● REVIEW

MicroRNAs as disease progression biomarkers and therapeutic targets in experimental autoimmune encephalomyelitis model of multiple sclerosis

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Abstract

Multiple sclerosis is an autoimmune neurodegenerative disease of the central nervous system characterized by pronounced inflammatory infiltrates entering the brain, spinal cord and optic nerve leading to demyelination. Focal demyelination is associated with relapsing-remitting multiple sclerosis, while progressive forms of the disease show axonal degeneration and neuronal loss. The tests currently used in the clinical diagnosis and management of multiple sclerosis have limitations due to specificity and sensitivity. MicroRNAs (miRNAs) are dysregulated in many diseases and disorders including demyelinating and neuroinflammatory diseases. A review of recent studies with the experimental autoimmune encephalomyelitis animal model (mostly female mice 6–12 weeks of age) has confirmed miRNAs as biomarkers of experimental autoimmune encephalomyelitis disease and importantly at the pre-onset (asymptomatic) stage when assessed in blood plasma and urine exosomes, and spinal cord tissue. The expression of certain miRNAs was also dysregulated at the onset and peak of disease in blood plasma and urine exosomes, brain and spinal cord tissue, and at the post-peak (chronic) stage of experimental autoimmune encephalomyelitis disease in spinal cord tissue. Therapies using miRNA mimics or inhibitors were found to delay the induction and alleviate the severity of experimental autoimmune encephalomyelitis disease. Interestingly, experimental autoimmune encephalomyelitis disease severity was reduced by overexpression of miR-146a, miR-23b, miR-497, miR-26a, and miR-20b, or by suppression of miR-182, miR-181c, miR-223, miR-155, and miR-873. Further studies are warranted on determining more fully miRNA profiles in blood plasma and urine exosomes of experimental autoimmune encephalomyelitis animals since they could serve as biomarkers of asymptomatic multiple sclerosis and disease course. Additionally, studies should be performed with male mice of a similar age, and with aged male and female mice.

Key Words: *animal model; blood plasma; blood serum; brain tissue; disease biomarkers; experimental autoimmune encephalomyelitis; microRNAs; multiple sclerosis; spinal cord; therapeutic targets; urine exosomes*

Introduction

Multiple sclerosis (MS) is an autoimmune neurodegenerative disease of the central nervous system characterized by a pronounced infiltration of inflammatory cells into the brain and spinal cord leading to demyelination, axonal damage, and impaired neuromuscular functions (Dendrou et al., 2015). The optic nerve also shows inflammatory infiltration and demyelination in MS (Horstmann et al., 2013). MS has a peak age of onset in humans between 20 and 40 years (Hawker and Frohman, 2004). It is 2 to 3 times more prevalent among females than males (Harbo et al., 2013), which may be due to hormonal or genetic factors, or being exposed to different environment influences than males. MS is the leading cause of non-traumatic disability among young adults in the US (Peterson and Trapp, 2005). About 85% of patients with MS have a relapsing-remitting pattern (RRMS) which is characterized by relapses (attacks) that last at least 24 hours, and are followed by a remission when symptoms become partly or completely relieved. About 50% of patients with RRMS will eventually transition to secondary progressive MS within 10 years, in which there is a progressive worsening of neurological function (increasing disability) (National Multiple Sclerosis Society). Moreover, about 15% of patients with MS are diagnosed with primary progressive MS characterized by a worsening neurologic function from the onset of symptoms, without early relapses or remissions (National Multiple Sclerosis Society). Focal demyelination is associated with RRMS, while progressive forms of the disease show axonal degeneration and neuronal loss (Bjartmar et al., 2000; Wujek et al., 2002).

Three tests are currently used in the clinical diagnosis and management of MS (Housley et al., 2015), namely the oligoclonal bands in the cerebrospinal fluid (Stangel et al., 2013), which are attributable mostly to immunoglobulins; the white matter/gadolinium-enhancing lesions detected by magnetic resonance imaging (Zivadinov and Leist, 2005; Fisniku et al., 2008), which correspond to active lesions with inflammation; and the John Cunningham virus antibody titers (Antoniol and Stankoff, 2014; Outteryck et al., 2014), which demonstrate exposure of the patient to this virus and the likely risk of developing progressive multifocal leukoencephalopathy following immunosuppressive therapy. However, while these tests have been used consistently in the clinic, they have limitations in regard to specificity and sensitivity (Li et al., 2006; Plavina et al., 2014). There is a need to identify new biomarkers for disease development and changes in

disease activity following therapy.

MicroRNAs (miRNAs) are short non-coding RNAs (~22 nt in length) that regulate gene expression by binding to the 3′ untranslated regions of target mRNAs and repressing protein translation or initiating mRNA destabilization/degradation (Grimson et al., 2007). Some miRNAs can bind to the 5′ untranslated regions of target mRNAs (Li et al., 2016). MiRNAs are dysregulated in many diseases and disorders including demyelinating and neuroinflammatory diseases (de Faria et al., 2012; Andersen et al., 2014). Total miRNA levels in whole brain lysates were markedly decreased at peak of disease and there was miRNA dysregulation in oligodendrocytes of experimental autoimmune encephalomyelitis (EAE) mice compared to control mice (Lewkowicz et al., 2015). A single miRNA can modulate a large number of functionally related genes, and they represent potential targets for developing neuroprotective strategies for MS. MiRNAs are involved in the differentiation and function of immune cells and play important roles in immune response (Chen et al., 2004).

We have performed a PubMed search for articles published January 2014–October 2019 on levels of miRNA expression in EAE animals to identify possible biomarkers of disease and how these might differ at various stages of disease course viz. asymptomatic (prior to onset, early stage biomarker), onset, peak disease, and chronic clinical points. Also we have examined these articles for whether overexpression or inhibition of specific miRNAs can alleviate EAE disease and thereby serve as therapeutic targets.

MicroRNAs in Experimental Autoimmune Encephalomyelitis Animals

The steps involved in the review and its contents are shown in **Figure 1**. A total of 20 articles were found in the review. The samples collected for analysis of miRNAs included blood serum, blood plasma and urine exosomes, brain tissue, spinal cord, lumbar motor neurons, retinal ganglion cells (RGCs). Most of the studies had induced EAE in female mice by immunizing with myelin oligodendrocyte glycoprotein (amino acids 35–55) peptide. One study had immunized female mice with myelin proteolipid protein (amino acids139–151) peptide, one study had immunized female mice with mouse spinal cord homogenate, and one other study had immunized female rats with guinea pig myelin basic protein (amino acids 69–88). Where reported, the ages of the mice ranged from 6–12 weeks and the ages of the rats between 8–12 weeks. The EAE clinical scores where reported indicated a progressive disease in six of the studies (Liu et al., 2014; Zhang et al., 2014, 2018a; Satoorian et al., 2016; Talebi et al., 2017; Juzwik et al., 2018), in three studies the disease profile was followed to a peak of disease at ~day 20 post immunization (p.i.) (Zhang et al., 2015; Singh et al., 2016; Shan et al., 2017), in two studies the clinical score reached a peak and then declined to baseline (Gerrard et al., 2017; Wan et al., 2019), and in two other studies a relapsing-remitting disease was indicated (Zhu et al., 2014; Zhang et al., 2019). In all of the studies, except that by Gerrard et al. (2017) which
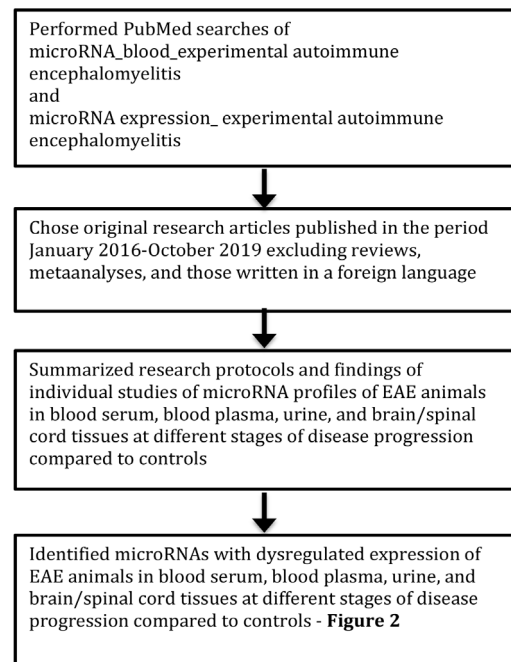

Figure 1 Flow diagram showing how the review was performed and its contents.

had used deep sequencing miRNA analysis, the miRNA levels were measured using real-time polymerase chain reaction analysis at different stages of disease which included pre-onset (before the appearance of symptoms), peak of disease (maximum clinical score of disability), and chronic/post-peak stage. MiRNA biomarkers at pre-onset (asymptomatic) stage are particular important as it would enable therapy to be initiated as soon as possible. Those miRNA biomarkers at peak disease have importance for testing disease-modifying therapies. Also miRNA biomarkers at the chronic stage would enable possible therapies for progressive MS-type disease to be evaluated. The findings of the studies are summarized as follows.

Blood serum
The levels of 9 miRNAs in serum samples collected from EAE mice on days 18–20 p.i. (peak of disease) were quantitated and miR-99b, miR-125a, and miR-146b were significantly higher in EAE mice than normal (control) mice. While the levels of miR-23b, miR-31, miR-193b, and let-7e tended to be increased in EAE mice, they were not significantly different from controls (Venkatesha et al., 2018).

Blood plasma
At pre-onset stage (day 6 p.i.) when EAE mice were phenotypically asymptomatic there was a significant increase in miR-155-5p level in plasma exosomes compared to control mice. At the onset stage (day 11–12 p.i.) the levels of miR-155-5p, miR-15a-3p, miR-16-5p, miR-200c-3p, miR-221-3p, and miR-30e-5p were significantly higher than controls, while at peak of disease (~day 20 p.i.) the levels of miR-155-5p, miR-27a-3p, miR-126a-5p, miR-350-3p, and miR-340-5p

were significantly increased in EAE mice (Singh et al., 2016).

Urine
A significant increase in the levels of miR-155-5p, miR-16-5p, miR-200c-3p, miR-429-3p, and miR-9-5p, together with a significant decrease in the level of miR-291a-3p, was found in urine exosomes of EAE mice at pre-onset stage (day 6 p.i.) compared to control mice. There was a significant increase in urine exosomes of miR-155-5p, miR-16-5p, miR-200c-3p, miR-221-3p, and miR-30e-5p at the onset stage (days 11–12 p.i.), while miR-9-5p and miR-350-5p in urine exosomes were significantly increased at the peak stage (~day 20 p.i.) (Singh et al., 2016).

Central nervous system tissues (brain and spinal cord)
The level of miR-23b was significantly decreased in central nervous system tissues during the acute phase on days 6, 11, 17, and 28 p.i., but there was no significant difference at the chronic stage on day 40 p.i. compared to control mice (Zhang et al., 2018a).

Brain tissues
A significant decrease in the level of miR-497 occurred in brain tissues of EAE mice on days 14 and 21 p.i. compared to day 0 (day of immunization) (Shan et al., 2017). In addition, the level of miR-26a was significantly decreased at peak phase of disease compared to controls (Zhang et al., 2015). During acute phase of EAE the level of miR-155 was significantly increased compared to control mice but was not significantly different in the remission phase of EAE (Zhang et al., 2014). The levels of miR-409-3p, miR-141, miR-873, miR-1967, and miR-18b were significantly increased on day 14 and day 20 p.i. compared to controls (Liu et al., 2014).

Spinal cord
At pre-onset (day 10 p.i.) there was no significant change in the level of miR-92a in spinal cord compared to control mice, but the level was almost 5-fold higher at peak of disease (day 20 p.i.). The level of miR-92a at post-peak of disease (day 25 p.i.) was less than at peak of disease and not statistically significant (Rezaei et al., 2019). The levels of miR-155-5p and miR-18a-5p in spinal cords of EAE mice were significantly increased at peak of disease (days 18–20 p.i.) and chronic phase (days 25–30 p.i.). Also while that of miR-150-5p in the spinal cords was also significantly increased at peak of disease, it was significantly decreased in the chronic phase compared to normal mice (Shakerian et al., 2018). The level of miR-142a-3p and miR-142a-5p was significantly increased in lumbar spinal cord at peak of disease (days 18–20 p.i.) and post-peak phase (day 25 p.i.) of EAE mice compared to control mice (Talebi et al., 2017). By comparison, the level of miR-181a and miR-181b in lumbar spinal cord was significantly decreased at peak of disease and chronic phase (days 24–30 p.i.). Interestingly, at the pre-onset phase (day 10 p.i.) the level of miR-181a was significantly increased while that of miR-181b was significantly decreased compared to control mice (Ghorbani et al., 2017). In cervical

spinal cords collected at late phase (days 31–33 p.i.) there was a significant increase in the level of miR-21-5p, miR-142-3p, miR-142-5p, miR-146a-5p, and miR-155-5p, and a significant decrease in the level of miR-153-3p and miR-219a-5p, compared to control mice. The clinical score profile indicated a single peak at days 12–13 p.i., then declining to clinical score 0 at days 16–17 p.i. and continuing to day 30 p.i. (Gerrard et al., 2017). In the spinal cord at pre-onset stage (day 6 p.i.) the level of miR-155-5p and miR-429-3p was significantly increased and that of miR-291a-3p was significantly decreased. At onset stage (day 11–12 p.i.) miR-155-5p expression was significantly increased, while at peak of disease (~day 20 p.i.) there was a significant increase in the level of miR-155-5p and miR-9-5p (Singh et al., 2016). A significantly increased level of miR-155 (~10-fold) and miR-223 (> 30-fold), together with a significantly decreased level of miR-124 (~50%), was found in spinal cord at day 14 p.i. compared to controls (Satoorian et al., 2016).

Lumbar motor neurons
In lumbar motor neurons of EAE mice collected by laser capture microscopy the levels of miR-146a-5p (~2-fold), miR-27a-3p (~5-fold), miR-23a-3p (~7-fold), and miR-223-3p (~40-fold) were significantly increased at peak disease compared to control mice (Morquette et al., 2019). In another study there was a significant increase in the levels of miR-223-3p and miR-7056-5p at onset, and of miR-340-5p, miR-142a-5p, miR-203-3p, miR-490-5p, miR-423-5p, miR-6540-5p, miR-370-5p, miR-205-5p, miR-1969, miR-7a-5p, miR-381-3p, miR-101a-3p, miR-374b-5p, miR-199b-5p, miR-novel-chr7_31864, miR-223-3p and miR-7056-5p at peak of disease. There was a significant decrease in the levels of miR-novel-chr7_35252, miR-335-5p, miR-novel-chr12_57357, miR-183-5p, miR-129-1-3p, miR-148a-3p, miR-127-3p and miR-6540-5p at onset, and of miR-335-5p, miR-novel-chr16_70802, miR-183-5p, miR-125b-1-3p, miR-92b-5p, and miR-127-3p at peak. The clinical scores indicated a progressive EAE disease (Juzwik et al., 2018).

Microvessels of spinal cord
Laser capture microscopy of microvessels was used to collect enriched endothelium RNA. At peak of disease (day 17 p.i.) the level of miR-146a was significantly increased (8-fold) compared to normal mice, but during the EAE first remission stage was not significantly different from normal mice (Wu et al., 2015).

Retinal ganglion cells
RGC layer was collected from retina by laser capture microscopy. At peak stage miR-146a-5p (~2-fold), miR-27a-3p (~7-fold), and miR-23a-3p (~30-fold) were significantly increased compared to presymptomatic control animals whereas expression of miR-223-3p was unchanged (Morquette et al., 2019). There was a significant increase in the levels of miR-148a-3p, miR-199b-5p, miR-7a-5p, miR-381-3p, miR-101a-3p, miR-340-5p, and miR-203-3p at peak, and of miR-374b-5p, miR-370-5p, miR-7a-5p, miR-7056-5p, miR-381-3p,

miR-142a-5p, miR-205-5p, and miR-1969 at chronic stage. The levels of miR-129-1-3p at peak and of miR-129-1-3p and miR-127-3p at chronic stage were significantly decreased compared to normalized presymptomatic levels (Juzwik et al., 2018).

Effect of Overexpression or Suppression of miRNA on Experimental Autoimmune Encephalomyelitis Disease

EAE mice treated with miR-146a mimic once a week for 6 weeks initiated on day 14 p.i. had significantly decreased cumulative clinical score starting at day 29 p.i., with functional improvement persisting until day 90 p.i. (Zhang et al., 2019). Transgenic mice with overexpression of miR-182 had a shorter induction period of EAE disease and more severe clinical symptoms than wild type mice. There was no effect on the induction period in mice with knockdown of miR-182 but clinical symptoms were less severe and the disease course was shortened (Wan et al., 2019). After immunization to induce EAE on day 7 post-virus injection, lentivirus (LV)-sh-miR-181c-infected mice (miR-181c knockdown mice) had reduced EAE symptoms and decreased clinical scores compared to LV-Ctr-infected mice (Zhang et al., 2018b). Mice injected with LV-miR-23b followed by MOG_{35-55} immunization on day 5 post-virus delivery had a delay in disease onset compared to mice treated with LV-Ctr (day 13 *vs.* day 11 p.i.), and the LV-miR-23b-treated mice had much less severe disease (Zhang et al., 2018a). Mice infected with LV-miR-497 mimic and EAE induced on day 7 after lentivirus injection had significantly lower clinical scores of disease from day 7 to day 20 than the mice infected with LV-miR-497 sponge and LV-Ctr (Shan et al., 2017). Mice with knockdown of miR-223 had a delayed onset of disease and less severe clinical symptoms than wild-type mice (Santoorian et al., 2016). Mice infected with LV-miR-26a and immunized with MOG_{35-55} on day 7 after lentivirus injection had a delay in disease onset and mild EAE compared to LV-Ctr-infected mice, whereas LV-anti miR-26a-infected mice developed severe disease (Zhang et al., 2015). In another study, mice administered miR-155 mimic on alternate days from day 5 to day 15 after immunization with MOG_{35-55} developed severe disease, whereas mice administered miR-155 inhibitor had less severe disease. Treatment of EAE mice with miR-155 inhibitor after the onset of clinical signs (when a score of 1.5 was recorded) increased clinical recovery from EAE (Zhang et al., 2014). Mice immunized with MOG_{35-55} on day 7 following injection of LV-miR-20b had milder EAE than mice receiving LV-Ctr. Injection of LV-miR-20b at day 14 p.i. (onset of disease) decreased the severity of EAE (Zhu et al., 2014). Furthermore, mice infected with LV-miR-873 and immunized with MOG_{35-55} on day 7 after lentivirus injection had a shortened induction period and developed more severe EAE disease compared to LV-Ctr-infected mice, while EAE mice treated with LV-miR-873-sponge had a delayed onset of disease and milder clinical symptoms (Liu et al., 2014).

Future Perspectives

At present there is no cure for MS. Pharmacological treatments are available to lessen the symptoms, reduce the relapse rate, hasten recovery from attacks, and delay the progression of relapsing-remitting MS. Treatments for relapsing-remitting MS include interferon-β, glatiramer acetate, fingolimod, dimethyl fumarate, and siponimod, although they all have adverse side effects. Ocrelizumab and siponimod are the only medications approved by the US Food and Drug Administration to treat both relapsing-remitting and progressive forms of MS (Mayo Clinic), with ocrelizumab approved to treat primary progressive MS (Food and Drug Administration) and siponimod approved to treat secondary progressive MS (Food and Drug Administration). Furthermore, the biomarkers currently used for diagnosing MS are unsatisfactory in terms of sensitivity and specificity, and cannot identify patients who are in the asymptomatic phase of MS (before the onset of clinical symptoms). Clearly a biomarker with high diagnostic accuracy that could recognize the asymptomatic stage of the disease would enable treatments to be started much earlier and have a more profound effect on modifying the severity of disease and its progressive course.

The EAE model is the most used and characterized animal model of MS. We have previously reviewed recent studies of pharmacological therapies in animal models of MS and found that many agents including glatiramer acetate and siponimod had neuroprotective effects (Martinez and Peplow, 2020a). Also by immunizing animals with MOG_{35-55} peptide it was possible to develop either a relapsing-remitting or progressive form of the disease. A combination of an immunomodulatory agent tuftsin and a drug promoting remyelination benzotropine improved MS-like pathologies in an EAE model (Thompson et al., 2018). Also a combination of the immunomodulator glatiramer acetate and an antioxidant drug epigallocatechin-3-gallate delayed disease onset and decreased clinical symptoms in an EAE relapsing-remitting mouse model (Herges et al., 2011) but not in an EAE progressive disease mouse model (Janssen et al., 2015).

MiRNAs may serve as diagnostic biomarkers and to monitor disease progression in patients with MS (Martinez and Peplow, 2020b). The present review of studies with EAE mice has confirmed miRNAs as biomarkers of disease and importantly at the pre-onset (asymptomatic) stage when assessed in blood plasma and urine exosomes, and spinal cord tissue (**Figure 2**). The expression of certain miRNAs has also been found to be dysregulated at the onset and peak of disease in blood plasma and urine exosomes, brain and spinal cord tissue, and at the post-peak (chronic) stage of EAE disease in spinal cord tissue (**Figure 2**). Interestingly, the miRNA expression of lumbar motor neurons at peak of disease showed that miR-223-3p was significantly increased (~40-fold) compared to control mice (Morquette et al., 2019). Another study confirmed this increase at peak of disease and also at onset of disease (Juzwik et al., 2018). MiR-223 was increased in spinal cord tissues on day 14 p.i. which approximates to the time of disease onset (Satoorian et al., 2016). Further-

more, miR-142a-5p was increased in RGCs at chronic stage (Juzwik et al., 2018) and also in spinal cord tissues at chronic stage (Talebi et al., 2017).

Comparing the findings in the present review with those in a previous review on microRNAs in blood and cerebrospinal fluid of human MS patients (Martinez and Peplow, 2020b) has shown some similarities in microRNA profiles. For example, miR-125a expression was increased in blood serum of EAE animals at peak of disease, and miR-125a-5p expression was increased in peripheral blood of MS patients compared to healthy controls. MiR-15a expression was increased in blood plasma of EAE animals at onset of disease, and miR-15b expression was increased in blood serum exosomes of RRMS patients compared to healthy controls. Both miR-15a and miR-15b belong to the miRNA-15 superfamily and often show similar changes of expression in disease states (Houshmand-Oeregaard et al., 2018). In addition, miR-150 expression was increased in spinal cord tissues of EAE animals at peak of disease, and miR-150 expression was

increased in cerebrospinal fluid of MS patients compared to controls. MiR-155 expression was increased in blood plasma of EAE animals at pre-onset, onset and peak of disease, and shown to be increased in white matter lesions of MS patients (Ma et al., 2014). Interestingly, expression of miR-125a-3p and miR-99b was increased in pediatric MS patients and miR-99b expression was increased in blood serum of EAE animals at peak of disease. Further comparisons of miRNAs in EAE animals and MS patients are needed, particularly using experimental animals at a more similar stage of development in their life cycle as MS patients.

Therapies using miRNA mimics or inhibitors were found to delay the induction and alleviate the severity of EAE disease (**Table 1**). Mice treated with miR-146a mimic had less severe clinical signs that persisted long after treatment ceased (Zhang et al., 2019). Overexpression of miR-23b delayed EAE disease onset and reduced the severity of disease (Zhang et al., 2018a). Similarly overexpression of miR-497 lowered the severity of disease (Shan et al., 2017), and overexpres-
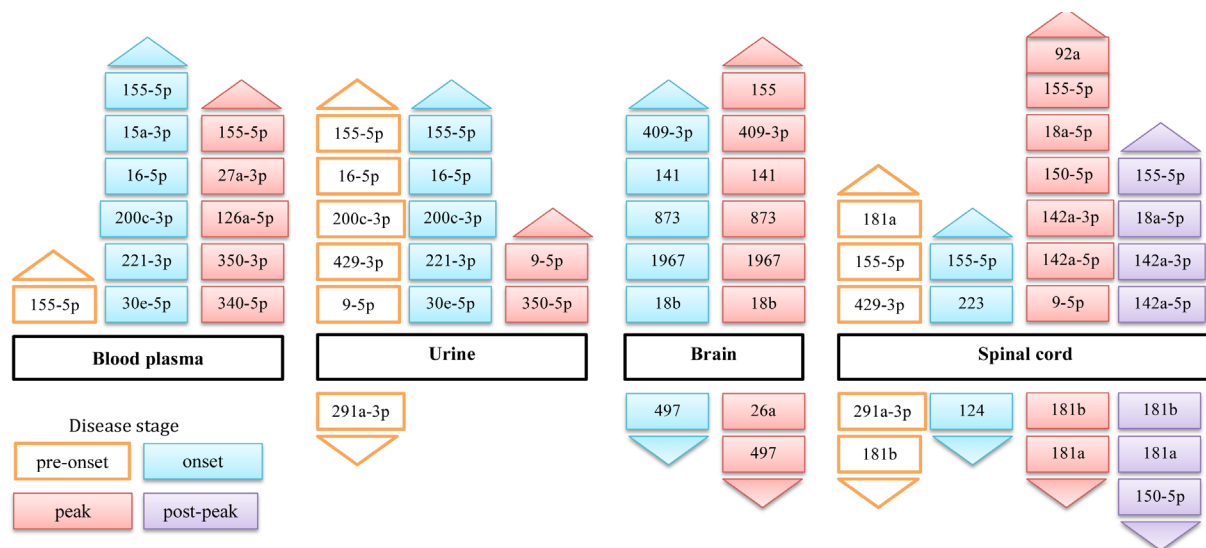

Figure 2 Changes in microRNA expression in blood plasma and urine exosomes, and brain and spinal cord tissues of experimental autoimmune encephalomyelitis mice during pre-onset (asymptomatic), onset, peak, and post-peak stages of disease as measured by real-time polymerase chain reaction assays.
Increased or decreased expression is indicated by arrows pointing upwards or downwards, respectively.

Table 1 Decreased severity of EAE disease following overexpression or suppression of specific microRNAs

Overexpression of microRNA	Suppression of microRNA	Decreased EAE clinical score	Decreased inflammatory infiltrate and demyelination of CNS	Reference
miR-146a		√		Zhang et al., 2019
miR-23b		√	√	Zhang et al., 2018a
miR-497		√	√	Shan et al., 2017
miR-26a		√	√	Zhang et al., 2015
miR-20b		√	√	Zhu et al., 2014
	miR-182	√		Wan et al., 2019
	miR-181c	√	√	Zhang et al., 2018b
	miR-223	√	√	Satoorian et al., 2016
	miR-155	√	√	Zhang et al., 2014
	miR-873	√		Liu et al., 2014

√ indicates this was observed in the study. CNS: Central nervous system; EAE: experimental autoimmune encephalomyelitis.

sion of miR-26a slowed the onset of disease and decreased its severity (Zhang et al., 2015). Administration of LV-miR-20b prior to EAE induction led to a milder EAE compared to mice receiving LV-Ctr (Zhu et al., 2014). Increased expression of miR-155-5p was found in blood plasma and urine exosomes and spinal cord (**Figure 2**). Treatment of EAE mice with miR-155 inhibitor starting before or after the onset of clinical signs reduced the severity of disease and increased clinical recovery (Zhang et al., 2014). Knockdown of miR-223 also delayed the onset of disease and lessened the severity of clinical symptoms (Santoorian et al., 2016), and mice with knockdown of miR-182 had less severe disease and a shortened disease course (Wan et al., 2019). Also knockdown of miR-181c caused less severe symptoms and decreased clinical scores (Zhang et al., 2018b). Mice treated with LV-miR-873-sponge had a delayed onset of disease and milder clinical symptoms (Liu et al., 2014). Such treatments have the potential of treating human patients with asymptomatic MS to delay or prevent disease onset, and to reduce disease severity and progression in patients with MS. Clinical trials with miRNA-based therapeutics have been initiated in the treatment of heart failure, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, hepatitis C virus, and wound healing (Hanna et al., 2019). On searching the EU Clinical Trials Register, it appears that while miRNA-based therapeutics have significant potential for treating and alleviating autoimmune diseases (Long et al., 2018; Salvi et al., 2019), no clinical trials are currently underway for miRNA therapeutics in patients with MS.

Further studies are warranted on determining miRNA profiles in blood plasma and urine exosomes of EAE animals since they could serve as biomarkers of asymptomatic MS and disease course. However, an important limitation of the studies reviewed was a lack of testing the dysregulated miRNAs as diagnostic markers of EAE disease by performing receiver operating characteristics analysis to determine areas under the curve as well as sensitivity and specificity values. Future studies should include this analysis so that the diagnostic importance of specific miRNAs can be fully assessed. As most of the studies reviewed had used female mice 6–12 weeks of age, studies should be performed with male mice of a similar age and also with older male and female mice 20–22 weeks of age, as the age of MS-affected patients is increasing due to increasing longevity (Vaughn et al., 2019). Also possible disease-modifying effects of miRNA mimics or inhibitors should be investigated in aged EAE animals.

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