Exploring genes that control microglial heterogeneity and transition

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Microglia, which comprise approximately 10% of total cells in the brain, are the resident immune cells in the central nervous system and contribute to maintaining the brain homeostasis through monitoring their microenvironment (Kettenmann et al., 2011). Recent studies have reported that microglia also regulate neural circuit formation after birth. The functional transition in microglia has been considered to correlate with their morphological changes over time. Although microglial morphologies were found to be modulated by expressions of particular genes, the mechanisms that underlie morphological changes in microglia have not been fully elucidated. Recently, we have reported that FAT atypical cadherin family protein 3 (FAT3) is a novel factor that stabilizes microglial processes regulated by hypoxanthine (Okajima et al., 2020). Since the timing of FAT3 expression coincides with that of microglial morphological changes during the postnatal stage, it is plausible that FAT3 regulates the microglial transition. In this review, we discuss the biological relevance among microglial heterogeneity and transitions. Moreover, we introduce our recent findings that link FAT3 to the aspect of microglial transition and functions during the postnatal stage.

Microglia are crucial for regulating early developing brain and neural circuit formation. For decades, the origin of microglia in the central nervous system had been thought to be derived from myeloid cells because the characters of microglia are close to that of macrophages. However, recent studies have revealed that erythromyeloid progenitors in the yolk sac migrate through the circulatory system to the brain and differentiate into microglia between 8.5 and 10 embryonic days of ages (Ginhoux et al., 2010). The macrophages in the blood are usually differentiated from the circulating monocytes, which originated from not yolk sac but bone marrow. On the other hand, some resident macrophages are generated in the yolk sac during primitive hematopoiesis. Thus, the origin of resident microglia is consistent with primitive macrophages. Microglia exhibit amoeboid shapes during embryonic and prenatal stages, followed by elongating and retracting the processes and changing to ramified shapes around 15 postnatal days of ages in mice. Until recently, it had been thought that amoeboid microglia are reactive forms and ramified microglia are resting forms; thus, microglia had been thought to possess two antithetical functional categories: pro-inflammatory (M1) and anti-inflammatory (M2) state. However, recent *in vivo* two-photon microscope analyses have revealed that ramified microglia are highly dynamic even in the presumed resting state. Moreover, it is hard to advocate the concept of polarization along an M1–M2 axis due to the recent finding of microglial heterogeneity. Therefore, microglial characters have been clarified as part of not dualistic but pluralistic aspects (Ransohoff, 2016).

The technological innovations, such as single-cell RNA-seq analyses, have identified various microglia and certain types of microglia-related cells (**Figure 1A**). For instance, it has been reported that some CNS-associated macrophages (CAMs) or border-associated macrophages (BAMs), which are present in non-brain parenchyma such as meninges, choroid plexus, and perivascular spaces, arise from the yolk sac and reside at the particular area with little proliferation under the normal condition (Goldmann et al., 2016; Van Hove et al., 2019). The aspects of CAMs/BAMs are similar to the resident microglia in the brain parenchyma; however, some distinct genes are expressed in each cell, respectively. So far, the CAMs/BAMs are thought to be one of the microglia-related cell populations in the central nervous system although the precise characters and gene expressions are distinct from resident microglia. These subjects have been discussed in many original papers and several reviews recently, so it is not described this topic here in detail (see one of the breaking reviews (Masuda et al., 2020)). Other types of microglia that emerged during the postnatal stages are proliferative area-associated microglia (PAM). The PAM exhibit amoeboid shape and emerge in the corpus callosum and cerebellar white matter around 1 week of age (Li et al., 2019). PAM is a microglial cluster characterized by enhancing particular gene expressions, such as CLEC7A and GPNMB, which peaked around P7. It has been known that CLEC7A acts as a pattern recognition receptor and control inflammatory responses. GPNMB is a type I transmembrane glycoprotein that modulates proliferation, metastasis, and inflammation. Furthermore, the expression of other genes, which are associated with microglial activation, are also increased in PAM. As PAM eliminate oligodendrocytes during postnatal myelination through the activation of phagocytosis, PAM are different types of reactive microglia and are crucial for maintaining brain homeostasis during postnatal stages. At the onset of various neurological diseases, microglia enhance inflammation. Under these pathological conditions, different types of microglia emerge and change the brain environment. One of the various microglial population is disease-associated microglia (DAM), which are observed in neurodegenerative disorders such as Alzheimer's disease and amyotrophic lateral sclerosis (Keren-Shaul et al., 2017). DAM are characterized by a common gene expression with reactive microglia after inflammatory stimulation. On the other hand, some patterns of gene expression are in stark contrast to that of reactive microglia. Furthermore, the genes expressed in surveillant microglia have been identified to be expressed in DAM), suggesting that DAM are certainly a different type of microglial population. Since DAM share several transcriptional markers with PAM, DAM could belong to the same character as PAM under the pathological conditions. A novel definition of these unique populations will be important for understanding the mechanisms of disease and developing drug discovery, and thus it is considered that many genes are implicated in regulating microglial heterogeneity. On the other hand, the whole picture of the genes, which regulate microglial morphology associated with their transition, has not been fully elucidated.

Recently, we identified another factor, atypical cadherin family protein FAT3, as a novel regulator that controls microglial morphology. The FAT family proteins are evolutionarily conserved from insect to human. It has been reported that Drosophila FAT (ft) and its homolog FAT-like (ft-like) mutations cause hyperplastic overgrowth in all larval imaginal discs and aberrant planar cell polarity. Ft on a cell is activated when it binds to the atypical cadherin Dachsous (Ds), which is derived from neighboring cells. Interaction of Ft with Ds is thought to regulate the Hippo pathways (Fulford and McNeill, 2020). In contrast to Drosophila Ft, mammalian FAT family proteins consist of four member proteins FAT1, FAT2, FAT3, which are resembled Drosophila Ft-like protein, and FAT4 is the ortholog of Ft (Aviles and Goodrich, 2017). FAT3 in humans is found on chromosome 11q14.3–q21 and a giant cadherin that encodes a protein of 4557 amino acids. FAT3 protein may interact with Ena/VASP proteins and associate with cell morphology. Several studies have suggested that Fat3 is related to common cascades with Drosophila Ft, such as the Hippo pathway, followed by regulating cellular proliferation and organ size. In fact, FAT1, which is a Drosophila Ft-like protein and show homology with FAT3 amino acid sequence, has been reported to interact directly with Mst1, a mammalian Hippo kinase, to form a multimeric signaling complex, suggesting that the Hippo pathway is an interesting candidate mediated by FAT3 in microglia. So far, loss of Fat3 gene has been reported to prevent neurite retraction in retinal amacrine cells and cause abnormalities in retinal development (Deans et al., 2011). Although Fat3 deficiency exhibits abnormal neuronal morphology, the roles of FAT3 in microglia had not been fully understood. Our recent studies have reported that microglial Fat3 contributes to the stabilization of their projections. FAT3 protein has an EVH1 (Ena/Vasp homology 1)-binding domain, which interacts with Ena/VASP proteins, and may be associated with actin cytoskeletons. We found that microglial FAT3 is induced by a high concentration of hypoxanthine, which is an intermediate to synthesize an inosine monophosphate. Expression of FAT3 induced by hypoxanthine significantly inhibits retraction of microglial processes, resulting in increasing the number of elongated microglial cell line BV2. Although the mechanisms of how hypoxanthine promotes FAT3 expression are still enigmatic so far, this observation demonstrated that one of the FAT3 functions is to stabilize the actin cytoskeleton in microglia (**Figure 1B**). Interestingly, microglial Fat3 is expressed around 2 to 3 weeks after birth. This timing is consistent with the microglial transition and synaptogenesis during the postnatal stages. In addition, some FAT3 proteins in the brain are localized at the synaptic sites (Okajima et al. unpublished data). Thus, it is plausible that that FAT3 is anchored in the synapses through interaction with several

scaffold proteins. In fact, FAT3 has putative EVH1- and PDZ-binding domains. Since several postsynaptic proteins have either PDZ domain (e.g. PSD95 and Shank) and EVH1 domain (e.g. Homer), these proteins could be targets for anchoring FAT3 at the postsynaptic sites (**Figure 1C**). This idea raises the biological question of why FAT3 is expressed in microglia. Because the timing of microglial FAT3 expression is consistent with that of synaptogenesis and synapse pruning, it is possible that microglial FAT3 regulates not only cellular morphology but also synapse formations via hetero- or homophilic interaction. As microglia does not eliminate the functional synapses, microglial FAT3 could work as a sensor for recognizing the functional synapses (**Figure 1D**). It could be an interesting idea that a neural activity transmits some signals to microglia via FAT3, followed by regulating proper synaptogenesis. Taken together, to understand the mechanisms that underlie microglial FAT3 may provide an informative clue to elucidate the microglial transition and heterogeneity associated with neural circuit formations.

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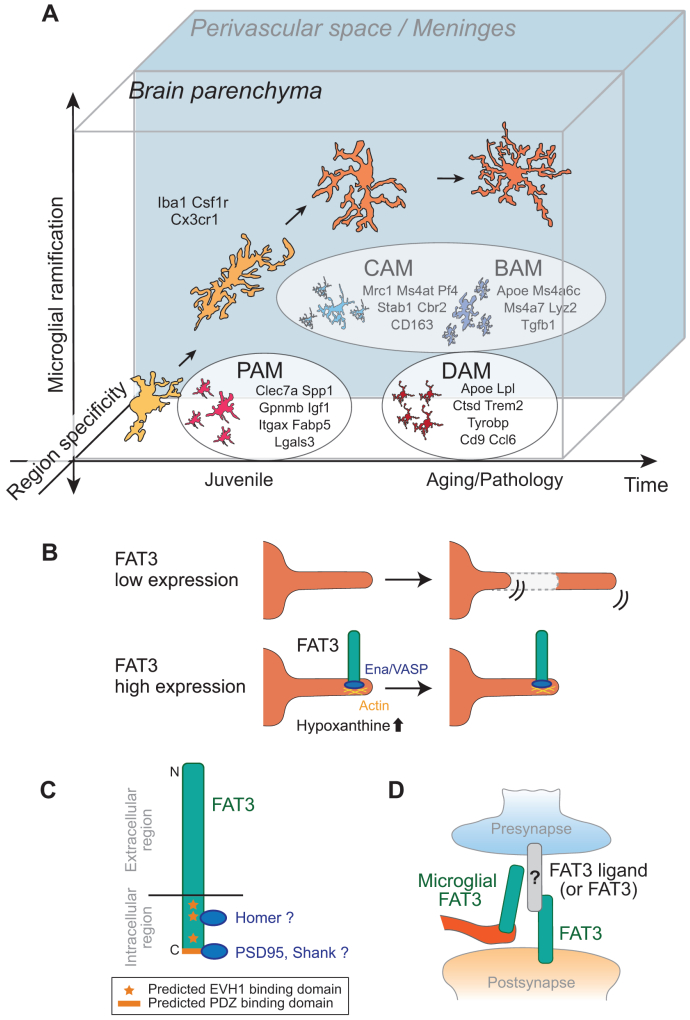

Figure 1 | **Microglial transition and putative functions of FAT3 *in vivo*.**
(A) Region- and timing-dependent microglial heterogeneity and transition are observed under physiological and pathological conditions. Many genes are associated with these processes. (B) FAT3 may stabilize actin organization through interacting Ena/VASP family proteins, followed by regulating microglial morphology. (C) Schematic structure of FAT3. FAT3 has predicted EVH1 binding domain and PDZ binding domain. (D) Another model of FAT3 functions *in vivo*. FAT3 is expressed in both neurons and microglia. Microglial FAT3 regulates synaptogenesis and synapse pruning via interacting with synaptic FAT3 ligand. BAM: Border-associated macrophage; CAM: CNS-associated macrophage; DAM: disease-associated microglia; FAT3: FAT atypical cadherin family protein 3; PAM: proliferative area-associated microglia.

References

Aviles EC, Goodrich LV (2017) Configuring a robust nervous system with Fat cadherins. Semin Cell Dev Biol 69:91-101.
Deans MR, Krol A, Abraira VE, Copley CO, Tucker AF, Goodrich LV (2011) Control of neuronal morphology by the atypical cadherin Fat3. Neuron 71:820-832.
Fulford AD, McNeill H (2020) Fat/Dachsous family cadherins in cell and tissue organisation. Curr Opin Cell Biol 62:96-103.
Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M (2010) Fate mapping analysis reveals that adult microglia derive from-primitive macrophages. Science 330:841-845.
Goldmann T, Wieghofer P, Jordão MJ, Prutek F, Hagemeyer N, Frenzel K, Amann L, Staszewski O, Kierdorf K, Krueger M, Locatelli G, Hochgerner H, Zeiser R, Epelman S, Geissmann F, Priller J, Rossi FM, Bechmann I, Kerschensteiner M, Linnarsson S, et al. (2016) Origin, fate and dynamics of macrophages at central nervous system interfaces. Nat Immunol 17:797-805.
Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B, Itzkovitz S, Colonna M, Schwartz M, Amit I (2017) A unique microglia type associated with restricting development of Alzheimer's disease. Cell 169:1276-1290.
Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91:461-553.
Li Q, Cheng Z, Zhou L, Darmanis S, Neff NF, Okamoto J, Gulati G, Bennett ML, Sun LO, Clarke LE, Marschallinger J, Yu G, Quake SR, Wyss-Coray T, Barres BA (2019) Developmental heterogeneity of microglia and brain myeloid cells revealed by deep single-cell RNA sequencing. Neuron 101:207-223.
Masuda T, Sankowski R, Staszewski O, Prinz M (2020) Microglia heterogeneity in the single-cell era. Cell Rep 30:1271-1281.
Okajima T, Gu Y, Teruya RI, Yano S, Taketomi T, Sato B, Chiba T, Tsuruta F (2020) Atypical cadherin FAT3 is a novel mediator for morphological changes of microglia. eNeuro doi: 10.1523/ENEURO.0056-20.2020.
Ransohoff RM (2016) A polarizing question: do M1 and M2 microglia exist? Nat Neurosci 19:987-991.
Van Hove H, Martens L, Scheyltjens I, De Vlaminck K, Pombo Antunes AR, De Prijck S, Vandamme N, De Schepper S, Van Isterdael G, Scott CL, Aerts J, Berx G, Boeckxstaens GE, Vandenbroucke RE, Vereecke L, Moechars D, Guilliams M, Van Ginderachter JA, Saeys Y, Movahedi K (2019) A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. Nat Neurosci 22:1021-1035.

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