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学位論文名	Involvement of Sema4D in the migration and morphology of murine microglia （マウスマイクログリアの移動や形態形変化におけるセマフォリン4Dの関与）
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論文内容の要旨

1. Abstract

Microglial cell usually display a ramified morphology (resting condition) in the healthy CNS but after infection of disease, activation takes place and the cells undergo a series of morphological, phenotypic and functional changes. Since its activation may result into secondary tissue damage, the mechanisms that induce its de-activation and subsequent ramification are of potential clinical relevance. The inhibitory signals that could prevent or transform cells from activating are still poorly understood. As semaphorins have been mainly described as inhibitory signals because they prevent cell migration and axon outgrowth, we hypothesized that Sema4D could be an inhibitory signal in microglia. We demonstrate, by RT PCR, the presence of Sema4D, plexinB1 and CD72 receptors in microglial cells. To study the effect of Sema4D on mobility and migration of microglia, we used two in vitro assays, a wound healing and a chemotaxis assay. Furthermore, we added Sema4D to the colture to observe changes in the morphology of the cells. The addition of Sema4D leaded to ramification, a process that was initiated within 24h. The lack of Sema4D induced the cells to thicken and loose their process in the knock out mice. These data suggest that Sema4D may play a significant role inhibiting microglia by keeping the cells in resting state

2. Results and Discussion

Mouse microglia express Sema4D

Primers designed to amplify Sema4D were selected using homologous regions of the mouse genes. mRNAs from microglia were reverse transcribed and the resultant cDNA templates were used to detect the expression of Sema4D. cDNAs prepared from HEK 293 transfected transiently with Sema4D, mouse brain and thymus were used as controls. PCR products for Sema4D were detected in microglial cells at an expected size of about 1Kb.

To visualize the location of Sema4D, we performed indirect immunofluorescence and confocal imaging using

mixed primary culture. In the amoeboid microglia, the expression of Sema4D was evident on the surface and around the nuclei whereas in the ramified microglia it was distributed throughout the cell body. Western blot of primary cells revealed a band for Sema4D with approximately 120 kDa.

Microglia express functional receptors for Sema4D

We next addressed the question whether microglia express functional receptors for Sema4D. CD72 and plexinB1 were detected in microglia using RT-PCR. Interestingly, we found that plexinB1 was up regulated in the activated conditions in quantinative PCRs ($p < 0.01$) and CD72 also showed an up regulated expression (not statistically significant).

Sema4D function on microglia cells : inhibition of cell migration

To study the role of Sema4D on the spontaneous migration of microglia, we used *in vitro* wound-healing. We analyzed how the cells repopulated the cell-free zone at 12h, 24h and 48h. To strengthen the idea that Sema4D could inhibit the migration of cells, we induced a stronger migration by adding lipopolysaccharide (LPS) or 10% FBS to the culture. In the presence of 100 ng/ml LPS, the number of cells that migrated into the wounded area was similar to that of 10% FBS. The addition of Sema4D to the culture inhibited the migration. We counted the number of migrating cells as a function of the surface from the original cell-free area (2 mm×0.6 mm=1.2 mm^2). To confirm that the effect of Sema4D was not due to the cell proliferation and to check if Sema4D could act as a chemorepulsive agent in microglial cells, we used a chemotaxis assay (Neuroprobe). Using a 48 well chemotaxis chamber, we investigated the effect of Sema4D on the migration of a microglial cell line (MG-5 cells). Sema4D at a concentration of 1 nM was sufficient to inhibit migration of microglia. Next, we investigated the effect of Sema4D on the morphology of microglia.

Sema4D induced morphological transformation of microglia into the ramified form

It has been suggested that the level of microglia activation can be different according to the severity of neuronal injury and the morphology varies from ramified (rasting condition) to intermediate stage (activated condition) and finally reach round-amoeboid morphology (brain macrophages). When cultured in 10% serum conditions, we could observe the majority of cells with shorter processes, showing from intermediate to round morphology. When Sema4D was added to the culture, microglia started to develop thin processes within 6h after the addition. Even in the presence of LPS, the addition of Sema4D induced the bear of processed that was more evident after 24h. Under these conditions, the cells developed longer and thinner processes resembling the morphology of resting ramified microglia. We noted that the transformation of amoeboid to ramified was bidirectional and ramified cells loose their processes after the removal of Sema4D.

Effect of LPS on the morphology of $Sema4D^{+/+}$ mice and $Sema4D^{-/-}$ mice

Since the addition of Sema4D in the supernatant of cultures induced signs of deactivation, this observation prompted us to study the phenomena *in vivo*. We injected LPS into the right side of the cortex and the animals were sacrificed after 48h. In the contralateral side in both $Sema4D^{+/+}$ mice and $Sema4D^{-/-}$ mice, microglia showed the typical famified morphology of resting cells, exhibiting multiple and branched processes and a small cell body. However, in the ipsilateral side, the $Sema4D^{-/-}$ mice showed shorter and thicker ramifications compared to the same side in the $Sema4D^{+/+}$ mice. Taken all data together, these findings indicate that Sema4D has an important role controlling the inflammation in the CNS and thus, protecting neurons and adjacent tissues from damage.

論文審査の結果の要旨

本研究はミクログリアの Sema4D による非活性化の影響を明らかにする目的で行われた。

その結果、Sema4D の添加により６時以内にミクログリアの細胞突起は多数に分岐し、細胞増殖を著しく低下させた。また Sema4D は細胞の遊走を抑制し、MG-5 細胞による一酸化窒素産生を顕著に低下させた。更にノックアウトマウスを用いて LPS の効果を検討した結果、Sema4D の欠如は細胞を肥厚させ、神経突起の分岐を減少させることが分かった。

以上のことより本研究はミクログリアの Sema4D による非活性化の作用を明らかにしたもので神経科学的に有益な示唆を与えるものであり、博士（学術）の学位取得に値すると認める。