in the abnormal expression pattern of neuronal subtype-associated genes in CP-composing neurons. RNA-sequencing analysis of *in vitro*-prepared CP-composing neurons cultured with or without isolated microglia demonstrated that type I interferon (IFN-I) and interleukin-6 (IL-6), which are belong to cytokine family, are the most likely candidates for modulators of neuronal subtype-associated gene expression. When their functions were perturbed using neutralizing antibodies against these cytokines, the abnormal alterations in the expression of neuronal subtype marker expression caused by excessive microglia was

significantly abolished. Taken together, these results indicate that the disturbed balance of neuronal subtype-associated gene expression in CP-composing neurons caused by ectopic microglia are attributed to IFN-I and IL-6 secreted from microglia (Figure 4).

Figure 4



In summary, microglia smartly exit the mid-embryonic CP via the CXCL12-CXCR4 system and these cells, if inadvertently present in the CP, would disturb the stabilization of the molecular properties of neuronal-subtype associated transcription factors expressed in postmigratory neurons via IL-6 and IFN-I. Thus, the developing cortex expels microglia from the mid-embryonic CP to appropriately fine-tune the expression of molecules needed for proper differentiation of postmigratory neurons, thus securing the establishment of functional cortical circuit in postnatal and adult brain.

3. Future Perspective

The findings from this study are expected to contribute to not only better understanding of the functions of microglia in the embryonic period but also the mechanisms of the brain formation and functional maturation in the postnatal to adult brain.

On the other hand, it has been reported that maternal inflammation in the abnormal state, such as infection, autoimmune diseases, diabetes, obesity and malnutrition, may cause unnecessary activation of microglia in the fetal brain. However, the molecular mechanisms are poorly understood. Detailed information provided from this study on microglial dynamics in the

normal embryonic brain may give insights to elucidate the molecular mechanism of fetal brain development in the abnormal state of maternal inflammation. This achievement will contribute to the establishment of the novel preventive and therapeutic platform against fetal brain dysplasia in the future.

4. Publication

Journal: Nature Communications

Title: Transient microglial absence assists postmigratory cortical neurons in proper differentiation

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DOI : 10.1038/s41467-020-15409-3

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Nat_Com_200402.pdf