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The role of non-coding RNAs in neuroinflammatory process in multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a central nervous system chronic neuroinflammatory disease followed by neurodegeneration. The diagnosis is based on clinical presentation, cerebrospinal fluid testing and magnetic resonance imagining. There is still a lack of a diagnostic blood-based biomarker for MS. Due to the cost and difficulty of diagnosis, new and more easily accessible methods are being sought. New biomarkers should also allow for early diagnosis. Additionally, the treatment of MS should lead to the personalization of the therapy. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) as well as their target genes participate in pathophysiology processes in MS. Although the detailed mechanism of action of non-coding RNAs (ncRNAs, including miRNAs and lncRNAs) on neuroinflammation in MS has not been fully explained, several studies were conducted aiming to analyse their impact in MS. In this article, we review up-to-date knowledge on the latest research concerning the ncRNAs in MS and evaluate their role in neuroinflammation. We also point out the most promising ncRNAs which may be promising in MS as diagnostic and prognostic biomarkers.

Keywords microRNA · lncRNA · miRNA · lincRNA · Long intergenic non-coding RNAs · Novel biomarker

Abbreviations

ALS	Amyotrophic lateral sclerosis
APC	Antigen-presenting cells
BBB	Blood-brain barrier
BDMCs	Bone marrow-derived dendritic cells
BMDMs	Bone marrow-derived macrophages
CCL2	C-C Motif Chemokine Ligand 2
CCR6	C-C Chemokine receptor 6
CDMS	Clinically definite MS
CIS	Clinically isolated syndrome
CNS	Central nervous system
CSF	Cerebrospinal fluid
CXCL1	C-X-C Motif Chemokine Ligand 1
CXCL10	C-X-C Motif Chemokine Ligand 10
CXCL13	C-X-C Motif chemokine 13
dCLNs	Deep cervical lymph nodes
DA	Dark Agouti
DCs	Dendritic cells
EAE	Experimental autoimmune encephalomyelitis
EVs	Extracellular vesicles
FACS	Fluorescence-activated Cell Sorting
ECM	Extracellular matrix
SELE	E-selectin
GM-CSF	Granulocyte Macrophage Colony- Stimulating Factor
IgG	Immunoglobulin G
IL	Interleukin
KO	Knockout
LPS	Lipopolysaccharide
LincRNA	Long intergenic non-coding RNA
LncRNA	Long non-coding RNA
miR, miRNA	MicroRNA

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MALAT1	Metastasis associated lung adenocarcinoma transcript 1
MMP9	Matrix metalloproteinase 9
MOG	Myelin oligodendrocyte glycoprotein
MRC1	Mannose Receptor C-Type 1
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSCs	Mesenchymal stem cells
n	Number
ncRNAs	non-coding RNAs
OPN	Osteopontin
PBMC	Peripheral blood mononuclear cells
p.i.	Post-immunization
PPMS	Primary progressive MS
PVG	Piebald Virol Glaxo
RIS	Radiologically isolated syndrome
ROS	Reactive oxygen species
RRMS	Relapsing-remitting MS
Smad7	Suppressor of mothers against decapentaplegic 7
SOCS	Suppressor of cytokine signalling proteins
Th17	T helper 17
TNFα	Tumour necrosis factor α
WT	Wild type

Introduction

Multiple sclerosis (MS) is a neurodegeneration disease in central nervous system (CNS) with chronic inflammatory immune-mediated demyelination. Inflammation, lesion formation, and blood brain barrier (BBB) impairment stand as three main components in MS pathogenesis. McDonald criteria are used for diagnosis of MS including clinical presentation, magnetic resonance imagining (MRI), and cerebrospinal fluid (CSF) testing [1]. There is currently a demand for and lack of a diagnostic blood-based biomarker for MS. Furthermore, MRI, the primary imaging technique, is all-consuming financially as well as time-dependent. Essentially, non-invasive/blood-based biomarkers are essential for a more accurate evaluation of the MS patient.

MicroRNAs (miRNAs) play a role in gene transcription repression. Elevated or reduced miRNA expression can have either inflammatory or protective/anti-inflammatory effects through influence on the immune response, BBB stability, production of cytokines, and reactive oxygen species (ROS). Various chronic inflammatory diseases, including diabetes, ischemic stroke, and MS, depicted the dysregulation of miRNAs [2–12]. However, the exact mechanism of the impact of miRNAs on neuroinflammation in MS remains largely unknown.

Long non-coding RNAs (lncRNAs) modulate diverse cellular processes at epigenetic, transcriptional, and post-transcriptional levels and manage protein activity. As non-coding molecules, they regulate several biological processes, including cell proliferation, migration, and cytokine release. Some degenerative diseases, including MS, recognized their alteration in abundance [5, 9, 13, 14].

As many studies showed the importance of both miRNAs and lncRNAs as potential biomarkers in MS, herein, we present up-to-date knowledge on the application of miRNAs and lncRNAs in the MS neuroinflammatory process [15–18]. We also indicate the most promising non-coding RNAs which can be clinically valuable to diagnose MS.

MiRNAs as diagnostic/prognostic biomarkers in neuroinflammation process of MS

A key pathological feature of MS is inflammation that is responsible for demyelination and axonal loss. The indispensable elements of the underlying pathomechanism are inflammatory Th1 and Th17 cells that can easily cross the BBB and initiate the inflammation process in CNS [19]. The autoreactive T-cells mediate the macrophage's recruitment leading to myelin and oligodendrocyte destruction [19, 20]. B cells as antigen-presenting cells (APC) activate CNS-infiltrating T cells, contributing to increased production of pro-inflammatory cytokines. Additionally, their ability to differentiate into memory cells and produce antibodies adds to their involvement in the pathogenesis of MS [21]. Those observations are consistent with the result of studies indicating the effectiveness of the anti-CD20 monoclonal antibodies therapy [22]. Neuroinflammation seems to be responsible for neurodegeneration in all stages of MS. It is responsible for the new lesions in the early stage of the disease, and the process decreases at the later stages [23]. Nowadays, there are attempts to improve the MS treatment and prognosis by introducing anti-inflammatory drugs and inflammatory biomarkers, respectively [24].

Studies described the role of miRNAs in in vitro and animal models of MS

In experimental autoimmune encephalomyelitis (EAE)—animal model of MS, Ghorbani et al. [25] aimed to specify the role of miR-181 isoforms. Transfection of primary macrophages and CD4 + cells with miR-181a and miR-181b mimics resulted in downregulation of *TNF-α* and *IL-6,* favouring anti-inflammatory (M2) over pro-inflammatory (M1) macrophage phenotype. Moreover, higher miR-181a and miR-181b expressions had an inhibitory effect on T cell differentiation toward the Th1 cell phenotype, demonstrating the anti-neuroinflammatory character of the miR-181 family. In the chronic phase, miR-181a/b directly suppressed

the expression of *the Smad7* gene and negatively correlated with the expression of miR-181a/b in the CNS EAE. Furthermore, Kleiter et al. [26] showed that *Smad7* determines polarization toward Th1 cells, and its suppression leads to reduced inflammation, which indicates its proinflammatory character in MS. Altogether, miR-181 isoforms showed a crucial role in accentuating immune responses through balancing the inflammatory gene expression, showing the anti-inflammatory properties of miR-181 in MS [25].

In vitro analysis of murine microglia after administration of miR-223 mimics resulted in decreased TNFα secretion and promoted M2 polarization. The miR-223 knockout (KO) mice showed CNS remyelination dysfunction, delayed EAE onset, impaired M2 polarization, and phagocytosis. These data confirm that the dysregulation of miR-223 plays a protective role in neuro-inflammation, making it a potential therapeutic target for MS [27].

The binding of SOCS3 protein to JAK kinase and the cytokine receptor results in the repression of STAT3 activation leading to an inflammatory response. The activated astrocytes, SOCS3, inhibit the JAK/STAT3 pathways, which leads to downregulation of receptor signalling and the inhibition of chemokine production, indicating its neuroprotective properties [28, 29]. In MS, CD4 +, CD8 +, and monocytes showed reduced expression of SOCS3, followed by increased STAT3 phosphorylation [25]. On the other hand, IL-17 produced by Th17 cells promotes the release of proinflammatory cytokines in astrocytes, and serum expression, grew along with EAE severity in mice [30]. In an in vitro study, the primary mice astrocytes transfection by miR-409-3p and miR-1896 imitates decreased SOCS3 and increased p-STAT3 expression. Brain tissue from EAE mice also showed similar results. Interestingly, the overexpression of both miRNAs affected the SOCS3, p-STAT3 expression, and significantly increased CD4 + T cell migration. The miR-409-3p and miR-1896 inhibition eased EAE course seen as the smaller lesions in CNS and decreased motor skill impairment. This study demonstrated that the co-upregulation of miR-409-3p and miR-1896 enhances neuroinflammation via regulation of the SOCS3/STAT3 pathway, increased cytokines production (IL-1β, IL-6, CXCL10, CCL2, CXCL1) and CD4 + migration. Downregulation of miR-409-3p and miR-1896 stands as a promising future therapeutic target [31].

Upregulated miR-142a-3p and miR-142a-5p were detected in EAE lumbar spinal cords, followed by increased CD4 + T cell infiltration and reduced myelin basic protein staining in mice [32]. Isolated splenocytes from EAE and anti-CD3/CD28-stimulated T cell line demonstrated the upregulation of MiR-142a-3p and miR-142a-5p, which indicates the role of splenocytes as the source of miR-142a. Transfection of naïve CD4 + cells with miR-142a-5p resulted in differentiation toward the Th17 phenotype and increased IFN-γ secretion. Transfection with miR-142a-3p did not significantly affect naïve cell differentiation [32]. In silico analysis showed *TGFBR-1* as the target gene for miR-142a-3p, while Luciferase assay identified *TGFBR-2* and *SOCS-1* genes for miR-142a-5p. EAE lumbar spinal cords samples showed decreased expression of the protective genes: *TGFBR-1, TGFBR-2,* and *SOCS-1* at the peak of the disease. It concludes that isoforms of miR-142a play a role in CD4 + differentiation and TGFBR-1 and SOCS-1 expression, thus characterizing them as a good diagnostic tool and future therapeutic target [32].

Bergman et al. [33] experimented with establishing the miRNA profile using next-generation sequencing (NGS) in an animal model of MS. More specifically, myelin oligodendrocyte glycoprotein (MOG)-induced EAE in Dark Agouti (DA) rats was used as a control against EAE-resistant Piebald Virol Glaxo (PVG) rats. Draining inguinal lymph nodes were harvested at naïve state on the 3rd, 7th, and 25th post-immunization (p.i.) day. A total of 329 different miRNAs were quantified, including 64 miRNAs expressed differently between DA and controls based on NGS. MiR-181a, miR-128, and miR-146a were found to be elevated at all time-points, whereas miR-223 and miR-125b-5p were only increased on day-7. Cell-type origin of miRNAs was also established: T cells primarily showed miR-181a and miR-128 expression, whereas non-lymphocyte fraction predominantly depicted the miR-199a-3p and miR-223 expression. The in silico analysis indicated miRNAs targeted gene expression, which showed that neuroinflammatory *CXCR3, PRKCD*, and *STAT1* genes could be the direct target of miR-181a and confirmed by in vitro experimental research. The study demonstrated immune responses that are partially modulated by several miRNAs. The identified miRNAs related to MS implicate their essential role in autoimmunity and put them as promising biomarkers [33].

A previously published in silico study showed that pre-exposed to IFN-γ dysregulated nine miRNAs in mesenchymal stem cells (MSCs), miR-467f, miR-466q miR-466 m-5p, and miR-466i-3p were upregulated in MSCs-derived extracellular vesicles (EVs). The proinflammatory biomarkers such as TNF and IL-1β expression were downregulated once activated microglia were transfected with miR-467f and miR-466q. Similar effects were seen in the amyotrophic lateral sclerosis (ALS) model [34], wherein primary SOD1G93A microglia transfected with miR-467f the *TNF* and *IL-1b* mRNAs expression was decreased, whereas only *TNF* was decreased when transfected with miR-466q. Moreover, further analysis revealed that these miRNAs inhibit their corresponding target genes (*MAP3K8* and *MK2)* that modify the p38 MAPK signalling pathway. These results were confirmed in vivo by transfecting EAE mice with the MSCs-derived EVs associated with decreasing neuroinflammation markers in the spinal cord tissue [35].

Another study focused on miR-106b-25 and miR-17–92 roles in MS pathogenesis showed that the lack of these miRNAs in CD4 + T cells might be protective factors against EAE in mice. Furthermore, depletion of those miRNAs in the spinal cord resulted in a significant reduction of inflammatory cytokines (GM-CSF, IFN-γ, IL-17) and decreased Th17 cells, which are identified as neuroinflammatory. The further pathological evaluation also revealed inflammatory cell infiltrates, demyelination, and axonal loss. The protective effects were seen when only miR-17–92 but not miR-106b-25 were attenuated. Thus, miR-17–92 may be an essential factor in modifying neuroinflammatory processes. However, the direct targets were not found [36] (Fig. 1).

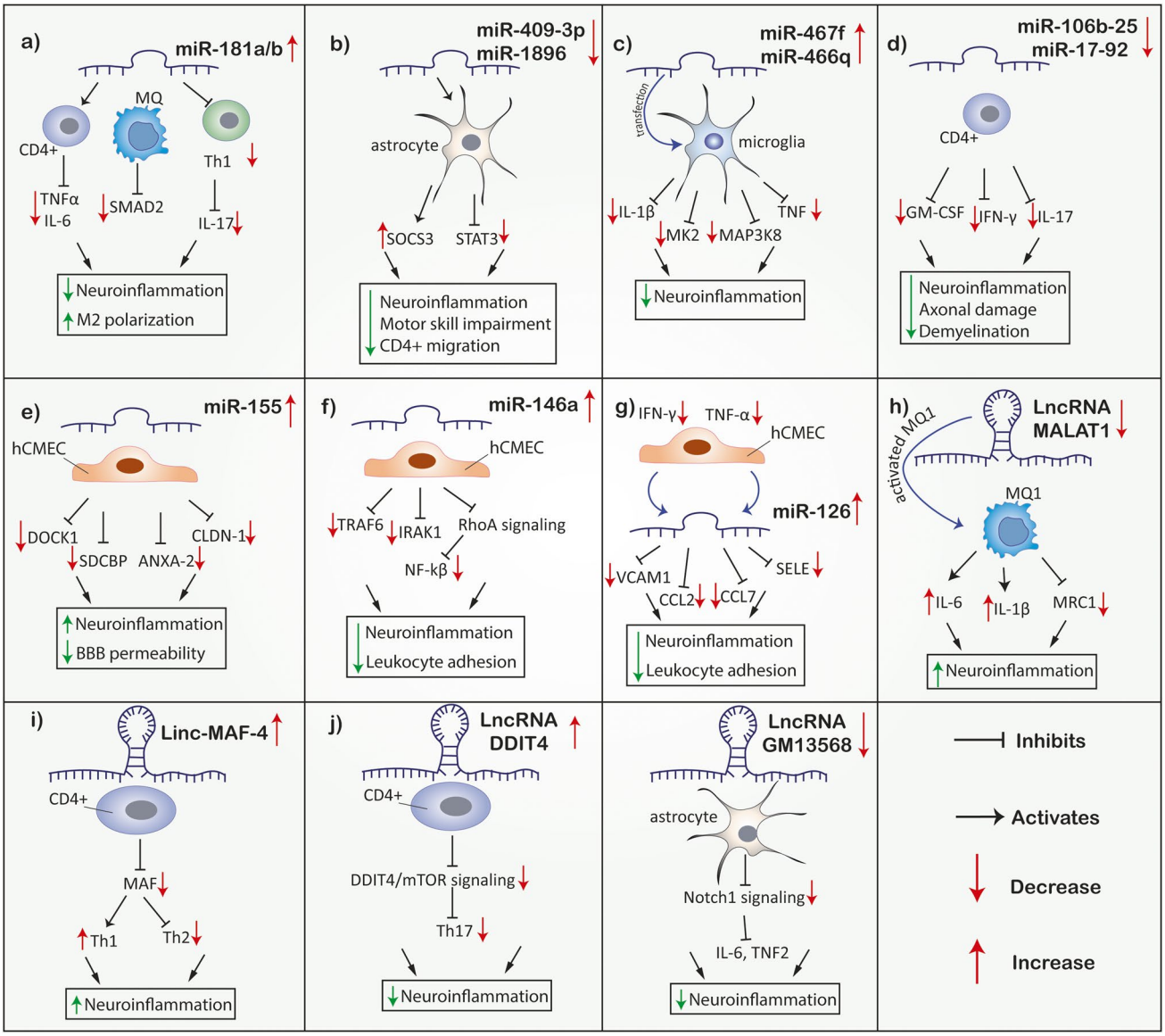

Fig. 1 The mechanism of ncRNAs contributed to neuroinflammation process in MS. Abbreviations: ANXA-2, Annexin A2; CCL2, C–C Motif Chemokine Ligand 2; CCL7, C–C Motif Chemokine Ligand 7; CD4+, T-helper cells; CLDN-1, Claudin 1; DOCK1, Dedicator Of Cytokinesis 1; GM-CSF, Granulocyte Macrophage Colony-Stimulating Factor; IL, interleukin; hMEC, Human Mammary Epithelial Cells; Interleukin; IFN, interferon; IRAK1, Interleukin-1 receptor-associated kinase 1; LincRNA, Long intergenic non-coding RNA; LncRNA, Long non-coding RNA; MALAT1, metastasis associated lung adenocarcinoma transcript 1; Map3k8, Mitogen-Activated Protein Kinase 8; miR, microRNA; M2, M2 type macrophage; Mk2, Mitogen-activated protein kinase miR-microRNA; MRc1, Mannose Receptor C-Type 1; MQ, macrophage; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; RhoA, Ras homolog family member A; SDCBP, Syndecan Binding Protein; SELE, Selectin E; SMAD2, SMAD Family Member 2; SOCS3, Suppressor of cytokine signalling 3; STAT3, Signal transducer and activator of transcription 3; Th1, T-helper cell 1; TNFα, tumour necrosis factor α; TRAF6, tumour necrosis factor receptor associated factor 6; VCAM1, vascular cell adhesion molecule 1

Studies described the role of miRNAs in patients with MS

MRI is the gold-standard imaging technique used in MS diagnosis and consequently prognosis [1]. The most considerable pitfalls of this imaging technique are low availability and the need for high financial expenditure. These limitations would be alleviated with diagnostic serum and blood biomarkers that would be both accessible and simpler in medical evaluation of the MS course. MiRNAs stand as promising biomarkers in this group of patients and meet the criteria mentioned above.

CSF samples were obtained from relapsing remitting MS (RRMS) and clinically isolated syndrome (CIS) patients. The immunology analysis, including CSF cell count, IgG index, CXCL13, MMP9, osteopontin (OPN) levels and the miRNAs expression were studied. The miRNA profiling in cell-free CSF showed substantial upregulation of two miRNAs: miR-145 and miR-150 between MS (CIS, n = 15; RRMS, n = 15) and noninflammatory neurologic disease controls (n = 13). Nevertheless, in a more extensive validation cohort (n = 420), only miR-150 remained statistically relevant. Further, miR-150 expression correlated with immunologic CSF parameters, which indicates its involvement in MS neuroinflammation. After a 52-month follow-up, CIS patients with increased miR-150 expression in CSF were more frequently converted to MS. Notably, miR-150 expression was significantly associated with clinical parameters like IgG index, CSF cells, CXCL13, MMP-9, OPN. Ultimately, miR-150 stands as a candidate biomarker for MS diagnostics and prognostic predictions [18].

Asymptomatic patients with white matter lesions characteristic for MS are diagnosed as the radiologically isolated syndrome (RIS) [37]. There is a need for new available predictive parameters to identify patients at risk of conversion into clinically definite MS (CDMS). In CSF, miR-144-3p, miR-448, and miR-653-3p were upregulated, and miR-483-3p in the plasma of the RIS-conversion group. Additionally, miR-448 in CSF and miR-483-3p in plasma were positively correlated with T2 lesions number. On the contrary, the miR-142-3p, miR-338-3p, miR-363-3p, miR-374-5b, and miR-424-5p were downregulated in plasma in this group of patients (RIS-conversion patients), and miR-363-3p had a negative correlation with T2 lesions. Bioinformatics enrichment analysis predicted cytokine-mediated signalling, adherence junction organization, and cell migration are the most significant gene ontology processes in neuroinflammation accompanying RIS to CDMS conversion. In sum, mentioned miRNAs stand as promising prognostic risk biomarkers of converting from RIS to MS. On the other hand, the study included a minimal sample size (n = 15), and further analyses with larger populations are required to verify those results [38].

Perdaens et al. [39] examined miRNA expression profiling in RRMS patients' serum, peripheral blood mononuclear cells (PBMCs), and CSF and compared it with disease prognosis. They confirmed the association of miR-146a-5p, miR-150-5p, miR-155-5p with MS and brought light upon seven new miRNAs that were previously uncharacterized in MS research (miR-15a-3p, miR-124-5p, miR-149-3p, miR-29c-3p, miR-33a-3p, miR-34c-5p, miR-297). In silico analysis predicted the signalling pathway in remitting MS to be more similar to controls than to relapsing MS. Multicompartment screening showed miR-34c-5p and miR-184 dysregulation in PBMCs while miR-181c-5p and miR-210-3p in CSF and PBMCs simultaneously. While three miRNAs were downregulated (miR-20a-5p, miR-33a-3, miR-214-3p), one was upregulated (miR-149-3p) in CSF of remitting MS subjects in comparison with relapsing ones. Relapsing and remitting MS showed downregulation of miR-15a-3p, miR-24-3p, miR-126-3p, miR-146a-5p, and miR-181c-5p in serum compared to healthy controls. Additionally, miR-214-3p expression was decreased in the relapsing MS group. Specific miRNAs for MS in comparison with symptomatic controls were identified by in silico analysis. In CSF of MS patients, the expression of miR-24-3p, miR-27a-3p, and miR-145-5p was significantly increased, which allows discrimination between MS and other neurological patients. Overall, this study reveals the use of mentioned miRNAs as possible future MS biomarkers [39].

The specific role of monocytes in the pathogenesis of MS is still poorly understood. Macrophages differentiate from monocytes and play a key role in neuroinflammation and MS disease progression [40]. Amoruso et al. [41] studied the miRNAs expression in monocytes in the MS group. The miR-146a, miR-223, miR-125a, miR-30c, and miR-23a were significantly upregulated in RRMS and PPMS groups. Additionally, the miR-181a was increased in RRMS. The expression level of miR-155 was downregulated in both groups; however, miR-124 was decreased only in PPMS patients. The phenotypic markers of monocyte polarization were assessed. The pro-regenerative cytokine-IL-10 was reduced, and CHI3L1 was elevated in PPMS monocytes. The miR-146a, miR-223, miR-125a, miR-181a, miR-124 have shown anti-inflammatory action in previous studies and miR-155 and miR-23a pro-inflammatory [25, 42–46]. Those deregulations indicate

that monocytes take part in alleviating neuroinflammation. Furthermore, mentioned miRNAs stand as potential predictors for monocyte polarization in MS.

Influence of miRNAs on blood–brain barrier function in MS

In preserving CNS homeostasis, the blood–brain barrier (BBB) plays a vital role by regulating the transport of molecules and cells across the extracellular CSF and the circulating blood. The BBB disruption is associated with MS, and it is thought to be the result and the cause of neuroinflammation in MS [47, 48]. The dysregulation of miRNAs followed by disturbed BBB integrity indicates miRNAs role in maintaining BBB function, e.g., by modulation of inflammatory cell adhesion [49, 50]. Collectively, discovering the association between miRNAs, BBB and neuroinflammation will allow a solid understanding of pathophysiology and show the emergence of novel biomarkers and potential therapeutic targets in MS (Fig. 2).

An extensive study aimed to evaluate the impact of pro-inflammatory miRNA-miR-155 on BBB integrity and neuroinflammation in MS by using in vitro and animal models. The study included mice with EAE and MS patients' brain samples and control subjects without neurological diseases. Analysis showed that miR-155 was significantly increased at the neurovascular unit in active MS lesions in the isolated microvessels. In mice, EAE and acute systemic inflammation model reduced CNS extravasation of systemic tracers with the loss of miR-155 expression. BBB permeability was assessed, and the miR-155 knockdown mice were found to possess a 50% less permeable membrane than negative counterparts and control. Moreover, i*n vitro* analysis showed that administration of miR-155 mimics increased BBB permeability via inhibiting DOCK-1, ANXA-2, CLDN-1and SDCBP gene expression. To sum up, the study suggested that in MS, the miR-155 acts as a unique inverse regulator of BBB function during neuroinflammation by modulation of brain endothelial cells [51].

The deep cervical lymph nodes (dCLNs) collect excess fluid, immune cells, and small molecules from the CNS via meningeal lymphatic vessels. Louveau et al. [52] studied the meningeal lymphatic flow in neuroinflammation in EAE mice. After ablation or disruption of the dCLNs lymphatic drainage, the EAE development was delayed. The lack of lymphatic drainage resulted in reduced communication within T cells and antigen-presenting cells (APC). It also suggests that dCLNs participate in the T cell pathway of neuroinflammation. A comprehensive understanding of how T cell encephalitogenicity is affected by CNS lymphatic drainage RNA-sequencing approach was

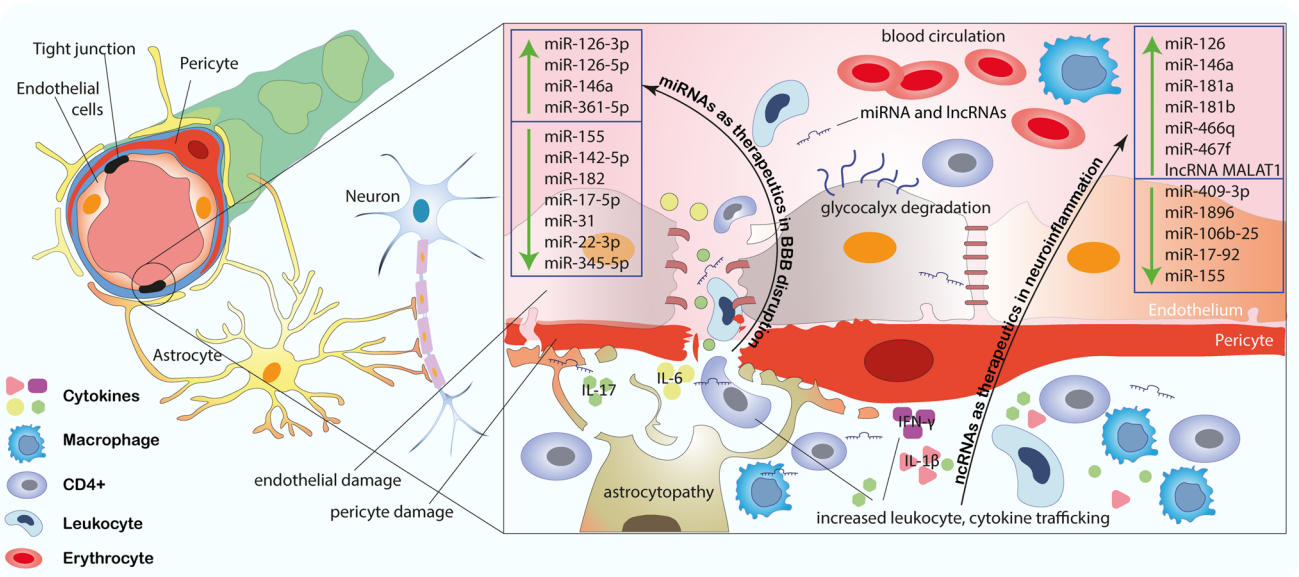

Fig. 2 The role of miRNAs and lncRNA in blood–brain barrier breakdown and neuroinflammation processes of MS. ↑ shows the mimic-use as protective against neuroinflammation, ↓ shows inhibitor-use as protective against neuroinflammation; Abbreviations: IL-interleukin; INF, interferon; miR, microRNA

conducted on dCLN-isolated antigen-specific T cells. Bioinformatic analysis revealed that previously downregulated genes were categorized to increase EAE development (CCR141), differentiation, and activation of T cells (TRADD42) and linked to migration and vascular transmigration (NOD243). Additionally, this study showed upregulation of miRNAs- Let-7 family, miR-142-5p, miR-182, miR-17–5 levels, and are thought to take part in T cell activation differentiation and migration neuroinflammation [52].

The migration of monocytes and T-cells across brain endothelial cells (BEC) stands as one of the pathophysiological mechanisms in MS. Cerruti et al. [50] analysed the role of miR-126-3p and miR-126-5p in the regulation of cell adhesion to BBB using a human BEC in vitro model. Administration of pro-inflammatory cytokines such as TNFα and IFNγ led to downregulation of miR-126 and enhanced monocytes and T-cells adhesion to BEC, whereas increased miR-126 expression in BEC significantly reduced PBMC adhesion. In silico analysis was confirmed in vivo analysis, which showed that miR-126 could target and inhibit genes (*VCAM1, CCL2, SELE,* and *CCL7*) related to leukocyte trafficking. Therefore, leading to increased inflammation. Thus, the study suggested that miR-126 can act as a protective factor and future biomarker for BBB permeability in MS [50].

Wu et *al*. [42] found upregulated miR-146a microvessels of MS-active lesions from human brain tissues and mice spinal cord with EAE. Inhibition of miR-146a caused increased cytokine-stimulated T cells adhesions, whereas transfection with miR-146a mimics resulted in decreased leukocyte adhesion. The NF-κB (p50/p65) activation and nuclear translocation depend on the binding of proinflammatory cytokines such as TNFα and IFNγ to their distinct receptors located on endothelial cells of the brain that eventually stimulate IRAK1 and TRAF6, the receptor-associated molecules [42]. Therefore, the study showed a novel mechanism of decreased neuroinflammation in MS using miR-146a mimic, which blocked NF-κB by suppressing IRAK1, TRAF6, NFAT5, and RhoA signalling. Inhibiting these genes led to downregulation of CCL2 and VCAM1, resulting in reduced leukocyte adhesion to brain endothelium [42].

Hoye et *al*. [53] detected upregulated miR-31 expression in dendritic cells (DCs) in CNS of EAE mice. The study performed by using microarray and bioinformatic analysis showed targets of miR-31: *HIAT1, SRP54B, TSPAN31*, and their role was not described in MS neuroinflammation, which makes them candidates for future studies. Taken all together, this data suggest the participation of miR-31 in inflammatory cells migration to BBB, specifically BMDCs. Potentially therapeutic inhibition of miR-31 would result in restraining DCs expression and migration to CNS along with decreased neuroinflammatory response in MS patients [53].

The only human study evaluating the importance of miRNAs in BBB permeability was done by Hemond et *al*. [54]. They aimed to establish key differences in miRNA expression among variable MS clinical phenotype groups by testing serum miRNA expressions in MS patients. Sixteen miRNAs showed statistical significance with three demonstrating differences between MRI phenotypes. The miR-22-3p and miR-345-5p were induced in high lesion burden phenotypes, which suggests inflammatory character of foregoing miRNAs. The miR-361-5p expression level was increased in patients with mild atrophy and low lesion volume, indicating a protective association against brain volume loss and neuroinflammation. Additionally, in silico analysis suggested the role of miR-22-3p and miR-361-5p in lymphocytes adhesion, extracellular matrix (ECM) integrity, and sealing BBB. Therefore, miR-22-3p, miR-345-5p, and miR-361-5p stand as potential prognostic MS biomarkers in differentiating MS phenotypes [54].

LncRNAs and long intergenic non-coding RNAs as diagnostic/prognostic biomarkers in neuroinflammation process of MS

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a lncRNA and plays a crucial role in MS [55]. The samples collected from MS patients and EAE mice lumbar spine showed MALAT1 downregulation in CNS amid autoimmune neuroinflammation. Activated splenocytes and macrophages (M1) showed significantly decreased MALAT1 expression. Analysis of anti-CD3 and anti-CD28 antibodies of activated T cells showed two-fold increase in MALAT1 expression following one-hour stimulation of splenocytes, followed by substantially reduced expression at 12, 24, and 48 h, suggesting that during acute phase MALAT1 expression was diminished in EAE mice models. In the M0 macrophages phenotype, MALAT1 downregulation showed upregulation of *IL-6* gene than cells transfected with scrambled sequences. Depressed MALAT1 gene expression in the M1 phenotype led to significant upregulation in IL-1b and IL-6 levels with a concomitant decrease in MRC1 expression. MALAT1 downregulation in the M2 phenotype appeared to significantly increase proinflammatory

iNos gene expression. Downregulation of MALAT1 was associated with an increase in shift towards Th1 and Th17 and enhanced T cell proliferation. Taken all together, MALAT1 could act as a potential anti-inflammatory effector in the context of autoimmune neuroinflammation, having gene modulating activity as well as implications for regulation of immune cells responsible for the chronic inflammation and stands as a potential MS biomarker [55].

Interleukin 9 (IL-9) is a key regulator of reactive astrocytes, inflammatory cytokines and autoimmune responses in MS and EAE. Liu et al. analysed the impact of lncRNA Gm13568 both in vitro and in vivo. The study involved animal model of EAE mice and IL-9 treated mouse primary astrocytes. It was found that the increased production of IL-9 in EAE mice led to Notch1/STAT3 signalling pathway activation and thus promoted the production of pro-inflammatory cytokines in astrocytes. Moreover, it was shown that the process is mediated by lncRNA Gm13568, which interacted with CBP/P300 to regulate the *Notch1* gene transcription. Further analysis showed that the inhibition of lncRNA Gm13568 resulted in decreased activation of the Notch1 signalling pathway and thus reduced production of pro-inflammatory cytokines in astrocytes. Hence, the data indicated that lncRNA Gm13568 affects the pathogenesis of EAE through the Notch1 signalling pathway and enhances the EAE process in the mice model. Consequently, the study suggested that lncRNA Gm13568 may be a promising target in MS treatment [56].

Th17 cells contribute to neuroinflammation in MS via cytokine production and recruiting proinflammatory lymphocytes. DDIT4/mTOR pathway stands as the regulator of Th17 cell differentiation. Zhang et al. investigated the role of the lnc DDIT4 in MS patients. They showed that the lncRNA DDIT4 was overexpressed in the PBMCs and naive CD4 + cells obtained from MS patients. Additionally, the overexpression of lncRNA DDIT4 supressed Th17 cell differentiation through inhibition of the DDIT4/mTOR axis. As expected, silencing lncRNA DDIT4 resulted in the opposite outcome. The results of this study indicated that lncRNA DDIT4 regulates Th17 cell differentiation and alleviates neuroinflammation in MS [57].

An influential study conducted by Gupta et *al*. on RNA-sequencing and bioinformatic analysis identified four long intergenic non-coding RNAs (lincRNAs) (ENSG00000260302, ENSG00000272512, ENSG00000223387, ENSG00000270972) as possible biomarkers for analysing phenotypic severity of the disease in MS patients in comparison of mild and severe phenotypes. Further, they validated their results by using digital droplet PCR in a confirmation cohort. In this study, these lincRNAs were significantly higher in the severe phenotype, demonstrating their prognostic value in MS [58].

Zhang et al. investigated the role of lincRNA MAF-4 in Th1 and Th2 differentiation in MS. The expression of lincRNA MAF-4 was significantly higher in mononuclear cells from peripheral blood from MS patients in comparison with healthy controls and correlated with the annual relapse rate in the MS patients group. To understand the molecular mechanisms in vitro analysis was performed. The transfection of CD4 + T cells with lincRNA MAF-4 favoured Th1 cell differentiation over Th2 by directly inhibiting the Th2 cell transcription factor—MAF, which increased the neuroinflammation. This study points the lincRNA MAF-4 as a key factor in regulating T-cell activity involved in demyelination in MS [59].

Current perspectives and limitations

To summarize and present the published data of miRNAs and lncRNAs involved in MS pathophysiology, we have generated a network graph showing ncRNAs and their targeted genes (Fig. 3), affecting neuroinflammation. Literature data (Table 1 and supplementary table 1) were transformed into a tabular network file and aggregated in R. Visualization of the network was performed using Cytoscape v3.9.0 [60]. Additional information regarding model organisms and ncRNAs and their inflammatory or anti-inflammatory effect shown in the studies was used for visual mapping of the nodes. The genes from analysed manuscripts were additionally evaluated using the DisGeNet v7.0 database for their association with MS [61]. According to this network (Fig. 3), we can conclude that miR-155 and miR-181a appeared in the highest number of studies as regulators in MS, since those miRNAs can target the greatest number of different genes (confirmed in the literature by experimental analysis). Furthermore, lncRNA MALAT1 was found to be associated with anti-inflammatory processes in MS. Importantly, as it is presented in Fig. 3, MALAT1 was studied not only in the in vivo analysis, but was also demonstrated in human studies.

In our analysis, the strongest association with neuroinflammation in MS showed genes: *TRAF6*, *IRAK1* and *SOCS-1*. *TRAF6* encodes proteins involved in proinflammatory signal mediation from members of the TNF receptor superfamily and the Toll/IL-1 family. *IRAK1* intermediates IL-1-induced upregulation of the transcription factor NF-κB. The expression of IRAK1 is induced by various

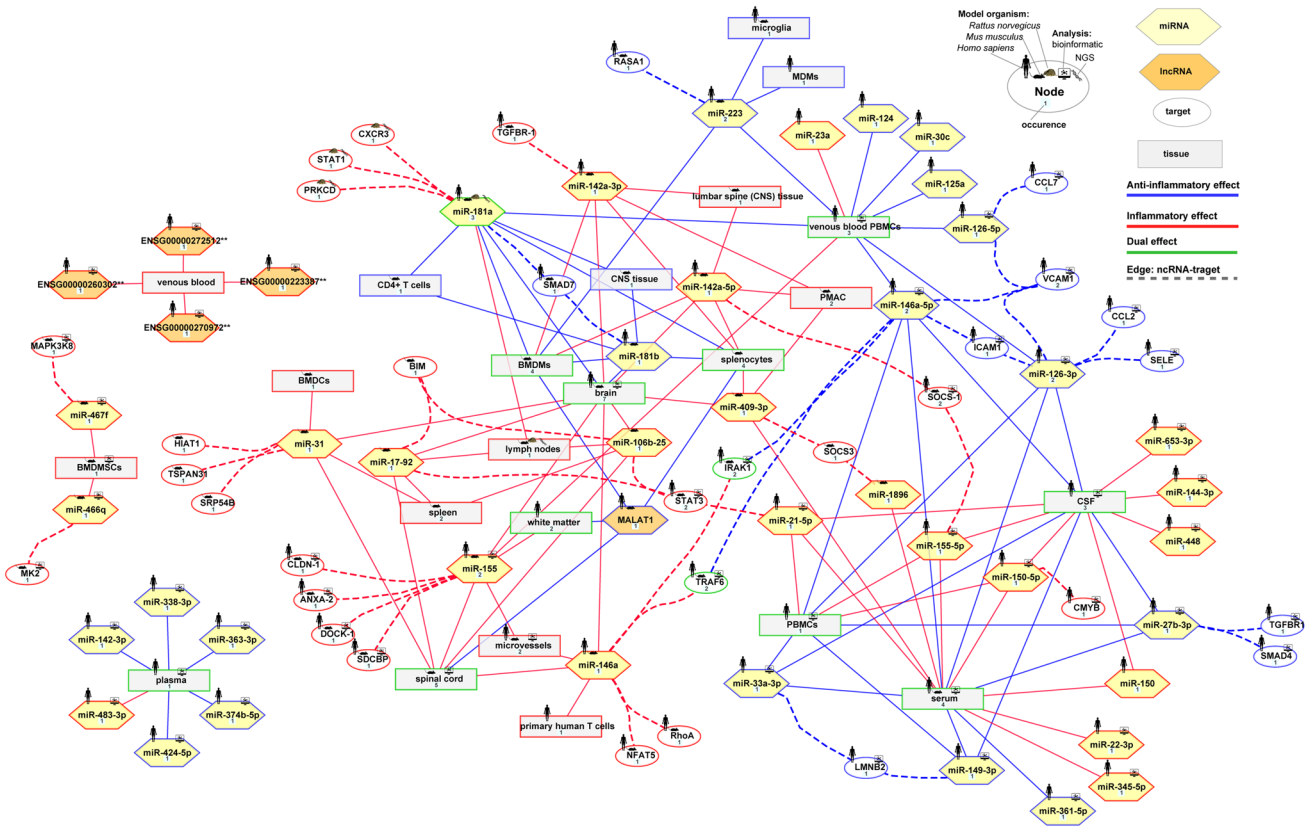

Fig. 3 Visualization of the literature regarding the role of miRNAs/lncRNAs and lincRNAs in MS neuroinflammatory process. Abbreviations: microRNAs, miRNAs; long non-coding RNAs, lncRNAs; long intergenic non-coding RNAs, lincRNAs

cytokines, including IL-6, IL-10, and IFN-γ. It regulates innate and adaptive immune responses and decreases pro-inflammatory state in MS. Using the DisGeNet database, we confirmed that those genes strongly relate to development of MS.

Measuring expression of ncRNAs in blood components and CSF may improve the prediction of clinical outcome. However, the use of lncRNAs and miRNAs as biomarkers in clinical practice still faces many limitations: (i) a small number of human studies describing the role of ncRNAs in the processes of neuroinflammation in MS; (ii) many of the studies described in this review require further validation and assessment of their results reproducibility; (iii) a number of studies that analysed the importance of ncRNAs in MS used EAE animal model, without confirmation in human studies; (iv) individual molecules examined in MS such as miR-155 and MALAT1 are not specific to MS only.

Conclusion

Several studies highlighted the promising role of miRNA and lncRNA as potential diagnostic and prognostic biomarkers in MS patients. Additionally, they may serve as potential therapeutic targets through inhibition or restoration of loss of function using mimic molecules that are similar to endogenous ones. Yet, the detailed mechanism of action of the described miRNAs and lncRNAs on neuroinflammation has not been fully explained and more studies need to be conducted. Importantly, a single miRNA or lncRNA may target multiple genes; thus, understanding the miRNA–lncRNA interaction network and functions and creating an effective and inexpensive way of making diagnosis and prognosis are prerequisites to apply ncRNAs in the future clinical practice regarding MS patients.

Table 1 Evaluating miRNAs and lncRNAs as neuroinflammatory biomarkers in MS

Reference	miRNA/lncRNA/lincRNA	Regulated genes	Inflammatory/Anti-inflammatory effect	Methodology	Species/Animal model (tissue, cell line) / Human study (serum/CSF/tissue/plasma)	Conclusion
[54]	miR-345-5p	Not mentioned	Inflammatory	Microarray analysis; bioinformatic analysis; qRT-PCR validation	Human blood serum	MiR-22-3p and miR-361-5p are both involved in BBB function including sealing the endothelial barrier, focal adhesion and endothelial-lymphocyte communication MiR-345-5p demonstrated a pathologic association with cerebral T2-hyperintense lesion volume displaying neuroinflammatory character
	miR-22-3p	Not mentioned	Inflammatory			
	miR-361-5p	Not mentioned	Anti-inflammatory			
[18]	miR-150	Not mentioned	Inflammatory	MiRNA isolation from CSF and cell-free plasma from MS patients Gene expression profiling qRT-PCR	CSF and cell-free plasma from MS patients	Upregulated miR-150 stand as future prediction factor for CIS patients converting into MS and marker of active neuroinflammation in MS
[39]	miR-33a-3p	LMNB2	Inflammatory	multiplex miRNA screening qRT-PCR validation in silico analysis	CSF, blood serum and PBMCs from MS patients	MiR-24-3p, miR-27a-3p, and miR-145-5p were most significantly upregulated in CSF in MS, which allows discrimination between MS and other neurological patients
	miR-126-3p	ICAM1, VCAM1				
	miR-146a-5p	ICAM1, VCAM1, TRAF6, IRAK1				
	miR-27b-3p	SMAD4, TGFBR1				
	miR-21-5p	STAT3				
	miR-150-5p	CMYB				
	miR-155-5p	SOCS-1				
	miR-149-3p	LMNB2				
[25]	miR-181a	SMAD7	Anti-inflammatory	EAE induced in C57BL/6 mice by injecting SC MOG 35–55 peptide emulsified in CFA at two sites, followed by two i.p injections of pertussis toxin administered on the day of immunization and 24 h later Splenocytes cell culture prepared from MOG-immunized mice 10 days after immunization qRT-PCR flow cytometry luciferase assay	Brain white matter from MS patients; splenocytes cell culture, BMDMs, CD4 + cells, CNS tissue from EAE C57BL/6 mice,	The anti-inflammatory action of miR-181 isoforms depends on diminish T cell differentiation towards Th1 phenotype and activating M2 macrophages via Smad7 gene
	miR-181b	SMAD7				

Table 1 (continued)

Reference	miRNA/lncRNA/lincRNA	Regulated genes	Inflammatory/Anti-inflammatory effect	Methodology	Species/Animal model (tissue, cell line) / Human study (serum/CSF/tissue/plasma)	Conclusion
[27]	miR-223	RASA1	Anti-inflammatory	EAE induced in C57BL/6 mice by SC injecting MOG 35–55 peptide emulsified in CFA at two sites, followed by two i.p. injections of pertussis toxin administered on the days 0 and 2 Lysolecithin-Induced Focal Demyelination induced in the corpus callosum of littermate male mice by injecting lysolecithin qRT-PCR western blot assay flow cytometry ELISA	PBMCs from RRMS patients; human MDMs cell culture; human adult microglia; EAE C57BL/6 mice; mouse BMDMs; mouse microglia	The miR-223 showed anti-inflammatory and repairing effects by taking part in clearing myelin debris and promoting remyelination
[32]	miR-142a-3p miR-142a-5p	TGFBR-1 SOCS-1	Inflammatory	EAE induced in C57BL/6 mice by SC injecting MOG 35–55 peptide emulsified in CFA at two sites, followed by two i.p injections of pertussis toxin administered on the day of immunization and 24 h later Harvested from MOG-immunized mice splenocytes, BMDMs and primary astrocytes were stimulated with MOG peptide or anti-CD3/anti-CD28 antibodies qRT-PCR Luciferase assays T cell culture Flow cytometry	Brain white matter from MS patients; lumbar spinal cord tissue from EAE female C57BL/6 mice; splenocyte cell culture from EAE mouse; BMDMs and primary mouse astrocyte culture; HEK293T cells	The miR-142 isoforms targets protective genes (TGFBR-1, SOCS-1) and change the pattern of T cells differentiation towards neuroinflammatory Th1 cells
[55]	MALAT1	Not mentioned	Inflammatory	EAE induced in C57BL/6 mice by SC injecting MOG 35–55 peptide emulsified in CFA at two sites, followed by two i.p injections of pertussis toxin administered on the day of immunization and 24 h later qRT-PCR flow cytometry	white matter from MS patients; spinal cord tissue from EAE female C57BL/6 mice; splenocytes culture, BMDMs, naïve CD4 + cell culture from C57BL/6 mice	Downregulation of MALAT1 intensify neuroinflammation through M1, Th1 and Th17 cells differentiation enhancement

Table 1 (continued)

Reference	miRNA/lncRNA/lincRNA	Regulated genes	Inflammatory/Anti-inflammatory effect	Methodology	Species/Animal model (tissue, cell line) / Human study (serum/CSF/tissue/plasma)	Conclusion
[51]	miR-155	DOCK-1, SDCBP, ANXA-2, CLDN-1	Inflammatory	EAE induced in C57BL/6 mice by SC injecting MOG 35–55 peptide emulsified in CFA at two sites, followed by two i.p injections of pertussis toxin administered on the day of immunization and 24 h later LCM was used to collect enriched brain endothelium RNA Microvessels were isolated from the mouse spinal cords Microarray analysis qRT-PCR ISH Western blot	white matter from MS patients, brain endothelium and LCM microvessels from C57BL/6 mice spinal cords, hCMEC/D3 line	The miR-155 overexpression was found to be significant for MS neuroinflammation processes affecting BBB by increasing permeability, altering adhesion pathways and inhibiting BBB protective genes
[42]	miR-146a	IRAK1, TRAF6, RhoA, NFAT5	Inflammatory	EAE induced in C57BL/6 mice by SC injecting MOG 35–55 peptide emulsified in CFA at two sites, followed by two i.p injections of pertussis toxin administered on the day of immunization and 24 h later LCM was used to collect enriched brain endothelium RNA Microvessels were isolated from the mouse spinal cords qRT-PCR Western blot	brain tissue and LCM microvessels from MS patients; primary human T cells; hCMEC/D3 line; Jurkat T cell line; cryostat and microvessels from EAE Biozzi ABH mice spinal cords	The miR-146a downregulate NF-κB signalling through repressing regulatory genes involved in NF-κB activation, regulates T cell adhesion to human brain endothelium and influence inflammatory signalling network what points its anti-inflammatory and protective character in MS
[31]	miR-409-3p miR-1896	SOCS3/STAT3 pathway SOCS3/STAT3 pathway	Inflammatory	EAE induced in C57BL/6 mice by SC injecting MOG 35–55 peptide emulsified in CFA qRT-PCR Western blot analysis Luciferase assay Flow cytometry	serum and brain tissue from EAE female C57BL/6 mice; primary mouse astrocytes; naïve CD4 + cells from mouse spleen;	MiR-1896 and miR-409-3p increase inflammatory cytokines production by down-regulating SOCS3/STAT3 axis

Table 1 (continued)

Reference	miRNA/lncRNA/lincRNA	Regulated genes	Inflammatory/Anti-inflammatory effect	Methodology	Species/Animal model (tissue, cell line) / Human study (serum/CSF/tissue/plasma)	Conclusion
[33]	miR-181a	CXCR3, PRKCD, STAT1	Inflammatory	EAE induced in DA rats by injected s.c. in the tail MOG in PBS, emulsified with IFA NGS qRT-PCR, FACS analysis qRT-PCR in silico bioinformatic analysis reporter assay	draining lymph nodes from EAE DA rats; HEK293T cells	MiR-181a, miR-128, miR-146a, miR-125b-5p miR-199a-5p, and miR-223 upregulation could be linked to clinical symptoms of MS in rats, seen as a prolonged and pathogenic immune response
[38]	miR-483-3p miR-144-3p miR-448 miR-653-3p		Inflammatory	PCR-SSO ELISA qRT-PCR western blot	CSF and plasma from MS patients	The miR-448 in CSF and miR-483-3p in plasma positively correlated with T2 lesions number and miR-363-3p had a negative correlation with T2 lesions in MRI of MS patients what indicates its role in MS neuroinflammation
	miR-142-3p miR-338-3p miR-363-3p miR-374b-5p miR-424-5p		Anti-inflammatory	reporter assay Enrichr—explore the gene ontology (GO) biological processes		
[41]	miR-146a-5p miR-223 miR-125a miR-181a miR-30c miR-124		Anti-inflammatory	qRT-PCR	PBMCs from MS patients	Pro-inflammatory miRNAs are downregulated and neuroprotective are upregulated in PBMCs from MS patients, what indicates its role in attempt to extinguish neuroinflammation
	miR-23a miR-155		Inflammatory			
[50]	miR-126-3p miR-126-5p	SELE, CCL2 CCL7, VCAM1	Anti-inflammatory	Flow-based leukocyte adhesion assay ELISA qRT-PCR In silico analysis	hCMEC/D3 cell line; PBMCs from MS patients	The miR-126-3p and miR126-5p simultaneously modulate VCAM1, SELE, CCL7 and CCL2 genes required for leukocyte migration and adhesion
[58]	ENSG00000260302** ENSG00000272512** ENSG00000223387** ENSG00000270972**		Inflammatory	RNA-*Seq* ddPCR In silico analysis	blood samples from MS patients Comparison of mild and severe phenotype	These lncRNAs were presented as future predictors for severe and mild courses in MS patients

Table 1 (continued)

Reference	miRNA/lncRNA/lincRNA	Regulated genes	Inflammatory/Anti-inflammatory effect	Methodology	Species/Animal model (tissue, cell line) / Human study (serum/CSF/tissue/plasma)	Conclusion
[53]	miR-31	HIAT1, SRP54B, TSPAN31	Inflammatory	EAE induced in C57BL/6 mice by SC injecting MOG 35–55 peptide emulsified in CFA at two sites, followed by two i.p. injections of pertussis toxin administered on the days 0 and 2 qRT-PCR miRAP flow cytometry FACS cell culture migration assay in in vitro BBB model In silico analysis	cells from harvested lymph nodes and ear skin from mice; brains, spinal cords, spleen tissue, BMDCs from EAE mice	The miR-31 participates in BMDCs migration through BBB
[35]	miR-467f miR-466q	Map3k8 Mk2	Inflammatory	EAE induced in C57BL/6 J mice by *s.c.* injecting of MOG 35–55 peptide in Freund adjuvant at two sites, followed by two i.v. injections of pertussis toxin administered on the days 0 and 2 microarray experiment cell culture qRT-PCR immunofluorescence in silico target analysis	bone marrow-derived MSCs isolated from C57BL/6 J mice with changed SOD1 expression; murine microglial cell line N9 (Neuro-Zone srl, Italy);	MSC-derived exosomes could affect neuroinflammation possibly through specific immunomodulatory miRNAs acting on microglia
[36]	miR-106b-25 miR-17–92	BIM, STAT3 BIM, STAT3	inflammatory	EAE induced in C57BL/6 J mice by *s.c.* injecting of MOG 35–55 peptide in Freund adjuvant at two sites, followed by two i.v. injections of pertussis toxin administered on the days 0 and 2 qRT-PCR flow cytometry	C57BL/6 WT and mutant mice;	MiR-106b-25 and miR17-92 clusters may represent a plausible therapeutic target in neuroinflammatory diseases

Table 1 (continued)

Reference	miRNA/lncRNA/lincRNA	Regulated genes	Inflammatory/Anti-inflammatory effect	Methodology	Species/Animal model (tissue, cell line) / Human study (serum/CSF/tissue/plasma)	Conclusion
[56]	lncRNA Gm13568	CBP/P300—Notch1 axis	inflammatory	EAE induced in C57BL/6 J mice by s.c. injecting of MOG 35–55 peptide in Freund adjuvant at two sites, followed by two i.v. injections of pertussis toxin administered on the days 0 and 2 cell culture qRT-PCR Western blot analysis	primary mouse astrocytes; spinal cords from EAE C57BL/6 J mice;	The lncRNA Gm13568 stand as factor in the Notch1/STAT3 signalling pathway and contributes to the neuroinflammation in MS animal model
[59]	linc-MAF-4	MAF	inflammatory	qRT-PCR cell culture	PBMCs from MS patients	linc-MAF-4 as a key factor in regulating T-cell activity involved in demyelination
[57]	lnc-DDIT4	DDIT4/mTOR	anti-inflammatory	qRT-PCR cell culture	PBMCs from MS patients	The lnc-DDIT4 inhibits Th17 cell differentiation

* Genes were predicted by using bioinformatic analysis [39]

** Represents the higher expression of lincRNAs in the severe subgroup of MS [58]

Abbreviations: ANXA-2-Annexin A2; BBB-blood-brain barrier; BCL2-B-cell lymphoma 2; BMDCs-bone marrow-derived dendritic cells; BMDMs-Bone marrow-derived macrophages; CCL2-C-C Motif Chemokine Ligand 2; CCL7-C-C Motif Chemokine Ligand 7; CCND1-Cyclin D1; CFA-complete Freund's adjuvant; CIS-clinically isolated syndrome; CLDN-1-Claudin-1; CNS-Central nervous system; CSF-cerebrospinal fluid; CXCR3 Motif Chemokine Receptor 3; DA-Dark Agouti; ddPCR-digital droplet PCR; DOCK-1-Dedicator Of Cytokinesis 1; EAE-Experimental autoimmune encephalomyelitis; ELISA-enzyme-linked immunosorbent assay; FACS-Fluorescence-activated Cell Sorting; GO-gene ontology; hCMEC-human cerebral microvascular endothelial cell; HEK- human embryonic kidney; i.p.-intraperitoneal; IRAK1-Interleukin-1 receptor-associated kinase 1; ISH-in situ hybridization; LCM-laser capture microdissection; lncRNA-long non-coding RNA; Long intergenic non-coding-lincRNA; RRC58-Leucine Rich Repeat Containing 58; MALAT1-metastasis associated lung adenocarcinoma transcript 1; Map3k8, Mitogen-Activated Protein Kinase Kinase Kinase 8; MDMs-monocyte-derived macrophages; Mk2, Mitogen-activated protein kinase miR-microRNA; miRAP-miRNA affinity and tagging purification; MOG Myelin Oligodendrocyte Glycoprotein; MRI Magnetic resonance imaging; MS multiple sclerosis; MYC Myelocytomatosis; NFAT5 Nuclear factor of activated T-cells 5; NGS Next-Generation Sequencing; PBMCs peripheral blood mononuclear cells; PCR-SSO polymerase chain reaction and subsequent hybridization with sequence-specific oligonucleotide probes; PMAIP1 Phorbol-12-Myristate-13-Acetate-Induced Protein 1; PRKCD Protein Kinase C Delta; qRT-PCR quantitative real-time polymerase chain reaction; RASA1-RAS P21 Protein Activator 1; RhoA-Ras homolog family member A; RRMS-Relapsing-remitting MS; SC subcutaneously; SDCBP Syndecan Binding Protein; SELE Selectin E; SMAD4 Mothers against decapentaplegic homolog 4; SMAD7 Mothers against decapentaplegic homolog 7; SOCS-1 Suppressor of cytokine signalling 1; SOCS3 Suppressor of cytokine signalling 3; STAT1 Signal transducer and activator of transcription 1; STAT3 Signal transducer and activator of transcription 3; TGF-β transforming growth factor β1; TGFBR-1 Transforming Growth Factor Beta Receptor 1; TLDA Targeted Taqman Low Density Array; TMEM167A Transmembrane Protein 167A; TRAF6 Tumour necrosis factor receptor associated factor 6; VCAM1 vascular cell adhesion molecule 1; VEGFA Vascular endothelial growth factor A; XIAP-X-linked inhibitor of apoptosis protein

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Availability of data and materials The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

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Consent to participate Not applicable.

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Competing Interests Authors declare no conflict of interest related to this work.

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