Review article

Altered expression of microRNAs and B lymphocytes during Natalizumab therapy in multiple sclerosis

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ABSTRACT

MicroRNAs (miRNAs) are a family of non-translated small ribonucleic acids (RNAs) measuring 21–25 nucleotides in length that play various roles in multiple sclerosis (MS). By regulating gene expression via either mediating translational repression or cleavage of the target RNA, miRNAs can alter the expression of transcripts in different cells, such as B lymphocytes, also known as B cells. They are crucial in the pathogenesis of MS; however, they have not been extensively studied during the treatment of some drugs such as natalizumab (NTZ). NTZ is a humanized immunoglobulin G4 antibody antagonist for integrin alpha 4 (α4) used in the treatment of MS. The drug reduces the homing of lymphocytes to inflammation sites. Integrin α4 expression on the cell surface of B cells is related to MS severity, indicating a critical component in the pathogenesis of the disease. NTZ plays an important role in modifying the gene expression in B cells and the levels of miRNAs in the treatment of MS. In this review, we have described changes in gene expression in B cells and the levels of miRNAs during NTZ therapy in MS and its relapse. Studies using the experimental autoimmune encephalomyelitis (EAE) model and those involving patients with MS have described changes in the levels of microRNAs in the regulation of proteins affected by specific miRNAs, gene expression in B cells, and certain functions of B cells as well as their sub-populations. Therefore, there is a possibility that some miRNAs could be studied at different stages of MS during NTZ treatment, and these specific miRNAs can be tested as markers of therapeutic response to this drug in future studies. Physiopathology, gene expression in B cells and their subpopulations can help understand this complex puzzle involving miRNAs and the therapeutic response of patients with MS.

1. Introduction

MicroRNAs (miRNAs or miR) are a family of non-translated small ribonucleic acids (RNAs) measuring 21–25 nucleotides in length that play different multifactorial roles in multiple sclerosis (MS). More than 2800 human mature miRNAs have been identified (Boxberger et al., 2019). Regulation of gene expression by either translational repression or cleavage of the target messenger RNA (mRNA) is the fundamental mechanism of miRNAs (Chen et al., 2013). miRNAs regulate approximately 90% of the protein-coding genes. These molecules play central roles in biological processes, such as immune cell lineage commitment, differentiation, proliferation, apoptosis, and maintenance of immune homeostasis. Its biogenesis begins when RNA polymerase II transcribes primary miRNA transcripts. miRNAs mature following a two-step process involving two enzymes with ribonuclease III activity, Drosha and Dicer (Madadi et al., 2019).

A cluster is a set of two or more miRNAs (Piket et al., 2019). miRNAs are secreted in extracellular fluids and transported to target cells by binding to proteins, such as argonautes, or via vesicles, such as exosomes. Extracellular miRNAs are important chemical messengers that mediate intercellular communication and are protected when transported to vesicles or proteins, which increases their stability in the extracellular milieu (O'Brien et al., 2018; Hannafon et al., 2015). Since its discovery, the accurate determination of miRNA expression levels is a prerequisite

for using small non-coding RNA molecules as novel biomarkers for disease diagnosis and prognosis. Quantitative Polymerase Chain Reaction (PCR) is the method of choice for measuring these expression levels of microRNAs (Madadi et al., 2019; Liguori et al., 2017). Like other small molecules and even neuropeptides, miRNAs may promote or restrict inflammatory signaling and exacerbate neuroinflammation (Chen et al., 2016). Neuroinflammation in this context includes MS, Alzheimer's disease, Parkinson's disease, prion disease, herpes encephalitis, ischemic stroke, and traumatic brain injury. Therefore, interest in using altered miRNA signatures as biomarkers for these disorders has increased exponentially (Slota and Booth, 2019).

Some diseases, such as MS, can generate miRNA profile dysfunctions through gene expression in the central nervous system (CNS) and the immune system (Chen et al., 2016). In MS, the myelin sheath surrounding nerve fibers of the CNS is damaged through a demyelinating inflammatory process. The damage disrupts communication between the brain and the rest of the body, leading to signs and symptoms that vary depending on the affected part of the CNS, including motor, sensory, visual, and autonomic dysfunctions. Hence, phenotypes and the pathogenic involvement in MS can vary between patients (Juźwik et al., 2018).

B lymphocytes (also known as B cells) in patients with MS are usually characterized by the expression of co-stimulatory molecules, which has also been demonstrated in the experimental autoimmune encephalomyelitis (EAE) model (Arneth, 2019). B cells present antigens and interact with T cells, which can facilitate the development of MS. They are also abundant producers of pro-inflammatory (such as interferon-gamma) and regulatory (such as interleukin 10 or IL-10) cytokines. In addition, B cells can form ectopic lymphoid structures or germinal centers, as observed in MS (Serafini et al., 2004; Juźwik et al., 2018). B cells can affect MS development and progression by targeting auto-antigens and regulating other immune cells that affect inflammatory responses (Kowarik et al., 2012). Moreover, humoral antibodies lead to tissue injury when they bind to brain cells and interfere with complement factor functions. Moreover, B cells can deplete anti-CD20 antibodies, causing MS relapse and further neurological deficiencies (Arneth, 2019). Thus, the possibility that the number of exosome miRNAs is increased in MS due to their ability to cross the blood-brain barrier (BBB) and be produced by injured cells reinforces that these small molecules could be analyzed as biomarkers of MS progression.

Moreover, the profile of different therapies is crucial in understanding MS. Since T and B cells as well as cytokines play essential roles in the pathogenesis of MS, immune therapies targeting them can achieve better treatment outcomes. Natalizumab (NTZ) is an important immunosuppressive drug (Jagot and Davoust, 2017). It is a humanized monoclonal immunoglobulin (Ig)-type G4 antibody antagonist for integrin alpha 4 (α4) used in the treatment of MS (Zintzaras et al., 2012). It acts by reducing the homing of lymphocytes to the sites of inflammation (Kappos et al., 2011). Disease activity in relapsing-remitting MS (RRMS) can be reduced with monthly infusions of NTZ (Zare-Shahabadi et al., 2013; Singer, 2017). The α4 molecule, when activated by B cells, increases the BBB permeability, which accounts for more significant cell migration, neuroinflammation, and the appearance of clinical symptoms. NTZ treatment can reverse the development of such symptoms mainly by decreasing cellular migration into the CNS, thus preventing neuroinflammation (Bargiela et al., 2017).

The relationship between the presence of B cells and plasma cells and Igs in the cerebrospinal fluid (CSF) and CNS tissues of patients with MS has been described in several studies (Arneth, 2019; Van Kaer et al., 2019). B cells are involved in antigen capture and presentation to T cells (Racke, 2008), cytokine production, antibody secretion, formation of tertiary lymphoid follicles in the CNS, demyelination, tissue damage, and remyelination (Serafini et al., 2004). The expression of integrin α4 on the surface of B cells corroborates with MS severity, indicating an essential component in the pathogenesis of the disease. Transcriptional expression studies have demonstrated that NTZ affects B cell activation and differentiation (Zare-Shahabadi et al., 2013). Nevertheless, few studies have

evaluated the relationship between changes in miRNA levels and B cells (including their functions and subpopulations). In addition, few studies have been conducted on the correlation between these factors and NTZ treatment.

Since miRNAs are not robustly used in clinical practice and there is a need for quick reliable diagnosis and early effective therapy for MS, the study of miRNA expression as possible biomarkers and their correlation with B cells is of substantial clinical interest in MS. Considering the efficacy of NTZ and its relationship with critical immune components in patients with MS, this study aimed to analyze changes in the levels of miRNA and B cell subpopulations during NTZ therapy in patients with MS, providing a basis for understanding the implications, importance of further delineating the disease mechanisms, and relevance for the search of biomarkers.

2. Materials and methods

This study analyzed the roles of miRNAs and B cells in NTZ therapy in the context of MS. We searched for published studies in the Medical Subject Headings (MeSH)/PubMed and SciELO/LILACS database from inception to January 2021. We used the following keywords: "Multiple Sclerosis," "MicroRNAs," "B-lymphocytes," "Experimental Autoimmune Encephalomyelitis," and "Natalizumab" (MeSH terms).

In this review article, all quantitative and qualitative studies evaluating the relationship between miRNA levels and B cells in MS were included. The function of B cells and the expression of their subpopulations were investigated and described. Preclinical and clinical studies were also analyzed, but case reports, theses, and conference summaries were excluded.

3. Results and discussion

3.1. MiRNAs and NTZ treatment in MS

miRNAs within serum exosomes can be used as biomarkers for MS is because in many inflammatory diseases, there is a significant increase in the circulating exosome concentration (Slota and Booth, 2019). Considering that exosomes reach and cross the BBB, they may originate from affected CNS cells in patients with MS. Therefore, clinical dysfunctions of MS can generate different levels of serum miRNAs. Serum exosome miRNA profiles can distinguish patients with MS from healthy controls and distinguish RRMS from progressive forms with high accuracy (Ebrahimkhani et al., 2017).

One example is the miRNA, hsa-miR-145, which can differentiate disease status with a specificity of 89.5%, sensitivity of 90.0%, and accuracy of 89.7%. Assessment of the 48-miRNA panel increased the diagnostic power to 96.3% (Keller et al., 2009). In addition to human studies, preclinical studies using the EAE model support possible new targets for miRNAs to be studied in MS (Juźwik et al., 2018). The EAE model allows the evaluation of different cells, such as lymphocytes, in the CNS tissue. The aggregation of B cells in the CNS and their expression during treatment with monoclonal antibodies in MS is yet unclear (Bell et al., 2019).

NTZ is administered as a monotherapy in adults with highly active RRMS. It is generally recommended for patients with insufficient response or those who cannot tolerate other MS-related therapies (Alroughani et al., 2019). In observational studies, approximately 60% NTZ-treated patients were free from disease activity (Prosperini et al., 2012; Sellner and Rommer, 2019). In the AFFIRM trial, NTZ treatment for 2 years, reduced the annualized relapse rate (ARR) by 68% (p < 0.001) and disability progression rate by 42% (p < 0.001) compared with that of placebo in patients with RRMS. It also improved the outcomes seen on magnetic resonance imaging (MRI) compared to those of placebo (Alroughani et al., 2019). The results of the SENTINEL trial support these data. The AFFIRM and SENTINEL trials have shown that NTZ, as a monotherapy or in combination with interferon beta-1a, significantly

reduced ARR, disability progression rate, and accumulation of new or enlarging lesions on MRI compared with placebo (Polman et al., 2006; Rudick et al., 2006).

NTZ was designed to bind to β1 and β7 subunits of integrin α4 on T cells and prevent interactions with their endothelial receptors (vascular cell adhesion molecule 1 and addressin-cell adhesion molecule 1) (Zare-Shahabadi et al., 2013). The migration of activated T cells across the BBB into the CNS is one of the key steps in the pathogenesis of MS. Activated T cells express integrin α4, which promotes migration across the BBB and initiates neuroinflammation. T cells onto the brain parenchyma and neuroinflammation are suppressed after NTZ therapy (Zare-Shahabadi et al., 2013). This phenomenon is reflected by a decrease in ARR, disability progression rate and a reduction in the number of neuroinflammatory lesions, as depicted by MRI (Alroughani et al., 2019).

However, adverse effects are known to occur following NTZ treatment. The development of powerful immunomodulatory drugs for the treatment of MS has led to a new population of progressive multifocal leukoencephalopathy (PML)-susceptible individuals (Kartau et al., 2019). The prevalence of PML worldwide is often estimated to be approximately two cases per 100,000 individuals, although this number varies by population (Kartau et al., 2019). Moreover, this group has historically been smaller than those affected by the human immunodeficiency virus (HIV) or cancers that affect the immune system. An expansion of immunosuppressive drugs in the last 15 years has led to a rapid increase in individuals affected by autoimmune treatment associated with PML. NTZ still poses a significant risk for the development of PML, depending on patient history as well as seropositivity for John Cunningham (JC) polyomavirus (JCPyV or JCV) (Bloomgren et al., 2012).

Once activated, JCPyV causes lytic infection in oligodendrocytes and astrocytes in the CNS, affecting neuronal stability (Berger et al., 2013). The infection of oligodendrocytes leads to extensive demyelination. As a result, the physical symptoms of PML are diverse and can include motor dysfunction, visual defects, and speech impairment (Ferenczy et al., 2012). Some individuals with PML may also develop seizures (Miskin et al., 2015). Interestingly, viral miRNAs are expressed inside infected cells and are delivered outside (Pegtel et al., 2010). Thus, viral miRNAs have been detected in the sera of patients with such infections (Jiang et al., 2018a, b). Like cellular miRNAs, they bind to host mRNAs and affect their translation (Kincaid and Sullivan, 2012). The JCPyV genome encodes a miRNA in the region that expresses a large T antigen (Takahashi et al., 2020).

JCPyV-encoded miRNA (miR-J1) has been detected in tissue and CSF samples from patients with PML (Seo et al., 2008). High miR-J1 expression was detected in the nuclei of JCPyV-infected cells in PML tissue samples via *in situ* hybridization (Takahashi et al., 2020). *In situ* hybridization for miR-J1-5p and -3p showed positive signals in 24/25 (96%) PML tissues positive for JCPyV by immunohistochemistry. Higher copy numbers of miR-J1 were detected in PML tissues than in non-PML tissues through real-time (RT) reverse transcription PCR (Takahashi et al., 2020). Moreover, the deletion or mutation of miR-J1 in recombinant JCPyV promoted the production of JCPyV-encoded proteins in cells transfected with JCPyV DNA, suggesting that polyomavirus-encoded miRNA may have a repressive role in viral replication in PML tissues (Takahashi et al., 2020). Even without directly correlating PML with NTZ in this study, *in situ* hybridization for viral miRNA may be a useful diagnostic tool for PML. It is not yet possible to state that this diagnostic tool will be effective in the specific context of PML induced by NTZ for MS treatment.

Considering that this drug was developed to act on T cells, an unexpected discovery was the demonstration of NTZ effects on B cells via transcriptional expression methods. The central point for the study of B cells in NTZ treatment is the overexpression of integrin α4 (Zare-Shahabadi et al., 2013; Lee-Chang et al., 2011). B cells are crucial in MS pathophysiology, and NTZ alters the expression of these cells, justifying the interest of studies in this scenario.

3.2. Correlation of NTZ therapy with B cells and miRNAs in MS

It is expected that the onset of MS is accompanied by alterations in the patterns of miRNAs, especially of B cells (Zare-Shahabadi et al., 2013; Sievers et al., 2012). miRNAs involved in B cell activation and differentiation (miR-106b-25 and miR-17-92) presented normalized in patients receiving NTZ therapy compared with those in untreated patients with MS. NTZ also increased circulating pre-B and B cells (Zare-Shahabadi et al., 2013). The mechanism of how this gain in circulating B cells helps in MS treatment is unclear (Zare-Shahabadi et al., 2013). Therefore, miR-106b-25 and miR-17-92 may play an important role in the evaluation of NTZ therapy, considering that they remained normalized compared to those in patients with MS who were not treated with the drug. Therefore, the role of these miRNAs in B cells activation and differentiation and the fact that NTZ has a broad action on these lymphocytes, acting antagonistically on alpha-4 integrin molecules, reinforce the possible relationship of miRNAs as possible biomarkers in MS (Zare-Shahabadi et al., 2013).

The expression levels of 1059 miRNAs were evaluated in B cells from untreated and NTZ-treated patients with RRMS and healthy volunteers (HV); 49 miRNAs were downregulated in untreated patients with MS compared to that of HV. A distinct pattern of 10 differentially expressed miRNAs was found in NTZ-treated patients compared to that of untreated patients. MiR-106b-25 and miR-17-92 levels were upregulated (Sievers et al., 2012). The miRNA/RNA interaction analysis revealed the most affected pathways to be the phosphoinositide 3-kinase (PI3-kinase), phosphatase and tensin homolog, and B-cell receptor (BCR) (Sievers et al., 2012). BCR induces the activation of PI3-kinase via the B cells antigen CD19 surface molecule during the transition from the pro-B-cell stage to the plasmablast stage. PI3-kinase activity is required for B-cell biology during the T cell-dependent immune response. The levels of these two miRNAs were downregulated in patients with MS after NTZ therapy (Sievers et al., 2012).

MiR-150 levels were increased in patients with MS compared to those with HV. miR-150 acts on the control of mature B cells by blocking the transcription factor, c-MYB, and increasing IgG index and the presence of oligoclonal bands. This miRNA was then compared with other candidate protein biomarkers, such as C-X-C motif chemokine 13, matrix metallopeptidase 9 (MMP-9), and osteopontin in patients with MS (Bergman et al., 2016). The profiling of miRNA levels was performed using TaqMan miRNA arrays from CSF of pooled patients with the clinically isolated syndrome (CIS), patients with RRMS, and HV with inflammatory and non-inflammatory neurological disease. It has shown the detection of 88 CSF miRNAs in an exploratory cohort. Subsequent validation in two other cohorts demonstrated higher miR-150 levels in patients with MS. Higher miR-150 levels were also observed in patients with CIS who converted to MS compared to non-converters and patients initially receiving NTZ treatment. Moreover, NTZ reduced miR-150 levels in the CSF, with a concurrent increase in plasma miR-150 levels. This change suggests that immune cells are a source of miR-150 and that their levels in CSF could be a novel candidate biomarker for NTZ therapy in MS (Bergman et al., 2016).

Evaluating B cells in peripheral blood samples from patients with RRMS, after 6 months of NTZ treatment, an increase in miR-150 expression level in the plasma was observed (Blume et al., 2018). Other miRNAs may also exhibit this pattern. miR-19b, miR-106b, miR-142-5p, miR-191, miR-383, miR-551a, and miR-598 were upregulated, while miR-15a and miR-15b were downregulated in B cells. miR-191 is a positive modulator of the transcriptional module comprising the transcription factors FOXP1 and EGR1 (Blume et al., 2018). Deletion and ectopic expression of miR-191 resulted in a developmental arrest in B-lineage cells. This indicates that fine-tuning of the combined expression levels of the miRNA factors, which in turn control B cell somatic recombination and cytokine-driven expansion, constitutes a prerequisite for efficient B cell development (Blume et al., 2018).

Table 1. Summary of the main miRNAs, their functions or pathways of action, their measurement method, testing period with NTZ, and differences in their expression.

Standards and functions of each miRNA

Name	Action	Measure Method	Citations of miRNAs in our review	Profile before NTZ treatment	Profile after NTZ treatment	Treatment time
miR-15a	Increases antibody production (Yuan et al., 2012).	Plasma was analyzed for miRNA level.	1. Ma et al. (2014)	Downregulated	Not analyzed	Not analyzed
miR-15b	Prevents cancer cell transformation by controlling oncogenes such as the insulin-like growth factor receptor 1 gene (Lovat et al., 2015).	Quantitative RT-PCR (qRT-PCR) and Northern blotting	1. Ma et al. (2014)	Downregulated	Not analyzed	Not analyzed
miR-17-92 cluster	A critical regulator of B-cell central tolerance at the immature B-cell stage (Dolati et al., 2018).	Not informed	1. Dolati et al. (2018)	Downregulated	Not analyzed	Not analyzed
miR-18a	Decreases cell proliferation by blocking activated protein kinase B (AKT) and extracellular-regulated kinase (ERK) pathways (Li et al., 2017).	Flow cytometry analysis	1. Ingwersen et al. (2014)	Downregulated	Upregulated	12 months
miR-19b	Maintains tolerance by controlling phosphatase and tensin homolog (PTEN) levels (Lai et al., 2016).	Northern blot analysis of miRNA and flow cytometry plots of lymphocytes from the spleen	1. Dolati et al. (2018) 2. Ma et al. (2014)	Upregulated Downregulated *(Ma X)*	Downregulated *(Dolati* et al.*)* Upregulated *(Ma* et al.*)*	6 months
miR-20b	Induces cell proliferation upon binding to PTEN (Zhou et al., 2014).	miRNA qRT-PCR	1. Ingwersen et al. (2014)	Downregulated	Upregulated	12 months
miR-29a	Prevents cancer cell transformation by controlling T-cell leukemia/lymphoma protein 1 oncogene (Pekarsky et al., 2006).	RT-PCR	1. Ingwersen et al. (2014)	Downregulated	Upregulated	12 months
miR-103	It associates with increased antibody titer by binding to nectins (Haralambieva et al., 2018).	Negative binomial generalized estimating equation (GEE) models were used for miRNA assessment, and the DIANA tool was used for gene/target prediction and pathway enrichment analysis	1. Ingwersen et al. (2014)	Downregulated	Upregulated	12 months
miR-106b	Increases cell proliferation by decreasing the expression of anti-proliferation factor 3 (BTG3) (Wei et al., 2017).	qRT-PCR	1. Dolati et al. (2018) 2. Ma et al. (2014)	Upregulated	Downregulated *(Dolati* et al.*)* Upregulated *(Ma* et al.*)*	6 months
miR-125a-5p cluster	Maintains BBB constriction by mobilizing endothelial cells/astrocytes and reducing intercellular adhesion molecule 1 expression (Reijerkerk et al., 2013).	Quantitative PCR (qPCR) analysis of miRNA expression	1. Dolati et al. (2018) 2. Ma et al. (2014)	Upregulated Downregulated (Ma X.)	Downregulated Not analyzed by *Ma* et al.	6 months
miR-132	Decreases production of cytokines such as TNF-a and lymphotoxin (Dolati et al., 2018).	Not informed	1. Dolati et al. (2018) 2. Miyazaki et al. (2014)	Upregulated	Further studies needed	Not informed
miR-142-5p cluster	Maintains B cell homeostasis by binding to B cell-activating factor-R. Its inhibition results in exacerbated proliferation (Zheng et al., 2018).	Flow cytometry analysis	1. Dolati et al. (2018) 2. Ma et al. (2014)	Downregulated	Upregulated	6 months
miR-150	Controls proliferation and differentiation of lymphocytes by decreasing expression of c-MYB (Xiao et al., 2016).	Expression profiling and northern blot analysis	1. Bergman et al. (2016) 2. Dolati et al. (2018) 3. Ma et al. (2014)	Upregulated	Reduced levels in the CSF with concurrently increased levels in the plasma *(Bergman* et al.*)* Downregulated *(Dolati* et al.*)* Upregulated *(Ma* et al.*)*	6 months
miR-181a	Increases apoptosis upon binding to B cell lymphoma protein 2 and induces myeloid leukemia cell differentiation protein (Ouyang et al., 2012).	qRT-PCR and immunoblotting	1. Sievers et al. (2012) 2. Dolati et al. (2018)	Upregulated	No difference	12 months
miR-191	Regulates B cell development, by acting on cell expansion and somatic recombination acts directly on the complex Forkhead box protein P1 and early growth response protein 1 (Blume et al., 2018).	qRT-PCR	1. Dolati et al. (2018) 2. Ma et al. (2014)	Downregulated	Upregulated	6 months
miR-320a	Contributes to increased BBB permeability and neurological disability (Dolati et al., 2018).	Not informed	1. Aung et al. (2015) 2. Dolati et al. (2018)	Downregulated	Not informed	Not informed

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Table 1 (*continued*)

Standards and functions of each miRNA

Name	Action	Measure Method	Citations of miRNAs in our review	Profile before NTZ treatment	Profile after NTZ treatment	Treatment time
miR-326	Induces differentiation for plasmablasts and increases antibody production by blocking ETS-1 protein (Xia et al., 2018).	RT-PCR	1. Ingwersen et al. (2014) 2. Ma et al. (2014)	Upregulated (Ingwersen J) Upregulated (Ma X.)	Downregulated It was not analyzed by Ma et al.	12 months
miR-383	It is related to increased proliferation and survival of lymphocytes, mainly by maintaining APRIL expression (Cui et al., 2017).	qRT-PCR	1. Dolati et al. (2018) 2. Ma et al. (2014)		Downregulated (*Dolati* et al.) Upregulated (*Ma* et al.)	6 months
miR-551a	Decreases cellular proliferation by blocking focal adhesion kinase (Anuj et al., 2019).	qPCR and immunoblotting	1. Dolati et al. (2018) 2. Ma et al. (2014)	Downregulated	Upregulated	6 months
miR-598	Acts in the suppression of proliferation. Its decrease results in the development and metastasis of several cancers, mainly by controlling IGF (Liu et al., 2018).	qRT-PCR	1. Dolati et al. (2018) 2. Ma et al. (2014)	Downregulated	Upregulated	6 months
miR-642	Decreases cell proliferation by blocking deoxyhypusine hydroxylase (Epis et al., 2012).	qRT-PCR	1. Dolati et al. (2018)	Downregulated	Upregulated	6 months
miR-let-7c	Decreases activation of B cells by decreasing the capacity of nutrient absorption by blocking c-MYC (Jiang et al., 2018a, b).	RT-PCR and Western Blot analysis	1. Dolati et al. (2018) 2. Ma et al. (2014)	Upregulated Not analyzed by *Ma X.*	Downregulated	6 months

Another important miRNA is the miR-142-5p (Ma et al., 2014). This molecule is suppressed in patients with MS (Ma et al., 2014), and its function is to maintain B cell homeostasis through the B-cell-activating factor-R (Zheng et al., 2018). After 6 months of NTZ treatment, their expression was also increased in patients with MS, which reduced the exacerbated proliferation of these cells (Ma et al., 2014). The most upregulated miRNAs were involved in maturation control (miR-19b and miR-191) or proliferation (miR-106b, miR-142-5p, miR-383, miR-551a, and miR-598) of B cells in this scenario. This indicates that NTZ treatment prevents the transmigration of these cells into the CNS and returns the expression of the regulators of cellular activities to normal levels (Ma et al., 2014). Other miRNAs that are involved in B-cell development are the miR-150, miR-180, and miR-17-92 clusters. The miR-17-92 cluster showed increased levels of activated B cells. The actions of miRNAs in this pathway remain unclear (Kacperska et al., 2016). We hypothesized that NTZ had an effect on the levels of these miRNAs, and these molecules may be analyzed as therapeutic biomarkers for the drug's response in the future.

Although these data demonstrate an active role of NTZ in the miRNA profile, their expression does not necessarily remain homogenous in all cases. Another study showed a decline in the expression of miR-19b, miR-106b, miR-142-5p, miR-150, miR-191, miR-383, miR-551a, and miR-598 in B cells of patients with RRMS following NTZ treatment (Dolati et al., 2018). Such differences may be related to the effect of NTZ on the immune cells. There is a clinical variability among individuals over time. The percentage of B cells varies from 10% to 40% of the total lymphocytes in treated patients (Dolati et al., 2018). There may be variability in the expression of miRNA over time at the individual level in NTZ-treated patients. This might be due to a fluctuation in the clinical response to the treatment, showing the importance of evaluating MS activity during treatment (Sievers et al., 2012).

Corroborating the importance of some miRNAs after an increase in B cells' proinflammatory response, increased secretion of lymphotoxin and tumor necrosis factor α (TNF-α) by B cells is associated with increased expression of miR-132 in patients with MS (Miyazaki et al., 2014). This mechanism is associated with the ability of miR-132 to suppress sirtuin 1 (SIRT1). This enzyme downregulates the transcription factor nuclear kappa B (TNF-κB) (Kauppinen et al., 2013). Through pharmacological blockade, the authors revealed that the inhibition of SIRT1 in normal B cells induced exaggerated lymphotoxin and TNF-α production. This suggests a novel function of miR-132, which could be explored for new possibilities in future treatments (Miyazaki et al., 2014).

Another critical molecule in the pathological function of B cells in MS is MMP-9 (Aung et al., 2015). This molecule maintains BBB permeability and reduces disruption and leukocyte infiltration. An increase in MMP-9 levels in B cells can be observed during disease relapse. At the same time point, MMP-9 expression was related to decreased miR-320a expression. These findings were demonstrated by transfecting human B cells from HV with a miR-320a inhibitor, which led to increased MMP-9 expression and secretion, assuming the same profile as MS pathological B cells (Aung et al., 2015).

Compared to HV, miR-181c was differentially regulated in patients with MS (Haghikia et al., 2012). The CSF of 53 patients with MS and 39 patients with other neurological diseases were analyzed. First, the global miRNA profile was screened for the reported miRNAs, followed by quantitative reverse transcriptase PCR to validate candidate miRNAs (Haghikia et al., 2012). With this quantitative analysis of CSF samples, the study found that miRNA 181c levels were downregulated in patients with untreated MS and were able to differentiate RRMS of secondary progressive MS (SPMS) courses with a specificity of up to 82% and a sensitivity of 69%. Therefore, a statistical analysis was conducted based on the combination of candidate miRNAs in a diagnostic test that resulted in the specificity and sensitivity values. The analyses showed significantly downregulated miR-181c levels in SPMS compared to those in RRMS (Haghikia et al., 2012). miR-181c is a potential candidate to be

evaluated in the EAE model and patients with MS during NTZ therapy in the future.

Other miRNAs are also possible candidates for evaluating the clinical alterations in patients with MS. The miRNAs miR-18a, miR-20b, miR-29a, miR-103, and miR-326 are the main possible biomarkers (Ingwersen et al., 2014). In a translational study, the EAE model and RRMS patients were analyzed during a longitudinal follow-up analysis of 1-year continuous NTZ therapy. The neuroinflammation process was accompanied by the downregulation of miR-18a, miR-20b, miR-29a, and miR-103. These miRNAs were responsible for the control of cell cycle progression, angiogenesis, and the transforming growth factor-beta pathway (Ingwersen et al., 2014). We highlighted miR-29a since this particular miRNA blocked the TCL1 oncogene, which is an important factor in the activation of B cells (Pekarsky et al., 2006). It was found to be downregulated in patients with MS. After 12 months of NTZ treatment, the drug improved its pattern by increasing its expression (Ingwersen et al., 2014).

MiR-326 is related to T helper 17 lymphocyte development and is upregulated in patients with MS and EAE models (Ingwersen et al., 2014). This miRNA induced the differentiation of plasmablasts and increased antibody production by blocking the ETS-1 protein (Xia et al., 2018). These changes can be directly correlated with clinical worsening in patients with MS. Interestingly, 1-year NTZ treatment reversed this profile, making it more similar to the HV pattern (Ingwersen et al., 2014). Observing such changes with more prolonged treatment periods clarifies the need to better explore the role of miRNAs in the clinical course of MS to verify the efficacy of the treatment and predilection of possible complications (Ingwersen et al., 2014). The main miRNAs, their functions or pathways of action, testing period with NTZ, and differences in expression are summarized in Table 1.

4. Conclusion

MS is a complex disease in which the cause is known to vary and not fully understood. It is also known that patients with MS have different expression levels of miRNAs and B cells in relation to proliferation, aggregation, and serum levels compared to HV. NTZ plays an important role in modifying B cell expression of miRNAs as well as genes in MS, as demonstrated in several preclinical and clinical studies. The role of miRNAs in MS involves modulating immune responses in the peripheral immune compartment and neuroinflammatory processes in the brain. Among all the miRNAs studied, miR-150 appears to be a potent marker of MS. The levels of this miRNA were found decreased in the CSF with a simultaneous increase in plasma miR-150 levels, suggesting that it may be a new candidate biomarker for NTZ therapy in MS.

miRNAs are obtained from the peripheral blood, but they can also be obtained from the CSF. Obtaining miRNAs from the CSF is not preferred because an invasive lumbar puncture procedure is needed and many centers in the world cannot evaluate miRNAs in the CSF. Therefore, some of the barriers to the use of miRNAs in clinical practice are the financial costs, availability, and difficulty of serial examinations via lumbar puncture. Another major challenge is the standardization of miRNA in general as a biomarker for diagnosis, therapeutic response, and prognosis since there are few clinical cohort studies to define these parameters in humans. Medicine will evolve and answer many of these questions through epigenetic studies and robust randomized controlled trials evaluating the correlation between miRNAs and their plasma or CSF levels with clinical outcomes (such as outbreaks of MS and disease progression with a clinical scale control) and/or neuroimaging tests).

We understand that there may be limiting factors regarding the clinical use of miRNAs, especially in underdeveloped countries where resources are scarce. However, as with some neoplasms (such as colon cancer, prostate cancer, and breast cancer) and other autoimmune diseases, the gene expression in B cells and their subpopulations can help understand this complex puzzle involving miRNAs and the response therapy of patients with MS. This article shows the correlation between

deregulated miRNAs during treatment with NTZ. We strongly suggest and encourage further clinical studies to better define the role of miRNAs in disease risk assessment, disease progression monitoring, and therapeutic responses to disease. The study of these molecules may help outline the molecular mechanisms of miRNAs in the pathogenesis of MS in the future.

Declarations

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