主論文の要旨

A mouse model of microglia-specific ablation in the embryonic central nervous system

（胎生期中枢神経系におけるミクログリア特異的除去マウスモデルの構築）

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【Introduction】

Microglia, a type of mononuclear cells in the central nervous system (CNS), originate from embryonic macrophage precursors in the yolk sac and invade the brain at embryonic day (E) 9.5–10.5. Microglia are thought to participate in various CNS developmental events. However, studies reporting functions of microglia during the embryonic period are significantly fewer than those during postnatal development, partly due to the lack of an effective microglial ablation system in the embryo. In addition to parenchymal microglia, several types of macrophages reside in CNS interfaces. Given that these CNS-associated macrophages (CAMs) are mostly of embryonic origin and colonize CNS interfaces during the embryonic stage like microglia, embryonic microglial ablation systems for studying their functions are required to minimize damage to CAMs.

Our previous study reported that Siglec-H is a microglia-specific marker that is almost negative for CAMs. Accordingly, systemic administration of diphtheria toxin (DT) to *Siglech*$^{dtr/dtr}$ mice, in which the gene encoding DTR was knocked into the *Siglech* locus, achieved specific ablation of microglia on postnatal day 7 or after. Given that the microglia-specific expression of Siglec-H was observed at the later embryonic stage in our previous study, we expected that this ablation system would be applicable to embryos.

【Method】

We mated *Siglech*$^{dtr/dtr}$ male mice with wild-type female mice to generate *Siglech*$^{dtr/+}$ embryos. A plug check was conducted in the morning, and the day when a vaginal plug was detected was regarded as E0.5. *Siglech*$^{dtr/+}$ embryos at E10.5, 12.5, 14.5, and 16.5 were used. Because intraperitoneal injection of DT into dams was not able to ablate microglia in their embryos in our preliminary experiment, we injected DT into the amniotic fluid of *Siglech*$^{dtr/+}$ embryos on the hypothesis that DT infused into the amniotic fluid might be transported to the CNS. Pregnant mice were anesthetized with isoflurane, and an abdominal incision was made. With illuminating embryos in the uterus by a flexible light source, DT (20 ng/μl) diluted with PBS was injected into amniotic fluid using glass micropipettes. The volume of DT solution was determined depending on the weight of embryos: 2, 5, 8, and 11 μl for E10.5, 12.5, 14.5, and 16.5, respectively (DT dose: approximately 280 ng/embryonic weight [g]). After several days, embryos were dissected from the uterus, fixed in Zamboni's fixative, and dehydrated in 25% sucrose. Then, coronal sections (16 μm thickness) were prepared and stained with antibodies. Fluorescent images were visualized using a confocal microscope.

【Result】

First, we confirmed that Siglec-H was specifically expressed by microglia but not macrophages in the early embryonic CNS. Then we tested *in utero* injection at E10.5, given

that microglia invade the brain at E9.5–10.5. We immunostained sections of cerebral cortex and spinal cords with anti-Iba-1 and anti-CD206 antibody and defined parenchymal Iba-1^{+}CD206^{-} cells and meningeal Iba-1^{+}CD206^{+} cells as microglia and macrophages, respectively. Immunochemistry and subsequent quantitative analyses found that the number of Iba-1^{+}CD206^{-} microglia in DT groups were decreased to 21.8% and 36.5% at E12.5 and 14.5, respectively, and then returned to the control level at E16.5 and E18.5 in the cerebral cortex. In contrast, there was no significant difference in the number of Iba-1^{+}CD206^{+} meningeal macrophages between the PBS and DT groups at any time point. In addition to cerebral cortex, we also examined spinal cord with injection of DT at E10.5 and obtained the same results. Therefore, microglia can be depleted for 4 days in the CNS after a single injection of DT at E10.5 *in utero*. Next, to test whether this microglial ablation model was also effective at later embryonic stages, we changed the injection time points. We injected DT at E12.5, E14.5, or E16.5 and analyzed the embryos after 2 days. We examined three regions (cerebral cortex, spinal cord, and hippocampus). Regardless of the injection time points, microglia were ablated 2 days after DT injection *in utero* in all these three regions, although meningeal macrophages were not affected. Finally, we tested ablation of microglia in specific regions where microglia are transiently accumulated to exert some functions. Previous studies showed that microglia accumulate in the intermediate zone/subventricular zone/ventricular zone (IZ/SVZ/VZ) of the cerebral cortex at E15.5–16.5. DT injection at E14.5 significantly decreased the microglial number in IZ/SVZ/VZ at E16.5. In addition to IZ/SVZ/VZ, microglia can also be ablated in dorsal root entry zone and region around corpus callosum, where microglia were also shown to transiently accumulate. Collectively, this ablation system can eliminate microglia throughout the CNS, including the specific regions where microglial density is transiently high, at any time point after microglial colonization in the CNS.

【Conclusion】

We have established a microglia-specific ablation model that is effective throughout the CNS at any time point of embryonic stages after microglial expansion to the CNS. Microglial dysregulation during the embryonic stage is thought to result in the abnormal formation of neural circuits, which eventually causes developmental brain disorders. To develop new therapeutic strategies, understanding microglial functions is necessary. Future studies using this ablation system may reveal novel or precise functions of embryonic microglia.