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Original Article

Prediction of probable impact of miR-34a and miR-215 on differentiation of naive CD4+ T cells to Th17 cells in multiple sclerosis

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Abstract

Background and aims: miRNAs, as a class of non-coding RNAs, take part in different cellular processes. Dysregulation of different miRNAs has been reported in numerous disorders to date. Multiple sclerosis (MS) is an autoimmune disease with high prevalence in Iran and Th17 cells play an important role in its pathogenesis. In the current study, we aimed to predict the possible role of miR-34a and miR-215 in the process of controlling Th17 differentiation, and hence, their possible impact on the onset and progression of MS.

Methods: We investigated probable interactions of miRNAs and genes that participate in Th17 cells differentiation using miRwalk database as an integrative one which utilizes 10 different algorithms to predict miRNA-mRNA interaction.

Results: Based on our findings, miR-34a and miR-215 were predicted to have a potential role in the induction of Th17 cells differentiation.

Conclusion: Conclusively, miR-34a and miR-215 may up-regulate Th17 cells of MS patients. Since bioinformatics data have shown that these miRNAs suppress negative regulatory genes in Th17 cells differentiation, we suppose that down-regulation of these miRNAs could ameliorate MS symptoms. Therefore, several therapeutic approaches may be considered for these miRNAs besides their application as valuable prognostic/diagnostic biomarkers in detection of various stages of MS.

Keywords: Multiple sclerosis, miRNA, Th17 cells

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Introduction

Multiple sclerosis (MS) is a common disease of the central nervous system, which occurs in young and middle-aged people (1). The prevalence rate of MS is about 1-2 in 1000, and this rate appears to be rising (2). This disease usually begins around the age of 30 and causes a series of disabilities in people. The incidence of this disease in women is twice as high as in men. However, men and women have the same prevalence in the primary progressive state of the disease. The main cause of this disease is not well known, but it seems that a combination of genetic and non-genetic factors such as virus, metabolism, or environmental factors may be involved, which lead to this autoimmune disorder (3). Isfahan province is one of the areas for which a moderate to high incidence of this disease has been reported, and studies show that the growth rate of this disease in Isfahan is increasing (4).

There are several phenotypes or patterns of the disease progression. These phenotypes include the following four subgroups. In the relapsing-remitting phenotype, the disease relapses unpredictably and then enters a silent phase without symptoms of a disease for several months or years. About 85% of MS patients initially show a relapsing-remitting form at the end of the second decade of their life (5). Additionally, 65% of people with relapsing-remitting phase develop a new phase of the disease called the secondary progression a few years after the onset of the disease, in which improving symptoms disappear and nervous problems increase (6). The primary progressive phenotype accounts for about 10%-20% of the affected population, with apparently no improvement in these people from the onset of the disease. Moreover, primary progressive MS tends to have a later age of onset than relapsing-remitting MS. The relapsing-remitting is a rare form of the disease in which patients develop a lasting neurological damage from the beginning of the disease

(7). Studies show that Th17 cells play an important role in the incidence of symptoms of many autoimmune diseases, including MS. Th17 cells are subtypes of $CD4^+$ T cells and play a role in the development of an autoimmune disease as a part of the adaptive immune system by producing IL-17A, IL-17F, IL-21, IL-22, and GM-CSF interleukins in the defense against extracellular infections caused by fungi and bacteria. IL-17 produced by Th17 cell induces proinflammatory cytokines and also proinflammatory chemokines that increase the chemotactic activity of inflammatory cells at the site of inflammation (9).

Studies have shown that by influencing the IL-17 pathway, the disease severity can be reduced (8-10). MiRNAs, a new group of non-coding and single-stranded RNAs with a length of approximately 22 nucleotides, are found in plants, animals, and some viruses. They are involved in shutting down RNA and regulating gene expression at the post-transcriptional level (11). These RNAs exert their effect by regulating and controlling the gene expression at the post-transcriptional level of mRNA. Therefore, they bind to the 3'-UTR region of their target protein and cause the destruction and inhibition of mRNA translation of their target protein (12). It has been observed that miRNAs play an important role in regulating the immune response. To date, few of the specific miRNAs that are important in regulating immune responses have been discovered or their role has been investigated (13).

The purpose of this study is to determine the bioinformatics of miRNAs that have the greatest effect on the differentiation pathway of Th17 cells.

Materials and Methods

This is a theoretical bioinformatics study that has been carried out using the miRWalk database. This study includes three sections of data collection from scientific papers, data analysis in the miRWalk database, and finally, the study of the results and conclusions, and the process is presented below. To date, many studies have been conducted to explore the signaling pathways involved in the differentiation of Th17 cells, and many of the positive and negative regulatory genes involved in this pathway have been identified. A series of these genes are considered as positive regulators and lead to differentiation into Th17 cells, which include IL-6, IL-23, STAT3, SMAD6/7, RORc, and mTOR. Some of them are also negative regulators, such as SOCS3, TSC1, SMAD2, and FOXP3, which direct the differentiation path toward other T cells, such as Th1, Th2, or Treg (14, 15) (Table 1).

The miRwalk database is a miRNA-mRNA interaction database that allows the simultaneous comparison of the prediction outcomes of the nine other miRNA-mRNA interaction databases. Therefore, if each of the ten miRNA-mRNA interaction databases predicts the likelihood of a mutant, 1 is placed in front of that database, otherwise, 0 is placed in front of it; therefore, each host finds a number from 0 to 10 that represents the number of databases that have predicted the probability of generating that interaction. In this method, interactions that have been confirmed through laboratory methods receive number of 5 or higher, which itself is evidence of the validity and reliability of this bioinformatic prediction method. Other bioinformatic databases that predict the miRNA-mRNA interactions include 1-DIANA-mT, 2-miRanda, 3-miRDB, 4-miRwalk, 5-RNAhybride, 6-PICTAR, 7-PITA, 8- RNA22, 9-TargetScan, and 10-miRwalk. In order to predict miRNAs involved in the differentiation of the Th17 subtype, first, a list of miRNAs expressed in blood samples, peripheral blood mononuclear cells, or tissues involved in various autoimmune diseases that had expression change and were proposed in at least two studies was prepared and then, using the miRwalk database, its inhibitory effect was investigated on the positive and negative regulators of Th17 subtype differentiation.

Results

Based on this study, we found that miR-215 and miR-34a could be proposed as the regulators involved in the differentiation of Th17 and instigation of MS. In examining miRNA-mRNA interactions, only those that were approved by at least 6 databases were considered.

The results indicate that miR-215 induces this cell line with its inhibitory effect on the negative regulators of the differentiation pathway to Th17 cells. The target miRNAs of this miRNA include SMAD2, TSC1, and SOC3 (16-21). The miR-34a precursor is transcribed on human chromosome 1. The precursors of miR-34b and miR-34c are transcribed on a region of human chromosome 11. The miR34a causes apoptosis of human white blood cells by inhibiting SIRT1 (20). This miRNA also plays a role in the differentiation of human B lymphocyte cells, which is adapted from other immune system cells. The miR-34a, like the previous miRNA, plays a role in the process of inhibiting the negative regulators of differentiation of Th17 cells. Negative regulators that are the target of this miRNA include SOCS3, PIAS3, and FOXP3 (22). The genes inhibited by these miRNAs are briefly illustrated in Figure 1.

Discussion

Th17 cells are a subset of the $CD4^+$ T cell line, whose role is protecting against extracellular infections caused by bacteria and fungi. Moreover, they play a role in the pathogenesis of autoimmune diseases such as MS (23). Therefore, the possible role of miR-34a and miR-215 in the process of differentiation of Th17 cells in patients with MS was proposed in the present study. Among the target factors of these two miRNAs are SMAD2, FOXP3, PIAS3, and SOCS3 that are the inhibitors of differentiation of naive T cells to Th17 cells. The performances of some of them are briefly reviewed below.

Table 1. The Most Important Positive and Negative Regulators of the Differentiation Pathway of Th17 Cells

Positive regulators	Negative regulators
IL17A	IFN-gamma
IL17F	PIAS3
IL23	IL12
IL23R	IL12R
IL6R	SOCS3
IL6	STAT5b
IL21	FOXP3
IL22	PPARg
IL21R	TSC1
IL1R	GATA3
RORC	STAT1
STAT3	STAT4
RORa	STAT6
Hif1α	SMAD3
SMAD6	SMAD4
SMAD7	SMAD2
MTOR	FOXO1

Note. These regulators can inhibit or induce differentiation into Th17 cells through different pathways (16, 17).

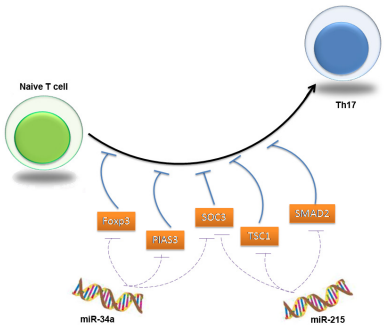

Figure 1.miRNA-mRNA interaction based on the miRWalk database . *Note*. In this Figure, the dots represent the potential targets for each miRNA. Red squares represent regulators for differentiation of Th17 cells that are likely affected by miRNAs. The black lines show an interaction between the miRNA and their target genes.

Studies show that SMAD2/3/4 transcription factors in the active form can bind to the FOXP3 promoter and induce its expression; therefore, the cytokine TGFβ acts on the induction of differentiation of the iTreg cell subtype. FOXP3 acts as one of the Th17 subtype inhibitors (24).
STAT proteins have a major role in the process of differentiating a variety of $CD4^+$ subtypes, each of which pushes the differentiation pathway into a specific subtype and inhibits differentiation into other subtypes. STAT3, STAT4, STAT5, and STAT6 are effective in the process of Th17, Th1, Treg, and Th2 differentiation, respectively (25).

Other factors that inhibit Th17 subtype differentiation in relation to the cytokine signaling pathway include the suppressor of cytokine signaling 3 (SOCS3) and the PIAS3 ligase. The SOCS3 protein is expressed under the influence of cytokine signals of IL-6, IL-21, IL-23 and by the STAT3 transcription factor, and acts as a negative regulator of STAT3 activity. Generally, SOCS proteins are one of the most important regulating proteins of the expression of STAT-dependent genes that in fact create a negative feedback in the STAT signaling pathway. Moreover, the specific deletion of SOCS3 in $CD4^+$ T cells is associated with an increase in phosphorylation of STAT3 and production of IL-17 (26).

Junker et al reported that the miR-34a expression is increased in the active phase (relapse) of MS (27). Increased expression of miR-34a has been seen in the white matter of MS patients (27). On the other hand, the increased expression of this miRNA has been observed in rheumatoid arthritis and inflammatory bowel disease (28,29). It has been observed in a study that the level of miR-215 expression in active plaques in MS patients has increased, while no change has been observed in its expression in other studies (30). In a recent study, the increase of miR-34a in a group of MS patients has been reported, which is correlated with increased number of Th17 cells (31). All the above-mentioned studies confirm our findings. Differences in the methods of study and the samples examined can be the cause of these differences in the results of the studies. Various studies show that in patients with MS, these miRNAs can be used as biomarkers to detect the disease in the early stages or before the onset of clinical symptoms in the person. Therefore, a significant increase in the expression of these miRNAs in the blood can be regarded as a biomarker to diagnose the severity of the disease or the activity level of Th17 cells. It is also predicted that the expression of these miRNAs in the T-cells of the affected individuals has increased, which in turn increases the population of the Th17 cells, and accordingly, by reducing the expression of this miRNA by, for example, the miRNA inhibitor in naive $CD4^+$ T cells, it is possible to inhibit the differentiation towards the Th17 cells and reduce the severity of the disease in this way.

Conclusion

In this study, we showed the probability of the role of miR-215 and miR-34a in differentiation of T cells towards Th17 cells by inhibiting transcription factors. With the help of miR-215 and miR-34a inhibitors, treatment in this field can be achieved, or the miRNA itself can be used as a biomarker for the diagnosis of MS. However, further and detailed experimental studies are needed to prove these suggested roles for miR-215 and miR-34a.

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Conflict of interests

None.

Ethical considerations

This study has been approved by the local Ethics Committee of Shahrekord University of Medical Sciences (ethic code: 93-9-5).

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