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Introduction of two candidate microRNAs, miR-21 and miR-146a, as biomarkers in MS

Farzaneh Rahvar[1*], Fawziah M. Mohammed[2], Mehrshad Seresht-Ahmadi[3], Nafise Poorhasan[3]

[1] Department of Medical genetic, national Institute for genetic Engineering and Biotechnology, Tehran, Iran.

[2] Faculty of Allied Health Sciences KuwaitnMedical Laboratory Sciences.

[3] Department of biology, Nourdanesh institute of higher education, Meymeh, Isfahan, Iran

[4] Personalized Medicine Research Center of AmitisGen, Tehran, Iran.

**Corresponding author: Farzaneh Rahvar, RDepartment of Medical genetic, national Institute for genetic Engineering and Biotechnology, Tehran, Iran. xxxxx*

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Abstract

The roles of miRNAs in autoimmunity are only beginning to be explored; they may be involved in regulating immune responses against self-tissues. miRNAs may contribute to disease progression and response to treatment in MS patients. Several studies on MS have analyzed the role or profile of miRNAs in different tissues including peripheral blood mononuclear cells (PBMCs). The current study evaluated mir-21 and mir-146a in CSF samples of patients with multiple sclerosis. Differential expression of the two miRNAs was detected in at least 80% of the CSF samples; however, additional functional studies and analyses of larger cohorts are needed to validate these results and to elucidate the real role of these miRNAs in the context of MS.

INTRODUCTION

MicroRNAs (miRNAs) represent a class of noncoding RNA molecules that play pivotal roles in cellular and developmental processes by regulating gene expression at the post-transcriptional level (1). miRNAs are endogenous, evolutionarily conserved, single-stranded RNAs, approximately 22 nucleotides in length, that suppress the expression of protein-coding genes by directing translational repression through base-pairing with complementary messenger RNA (mRNA) and/or by promoting degradation of target mRNA degradation (2). They play an important role in diverse biologic processes such as fetal development, cell proliferation and differentiation, apoptosis, oncogenesis, metabolism, angiogenesis, and inflammation. The expression of miRNAs is initially controlled at the level of transcription by transcription factors that regulate the production of miRNA-containing primary transcripts in specific cell types during development or in response to different environmental signals (3). Dysregulation of miRNA expression and function is associated with a variety of human diseases, including cancer, neurodegeneration, and autoimmunity. Multiple sclerosis (MS) is an autoimmune disease characterized by chronic inflammatory demyelination in the central nervous system (CNS), which can result in cognitive decline and permanent disability in young adults (4). The etiology of MS has been widely studied, including virus infection, genetic predisposition, lack of vitamin D, occupational exposure, and toxins. It is accepted that MS is an inflammatory and neurodegenerative disease (5). Studies have revealed that miRNAs may contribute to MS progression and response to treatment. Several studies on MS have analyzed the role or profile of miRNAs in different tissues including peripheral blood mononuclear cells (PBMCs), CD4+ cells, and MS brain lesions. They can also be released extracellularly into body fluids such as plasma or cerebrospinal fluid (CSF), where they remain stable (6). CSF is in direct contact with the extracellular space of the brain and can mirror biochemical changes affecting the brain. Some recent studies have evaluated the presence of miRNAs in CSF and their usefulness as potential biomarkers of MS (7). Emerging results indicate that miR-21 and mir-146a promote inflammation and play important roles in the pathogenesis of autoimmune diseases including type 1 diabetes (T1D), psoriasis, multiple sclerosis (MS), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). The current study evaluated mir-21 and mir-146a in CSF samples from patients with multiple sclerosis.

METHODS AND MATERIALS

In this study, 10 CSF samples were collected from

MS patients over 18 years of age and 5 normal CDF samples. Following consensus conditions for CSF collection, CSF was centrifuged immediately after a lumbar puncture at 400×g for 15 min to obtain cell-free CSF. Cell-free CSF aliquots were stored at − 40 °C until RNA extraction. Total RNA was extracted from CSF using a microRNA Purification Kit (Norgen Biotek, Canada) and following the manufacturer's instructions. cDNA synthesis was performed by microScript microRNA cDNA Synthesis Kit (Norgen Biotek, Canada). For this purpose, a poly (A) tail was first added to the RNA template, followed by cDNA synthesis using an adapter primer. The cDNA qPCR amplification was done using a universal PCR reverse primer and the forward primer that contains the sequence of mir-21 and mir-146/a (Table 1). Real-time PCR was performed using SYBR® Premix Ex Taq™ (Takara Bio, Japan).

Table 1. Forward primer sequence

MicroRNA	Forward primer
Mir21	5′-GCCCGCTAGCTTATCAGACTGATG-3′
Mir-146a	5′-TAT-TGG-GCA-AAC-AATCAG-CA-3′

RESULTS

The differential expression between the patient group and the healthy group for miR-21 and miR-146a genes was calculated using the 2^-ΔΔct method; it was detected in at least 80% of the CSF samples, and increased expression of miR-21 and miR-146a was found in patients.

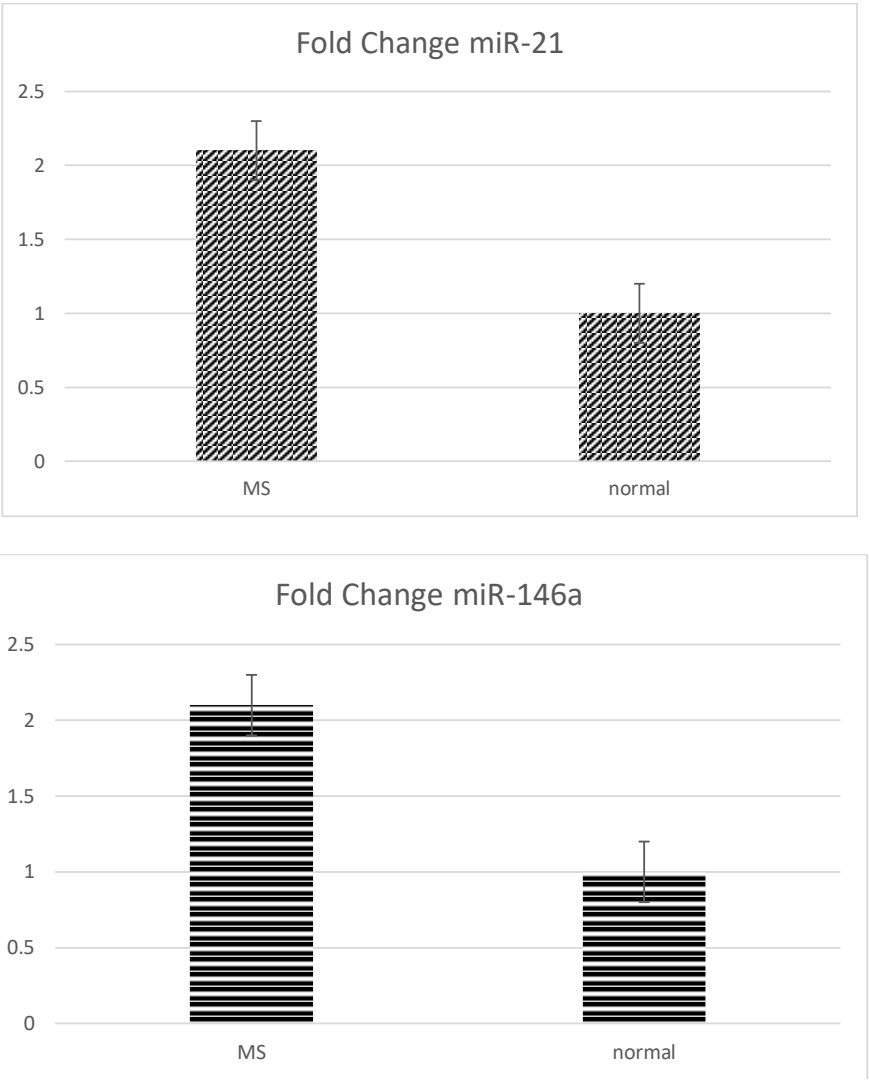

Fig. 1. Fold change gene expression of miR-21 and miR-146a in normal and MS CSF samples. Results show increased expression of miR-21 and miR-146a in patients

DISCUSSION

The roles of miRNAs are only beginning to be explored in the context of autoimmunity; they may be involved in regulating immune responses against self-tissues. Immune responses are normally targeted against microbial pathogens and not self-antigens by mechanisms that are only partially understood (8). Over the past few decades, multiple mechanisms have emerged that operate to prune the lymphocyte repertoire of self-reactive specificities and maintain immunological tolerance. miRNAs play an important role in diverse biologic processes, such as fetal development, cell proliferation and differentiation, apoptosis, oncogenesis, metabolism, angiogenesis, and inflammation. The expression of miRNAs is initially controlled at the level of transcription by transcription factors that regulate the production of miRNA-containing primary transcripts in specific cell types during development or in response to different environmental signals (9). Circulating miRNAs are being widely studied as potential biomarkers for the diagnosis and prognosis in different diseases due to their stability and ease of measurement in tissues and biological fluids (10). Most of these studies are related to cancer research and have demonstrated the capability of circulating miRNAs as new and reliable diagnosis and prognosis biomarkers to detect and identify different cancers. Recent studies have elucidated the role of miRNAs in neurodegenerative diseases, such as MS, and their capacity to predict disease subtypes (as well as response to specific treatments) with a high degree of accuracy (11). Several studies have analyzed miRNA expression in cell-free CSF, a biological fluid that can mirror events occurring in the CNS. The current study evaluated the differential gene expression of two microRNAs, miR-21 and miR-146a, in the CSF samples of people with MS compared to healthy people. The results showed a significant increase in the expression of these two genes in patients compared with healthy individuals. Junker et al. reported a set of 28 miRNAs deregulated in brain tissue with active MS lesions. The current results confirmed the presence in CSF of two of the miRNAs previously reported as deregulated in active brain lesions. Fenoglio et al. found miR-21 and miR-146a/b to be over-represented in PBMCs of relapsing-remitting MS patients compared with the controls (12). They suggested that this upregulation was specific to the acute phase of MS and contributed to the differentiation and regulation of CD4+ T cells, which are involved in the CNS inflammatory processes that take place in MS (13). The data points to the validity of the hypothesis that the overexpression of these miRNAs in CSF is induced to counteract the pro-inflammatory milieu in MS, and they might be released into the CSF in an attempt to reduce the damage to the brain. However, additional functional studies and analyses of larger cohorts are needed to validate these results and to elucidate the real role of these miRNAs in the context of MS.

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