The Role for Exosomal microRNAs in Disruption of Regulatory T Cell Homeostasis in Multiple Sclerosis

Kimitoshi Kimura[1,2], Hirohiko Hohjoh[3] and Takashi Yamamura[1,4]

[1]Department of Immunology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan. [2]Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto, Japan. [3]Department of Molecular Pharmacology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan. [4]Multiple Sclerosis Center, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan.

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ABSTRACT: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system, in which myelin and oligodendrocytes are the main targets recognized by inflammatory CD4+ T cells reactive to myelin peptides. Regulatory CD4+ T (Treg) cells normally keep homeostasis of the immune system by inhibiting detrimental effects of inflammatory T cells. However, Treg cells are reduced in patients with MS for unknown reason. This commentary highlights a novel function of circulating exosomes to inhibit the differentiation of Treg cells in MS. Our recent work has demonstrated that the circulating exosomes, a member of extracellular vesicles, of patients with MS exert this effect by transferring *let-7i* to naive CD4+ T cells. The transferred *let-7i* subsequently causes a decreased expression of insulin like growth factor 1 receptor (IGF1R) and transforming growth factor β receptor 1 (TGFBR1), leading to the inhibition of Treg cell differentiation. Thus, extrinsic microRNAs transferred by exosomes might have an active role in triggering autoimmune diseases. We hypothesize that extracellular vesicles including exosomes can be a communication tool between the gut microbiota and the host immune system. Further research in this area will expand the knowledge about the precise mechanism of autoimmune diseases and can lead to a new therapeutic approach.

KEYWORDS: Multiple sclerosis, exosome, miRNA, *let-7i*, Treg cell

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CORRESPONDING AUTHOR: Takashi Yamamura, Department of Immunology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187-8502, Japan Email: yamamura@ncnp.go.jp

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Multiple sclerosis (MS) is an autoimmune disease, which typically affects young adults at around 30 years of age.[1] Accumulating neurologic deficits are a major problem in MS, which makes normal daily living difficult in the late stage. More precise understanding of the pathogenesis is needed for the development of a new therapeutic approach. Inflammatory CD4+ T cells, such as T helper 1 (T_H1) and T_H17 cells, have a pivotal function in MS pathogenesis.[1] However, regulatory CD4+ T (Treg) cells intrinsically control the excessive activity of these inflammatory cells and thus suppress the development of autoimmune diseases.[2,3] It has been recognized that Treg cells are decreased in frequency and functionally impaired in patients with MS, although the mechanism is still unclear.[4,5]

Recently, we found a novel pathogenic mechanism for MS, involving the exosomes in the plasma.[6] Exosomes are extracellular vesicles smaller than 150 nm in diameter.[7] They are secreted by various kinds of cells, delivered throughout the body in the circulation, and then taken up by target cells. Exosomes contain microRNAs (miRNA), proteins, and lipids, which could be delivered to target cells. Among these exosomal contents, miRNAs are unique in that they directly regulate translation of messenger RNAs (mRNA). MicroRNAs are small noncoding RNAs, which are involved in posttranscriptional regulation of gene expression. The critical role of miRNAs in autoimmunity has been formally proven in a number of studies. Dicer1 and Drosha sequentially process primary miRNAs (pri-miRNA) into mature miRNAs, and *Dicer1*$^{-/-}$ and *Drosha*$^{-/-}$ mice develop spontaneous autoimmunity.[8] The differentiation of T_H17 cells is promoted by *miR-21*, and deletion of this miRNA results in resistance to experimental autoimmune encephalomyelitis (EAE) in mice, a model of MS.[9] *Mir-183-96-182* cluster enhances pathogenicity of T_H17 cells.[10] The suppressive activity of Treg cells is impaired in the presence of interleukin (IL)-6. This phenomenon was found to be at least partially mediated by *miR-17* induced by IL-6.[11] Moreover, miRNA-containing exosomes secreted by Treg cells contribute to their suppressive function on proliferation and cytokine secretion of T_H1 cells.[12]

Expression profile of miRNAs is known to be altered in immune cells, brain, blood, and cerebrospinal fluid in patients with MS, as compared with healthy controls.[13] Several studies reported that some miRNAs in the circulation could be used as markers for diagnosis or for evaluation of disease stage and severity in MS.[14] Among miRNAs detected in the circulation, exosomal miRNAs are especially important and should be differentiated from others because they could function in target cells. In the field of cancer, for example, extracellular vesicles secreted from breast cancer cells reportedly trigger the destruction of blood-brain barrier via *miR-181c* and promote brain metastasis.[15] We and others reported that the expression profile

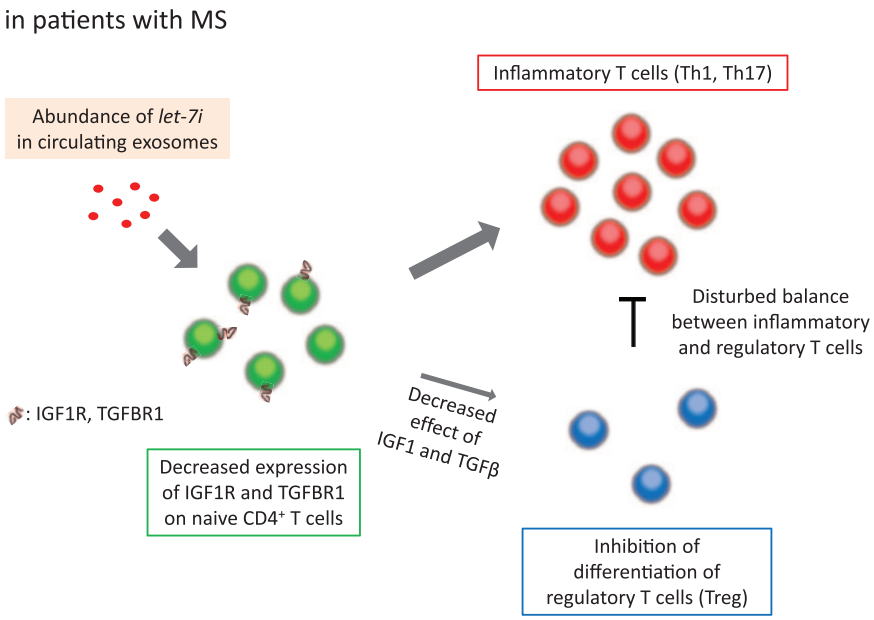

Figure 1. Circulating exosomes inhibit the differentiation of Treg cells in MS. *Let-7i* is upregulated in exosomes from patients with MS and in turn decreases the expression of IGF1R and TGFBR1 on naive CD4+ T cells. This results in lower frequency of Treg cells in MS. IGF1R indicates insulin like growth factor 1 receptor; TGFBR1, transforming growth factor β receptor 1; Treg cells, regulatory T cells.

of miRNAs in the circulating exosomes is dysregulated in patients with MS.[6,16,17] Furthermore, we clarified that the differentially expressed miRNAs are involved in the disease pathogenesis.[6] In our own research, we first found that the frequency of Treg cells (IFN-γ$^-$IL-17A$^-$Foxp3$^+$CD4$^+$ T cells) among CD4$^+$ T cells was lower after culture in the presence of exosomes from patients with MS (MS-exo) compared with those from healthy controls (HC-exo). Transfection of *let-7i*, one of the upregulated miRNAs in MS-exo, also decreased Treg cell frequency, and the effect of MS-exo disappeared when T cells were treated with *let-7i* inhibitor prior to culture. Further analysis revealed that *let-7i* decreased the expression of insulin like growth factor 1 receptor (IGF1R) and transforming growth factor β receptor 1 (TGFBR1) on naive CD4$^+$ T cells and then inhibited their differentiation into Treg cells. The expression of these receptors was decreased on circulating naive CD4$^+$ T cells in patients with MS and positively correlated with the frequency of Treg cells in the blood. Moreover, the frequency of Treg cells was lower in the group with a higher amount of exosomal *let-7i*. Collectively, these results indicate that circulating exosomes have a key role in homeostasis of Treg cells via *let-7i-IGF1R/TGFBR1* axis and contribute to the pathogenesis of MS (Figure 1).[6]

Several studies experimentally proved pathogenic function for some cell-intrinsic miRNAs that were altered in patients with MS.[13] Although whole circulating miRNAs and exosomal miRNAs had been reported as biomarkers, the possible pathogenic function of these extracellular miRNAs had not been studied before our study. Target prediction and pathway analysis are frequently used to assess the function of miRNAs. However, the profile of expressed genes is totally different depending on types of cells, and consequently the gene set affected by a certain miRNA is also different. Therefore, the actual function can be known only by experiments manipulating the amount of miRNAs in the cells of interest. Regarding *let-7i*, which was found to be upregulated in circulating exosomes in MS,[6] it could affect dendritic cells and indirectly decrease Treg cells.[18] *Let-7* family could also promote differentiation of IFN-γ–producing NKT1 cells[19] and inhibit production of IL-10 by T cells.[20] Further studies are needed to verify these possibilities.

Although a critical function of exosomes has been shown in various diseases, there remain several major problems. The precise origin and target of exosomes are key determinants of their function *in vivo*. However, it is difficult to address this question once exosomes are released in the circulation. Interestingly, miRNAs with specific sequence motifs are likely to be sorted into exosomes,[21] which results in differential miRNA profiles between the parent cells and the secreted exosomes. Therefore, the parent cells cannot be suggested simply by similarity of their miRNA profile with that of exosomes. As for targets of exosomes, the type of integrins on exosomes from metastatic tumor cells are involved in organ tropism of parent tumor cells.[22] However, several pathways other than integrin interaction are assumed, and there is no efficient way to detect target cells of exosomes. Some genetic modification approaches are now being used to detect intercellular transfer of RNAs and proteins.[23] For example, transgenic mice, which express CRE recombinase in the parent cells and have a reporter gene with floxed STOP codon in the cells of interest, can be used to search for extracellular vesicle–mediated transfer *in vivo*.[23] However, such approaches are not easily applicable to human studies. Methodological breakthrough would enable high-resolution analysis of a single exosome particle and contribute to

solving these problems, just like flow cytometry for single-cell analysis.

Notably, microvesicles, which are another type of extracellular vesicles typically larger than exosomes, also contain miRNAs and could function as an intercellular mediator.[7] They are generated by direct outward budding and fission of the plasma membrane, in contrast to exosomes, which are first formed as intraluminal vesicles comprising multivesicular endosomes (MVE) and then secreted after fusion of MVEs with the plasma membrane.[7] Several mRNAs and proteins other than miRNAs also participate in intercellular communications mediated by extracellular vesicles including exosomes. For example, ovarian cancer cells produce extracellular vesicles containing *MMP1* mRNA and they are involved in peritoneal dissemination.[24] B cells secrete exosomes loaded with major histocompatibility complex class II, which present antigens to corresponding T cells.[25] Comprehensive analysis of various kinds of extracellular vesicles and their contents should further clarify the pathogenesis of disabling diseases such as MS.

Increasing amounts of evidence suggests that gut microbiota has a fundamental role in cancer, neurodegenerative diseases, and autoimmune diseases. We reported that the severity of EAE in mice is changed by modifying gut flora,[26] and that the microbiome is altered in patients with MS.[27] Regarding extracellular vesicles, *Bacteroides fragilis* delivers immunomodulatory molecules packaged in outer membrane vesicles to intestinal dendritic cells and induces Treg cells.[28] In contrast, host intestinal epithelial cells are known to modulate gut microbiota by secreting miRNAs, which enter commensal bacteria and directly regulate bacterial gene expression.[29] Some of these miRNAs are included in the secreted extracellular vesicles.[29] Besides bacteria, parasite infection in the gut could ameliorate the disease course of MS.[30] Previous studies showed that nematode in the intestine secretes vesicles that contain miRNAs and then modulates the function of host immune cells in mice.[31] Interestingly, some nematode miRNAs are found in the circulation of infected mice.[31] They could affect various kinds of cells including immune cells throughout the body. Extracellular vesicles may be critically involved in host-gut microbiota interaction. Further studies are needed to investigate the relevance in MS.

Author Contributions

KK, HH and TY drafted the manuscript.

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