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Review

Pathophysiology of translational regulation by microRNAs in multiple sclerosis

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ABSTRACT

MicroRNAs (miRNAs) comprise a group of several hundred, small non-coding RNA molecules with a fundamental influence on the regulation of gene expression. Certain miRNAs are altered in blood cells of multiple sclerosis (MS), and active and inactive MS brain lesions have distinct miRNA expression profiles.

Several miRNAs such as miR-155 or miR-326 are considerably overexpressed in active MS lesions versus controls, and mice lacking these miRNAs either through knock-out (miR-155) or by in vivo silencing (miR-326) show a reduction of symptoms in experimental autoimmune encephalomyelitis (EAE), a model system for multiple sclerosis.

This review describes miRNAs regulated in the blood or in brain lesions of MS patients in the context of their previously described functions in physiology and pathophysiology.

1. Introduction

Recent work on microRNAs (miRNAs) has added a new level of complexity to our understanding of gene expression and new insights into their contribution to many diseases associated with the immune system and inflammation.

Mature miRNAs are single-stranded RNA molecules approximately 22 nucleotides in length, which derive from larger hairpin-folded RNA precursors called premiRNA. (For review see [1].) The function of mature miRNAs is translational inhibition or degradation of a target messenger RNA (mRNA), which is achieved by binding miRNAs to specific target sequences in the 3′UTR of their target mRNA [2,3]. Notably, one miRNA may have several hundred mRNA target genes [4,5] and the expression of more than one-third of all mammalian genes might be regulated by miRNAs [6].

Several hundred miRNAs have so far been identified in humans [7], although the number of predicted small non-coding RNAs is more than one thousand. Two important nucleases involved in the processing of miRNAs are Dicer and Drosha. The removal of the Dicer gene is frequently used in *in vivo* models to define the involvement of miRNAs in a particular regulatory process. It has become clear in recent years that human miRNAs are for the most part highly conserved in other species, especially other mammals [8–10].

Posttranscriptional regulation by miRNAs plays an important role in development but also many biological processes in adulthood can be influenced. Several miRNAs are highly expressed in specific cell types and during particular stages of development [11,12].

It is increasingly recognized that miRNAs play essential roles in the immune system [13–15] anfor correct function in the mature CNS [16,17]. It was found that miRNAs are critical for the maintenance of immune tolerance, as can be seen in mice with a deletion of Dicer-mediated miRNA processing in regulatory T cells (Treg cells). These mice develop a fatal autoimmune disease which is very similar to the autoimmune syndrome of animals with a complete deficiency of Treg cells [18,19]. Moreover, inflammatory cytokines can regulate the expression levels of miRNAs in a distinct cell type as shown, for example, in astrocytes [20].

The involvement of miRNAs in the pathogenesis of diverse diseases such as cancer, cardiac failure, and neurodegenerative diseases such as Alzheimer's and Parkinson's disease, has been demonstrated [17,21–25].

MiRNAs are suited for therapeutic targets, as could be demonstrated with animal models of diverse diseases [26–28] and might presumably lead to new therapeutic strategies for autoimmune diseases such as multiple sclerosis (MS).

Only recently miRNA expression was detected in the context of MS. Six papers have been published in which profiles of miRNAs were found in the peripheral blood of MS patients [29–34] and

Abbreviations: miRNA, microRNA; MS, multiple sclerosis; Treg cells, regulatory T cells

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one report examines expression profiles of miRNAs in brain lesions of different activity in MS patients [20].

Dysregulation of inflammation, demyelination and axonal damage constitute the basis of pathology in multiple sclerosis, but it is still unclear how the disease is initiated and becomes chronic. A complex interaction between infiltrating immune cells and brain-resident cells leads to the development of lesions [35–37]. Activation of macrophages and microglia plays a crucial role in the active phase of the inflammation in the lesions during the breakdown of the myelin sheath [37–39]. So far, approaches searching for new insights into the pathogenesis of multiple sclerosis have been based on the examination of transcripts or the analysis of proteins either *in vitro* or *in situ*, ranging from the examination of the transcriptome of MS lesions [40] to the scrutiny of proteome of MS lesions [41]. Posttranscriptional regulation and modification of the translational level is increasingly recognized.

This review will discuss the potential involvement of miRNAs, with their prospective regulatory function in the pathogenesis of MS. The main focus will be on the characterization of miRNAs that has so far emerged from investigations into miRNA alterations in MS.

2. MiRNA profiling of blood and brain lesions from MS patients

In the past few years it has been shown that different patterns of early active inflammatory lesions exist in the brain of MS patients [42]. With the profound heterogeneity in clinical course, neuroradiological appearance of the lesions, involvement of susceptibility gene loci, and response to therapy, it would seem that multiple sclerosis comprises a large number of heterogeneous diseases. Apart from this, Neuromyelitis optica is defined as a separate inflammatory demyelinating disorder [43].

2.1. Will it be possible to define different disease entities with specific miRNA profiles?

Recently, specific lesion profiles of active and inactive lesions were described in the brains of MS patients [20]. Different miRNA profiles were found, depending on the activity of the lesions. Apart from this miRNA profiles were quantified in the blood of MS patients and showed differentially expressed miRNAs in MS patients versus healthy subjects [29–34].

The abundance of miRNAs in the peripheral blood might be influenced by the starting material (i.e. whole blood or purified blood leukocytes), the method of normalization, the method of quantification (array technology or quantitative PCR) or different therapies of the patients. All of these factors might influence differences between the studies of miRNA expression in the blood of MS patients. Brain lesion profiles might be influenced by the cellular composition and the regulation of miRNAs in the invading inflammatory cells as well as their regulation in brain-resident cells.

Despite these difficulties in miRNA profiling, there is some overlap between the studies. Interestingly, some of the miRNAs which were found to be regulated in active or inactive brain lesions also occurred in the blood of MS patients. Table 1 provides an overview of miRNAs, which were found to be regulated in MS in more than one expression study up till now.

The miR-326 upregulated in relapsed patients [31] was one of the most upregulated miRNAs in active MS lesions, especially in a very active form of the disease called the Marburg variant of MS [20]. The Marburg variant of MS describes a very fulminant disease course which often leads to the death of the patient and which displays all the histopathological features of active MS lesions, including the infiltration of immune cells, the phagocytosis of myelin and the degeneration of axons.

Lindberg and colleagues showed miRNA alterations in $CD4^+$ T cells, $CD8^+$ T cells and B cells from 23 untreated MS patients as compared to healthy controls [33]. Ten miRNAs were identified as being regulated in $CD4^+$ T cells from patients, in comparison to analogously prepared cells from healthy subjects. In $CD8^+$ T cells, four miRNAs were conspicuous and six miRNAs were differentially expressed in B cells in MS subjects. In subjects with MS, miR-497 was upregulated in $CD4^+$ T cells and B cells, whereas it was downregulated in $CD8^+$ T cells. Moreover, miR-193a was upregulated in $CD4^+$ T cells and miR-34a was downregulated in these cells. Finally miR-629 was upregulated in $CD8^+$ T cells, but miR-30a-3p appeared to be downregulated. Some of the miRNAs found to be regulated in blood cell subsets in MS patients in comparison to controls also appeared in active or inactive MS brain lesions. MiR-497, miR-629 and miR-30a-3p were upregulated in inactive lesions and miR-193a and miR-34a were upregulated in active lesions as compared to controls [20].

A recent study by Keller and colleagues described diverse miRNA expression profiles in whole blood cells of MS patients compared with healthy controls, and identified a number of miRNAs that were up- or downregulated [32]. Of the ten most significantly regulated miRNAs in this study, miR-142-3p and miR-223 were upregulated in active MS lesions [20].

De Santis and colleagues isolated $CD4^+CD25^{high}$ Treg cells from 12 MS patients and 14 healthy donors and examined the expression of 723 miRNAs by microarray analysis [30]. The expression of 23 miRNAs was altered more than 1.5 fold in MS versus healthy Treg cells. Most of these miRNAs were upregulated in MS versus healthy controls and only a few of them appeared to be downregulated. MiR-223 was also reported in this study as being upregulated in Treg cells from MS subjects. Among the other upregulated miRNAs, miR-15a and miR-22 were overexpressed in active lesions in the brains of MS patients [20] and miR-148a was upregulated in inactive lesions in comparison to normal brains [20].

Cox and colleagues measured miRNAs using microarray analysis in the peripheral blood of 59 untreated MS patients and 37 controls [29]. Of these 59, 18 had a primary progressive, 17 a secondary progressive and 24 a relapsing-remitting disease course. They found that miR-17 and miR-20a were significantly underexpressed in all MS subtypes.

It is reasonable that in MS, activity of the disease and the occurrence of relapses or phases of remission might alter the miRNA profile in the peripheral blood of MS patients. Evidence for this derives from two different recent reports which demonstrated miRNA alterations in the blood of MS patients during relapses in comparison to healthy controls [31,34]. Otaegui and colleagues showed an upregulation of three miRNAs (miR-18b, miR-493 and miR-599) during relapses [34]. However their results did not reach clearly significant levels, which might be due to the small number of patient samples examined. The expression of miR-326 was reported to be elevated in the peripheral blood leukocytes of patients with MS, especially during relapses [31]. This miRNA participates in the differentiation of Th-17 cells in a mouse model, which could also be demonstrated in this study [31]. Th-17 cells might play an important role in the pathophysiology of MS [44,45].

Cox and colleagues showed in their study that miR-17 and miR-20a modulate T cell activation genes in a knock-in and knock-down T cell model [29]. They also described these miRNAs as significantly downregulated in MS whole blood cells. These two miRNAs belong to a cluster of 7 different miRNAs, the so-called miR-17-92 cluster. Interestingly, miRNAs of this cluster were upregulated in the study by Lindberg and colleagues [33]. The described regulation was contrary in these two studies, which could be due to differences in the studied material (one of the studies used whole blood, in comparison to B cells and $CD4^+$ and $CD8^+$ T cells purified from peripheral blood mononuclear cells), the method of quantifi-

Table 1
miRNAs regulated in multiple sclerosis.

miRNA[a]	Regulated in tissue	Detection method	Ref.
miR-629	Upregulated in inactive MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Upregulated in CD8+ T cells from blood samples from MS patients versus healthy controls	qPCR	[33][g]
miR-497	Upregulated in inactive MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Upregulated in CD4+ T cells and B cells from blood samples from MS patients versus healthy controls, downregulated in CD8+ T cells from blood samples from MS patients versus healthy controls	qPCR	[33][g]
miR-422a	Upregulated in whole blood samples from MS patients in comparison to healthy donors	Microarray analysis	[32][f]
	Downregulated in B cells from blood samples from MS patients versus healthy controls	qPCR	[33][g]
miR-326	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Upregulated in peripheral blood leukocytes of MS Patients with relapses versus comparable cells from normal controls	qPCR	[31][c]
miR-223	Upregulated in whole blood samples from MS patients in comparison to healthy donors	Microarray analysis	[32][f]
	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Upregulated in CD4+CD25^{high} bona fide Treg cells from MS Patients versus comparable cells from healthy donors	Microarray analysis	[30][b]
miR-193a	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Upregulated in CD4+ T cells from blood samples from MS patients versus healthy controls	qPCR	[33][g]
miR-155	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	miR-155-/- mice were highly resistant to experimental autoimmune encephalomyelitis (EAE)		[48]
miR-148a	Upregulated in inactive MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Upregulated in CD4+CD25^{high} bona fide Treg cells from MS Patients versus comparable cells from healthy donors	Microarray analysis	[30][b]
miR-142-3p	Upregulated in whole blood samples from MS patients in comparison to healthy donors	Microarray analysis	[32][f]
	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
miR-126	Upregulated in CD4+ T cells from blood samples from MS patients versus healthy controls	qPCR	[33][g]
	Downregulated in whole blood samples from MS patients versus healthy controls	Microarray analysis	[29][d]
	Upregulated in inactive MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
miR-93	Upregulated in CD4+CD25^{high} bona fide Treg cells from MS Patients versus comparable cells from healthy donors	Microarray analysis	[30][b]
	Downregulated in whole blood samples from MS patients versus healthy controls	Microarray analysis	[29][d]
miR-34a	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Downregulated in CD4+ T cells from blood samples from MS patients versus healthy controls	qPCR	[33][g]
miR-30a-3p	Upregulated in inactive MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Downregulated in CD8+ T cells from blood samples from MS patients versus healthy controls	qPCR	[33][g]
miR-27a	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Downregulated in whole blood samples from MS patients versus healthy controls	Microarray analysis	[29][d]
miR-22	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][d]
	Upregulated in CD4+CD25^{high} bona fide Treg cells from MS Patients versus comparable cells from healthy donors	Microarray analysis	[30][b]
miR-20b	Downregulated in whole blood samples from MS patients in comparison to healthy donors	Microarray analysis	[32][f]
	Downregulated in whole blood samples from MS patients versus healthy controls	Microarray analysis	[29][d]
	Upregulated in inactive MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
miR-15a	Upregulated in CD4+CD25^{high} bona fide Treg cells from MS Patients versus comparable cells from healthy donors	Microarray analysis	[30][b]
	Upregulated in active MS brain white matter lesions versus normal brain white matter	qPCR	[20][e]
	Downregulated in whole blood samples from MS patients versus healthy controls	Microarray analysis	[29][d]
let-7i	Downregulated in whole blood samples from MS patients versus healthy controls	Microarray analysis	[29][d]
	Upregulated in CD4+CD25^{high} bona fide Treg cells from MS Patients versus comparable cells from healthy donors	Microarray analysis	[30][b]

[a] Only those miRNAs were included which appeared to be regulated in more than one MS miRNA expression study or which were relevant in an EAE model.
[b] Subjects: 12 patients with relapsing-remitting MS with a stable disease course; 14 healthy controls (for validation 10 MS patients and 10 controls); treatment: no immunomodulatory treatment.
[c] Subjects: 43 patients with relapsing-remitting MS (25 in relapse, 18 in remission); 11 patients with NMO (5 in relapse, 6 in remission); 42 controls; treatment: no drug treatment.
[d] Subjects: 59 patients with different disease course (18 primary progressive MS, 17 secondary progressive MS, 24 relapsing-remitting MS); 37 healthy controls; treatment: no immunomodulatory therapy within the previous 3 months.
[e] Brain specimens: 16 active lesions and 5 chronic inactive lesions from 20 autopsy cases with MS, 9 control white matter specimens from 9 control cases without any known neurological disease; treatment: not determined.
[f] Subjects: 20 patients with relapsing-remitting MS; 19 healthy controls; treatment: 9 patients with glatiramer acetate, 10 patients with interferon-b, 1 patient without any disease-modifying therapy.
[g] Subjects: 23 patients with relapsing-remitting MS; treatment: no immunomodulatory or MS-specific treatment within the previous 6 months.

cation and the methods used to normalize miRNA expression levels. Despite the contrary results, the abnormality in the expression of this miRNA cluster shows its significance in the pathogenesis of MS. It has already been demonstrated that the miR-17-92 cluster contributes to the development of autoimmunity in the presence of serum auto-antibodies and a lymphoproliferative disease in mice [49]. In this mouse model, the number of mature CD4$^+$ T cells was markedly increased and they had a highly activated profile, indicating a failure of peripheral tolerance.

3. Contribution of miRNAs to myelination

Destruction and insufficient regeneration of the myelin sheath are characteristic hallmarks of MS. While in the early phase of MS, remyelination can be observed relatively frequently, in later stages of the disease this process is less effective. Up to now it is not clear whether all the mechanisms which regulate developmental myelination have the same effects during remyelination. For example, in contrast to developmental myelination, Notch signaling seems not to be a rate-determining factor in experimental remyelination [50]. Despite this some of the mechanisms which exert influence on developmental myelination have conserved roles in remyelination. For instance the regulation of Lingo-1 has influence on developmental myelination [51]. Lingo-1 is expressed in oligodendrocytes and it is a strong negative regulator of the differentiation of oligodendrocytes as well as axonal myelination. Loss of Lingo-1 led to a considerably enhanced myelination in a mouse model, whereas overexpression of Lingo-1 caused an inhibition of oligodendrocyte differentiation and myelination [51]. It also modulates the remyelination in animal models of demyelination [52].

It has recently been reported that miRNAs play a relevant role in the process of developmental myelination. Whether this can be

transferred to remyelination in MS has to be determined in further studies.

The role of miRNAs in the generation of myelin was demonstrated in a mouse model in which conditional ablation of the enzyme Dicer in the oligodendrocyte lineage cells resulted in a fundamental disturbance of myelination [53–55]. Particularly two miRNAs, miR-219 and miR-338, are involved in myelination, as could be demonstrated in these studies. It was shown that the generation of miRNAs was not necessary for the generation of oligodendrocyte precursors. Only the post mitotic stadium of oligo dendrocyte lineage cells is significantly affected. Absence of miRNAs resulted in blocked myelination, although a great number of oligodendrocyte precursors expressing platelet-derived growth factor receptors (PDGFrs) were observed [53,55]. A recently suggested model describes these miRNAs as "brakes" that defer the proliferation of oligodendrocyte lineage cells, thereby triggering myelination [56] and Fig. 1. Expression of Dicer is consistently increased during oligodendrocyte differentiation [57]. Demyelination, oxidative damage, lipid accumulation, inflammation, astrocytosis microgliosis, and ultimately neuronal degeneration and axonal damage were observed due to the lack of miR-219 and its target ELOVL7 (elongation of very long chain fatty acids protein 7) in a mouse model with a deletion of Dicer in mature oligodendrocytes [54].

Interestingly, having an impaired ability to differentiate over time, oligodendrocyte lineage cells are present in demyelinating multiple sclerosis lesions [58,59]. Remarkably, miR-219 and miR-338 are significantly heavily downregulated in chronic inactive demyelinated MS lesions [20].

The functional importance of high levels of miR-219 and miR-338 in oligodendrocyte lineage cells has been demonstrated. More studies are needed to show the functional importance of low levels of miR-219 and miR-338 and others in demyelinated multiple sclerosis lesions.

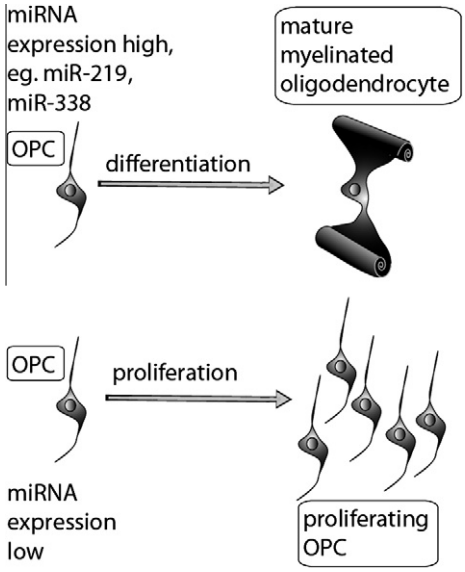

Fig. 1. Involvement of miRNAs in myelination: miRNAs modulate oligodendrocyte proliferation and differentiation. High miRNA levels of, eg. miR-219 or miR-338 and others, lead to the differentiation of oligodendrocyte precursor cells (OPC) by targeting factors like PDGFRa, Sox6, FoxJ3, and ZFP238. None of these proteins are affected by miRNA inhibition if miRNA levels are low, as this promotes OPC proliferation.

4. MiRNAs modulating the innate immune response

In the development and course of MS, many different components of the innate and adoptive immune system play fundamental roles (for review, see [60]).

Some miRNAs which appeared to be regulated in MS lesions or MS patient blood were recently described as having regulatory functions in the immune system [14] or in other autoimmune disorders [61]. In rheumatoid arthritis (RA), miR-155 and miR-146a were upregulated in synovial tissue [62] in comparison to controls. Both of them were also upregulated in active MS lesions [20].

MiRNAs have the potential to regulate TLR signaling [63]. In an early study, miRNA profiles of the human monocyte cell line THP1 were determined after treatment with the Toll-like receptor 4 (TLR4) ligand, lipopolysaccharide (LPS) [64], which revealed a strong upregulation of miR-146, miR-155 and miR-132. MiR-146a was upregulated after treatment with many different inflammatory stimuli, such as other TLR ligands and cytokines like IL1b, whereby its induction is obviously NF-κB dependent. Notably, astrocytes express several TLRs [65] and their miRNA levels change strongly after many different inflammatory stimuli [20]. MiR-146a was remarkably inducible *in vitro*, especially after stimulation with IL1β [20].

TRAF-6 and IRAK-1, two key TLR4 signaling proteins were shown to be mRNA targets of the miR-146a [64] and Fig. 2. It was recently reported that miR-146a functions as a negative regulator of IL8 and RANTES production, whereas negative regulation of TRAF-6 and IRAK-1 was not seen [66]. It seems likely that miR-146a represents a negative regulator of TLR signaling [64]. In murine bone marrow-derived macrophages, miR-155 was upregulated in response to the TLR3 ligand poly-inositolcytidine (poly(I:C)) and interferon beta [67]. The group identified upregulation of miR-155 as dependent on the MAP kinase JNK [67]. The induction of this miRNA was also reported from other groups [68–70]. In the lung, diverse miRNAs, including miR-214, miR-21 miR-223 and miR-142-3p, are upregulated after a short time period of only one to three hours after treatment with LPS [71]. These miRNAs were also found to be upregulated in active MS lesions in comparison to controls [20]. Involvement of TLR signaling and its effect on miRNA levels was investigated in another study in which infection of cholangiocytes with cryptosporidium parvum or treatment with LPS led to downregulation of the miR-let 7i. This miRNA appeared to be regulated in two independent miRNA MS profiling studies [29,30]. It was shown to be downregulated in whole blood samples from MS patients [29] and upregulated in $CD4^+CD25^{high}$ bona fide Treg cells from MS patients versus healthy donors [30]. A predicted target of this miRNA is TLR4 itself, and overexpression of miR-let 7i accompanied by reciprocal expression of this TLR4 has been previously observed [72].

5. MiRNAs modulating the adaptive immune response

Early studies connecting miRNAs to T cell function showed abnormalities in Dicer-deficient T cells. Dicer knockout resulted in a deviant helper T cell differentiation and cytokine production. $CD8^+$ T cell differentiation was defective in the absence of Dicer, and a low number of $CD4^+$ T cells was produced which were limited in their proliferation after stimulation and which had an increased apoptosis rate [73,74].

MiRNA profiling of $CD4^+$ $CD25^+$ Treg cells revealed an upregulation of distinct miRNAs, such as miR-21, miR-146a, miR-223, miR-214, miR-125a and miR-155, in comparison to naive T cells [75]. Other miRNAs, such as miR-150 and miR-142-5p, were downregulated in Treg cells [75]. Expression of most of these miRNAs could be induced by overexpression of the Treg-specific transcription factor FoxP3. This miRNA profile appeared to be similar to the profile of activated $CD4^+$ T helper cells [75].

Treg cells are thought to play an eminent role in the pathogenesis of MS, which could be demonstrated in MS patients as well as

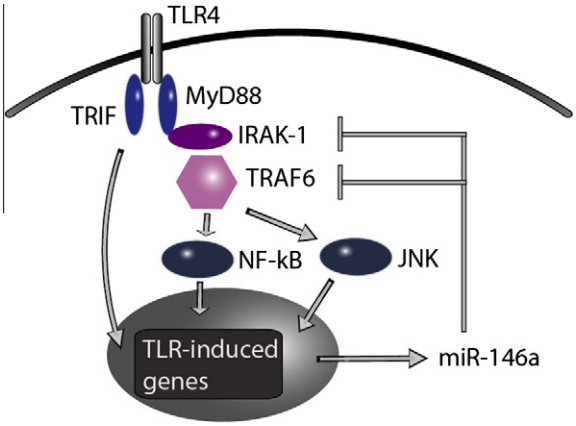

Fig. 2. miR-146a modulates TLR signaling: In addition to other miRNAs, miR-146a is potentially involved in the modulation of TLR signaling. Two important molecules of TLR4 signaling, TRAF-6 and IRAK-1, are targets of this miRNA. The overexpression of miR-146a caused by the upregulation of TLR-induced genes might thus influence the signaling in a reciprocal way.

in animal models of the disease (For review see [76]). Remarkably, miR-21, miR-146a, miR-223, miR-215 and miR-155 were all upregulated in active MS lesions [20]. A direct connection between this striking similarity of miRNA profiles of $CD4^+$ $CD25^+$ Treg cells and active MS lesions remains to be proven.

One recent study focused on alterations of miRNAs in $CD4^+$ $CD25^+$ Treg cells in MS patient blood versus controls [30]. In this study, 23 human miRNAs were identified as being differentially expressed between $CD4^+CD25^{high}$ *bona fide* Treg cells from MS patients vs. healthy donors. Some members of the miR-106b-25 cluster were found to be downregulated in MS patients when compared to healthy donors in $CD4^+CD25^{high}$ $CD127^{dim/-}$ Treg cells. The group focused on miR-106b and miR-25 from this cluster, which were previously shown to modulate the TGF-beta signaling pathway through their action on CDKN1A/p21 and BCL2L11/Bim [77]. TGF-beta is involved in Treg cell differentiation and maturation [78–81]. Therefore, the deregulation of this miRNA cluster may alter Treg cell activity in the course of MS by altering TGF-beta biological functions. Some of the miRNAs which were described by De Santis and colleagues were also previously found in other studies describing altered miRNA expression in MS (see Table 1).

Autoreactive T cells against myelin proteins occur in all healthy individuals [46,47].

Do altered miRNAs in these cells provide one missing link discriminating aggressive versus non-aggressive T cells in MS patients versus healthy subjects?

Some miRNAs regulated in MS have been described as having an influence on T cell pathogenicity. MiR-155 was shown to have an impact on Th-17 development [48], as did miR-326 [31]. Both of them are highly upregulated in the brain of MS patients [20].

Aspects of the impact of miRNAs involved in the pathophysiology of MS on the proliferation, activation and differentiation of $CD4^+$ T cells are depicted in Fig. 3.

In the following, a selection of miRNAs prominently involved in the regulation of immune cells or brain resident cells, which were found to be regulated in studies with MS patient blood or MS brain lesions, will be discussed in detail.

5.1. MiR-155

MiR-155 was among the most upregulated miRNAs in active MS lesions compared to control brain specimens [20]. In this study its level is more than 10-fold higher in MS lesion tissue than in respective control tissue.

MiR-155 is a miRNA with many different functions in the immune system. MiR-155-deficient mice display considerable immune defects. Notably, the B cell response and the Th2 helper T cell response are affected [68,69]. MiR-155 is important for production of antigen-specific IgGs [68,69,82]. Apart from this, miR-155 expression is dramatically increased in $CD4^+$ T cells upon their activation, suggesting a functional importance for this miRNA in activated T cells [83]. Many targets of miR-155 with potential immunological relevance have been identified by expression profiling. Transcription factors such as c-Maf, cytokines and signal proteins are targets of miR-155 (for review see [84]). C-Maf functions as a promoter of Th2 cell development [68], while SOCS1, another target of miR-155, is repressed by miR-155 in both $FoxP3^+CD4^+$ Treg cells and $FoxP3^-$ $CD4^+$ T cells, and impacts Treg cell fitness [85]. A complex picture of how miR-155 affects T cell developmental pathways is imminent and it appears to involve many targets and pathways. This may also be true for miR-155's positive role in Th17 cell development [48].

Interestingly, depletion of miR-155 leads to a reduction of tumor necrosis factor alpha (TNFα) mRNA, suggesting a positive regulation of TNFα production by miR-155 [69]. Consistently, upregulation of miR-155 provoked by exposure to LPS also enhances TNFα production [70].

In active lesions in the brain of MS patients, macrophages and activated microglia are the predominant cell types. MiR-155 plays an important role in macrophage response to inflammatory stimuli [67]. This is partially achieved by targeting SMAD2 and thereby influencing the TGF-beta signaling [86]. It was recently shown that this miRNA has a direct effect on the regulation of macrophage phenotype by targeting IL13Rα1 [87]. Expression of miR-155 is upregulated by inflammatory cytokines such as TNFα or IL-1 in astrocytes [20]. Along with other miRNAs, miR-155 targets the surface protein CD47 [20], which is downregulated in chronic active [88] and active [20] MS lesions. CD47 inhibits the phagocytic activity of macrophages [89–91] and cytokine production of dendritic cells via signal regulatory protein-α [92]. Owing to its inhibitory effect on macrophages, CD47 has been regarded as a 'don't eat me signal' and as a 'marker of self' [90,91]. By having an influence on the expression of CD47, miR-155 might have a direct effect on the activity of macrophages in active MS lesions [20].

The importance of CD47 as a marker of self is also supported by the observation that interspecies incompatibilities of CD47 contribute to the rejection of xenogeneic grafts by macrophages [93]. In line with this, miR-155 overexpression was observed to be upregulated in acute rejection reactions of renal allografts compared to normal renal allografts [94].

One of the properties of miR-155 is the promotion of T cell-dependent tissue inflammation. Mice with a deficiency of this miRNA are resistant to the development of active EAE, a model system for multiple sclerosis [48].

MiR-155 and its star-form partner miR-155* were induced at different time points during stimulation of Toll-like receptor 7 (TLR7) in plasmacytoid dendritic cells (PDCs), and it was demonstrated that they regulate the interferon production in PDCs [95]. In doing so, miR-155* augmented interferon-α/β expression by suppressing IRAKM, whereas miR-155 inhibited their expression by targeting TAB2.

Due to its various functions in the immune system, it is likely that miR-155 also has different functions in MS pathology. This might be true also for other miRNAs regulated in the blood or brain tissue of MS patients.

5.2. MiR-326

MiR-326 might also be critically involved in the pathogenesis of MS. It is one of the most upregulated miRNAs in MS lesions and it

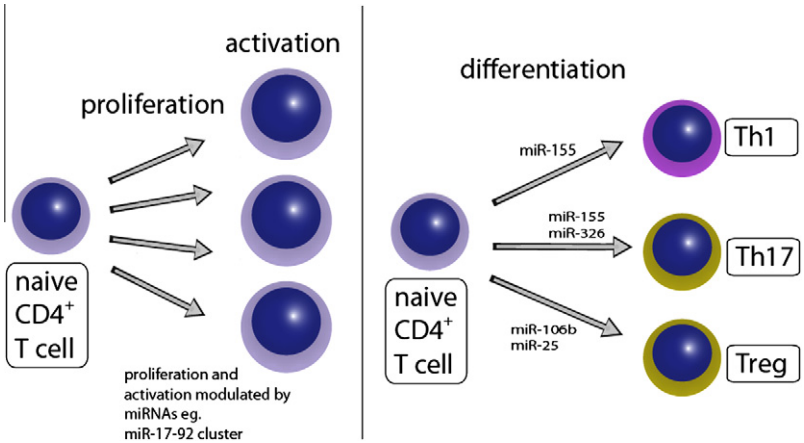

Fig. 3. miRNAs in the differentiation and function of CD4$^+$ T cells: Diverse miRNAs have been described in modulating the proliferation and activation of naïve CD4$^+$ T cells. The miR-17-92 cluster is one important example for this modulation. In a mouse model, the miR-17-92 cluster contributes to the development of autoimmunity and induces a lymphoproliferative disease [50]. In this model, the number of mature CD4$^+$ T cells was markedly increased and they had a highly activated profile. MiRNAs were also described in the differentiation of naïve CD4$^+$ T cells. MiR-155 promotes the differentiation of inflammatory T cells, including the T helper 17 (Th17) cell and Th1 cell subsets. The transcription factor C-Maf is targeted and downregulated by miR-155. By the lack of this transcription factor, which has an important role in the development of Th2 cells, the differentiation is shifted towards the Th1 phenotype. By targeting Ets-1, a known negative regulator of Th-17 differentiation, miR-326 is able to promote Th-17 differentiation. MiR-106b and miR-25 were shown to regulate the TGF-beta signaling pathway by targeting CDKN1A/p21 and BCL2L11/Bim [49]. Treg cell differentiation and maturation is modulated by TGF-beta [78–81].

was found to be upregulated during relapses in the blood of MS patients [31]. In an animal model, inhibition of miR-326 was shown to reduce EAE symptoms by preventing Th17 cell differentiation through a T cell-intrinsic mechanism [31]. It was shown that miR-326 targets Ets-1, a known negative regulator of Th-17 cells. MiR-326 promoted the generation of Th-17 cells both *in vivo* in mice and *in vitro* [31].

5.3. MiR-223

Another example of a miRNA which could be involved in the pathogenesis of MS is miR-223. Originally, it was described in the regulation of granulopoesis. Mice with a deficiency of miR-223 have an increased number of granulocyte precursors which are hypermature and particularly sensitive to activating stimuli [96]. Hence it could be shown that miR-223 was an important negative regulator of granulopoesis by targeting the transcription factor Mef-2c. Another study indicates that miR-223 is critical in macrophage differentiation [97]. By targeting transcripts of the kinase IKKα, miR-223 together with miR-15a and miR-16 modulate the NF-κB pathway. Thus a downregulation of these miRNAs might prevent macrophage hyperactivation. MiR-223 also occurs in CD4$^+$ CD25$^+$ Treg cells, where it appeared upregulated in comparison to naive T cells [75].

Up to now, three studies showed an upregulation of this miRNA in the context of MS (see Table 1). It was found upregulated in the blood of MS patients compared to healthy controls [32], as well as in CD4$^+$CD25^{high} *bona fide* Treg cells from MS patients [30] versus comparable cells from healthy donors, and also in active MS lesions in the brain compared to normal brain specimens [20].

5.4. MiR-142-3p

MiR-142 is located on chromosome 17 at a site which is associated with a translocation in B cell lymphoma [98,99]. This miRNA is among the most highly expressed miRNAs in many hematopoietic lineages, T cells and B cells as well as in stem cells, and its expression also depends on the activation state of the cell type [100,101].

A low level of intracellular cAMP is needed for the activation of conventional CD4$^+$ T cells, whereas naturally occurring Treg cells contain high levels of cAMP and exert their suppressor function by transferring cAMP into conventional T cells through gap junctions [102]. MiR-142-3p also has the potential to target AC9 mRNA in T cells, which restricts cAMP production [103]. This effect might not be restricted to T cells but may also play a role in other cell types.

5.5. MiR-148a

So far, two studies have demonstrated the connection between miR-148a and MS. This miRNA was upregulated in the brain in inactive MS lesions versus controls [20] and in CD4$^+$CD25^{high} *bona fide* Treg cells from MS patients versus controls [30].

Recently, interesting functions of miR-148a in the immune system were observed. MiR-148a could inhibit LPS-induced upregulation of MHC II expression on DCs [104]. Moreover it was able to inhibit DC production of cytokines, especially including IL-12 after various stimuli [104]. It seems that miR-148a acts as a negative regulator for the innate response and APC function of DCs.

Another study provided evidence that dysregulation of miR-148a is involved in SLE, which inhibits DNA methyltransferase 1 (DNMT1) expression and promotes DNA hypomethylation in lupus CD4$^+$ T cells [105]. This in turn leads to the overexpression of autoimmune-associated methylation-sensitive genes, such as CD70 and LFA-1, via promoter demethylation.

Adoptive transfer of T cells made autoreactive by treatment with DNA methylation inhibitors or by transfection with LFA-1 is sufficient to cause a lupus-like disease in mice [106,107].

5.6. MiR-126

Another miRNA, miR-126, which is dysregulated in the blood [29,33] and inactive brain lesions of MS patients [20], was also reported to target DNMT1, thus regulating the activity of CD4$^+$ T cells in SLE [108]. In other studies it was shown that miR-126 [109,110] and its host transcript Egfl7 [111] are expressed in endothelial cells.

In vivo application of a complementary sequence to miR-126, a so-called antagomiR, showed a markedly reduced angiogenic response in an experimental ischemia mouse model in which ischemia was induced in the hind limb by ligation of an artery [112]. It

was suggested that miR-126 increases the sensitivity of activated endothelial cells to VEGF or other growth factors by directly repressing negative regulators of the VEGF pathway, including the Sprouty-related protein, SPRED1, and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2) [113].

5.7. MiR-497

What the function miR-497 is in autoimmune disease has yet to be investigated. It was upregulated in $CD4^+$ T cells and B cells, but downregulated in $CD8^+$ T cells in MS patients compared with the expression in control subjects [33]. Apart from this, it was upregulated in inactive MS brain lesions versus controls. Recently it was suggested that miR-497 promotes ischemic neuronal death by negatively regulating the antiapoptotic proteins bcl-2 and bcl-w [114]. Because of the regulation of this antiapoptotic pathway, miR-497 may contribute to the pathogenesis of ischemic brain injury in stroke [114]. Future studies will have to clarify whether this also plays a role in degenerative processes of the MS brain.

5.8. MiR-34a

Two different studies showed a regulation of miR-34a in MS. MiR-34a was upregulated in active MS lesions and downregulated in $CD4^+$ T cells in blood samples from MS patients versus controls.

Only recently, miR-34a together with other related miRNAs (miR-34b and miR-34c) were identified as transcriptional targets of p53. Furthermore, He and colleagues demonstrated that they were able to target mRNA transcripts, which are important in cell cycle progression and cell survival [115]. They showed that miR-34 family miRNAs mediate growth arrest in a variety of cell types. Interestingly, a specific role for miR-34a during neuronal differentiation has been uncovered lately. Upregulation of miR-34a during mouse neuronal stem cell differentiation was found and this appeared in parallel to the development of postmitotic neurons [116]. Some potential miR-34a target transcripts might be involved in the transition towards postmitotic neurons. Previously it was shown that miR-34a targets silent information regulator 1 (SIRT1) [117]. MiR-34 inhibition of SIRT1 leads to an increase in acetylated p53 and expression of p21 and PUMA, which are transcriptional targets of p53 [117]. As miR-34a itself is a transcriptional target of p53 this interaction suggests a positive feedback loop between p53 and miR-34a [117].

6. Concluding remarks and future perspectives

Although the examinations of blood and tissue from patients are always only snapshots of miRNA expression, and the behavior of miRNAs during a certain process in MS is difficult to determine, there are until now several overlaps between the expression profiles of miRNA in MS in the published studies (for summary see Table 1).

The key question as to whether it will be possible to define different disease entities of MS with specific miRNA profiles remains open up to now. There is a good chance that future miRNA profiling studies will provide an answer to this question.

Whether those miRNAs described in the recent MS profiling studies might serve as valuable diagnostic markers for a specific state of the disease will be evaluated in future studies. Moreover, their value as therapeutic markers for the selection of individual therapies or as proof of the therapeutic effects of particular drugs still needs to be determined.

Many of the miRNAs regulated in the blood or CNS lesions of MS patients have been identified as key regulators of immune cell development and function, as well as master gene regulators of physiological and pathological processes of other brain-resident cells, such as oligodendrocytes or astrocytes. The second key question - as-yet unanswered - is whether altered miRNAs in the immune cells provide a missing link towards discriminating aggressive versus non-aggressive cell subsets in MS patients versus healthy subjects.

The observation that a single miRNA can have several effects on different cellular lineages indicates that functional miRNA targets may vary in individual cell types and their diverse activation and differentiation stages. Thus one miRNA might have many roles, starting from guidance of cell development to the activation and modulation of immune cells.

One major goal in the next few years will be to understand the pathophysiology of the human disease MS and to find appropriate pharmaceuticals to combat the disease. A better understanding of MiRNAs is essential for both of these efforts.

7. Disclosure Statement

The author declares no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three (3) years of beginning the work submitted that could inappropriately influence (bias) this work.

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