学位論文の要旨

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論文内容の要旨

INTRODUCTION

Microglia are the resident immune cells in central nervous system (CNS), belong to the mononuclear phagocyte lineage. Microglia have crucial roles in CNS development and homeostasis, and are expected to be a therapeutic target for neurodegenerative diseases or neuroinflammatory diseases. Microglia arise from primitive yolk sac macrophages and develop independent of the hematopoietic system. Furthermore, a recent study demonstrated that other tissue-resident macrophages also originate from yolk sac progenitors. This evidence suggests that the developmental fate of tissue macrophages is influenced by the environment in which cells are placed.

MicroRNAs (miRNAs) are small non-coding RNAs that function as guide molecules in RNA silencing. miRNAs are also enriched in CNS and play crucial roles in the development and plasticity of the brain. Certain miRNAs have been reported to regulate differentiation, activation, and polarization of microglia. In addition to colony-stimulating factors including interleukin-34, which dictate the developmental fate of microglia, miRNAs enriched in the CNS may also play a role in the acquisition of a distinct microglial phenotype.

A murine bone marrow chimera model suggests that hematopoietic cells have the potential to develop into microglia. We developed an *in vitro* model in which lineage-negative bone marrow (LN^{-}) cells co-cultured with astrocytes differentiated into microglia-like cells. In this model, bone marrow-derived cells showed two morphological forms, namely, small round cells having relatively small, round-shape soma or large flat cells having polymorphic soma and

pseudopodia. Only small round cells expressed triggering receptor expressing on myeloid cells-2 (TREM-2) that is predominantly expressed on microglia. This indicated that small round cells had phenotypical similarity to microglia and thus we defined these cells as microglia-like cells.

In the present study, we sought to identify miRNAs that affect the phenotype of microglia using an *in vitro* co-culture model and immortalized microglial cell line. We show that miR-101a modulates microglial morphology and inflammation.

MATERIALS AND METHODS

Primary mixed glial cell cultures were prepared from the brains of postnatal 3–5-day-old B6 mice. Astrocytes were prepared after removal of $CD11b^+$ cells and cultured for 5 days to form a confluent monolayer. To isolate LN^- cells, bone marrow cells were collected from GFP mice and LN^- cells were negatively selected using antibodies against lineage-specific markers. LN^- cells were seeded on astrocytes and cultured for 7 days in the absence or presence of miRNAs or their inhibitors. All experimental procedures with animals were approved by the Ethics Committee for the Treatment of Laboratory Animals of the National Institute of Neuroscience, National Center of Neurology and Psychiatry, approval ID: 2014011. Animals were maintained in specific pathogen-free conditions, and all care and use were in accordance with institutional guidelines.

We screened 739 miRNA inhibitors using the miRNA inhibitor library. Microglia-like cells were identified as small, round cells that were immune-positive for CD11b, Iba1, CX3CR1 and TREM-2. We picked up miRNA inhibitors that affect the numbers and the shapes of microglia-like cells.

Cytokine productions were analyzed in the presence of each miRNA inhibitors or mimics using ELISA. We isolated mRNA and miRNA from microglia cell line MG6 and brain cells. We performed reverse transcription and real-time PCR to analyze gene expressions.

We used the TargetScan algorithm (TargetScanMouse 6.2, http://www.targetscan.org/mmu_61/) to identify target genes of miR-101a, and DAVID algorithm to identify KEGG pathways enriched in miR-101a target genes (DAVID; https://david.ncifcrf.gov/).

RESULTS & DISCUSSION

Among 739 miRNA inhibitors, 38 showed cytotoxic effect on co-culture and were excluded from the analysis. There were 27 hits among 701 inhibitors: 22 inhibitors increased the number of SR cells and 5 inhibitors decreased them. We thereafter focused on the 5 miRNA

inhibitors (miR-101a, miR-139-3p, miR-214*, miR-218, and miR-1186) that decreased the number of small round cells. Since it was expected that miRNA mimics would have the opposite effect of miRNA inhibitors, we assessed the effect of five miRNA mimics using the same co-culture system and found that miR-101a was the only miRNA whose inhibitor and mimic exerted opposite effects.

We next investigated the effect of miR-101a on the expression of microglial surface markers. miR-101a inhibitor did not affect the number of $CD11b^+$ or $Iba1^+$ small round cells. In contrast, miR-101a inhibitor decreased the number of both $TREM\text{-}2^+$ and $CX3CR1^+$ cells, whereas miR-101a mimic increased these numbers. These data indicate that miR-101a positively regulates the development of microglia-like cells.

We investigated the effect of miR-101a on the character of microglia-like cells. miR-101a treatment significantly decreased the production of IL-1β from MG6 cells compared to control-treated cells. In contrast, transfection of the miR-101a mimic significantly increased the production of IL-6 and TNFα from MG6 cells in response to LPS. These results indicate that miR-101a modulates expressions of proinflammatory cytokines in microglia.

Finally, we sought to identify target genes of miR-101a using the pathway analysis. DAVID pathway analysis revealed that many target genes of miR-101a were included in MAPK pathway. The MAPK signaling pathway is closely associated with the production of inflammatory cytokines. We investigated the effect of miR-101a on the expression of several molecules involved in the MAPK pathway and identified MAPK phosphatase-1 (Mkp-1) as a target gene that was downregulated by miR-101a. It was speculated that miR-101a regulates microglial inflammation by controlling Mkp-1 expression. These results suggest that miR-101 augments the production of both IL-6 and TNFα from microglia by inhibiting MKP-1.

Our data showed that miR-101a regulates the development and proinflammatory cytokine expressions of microglia-like cells via the MAPK pathway. Since microglia is involved in various CNS diseases, miR-101a might be a therapeutic target for these diseases. We would like to analyze the *in vivo* effect of miR-101a using disease model mice.

CONCLUTION

We have demonstrated that miR-101a, which is enriched in the brain, promotes the differentiation of bone marrow-derived microglia-like cells and cytokine production from microglia. The results shed new light on the molecular basis of microglial differentiation.