MicroRNAs as the Important Regulators of T Helper 17 Cells: A Narrative Review

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ABSTRACT

T helper (Th)-17 cells are a distinct and important subset of Th cells and their functions are due to the ability of production and secretion of key cytokines in the immune system such as interleukin (IL)-17, IL-22, IL-21, and tumor necrosis factor-α (TNF-α). According to these cytokines, these cells have vital roles in the pathogenesis of the disease such as rheumatoid arthritis (RA) and osteoarthritis (OA). Nowadays, microRNAs (miRNAs) are defined as essential regulators of cell function by targeting transcription factors and other elements that act in cells to control gene expression. The purpose of this study was to detect and investigate articles evaluating the function of miRNA in Th-17 cell performance. The language was restricted to English and the search was done in PubMed, Web of Science and Embase. In this review, we first explain the role of effective factors in the function of Th17 lymphocytes, and then, we summarize the performance of several miRNAs involved in the activation and appropriate functions of Th17 cells in the immune system.

Keywords: Cytokines; MicroRNAs; Th17 cells

INTRODUCTION

Since microRNAs (miRNAs) play an important role associated with cytokines and transcription factors, we first briefly review the function of cytokines and transcription factors related to T helper (Th)-17 cells, and then specifically, we will address specific miRNAs related to Th17 cells functions.

CD4⁺ T or Th cells are a major group of T cells involved in a variety of immune system functions, including regulation of proliferation and differentiation of B cells and CD8⁺ T cells, antibody production, and controlling innate immune system functions. Naïve T helper cells activated in the two-signal hypothesis process (engagement with antigen-MHC class II on the surface of antigen-presenting cells (APCs) as the first activation signal and co-stimulation with cytokines as the second signal) and then differentiated into diverse subgroups.⁴ In addition, a set of other variable factors such as diverse pathogen-associated molecular patterns (PAMPs), T cell receptor (TCR) signaling strength, cytokine concentration, signal transducer and activator of transcription factors (STATs), lineage-specific

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transcription factors, and lineage-associated TFs are further deemed to be involved in the activation of naïve T cells.³

T-box proteins expressed in T cells (T-bet), GATA binding protein (GATA3), aryl hydrocarbon receptor (AhR), retinoic acid-related orphan receptor gamma t (RORγt), and forkhead box P3 (FoxP3) are major lineage-specific transcription factors (TFs) concerning Th cells differentiation.² Before the activation of these TFs, the STATs family relays the signal by cytokines, promoting the differentiation of naïve Th cells into specific T cell lineage. For instance, Th1 cells secrete interferon-γ (IFN-γ) and interleukin (IL)-12 resulting in the activation of T-bet and STAT4 as their main transcription factors. T-bet is the main target of IFN-γ and STAT4 is regulated through IL-12; therefore, these two cytokines are the most important regulators for Th1 cells differentiation.¹

GATA3 and STAT6 are master transcription factors involved in Th2 cells differentiation, triggering the production of IL-4, IL-5, IL-6, IL-10, and IL-13.² STAT6 is the main target of the IL-4 signaling pathway activating GATA3 as a key in mounting the humoral immune system against extracellular pathogens and also in allergic reactions and atopy.¹

Th17 cells are necessary for immune system responses against bacterial and fungal attacks on mucosal surfaces. These cells are one of the main players existing on the site of inflammation and important culprits behind the pathogenesis of autoimmune and allergic diseases.⁴,⁶ This subset of Th cells secrete IL-17A and IL-17F in addition to IL-22, cause the production of neutrophils through stimulating the expression of granulocyte-colony-stimulating factor (GCSF) and CXCL-8 or IL-8.⁸,⁹ Th17 cells production enhanced if IL-4 and IFN-γ signaling pathways are blocked. Furthermore, the differentiation of these cells is not dependent on the transcription factors involved in Th1 or Th2 cells differentiation such as T-bet, STAT1, STAT4 and STAT6.¹

Figure 1 shows the role of important cytokines and transcription factors in the differentiation of Naïve T-cells in three subcategories.

Figure 1. Role of cytokines and transcription factors in T helper (Th)-17 cells differentiation

Fundamental Role of IL-6/IL-23/STAT3/ RORγt

Cytokines such as IL-1β, IL-6, IL-23, and TGF-β are major factors regarding the induction of the differentiation of naïve T helper cells to Th17 cells and play important roles in the pathogenesis of the inflammatory disease.²,¹⁰

RORγt, encoded by the gene RORC, is known as the key transcription factor for a divergence to Th17 cells and IL-17 production.¹ In addition to RORγt, RORA is similarly expressed in Th17 cells, maintained at a constant level, and a necessary component in Th17 cell expansion. Signaling pathway mediated by IL-6 receptor (IL-6R with gp130) and IL-23 receptor activates the phosphorylation and dimerization of STAT3 following engagement with IL-6 and IL-23. As a result of such stimulation, STAT3 translocates to the nucleus, promoting the activation of the RORγt gene.²,⁴ It is specified that RORγt expression is sufficient to activate IL-17 production, independent of other cytokines. Therefore, this factor can be considered as the master regulator in the control of Th17 cells in case any defect in this factor halts the differentiation into the Th17 subset.³ IL-6 as an inflammatory biomarker can induce the production of IL-21 in Th17 cells, thereby resulting in IL-23R expression, exposing Th17 cells to the IL-23 cytokine network.³,¹¹ While it is reported that Th17 cell generation depends on the IL-21
MicroRNAs as the Important Regulators of T Helper 17 Cells

signaling pathway, some studies have shown that Th17 production can be accomplished in the absence of IL-21 and its signaling pathway.\(^3\)

The maintenance of the Th17 cell state is contingent on the activation of STAT3 and RORγt and the resultant suppression of T-bet and FoxP3 in Th-1 and Treg cells.\(^3,12\) Genomic screening based on the elimination of STAT3 function limits the differentiation and plasticity of Th17 cells as illustrated by CHIP-seq or genome-wide chromatin immunoprecipitation sequencing.\(^1,3\)

AP-1, IRF4, and BATF Inevitable Acts

Interferon-regulatory factor 4 (IRF-4), formerly known to be involved in the regulation of GATA3 for Th2 cell differentiation, is also necessary for the differentiation of Th17 cells. IRF4 is an essential factor mediating the transition from Treg to Th17 cells and playing an important role in Th17 cell plasticity.\(^3\)

Basic leucine zipper transcription factor, ATF-like (BATF), a basic leucine zipper transcription factor ATF-like, is associated with the AP-1 family of transcription factors and similarly important for the differentiation of Th17 cells. Any defect in this factor leads to the highly downregulation of IL-17 production in Th17 cells. Upon binding to JunB, BATF adjusts the stimulation of several Th17 specific transcription factors through interaction with IL-17, IL-21, and IL-22 promoters. IRF4/BATF activation following T cell receptor signaling on the promoter of IL-17 gene creates an opportunity for RORγt function, also IRF4 and BATF deletion in Th cells causes the reduction in the expression of RORγt and more expression of transcription factors such as FoxP3.\(^2,3\)

AhR and IL-23 and Their Influence on Th17

Effector functions of Th17 cells are dependent on IL-23 (a heterodimer composed of p40 shared by IL-12 and p19 subunit), a cytokine with an adjunct role in this process and subsequently in inflammatory response’s and disease;\(^1,13\) however, it is to be noted that IL-23 is required for AhR transcription factor regulation. AhR is a cytosolic receptor initiating the differentiation toward Th17.\(^1\) Activated AhR fine-tunes the development and differentiation of Th17 cells and IL-22 production.\(^2,3\) AhR interaction with 6-formylindolo[3,2-b] carbazole (FICZ) upregulates IL-22 and IL-17 in vitro.\(^2\) It is also reported that AhR possibly plays a role in the plasticity of Th17 by activating STAT1 and STAT5.\(^3\)

Runx1, FoxP3 and T-bet and Their Correlation with Th17

Runt-related transcription factor 1 (Runx1) is a member of Runt-domain class of transcription factors that controls the differentiation of CD4+ Th cells and acts in concert with RAR related orphan receptor C (RORc) and RORγt, promoting Th17 cell differentiation and IL-17 production.\(^3\) In addition, T-bet attachment to Runx1 can inhibit Th17 cell differentiation by preventing the association between Runx1 and RORc. It is important to note that Runx1 overexpression could reverse the effect of T-bet on Th17 cells.\(^3\)

The interaction between FoxP3 and RORγt leads to the inhibition of RORγt function due to the interaction between exon 2 of FoxP3 and RORγt. It is noteworthy that IL-23, IL-21, and IL-6 are somehow associated with STAT3, resulting in RORγt activation through inhibiting the FoxP3 function.\(^3\)

T-bet is a key transcription factor playing major roles in the pathogenic generation and function of Th17 cells. Interestingly, T-bet suppression inhibits Th17 cell expansion in a definite microenvironment via IL-23R downregulation.\(^1,3\)

Interferon regulatory factor 8 (IRF-8) is yet another transcription factor that suppresses RORγt; therefore, IRF-8 depletion promotes the differentiation of Th17 cells.\(^2\)

Signaling Pathway of IL-1/IL-1R and Its Association with Th17 Cells

There is an essential signaling pathway for the commitment and differentiation of Th17 cells by the attachment of IL-1 to its receptor (IL-1R). While this cytokine is capable of independently promoting IL-17 and RORγt in naïve Th cells, its effect can be augmented by the synergistic influence of cytokines such as IL-6 or IL-23.\(^3\) Single Ig IL-1-related receptor (SIGIRR) is a negative regulator of the IL-1 receptor, inhibiting Th17 cells differentiation and function. IL-1β may be essentially required for the differentiation of Th17 cells mainly in retinoic acid-rich environments inhibiting the development of Th17 cells.\(^3\)

TGF-β Function and Its Interaction with Th17

TGF-β potentially inhibits Th1 and Th2 cell differentiation by suppressing T-bet (for Th-1) and GATA3 (for Th-2), resulting in the inhibition of STAT5 signaling related to IL-2 cytokine.\(^1\) Low concentration of TGF-β is indirectly associated with the
development of TH17 responses, whereas at high TGF-β concentrations, the expression of IL-23R and RORγt is restrained and naïve Th cells differentiate into regulatory T cells.1

TGF-β is also essential for the optimal differentiation of Th17 cells and is involved in the downregulation of IFN-γ and IL-4 signaling pathways as indicated by reports in which Tgfb1 deletion reduces the frequency of Th17 cells and release of IL-17.3

Several studies have shown that TGF-β signaling is related to the activation of smad family proteins (Smads) and STAT3 transcription factors, both of which are required for effective Th17 cell development.1

Similar to TGF-β, all-trans retinoic acid is a metabolite of vitamin A (ATRA) which inhibits RORc expression, promotes FoxP3 (master regulator of Treg cells) expression, and negatively regulates Th17 cell differentiation.1

Other Important Cytokine Networks

IFN-γ and IL-27 regulate STAT1 and IL-12, subsequently fine-tuning STAT4, and curbing Th17 development. Surprisingly, STAT4 deletion further inhibits the polarization of Th17 cells.1

STAT5 signaling of IL-2 attenuates the polarization of Th17 and has a direct negative influence on the gene expression of IL-17.1

IL-21 regulates STAT3 and TGF-β signaling pathways and is sufficient for the induction of RORγt gene expression and polarization of Th17 in the absence of IL-6 cytokine.1

In various inflammatory conditions, IL-1β is necessary for the polarization of Th17 cells by assisting in the production of inflammatory cytokines, which results in the formation and activation of the inflammasome. This cytokine induces IRF4 signaling associated with the production of Th17 cells as indicated in a study where Irf4 deletion diminished the capacity of Th17 cells formation.1

Figure 2 shows the role of different cytokines and transcription factors associated with the cytokines involved in the activation or inhibition of Th17 cell performance.

Figure 2. Summary of cytokines and their related transcription factors on T helper (Th)-17 cells function

The Role of miRNA in Th17 Cell Functions

MiRNAs are important mediators playing a significant role in regulating the function of Th17 cells. These regulators are a large family of small non-coding RNAs about 17 to 22 nucleotides long-playing significant roles in post-transcriptional changes and the regulation of gene expression.14-16

The functional mechanism of miRNA is mainly through binding and sequestration of mRNAs, degradation of mRNAs, and reduction in their translation to perform fundamental functions in immune response homeostasis, immune system, and inflammatory response regulation, and in the development of various immune cells.17-19 A particular miRNA might act with various objects, and a distinct mRNA could be directed by numerous miRNA.20 Aberrant regulation by these RNAs ensues various diseases such as cancer, diabetes, neurological disorders, and cardiovascular and autoimmune diseases. These molecules can be considered as ideal targets for diagnosis, prognosis, and treatment of diseases.14,21

In the following sections, we attempted to evaluate the performance of the well-characterized miRNA involved in the development and performance of Th17 cells. Owing to the wide variety of such miRNA and their different roles, we decided to write this review article based on the type of miRNA and their performance.
MicroRNAs as the Important Regulators of T Helper 17 Cells

Mir-155

Jumonji and AT-Rich Interaction Domain Containing 2 (JARID2) gene is a member of the Jumonji family acting as a chromatin modifier by removing methyl groups from the lysine residues at the histone tail and triggering gene expression; this gene has been shown as a potential target for miRNA-155. Unlike other members of this group, Jarid2 has a histone demethylase activity in its catalytic domain and is associated with Polycomb Repressive Complex2 (PRC2) in thymocyte cells. PRC2 is crucial for cell differentiation and gene expression through changes in chromatin state and plays an important role in the differentiation of embryonic stem cell (ESC) into Th cells. On the other hand, JARID2 plays a role in controlling the expression of IL-22, IL-9, IL-10, TBX21, and ATF3 genes, all of which are important in the regulation and control of Th17 cells. MiRNA-155 plays a major role in the expression and translation of the cytokine genes in Th17 cells through the regulation of the JARID2 gene post-translational modifications.18

Another target of miRNA-155 is the suppressor of cytokine signaling-1 (SOCS1), which has a significant role in the differentiation of Th17 cells and the production of IL-17. The STAT3 gene, activated by IL-6, is essential for regulating Th17 cell function, and controlling the production of IL-17.17 SOCS1 is a negative regulatory factor of the JAK / STAT signaling pathway, thereby having a role in the differentiation of Th17 cells. MiRNA-155 induces the differentiation of Th17 cells and promotes IL-17 synthesis by suppressing the gene SOCS1. It has further been proved that a defect in the function of miRNA-155 may impair the production of IL-17 and IL-22.17 It is also likely that miRNA-155 affects the induction of RORγt factor, a major player in the differentiation of Th17 cells.10

The expression of miRNA-155 in heart tissue and CD4+ T cells is ameliorated during an inflammatory heart disease known as experimental autoimmune myocarditis (EAM). Th17 cells derived from the naïve T cells are crucial in the pathogenesis of this disease. The differentiation of this group of cells is dependent on TGF-β-like cytokines which are similar to IL-6 and require the transcription factor RORγt signaling pathway. Differentiation and activation of Th17 cells result in the production of cytokines such as IL-6, IL-23, IL-1β, and TNF-α that have a significant role in inflammatory processes. In this disease, SOCS1 is targeted and inhibited by miRNA-155, leading to the activation of JAK / STAT, and IL-6 signaling pathways for the induction of Th17 cell proliferation.22

The E26 avian leukemia oncogene 1 (Ets-1) is yet another player with important roles in the differentiation and development of Th17 cells. IL-23 is a target of Ets-1 whose role in Th17 cell differentiation has been corroborated. IL-2 gene, which is an essential factor in the development of the Th cells, is another potential target of Ets-1 suppressing the production of IL-23 so IL-2 regulates the differentiation of Th17 cells. Consequently, the deficiency in miRNA-155 may inhibit the development of Th17 cells.23

The expression of miRNA-155 is augmented in the process of glomerulonephritis, which is related to the levels of anti-neutrophil cytoplasmic antibody (ANCA). In this disease, SOCS1, Src homology 2 domains containing inositol-5-phosphatase 1 (SHIP-1) and Ets-1 both involved in the development of Th17 cells and targeted by miRNA-155. The defect in the functional pathway of miRNA-155 target genes impairs the production of Th17 cells. As a result, it is possible to ameliorate the symptoms of the disease by targeting this factor in the affected people.24

The function of Th17 cells in various cancers has been investigated and extensive research has been conducted on cancers such as cervix cancer. The expression of miRNA-155 in cervix cancer is significantly increased, improving the production of cytokines such as IL-6, IL-17, and IL-23, and the high expression of RORγt. Studies have shown that miRNA-155 negatively regulates the SOCS1 gene which plays a major part in cell differentiation and tumor formation and results in differentiation towards Th17, enhanced production of IL-17, and Th17 cell-associated transcription factors including RORγt (related to TGF-β) and IL-6 and STAT3 (IL-6 binding factor involved in Th17 differentiation).25

Acute coronary syndrome (ACS) is one of the important diseases studied concerning the role and function of Th17. Th17 cells are highly involved in systemic and vascular inflammation and the stability of the plaques formed in this disease. The expression of miRNA-155 in peripheral blood mononuclear cells (PBMCs) is inversely correlated with the number of Th17 cells. The expression of this miRNA can be regulated in association with the activation of NF-kB and C-Jun kinases, or it is probably increased with B cell receptor (BCR) or T-cell receptor (TCR). The results of a study indicated that in patients with ACS,
the number of Th17 cells is negatively controlled by miRNA-155.26

"Dicer" is an essential enzyme in the generation of mature miRNA. This enzyme possesses RNase activity and plays a crucial role in the production of miRNA during the differentiation and maturation of thymocytes into the T-helpers subtype.27 As far as the authors of the present study are concerned, Th17 cells are involved in host defense and are widely conducive to the pathogenesis of inflammatory and autoimmune diseases. When inflammation occurs, the expression of miRNA-155 is increased by factors such as TNF-α and lipopolysaccharides; RORγt factor is further induced and proceed the balance towards the differentiation of Th17 cells. It has been demonstrated that in inflammatory conditions, miRNA-155 induces the differentiation of Th17 by targeting SOCS1, which inhibits the JAK / STAT signaling pathway.27 Studies have shown that a SOCS1 analogous molecule called tyrosine kinase inhibitor peptide (Tkip) can block the IL-6-dependent signaling pathway of STAT3 and inhibit the induction of Th17 cells and IL-17 production due to RORγt; consequently, this molecule controls the extent of experimental autoimmune encephalomyelitis (EAE) in both acute and chronic phases of the disease.28

MiRNA-155 is expressed in various immune cells such as macrophages, dendritic cells, and various types of T lymphocytes. The expression of this miRNA is increased during the innate and adaptive immune responses to inflammatory mediators. Deficiency or defect in the synthesis of miRNA-155 reduces the number of Th17 cells, macrophages, and neutrophils, accelerating the locomotor recovery following the spinal cord injury. In this process, the main target of miRNA-155 is the SOCSJ gene.29

Th17 cells also play a significant role in transplant rejection. Inflammation is one of the mechanisms of the immune system involved in the transplant rejection process, and Th17 cells are efficient in triggering and regulating inflammation. The important role of Th17 cells is regulation and controlling the inflammation process that is perfectly exemplified by the chronic rejection of cardiac transplantation. Studies have indicated that the expression of miRNA-155 is specifically enhanced in cells infiltrating the cardiac allograft tissue. The inhibition of miRNA-155 inhibits the differentiation of Th17 cells and reduces the production of IL-17, thereby suppress the inflammation process. Various transcription factors targeted by the miRNA-155 were investigated, leading to results similar to those of the previous studies and revealing the SOCSJ gene as one of the main targets of this miRNA.30

In patients suffering from autoimmune disorders such as multiple sclerosis (MS), the miRNA-155 is overexpressed in T cells, B cells, and dendritic cells. The production of IL-17 in the lymph node, spleen, and central nervous system (CNS) was evaluated in EAE mouse models, results showing high levels of IL-17 due to the increase in the number of Th17 cells. In addition, symptoms relieving and reduction in inflammation was reported in the brain of mice deficient in miRNA-155 due to the reduced levels of IL-17 and the production of Th17 cells.27

Polarization of Th17 cells will be impaired in miR-155 deficient T cells and production of two cytokines, IL-17 and IL-22 decreased significantly in RA pathogenesis.31 This miRNA stimulate differentiation of Th17 cells in rheumatoid arthritis progression via induction of a key transcription factor RORγt and STAT3.32 By targeting SOCS1, Th17 cells production stimulated due to the function of mir-155 in chronic inflammatory disease such as RA.27

Figure 3 shows different targets of miRNA-155 and their role in Th17 cell functions.

Further studies are required to identify the miRNA-155 target genes and their functions in these inflammatory statuses.

**Mir-146**

MiR-146a is one of the most studied miRNAs for its multiple functions in the regulation of the innate and adaptive immune responses. This miRNA suppresses several effectors in the TLR4 dependent pathway, such as TNFR-associated factor 6 (TRAF6), Interleukin-1 receptor-associated kinase 1 (IRAK1), IRAK2, IRF3, and IRF5 and lack of mir-146 leads to excessive production of IL-6 and TNFα and increase the number of Th17 cells.33,34 mir-146a has been recognized as a vital regulator that decrease the expression of inflammatory genes with the contrasting action to miR-155.31

One important immunological pathway in Th17 cells is the NF-kB pathway that can be regulated by mir-146. In this manner, mir-146 attenuates the TRAF6 and IRAK1 factors so inhibit the expression of NF-kB and lastly prevent inflammation. On the other hand, the
expression of mir-146 can affect IL-17 production and increase its secretion. Studies showed that in the absence of mir-146, expression of IL-17, and RORγt as a key transcription factor for Th17 cell differentiation were increased.

**Mir-21**

In addition to Th-1 cells, Th17 cells are important mediators in delayed-type hypersensitivity (DTH), developing severe inflammation in this process. MiRNA-21 is another miRNA involved in the regulation of the function and differentiation of Th17 cells. The major target of this miRNA is the SMAD7 gene, which is involved in suppressing the differentiation of Th17 cells. SMAD7 is suppressed by miRNA-21, hence the increase in the proliferation of Th17 cells happened. TGF-β is a major cytokine involved in the differentiation of Th17 cells. Studies have shown that SMAD7 is a negative regulator of TGF-β signaling able to control the differentiation of Th17 cells. The excessive expression of miRNA-21 is associated with several autoimmune diseases such as MS, systemic lupus erythematosus (SLE), and psoriasis. MiRNA-21 augments the proliferation and production of Th17 cells owing to its SMAD7 inhibitory properties.

**Fig. 3. Mir-155 and its targets in T helper (Th)-17 cells function**

**Fig. 4. Targets of mir-21 and their functions on T helper (TH)-17 cells**

Another target of this miRNA is Phosphatase and tensin homolog (PTEN). PTEN is an important inhibitor of the PI3K/Akt signaling pathway and Akt phosphorylation is a major inhibitor of cell proliferation. Expression of miRNA-21 elevated in TH-17 cells and could suppress the expression of PTEN therefore Akt signaling activated and Th17 cell proliferation inhibited in this manner.

Recent studies have suggested that miRNA-21 has a major action in the development of inflammatory diseases and the induction of specific cell differentiation. This miRNA regulates the differentiation of Th17 cells by the negative regulation of STAT3 and it is proven that STAT3 is a key factor in signaling pathways related to cytokines such as IL-6, IL-22, and IL-23 that are critical for Th17 cells development.

Remember that owing to the existence of various targets for miRNA-21, different outcomes can be achieved. Figure 4 briefly summarizes some of the targets associated with miRNA-21 and their role.

**Mir-210**

MiRNA-210 is a specific miRNA in hypoxia, shown to be associated with TCR-CD28 signal, particularly in Th17 cells. Hypoxia-inducible factor 1α (HIF-1α), a key factor in the differentiation of Th17 cells, is negatively regulated by miRNA-210. TCR
induces the production of miRNA-210 in T cells under hypoxia and this action is augmented by the antigenic stimulation. In this case, HIF-1α is a target for miRNA-210, and removal of this miRNA can trigger the differentiation of Th17 cells along with the lack of oxygen.\textsuperscript{44}

TGF-β and IL-23, as two key cytokines are involved in the differentiation of Th17 cells, increasing the expression of miRNA-210 by HIF-1α induction through epigenetic mechanisms and hyperacetylation. In this pathway, HIF-1α interacts with P300 (an enzyme with acetyltransferase activity) in CD4+ T cells, causing the hyperacetylation of histone H3 in the promoter region of miRNA-210.\textsuperscript{45}

**Other miRNAs**

**Mir-15b**

Mir-15b expression has been shown to decrease in patients with MS. Overexpression of miRNA-15b in the EAE mice model alleviates the symptoms while its elimination causes the exacerbation of the symptoms. This miRNA targets the N-acetyl glucosamine transferase enzyme, which plays a regulating role in the expression of the \textit{RORγt} gene through the glycosylation of NF-kB, which increases the differentiation of Th17 cells, and enhances TCR activity. NF-kB member, c-(REL proto-oncogene, NF-KB subunit) Rel and P65 regulate the differentiation of Th17 cells by binding to the \textit{RORγt} promoter. Consequently, miRNA-15b regulates Th17 cells by inhibiting the enzyme and NF-kB, thereby alleviating the symptoms of the disease.\textsuperscript{14}

**Mir-590**

Mir-590 is another miRNA detected excessively in the peripheral blood and cerebrospinal fluid of patients with MS. The expression levels of this miRNA are directly associated with the severity of the disease. The differentiation and development of Th17 cells are enhanced by this miRNA, increasing the pathogenicity of Th17 cells. One of the factors targeted by miRNA-590 is the transducer of erbB (Tob1), a negative regulator of Th17 cell differentiation in patients with MS. On the contrary, miRNA-590 positively regulates IL-17A and RORc mRNA expression, enhancing the phosphorylation of STAT3 and an increase in Th17 cell differentiation happens which deteriorates the symptoms of the disease.\textsuperscript{46}

**Mir-873**

Mir-873 is a significant player in the production of Th17 cells with pivotal roles in regulating Forkhead box protein O1 (Foxo1). As a member of the Forkhead family, Foxo1 is a negative regulator of Th17 cell differentiation suppressed in SLE disease through the inhibitory function of miRNA-873, resulting in the increased differentiation of Th17 cells. In addition, the production of IL-17 in these patients increased due to the stimulation of Th17 cells along with an increase in the activity of Th17 cells, all of which lead to the severity of SLE disease.\textsuperscript{47}

**Mir-301a**

Mir-301a acts as an inhibitor of protein inhibitor of activated STAT3 (PIAS3) and is highly expressed in PBMCs and Th17 cells, increasing the number and function of Th17 cells through its inhibitory effects.\textsuperscript{48} Interestingly, IL-23 and IL-6 signaling pathway was obstructed with down-regulation of miR-301a by specific antagonir leading to a noticeable decline in phosphorylation of STAT3 and decreased the number of Th17 cells.\textsuperscript{49}

**Mir-425**

Mir-425 is a regulator known to be involved in inflammatory bowel diseases (IBD) such as crown’s disease and ulcerative colitis. This miRNA is overexpressed in Th17 cells and promotes the differentiation of T-helper cells towards Th17. The expression of this miRNA in Th17 cells was reported to be higher than that in Th-1, Th-2, and Treg cells. Several transcription factors such as Foxo1 have been proposed to be targeted by this miRNA. MiRNA-425 reduces the inhibitory effects of Foxo1 by reducing the expression of this factor, leading to the increased production of Th17 cells and IL-17 secretion in IBD.\textsuperscript{50}

**Mir-181c**

Mir-181c is one of the main miRNAs that highly involved in the regulation of Th17 cell function and the pathogenesis of EAE. It has been shown that Smad7 is an inhibitory factor in the TGF-β signaling pathway during the differentiation of Th17 cells. Functional knockout mir-181c reduces T cell sensitivity to TGF-β signaling and increases the expression of IL-2 and this cytokine is a negative regulator for Th17 cell differentiation and IL-17 production and as a result,
MicroRNAs as the Important Regulators of T Helper 17 Cells

Mir-206
Mir-206 is one of the miRNAs related to Th17 cells, the main offender behind the process of inflammation caused by dermatomyositis. This miRNA exerts its effect through targeting the transcription factor Kruppel like factor 4 (KLF4) which is involved in cell proliferation, malignancies, and tumor suppression. KLF4 is suppressed by miRNA-206 and reduces the number of Th17 cells. In dermatomyositis disease, the reduction in the expression of miRNA-206 augments the expression of KLF4 and the production and differentiation of Th17 cells, which is associated with the severity of the disease.

Mir-365 and Let-7a
Mir-365 and Let-7a are two important factors in Th17 cell function. IL-6 is an inflammatory cytokine with an important role in the differentiation of Th17 cells. This cytokine inhibits the differentiation of Treg cells by inhibiting TGF-β and stimulates the differentiation of Th17 by inducing the RORγt transcription factor. MiRNA-365 and Let-7a are involved in the negative regulation of IL-6 levels, while the latter is a more potent inhibitor compared with the former. These two miRNAs have been reported to ameliorate liver inflammation as a result of the low production of Th17 cells and IL-6 secretion in hepatitis. In addition, Let-7a inhibits the expression of the genes associated with IL-17, IL-21, and IL-23R, and is one of the main targets of the IL-6 signaling pathway with STAT3.

Mir-145
Mir-145 is an inhibitor of Th17 cell differentiation. CD28 (a costimulatory molecule associated with B7 molecule in the activation of T cells) and the nuclear factor of activated T-cells (NFAT) are two important factors in the activation and differentiation of various CD4+ cells such as Th17 cells. These cells also play a crucial role in the pathogenesis of myasthenia gravis (MG) disease. miRNA-145, an important miRNA, targets CD28 and NFAT at their 3’ UTR and inhibits the differentiation of Th17 cells. Mutation in this miRNA resulting in harmful responses during the progression of the disease and induces the differentiation of Th17 cells, causing the production of IL-17 which is associated with the severity of the MG.

Mir-448 and mir-20b
Mir-448 and mir-20b are frequently expressed in Th17 cells. MiRNA-448 has been shown to augment the production of IL-17A and the expression of RORγt. On the other hand, miRNA-448 increases the differentiation of Th17 cells through inhibiting Protein tyrosine phosphatase non-receptor 22 (PTPN22) with anti-inflammatory properties. IL-1β acts as a stimulator for the proliferation and differentiation of Th17 cells, increasing the expression of miRNA-448 by the NF-kB signaling pathway. In addition to miRNA-448 function, miRNA-20b plays a role in the differentiation of Th17 cells through inhibiting RORγt and STAT3. It has been shown that miRNA-448 and miRNA-20b have opposite effects in terms of regulating the function of Th17 cells.

Mir-16
Mir-16 is highly expressed in inflammatory diseases such as RA. The important roles of Th17 cells and their cytokines in inflammatory and autoimmune diseases have been confirmed. The expression of this miRNA is directly associated with the expression of RORγt, implying the high expression levels in Th17 cells. MiRNA-16 acts by targeting the 3’UTR of the TNF-α gene, a key inflammatory cytokine in RA, and positively regulates the signaling pathway of this cytokine. The improved production of TNF-α increases the differentiation of Th17 cells and activates the RORγt factor in this process.

Mir-223
Mir-223 can effectively control the differentiation and function of Th17 cells. The inhibition of this miRNA has been shown to increase the production of IL-6, leading to the differentiation of Th17 cells. One of the major targets of this miRNA is the RING finger and C3H zinc finger protein 1 (Roquin-1), that having a role in regulating cellular proliferation and in the production of Th17 cytokines. One study on this miRNA has shown that the deletion of miRNA-223 in myeloid dendritic cell (mDC) leads to the increase in Programmed death-ligand 1 (PD-L1) expression while the production of cytokines such as IL-1, IL-23, and IL-6 by Th17 cells is reduced and Th17 cells are inactivated.
Mir-495 and mir-1192

Indoles such as indole-3-carbinol (I3C), 3,3'-diindolylmethane (DIM), and the Aryl hydrocarbon receptor AhR (6-formylindolo carbazole (FICZ)) have effective antioxidant activities and can regulate cellular events through controlling the gene expression, cell cycle, and apoptosis. These factors are further capable of controlling the differentiation of Th17 cells. These indoles are AhR ligands that their activation regulates the differentiation of Th17 cells. I3C and DIM can reduce DTH response and disrupt Th17 differentiation, whereas FICZ induces DTH and increases Th17 differentiation. I3C and DIM indoles specifically decrease IL-17 secretion by increasing the expression of miRNA-495 and miRNA-1192, while FICZ indole represents the opposite effects and increases the expression of IL-17 through the miRNA mentioned above. The type of AhR ligand and the ligand-binding ability can result in different outcomes concerning controlling the differentiation of Th17 cells.59

Figure 5 Summarizes the interactions between important miRNA and their specific targets on Th17 cells function. Table 1 mentioned the role of each miRNA on the Th17 cell function.

In conclusion, it has been suggested that miRNA plays a crucial role in the regulation of the gene expression process and cell function. Therefore, their identification has paved a new way for a better understanding of the pathogenesis, control, treatment, and diagnosis of diseases. To the best of our knowledge, this is the first review article written about the function of miRNA in Th17 cells' performance and activation. In this review, we concluded that miRNA including mir-15b, mir-590, mir-873, mir-301a, mir-181c, mir-448, mir-16, and mir-425 can active Th17 cell, but miRNA such as mir-210,

Table. 1. Effects of miRNAs due to their targets on T helper (Th)-17 cell function

| MiRNA  | Target   | Effect on Th17 cells |
|--------|----------|----------------------|
| mir-155 | Jarid2   | Activator            |
| mir-155 | Socs1    | Activator            |
| mir-155 | Rorγt    | Activator            |
| mir-155 | Ets1     | Activator            |
| mir-155 | C-Maf    | Activator            |
| mir-155 | SHIP1    | Activator            |
| mir-21  | SMAD7    | Activator            |
| mir-21  | PTEN     | Inhibitor            |
| mir-21  | STAT3    | Inhibitor            |
| mir-15b | Rorγt    | Activator            |
| mir-590 | Tob-1    | Activator            |
| mir-590 | Rorc     | Activator            |
| mir-873 | Foxo1    | Activator            |
| mir-301a| PIAS3    | Activator            |
| mir-181c| SMAD2    | Activator            |
| mir-181c| SMAD3    | Activator            |
| mir-448 | NFκB     | Activator            |
| mir-16  | TNFa     | Activator            |
| mir-448 | PTPN22   | Activator            |
| Mir-445 | Foxo1    | Activator            |
| mir-20b | Rorγt    | Inhibitor            |
| mir-146 | IRAK1    | Inhibitor            |
| mir-146 | TRAF6    | Inhibitor            |
| mir-223 | Roquin1  | Inhibitor            |
| mir-206 | KLP4     | Inhibitor            |
| mir-365 | STAT3    | Inhibitor            |
| Let-7a  | STAT3    | Inhibitor            |
| mir-145 | NFAT     | Inhibitor            |
| mir-210 | HIF-1α   | Inhibitor            |
MicroRNAs as the Important Regulators of T Helper 17 Cells

Figure 5. Important miRNA and their specific targets roles on T helper (Th)-17 cells

mir-145, let-7a, mir-365, mir-206, mir-146, mir-20b, and mir-21 inhibit them. Moreover, mir-155 can activate the function of Th17 cells due to its targets mentioned in our article. When we study miRNA roles, it is important to know which factor or gene is targeted by one MiRNA, Since a miRNA has different properties due to different targets. According to this fact, further studies are needed to better understand the role and function of these agents.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to be mentioned

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