MicroRNAs in gray and white matter multiple sclerosis lesions: impact on pathophysiology

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Abstract

Multiple sclerosis (MS) is a chronic disease of the CNS, hallmarked by inflammation and demyelination. Early stages of the disease frequently show active lesions containing numerous foamy macrophages and inflammatory cells. Disease progression is highlighted by increasing numbers of mixed active/inactive or inactive lesions showing sparse inflammation and pronounced astrogliosis. Furthermore, gray matter lesions increase in number and extent during disease progression. MicroRNAs (miRNAs) comprise a group of several thousand (in humans more than 2000), small non-coding RNA molecules with a fundamental influence on about one-third of all protein-coding genes. Furthermore, miRNAs have been detected in body fluids, including spinal fluid, and are assumed to participate in intercellular communications. Several studies have determined miRNA profiles from dissected white and gray matter lesions of autopic MS patients. In this review, we summarize in detail the current knowledge of individual miRNAs in gray and white matter lesions of MS patients and present the concepts of MS tissue lesion development based on the altered miRNA profiles.

Keywords: miRNA; multiple sclerosis; demyelinated lesions; white matter lesions; gray matter lesions; inflammatory lesions; chronic inactive lesions

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Introduction

The recent past has seen rapid advances in the study of microRNAs (miRNAs) in many diseases, including multiple sclerosis (MS). These advances result in part because miRNAs are stable even in formalin-fixed paraffin-embedded tissue and can therefore be reliably detected in archived tissue [1]. Thus, in MS, miRNA profiles have now been determined from normal-appearing white matter (NAWM), white matter lesions (WML) and different types of gray matter lesion (GML) (subpial and leukocortical) and show characteristic alterations [2–6]. Concepts concerning pathomechanisms in MS lesions have been developed based on these miRNA profiles, which is the basis of this review.

Multiple sclerosis

MS is a chronic inflammatory and demyelinating disease of the CNS. The neuropathological hallmarks of the disease are in both WML and GML. During the early phase of disease, the lesions are located mainly in the white matter and frequently exhibit pronounced inflammatory alterations [7]. Activated, foamy macrophages and microglia play a crucial role in the active phase of inflammation during the degradation of myelin sheaths [8–10]. In the early phase of lesion development, during myelin phagocytosis (early active lesions), four histomorphological subgroups can be defined based on the abundance of complement factors, or abundance/loss of various myelin proteins, and oligodendrocyte apoptosis [11,12]. Inactive lesions dominate the histological picture of later disease stages [13]. New active WMLs may also develop, but GMLs increase disproportionately in number and size during these disease stages [7,8]. Three lesion subtypes, i.e. subpial, intracortical and leukocortical, are distinguished depending on their localization within the gray matter [14,15]. Only early GMLs show significant inflammatory changes [16], which means that most visible GMLs are chronic in nature and therefore most similar to inactive WMLs [14]. Inactive MS lesions are sharply delineated from NAWM and show demyelination, more or less pronounced loss of oligodendrocytes and virtually no lymphocytic and histiocytic infiltrate in the lesion’s center, whereas frequently only slight inflammation is seen in the lesion’s rim (mixed active/inactive lesions versus inactive lesions) [13]. Notably, GMLs generally show less inflammation than WMLs [14,17].
The capacity of remyelination is highest in early disease stages [2], nonetheless even completely remyelinated lesions (so-called shadow plaques) have thinner myelin sheaths than regularly myelinated axons [11]. The consequences of chronic inflammation, myelin loss and the reduction of oligodendrocytes are astrocytic scar formation and microgliosis, as well as a decrease in remyelination capacity that in turn results in axonal transport deficiency and axonal loss [11]. Interestingly, despite axonal loss, neuronal cell bodies are somewhat better preserved [18–20]. Nonetheless, patients show increasing disabilities during the course of MS, which is significantly correlated with axonal reduction [21]. Although much research has been carried out in the field, knowledge of the exact mechanisms of MS progression and, hence, therapeutic approaches are still lacking.

MicroRNAs

Nowadays, researchers try to identify genetic and epigenetic alterations in order to gain a better insight into pathophysiological processes. MiRNAs are small non-coding RNAs, normally composed of 19–23 nucleotides. They are released from larger RNA sequences, so-called pre-miRNAs via the enzyme dicer (for a review see [22]). A single RNA strand from either the 5′ or the 3′ end of the hairpin structure of the pre-miRNA is integrated into the RNA-induced silencing complex process, which leads to activation of the mature miRNA [22]. Binding of the miRNA’s seed region (nucleotides 2–7 of the miRNAs) to their target gene is based on complementarity of their RNA strands [23].

By binding to these distinct target sequences in their target’s 3′UTR, miRNAs are capable of influencing protein expression post-transcriptionally, via inhibition of translation or degradation of the targeted mRNA transcripts [24,25]. On the one hand, a single miRNA can regulate a multitude of targets, whereas on the other hand, mRNA targets are generally regulated by diverse miRNAs [26,27]. Due to similarities in their mature sequences and/or in the structure of their pre-miRNAs, some miRNAs are subsumed into groups called miRNA families [28,29]. This is of particular interest because miRNAs of the same family suppress the same target genes due to their similar seed sequences [30]. Therefore, miRNAs can control and modulate many different signaling pathways. In fact, it is estimated that more than 60% of human genes are regulated this way [31]. In addition, miRNAs can be sequestered into the blood or cerebrospinal fluid (CSF) via exosomes, where they are presumably involved in intercellular communication [32].

Diverse regulated miRNAs have been identified in MS lesions of different activity and localization

To date, there are relatively few studies of miRNA profiles in CNS tissue (WML, GML or NAWM) of MS patients, as opposed to similar studies investigating miRNA alterations in CSF and blood [2–6,33,34]. Despite their scarcity, these studies found that miRNAs were distinctively and reproducibly altered in MS-affected CNS tissues, depending on the localization (white or gray matter) as well as on the inflammatory activity of the lesions (Table 1). One recently published study also evaluated miRNA levels in chronic inactive lesions [35]. The results of this study were omitted from Table 1 and Figures 1 and 2 because miRNA alterations were compared with corresponding levels in NAWM. Therefore a direct comparison with the results of the abovementioned studies, in which MS tissue was matched with non-MS tissue, is elusive.

Remarkably, each type of lesion has its own distinctive set of upregulated and downregulated miRNAs, and even NAWM shows alterations in miRNA expression profiles compared with healthy controls [2–6]. As we will show below, despite methodological differences (see supplementary material, Table S1), there is good overlap of miRNAs between the individual studies and different lesion types that are repeatedly detected. Considering the targets of these miRNAs, one can draw conclusions on the pathophysiological processes in which miRNAs are involved [36,37]. However, it is necessary to assign the alterations in miRNA levels to the responsible sources, local or infiltrating cells, because the specific miRNA targets may differ substantially between different cell types. In this, both local cells, such as microglia, astrocytes, oligodendrocytes and neurons, as well as invading leukocytes (including lymphocytes and macrophages) have to be taken into account [37]. Whether miRNAs are cell bound or exosomal probably also plays a role, as recent studies on CNS pathologies have shown [38]. Furthermore, it should be noted that most transcripts have numerous possible miRNA binding sites predicted by computer algorithms (such as Targetscan [23] or PICTAR [39]). Only a part of these are biologically active and play roles in (patho-)physiology [40]. Therefore, the predicted miRNA targets have to be confirmed experimentally [41]. Downregulated miRNAs might be of particular interest, because decreased miRNA levels normally result in increased expression of their targets, which in turn might have a great impact on the development of lesions.

The impact of miRNAs on different features of MS

Reactive gliosis

Activated microglial cells and foamy myelin phagocytizing macrophages in NAWM and active lesions, respectively, display typical characteristics of MS. The level of activity of these histiocytic cells seems to have a great impact on inflammation and tissue injury [42–45]. In this context, miR-155 is upregulated in all types of lesion, including active and inactive lesions as well as NAWM (Table 1, Figure 3). It is upregulated in astrocytes via the proinflammatory cytokines TNF-α and IL-1 [5] and
seems to be a key regulator of the response of macrophages to inflammatory stimuli [46], mediated through interaction with SMAD2 and consecutive activation of TGF-β signaling [47]. Furthermore, miR-155 targets IL-13Ra1, which influences macrophage phenotypes [48]. Nevertheless, it is likely that macrophage activity is also regulated indirectly via the impact of miRNAs on local cells. Junker and colleagues [5] discovered that the 10 most strongly regulated miRNAs in active lesions (including miR-155) were located in glial cells and targeted the immune-modulating molecule CD47. This is a ‘marker of self’ and its reduced expression on cell surfaces followed by decreased interactions with its binding partner signal regulatory protein-α (SIRP-α) results in increased prophagocytic signals to macrophages (Figure 1).

Additionally, miR-124, which is downregulated in a mouse model of MS, experimental autoimmune encephalomyelitis (EAE), influences the activity of macrophages and microglial cells by targeting the transcription factor CCAAT/enhancer-binding protein-α and its downstream target PU.1 [49]. Noorbakhsh and colleagues [6] identified three miRNAs upregulated in NAWM (miR-338, miR-155, miR-491) that are all directed against neurosteroid synthesis enzymes, leading to a reduced level of

Table 1. miRNA profiles in MS lesions

| miRNAs in white matter | miRNAs in inactive MS lesions | miRNAs in NAWM |
|------------------------|-------------------------------|----------------|
| Upregulated in lesions | Downregulated in lesions     | Upregulated    |
| miRNAs study           | miRNAs study                  | miRNAs study  |

- miR130a a miR139 a miR180-3p b miR133a b miR125b-1* c miR122-5p b
- miR142-3p a miR181c a miR122-5p b miR140 a miR133a-3p b miR122b-5p d
- miR126 a miR185-5p a miR151a-5p / miR151b-5p a,b miR142-5p c miR126a-3p b
- miR146a a miR23b a miR154-5p b miR151a-5p c miR126c c
- miR146b a miR328 a miR154-5p b miR151a-5p c miR126d c
- miR130a-3p / miR130a-3p a,b miR1318a-3p b miR181b c miR128c c
- miR135a-5p / miR135a-5p a,b miR181a-3p b miR194-5p c miR129-2-3p b
- miR165a a miR656 b miR181b c miR129-3p c
- miR139a a miR487a a miR181b c miR129-2-3p c
- miR199a a miR142-3p b miR181b c miR130a c
- miR200a a miR150-5p b miR181d-5p a,b miR133e c
- miR21 a miR152 a miR18a-5p b miR135f d
- miR214 a miR155-5p b miR190a-5p / miR190b a,b miR154a-5p b miR135g c
- miR22 a miR195-5p a,b miR194-5p c miR154b c miR135h c
- miR223 a miR1972 c miR197-3p c miR154c c miR135i c
- miR23a a miR206 a miR213 b miR154d c miR135j d
- miR27a a miR200c a miR219a-2-3p b miR154e c miR135k c
- miR320 a miR204-5p / miR204 a,b miR219a-3p b miR154f c miR135l d
- miR326 a miR214 a miR23b-3p / miR23b a,b miR154g c miR135m d
- miR34a a miR23a-3p / miR23a a,b miR328 b miR92a c miR154h c
- miR650 a miR2682-5p b miR330-3p / miR330 a,b miR329-5p b miR155a c
- miR28 a miR337-5p b miR338 a,b miR155b c
- miR30a-3p a,b miR338 b miR155c c
- miR30a-5p a,b miR33a-5p b miR155d d
- miR30d a miR340-5p / miR340 b miR155e d
- miR30c-3p b miR425-5p b miR155f c
- miR3065-5p b miR574-3p b miR155g d
- miR3144-3p a miR642 a miR155h d
- miR34a-5p b miR885-5p b miR155i d
- miR1362-5p b miR1362-5p b miR155j d
- miR1365 a miR1365 b miR155k d
- miR1378i b miR1378i b miR155l d
- miR428b b miR428b b miR155m d
- miR4454+ b miR4454+ b miR155n d
- miR497-5p / miR497 a,b miR497-5p / miR497 a,b miR155o d
- miR532 a miR532 a miR155p d
- miR574-5p b miR574-5p b miR155q d
- miR612 b miR612 b miR155r d
- miR629 a miR629 a miR155s d
- miR660 a miR660 a miR155t d
- miR664a-3p b miR664a-3p b miR155u d
- miR7c-5p / let-7c a,b miR7c-5p / let-7c a,b miR155v d
- miR9-5p / miR9 a,b miR9-5p / miR9 a,b miR155w d
- miR9b-3p b miR9b-3p b miR155x d
- miR99a-5p b miR99a-5p b miR155y d

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Two of these three miRNAs were found to be upregulated in EAE mice, accompanied by reduced levels of allopregnanolone. Substitution of allopregnanolone attenuated multiple features of EAE, including neuroinflammation, demyelination, axonal damage and neurobehavioral deficits [6].

Table 1. Continued

| miRNAs in gray matter subpial MS lesions | miRNAs in gray matter leuco cortical MS lesions | miRNAs in hippocampal MS lesions |
|----------------------------------------|-----------------------------------------------|----------------------------------|
| Upregulated in lesions miRNAs study   | Downregulated in lesions miRNAs study         | Upregulated in lesions miRNAs study |
| b miR1180-3p                          | b miR1180-3p                                   | b miR124 e hsa-miR138 e          |
| b miR122-5p                           | b miR1260a                                     | b miR143 e hsa-miR181a e         |
| b miR1260a                            | b mir1285-5p                                   | b miR24 e hsa-miR181c e          |
| b mir1285-5p                          | b mir139-3p                                    | b mir30d e hsa-miR204 e          |
| b mir139-3p                           | b mir194-5p                                    | b mir379 e                       |
| b mir1972                            | b mir219a-2-3p                                 | let-7f e                         |
| b mir2682-5p                          | b mir219a-5p                                   | let-7 g e                        |
| b mir3065-5p                          | b mir219a-5p                                   |                                  |
| b mir3144-3p                          | b mir219a-5p                                   |                                  |
| b mir2602-5p                          | b mir219a-5p                                   |                                  |
| b mir32-5p                            | b mir219a-5p                                   |                                  |
| b mir328-5p                           | b mir219a-5p                                   |                                  |
| b mir3574-3p                          | b mir219a-5p                                   |                                  |
| b mir320e                            | b mir660-5p                                    |                                  |
| b mir378i                            | b mir548ah-5p                                  |                                  |
| b mir4286                            | b mir548ah-5p                                  |                                  |
| b mir432-5p                           | b mir574-5p                                    |                                  |
| b mir4488                            | b mir574-5p                                    |                                  |
| b mir548ah-5p                         | b mir612                                       |                                  |
| b mir574-5p                           | b mir612                                       |                                  |
| b mir612                             | b mir660-5p                                    |                                  |
| b mir888-5p                           | b let-7f                                        |                                  |
| b let-7e-5p                           | b let-7f                                        |                                  |

a, Junker et al [5]; b, Fritsche et al [3]; c, Noorbakhsh et al [8]; d, Guerau et al [4]; e, Dutta et al [2].

Figure 1. Hypothetical model of miRNA-regulated macrophage activity in MS lesions. In active MS lesions, miRNA-155, miRNA-34a and miRNA-326 are upregulated in comparison with control white matter. These miRNAs target the 3'-UTR of CD47 and might thereby reduce CD47 expression. These CD47-regulating miRNAs were found in astrocytes. Their expression in other brain resident cells needs to be explored. Reduced expression of CD47 might release macrophages/microglia from inhibitory control normally mediated by interaction of SIRP-α on macrophages/microglia with CD47 on potential targets. Reduced signaling via SIRP-α might then promote phagocytosis of CD47low target cells, and possibly also of susceptible bystander cells, e.g. oligodendrocytes. ‘Unleashed’ phagocytosis will be directed particularly against opsonized (e.g. antibody-coated) targets, because reduced CD47 is known to promote phagocytosis of antibody-coated cells. Reproduced from Junker et al [5] with permission.
Demyelination and remyelination

There are no existing studies that have directly determined the effect of miRNAs on demyelination or remyelination in MS. Nevertheless, some investigations have highlighted the role of miRNAs on myelination under non-MS conditions in vitro and in vivo. For this purpose, animal models were used. Thus, the distinctive miRNAs were mainly from mouse or rat. Due to the high degree of conservation between species, the results from these studies are likely to be transferable to humans.

It was shown that conditional ablation of dicer in oligodendroglial precursors led to disturbed myelin production [50–52]. Furthermore, multiple miRNAs were involved in rapid coordination of gene expression during oligodendroglial differentiation (miR-219, miR-338 [50], miR-23 [53] and miR-9 [54]), proliferation (miR-17-5p and miR-19b [55]) and myelination (miR-23a, miR-27a) [53,56]. Moreover, binding of miR-219 to elongation of very long chain fatty acids protein 7 (ELOVL7) is conducive to maintaining the redox and lipid homeostasis in mature oligodendrocytes [51]. Another miRNA, miR-27a, is needed for generation of mature oligodendrocytes. Increased levels of this miRNA lead to failed remyelination [56] and are detected during demyelination [56] and in active MS lesions [5]. miR-23 takes part in oligodendroglial differentiation and myelination by regulating lamin B1 [57]. In addition, transgenic mice overexpressing miR-23a showed increased myelin thickness, confirming the capacity of miR-23 to enhance myelin synthesis and oligodendroglial differentiation [53]. On the other hand, suppression of miRNA expression blocked myelination, even though large numbers of oligodendroglial precursors expressing platelet-derived growth factor receptors were detected [50,52]. Disturbances in oligodendrocyte differentiation were noticed in demyelinating lesions during the course of MS [58,59], leading to the hypothesis that miRNAs are involved in defective remyelination. It is noteworthy that only the above-named miRNAs, miR-219, miR-388, miR-23 and miR-9, are dysregulated in MS lesions [3,5]. Downregulation of miR-219 and miR-338 compared with NAWM was also confirmed in inactive lesions [35].

Overexpression of miR-338 and miR-219 in cultured oligodendroglial precursor cells promoted their differentiation to pre-oligodendrocytes. This could be useful in cellular therapies of myelinopathies [60,61]. Milbre and colleagues [62] established a method to introduce miRNAs into oligodendrocytes in vitro and in vivo to control their differentiation, maturation and myelination. The study confirmed that miR-219 and miR-338 increased oligodendrocyte differentiation and myelination in rats. These results thus underline the therapeutic potential of miRNAs in influencing remyelination.

Neuronal and axonal changes

miRNAs are also important players in the regulation of neuronal and axonal proteins. A recent study detected statistically increased expression of five neuronal...
miRNAs in GML (let-7e-5p, miR-4286, miR-432-5p, miR-4488 and miR-574-5p), and one additional miRNA that was relatively upregulated in GML compared with chronic inactive WML (miR-330-3p), which all targeted the synaptic protein Syt7 [3]. In addition, there was a clear disturbance of the axonal transport of Syt7, which accumulated retrogradely in neuronal cell bodies. It was hypothesized that upregulation of the abovementioned miRNAs modulates the endogenous synthesis of Syt7 [3] (Figure 2).

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miRNAs of the let-7 family have been repeatedly described to modulate neurogenesis and axonal guidance [63–67] and dysregulation of miR-let-7 has been associated with neurodegenerative processes [68,69]. Knockdown of miR-let-7a seems to display neuroprotective effects in cerebral ischemia [70]. Moreover, miRNAs of the let-7 family and others were detected in lumbar motoneurons of EAE mice [71] at the peak of their symptoms. In addition, miR-330 was associated with neuroprotective effects in Alzheimer’s disease, exhibiting effects on the release of amyloid beta protein, oxidative stress and mitochondrial dysfunction [72]. Furthermore, miR-330-5p competes with Rpph1 in control of CDC42 expression and is

Figure 3. miRNAs of different MS lesions dysregulated in the same way. Blue rectangles represent upregulated and red rectangles downregulated miRNAs. Lines connect miRNAs dysregulated in the same way in different MS lesion types. Active white matter lesions (AP) show 28 dysregulated miRNAs (20 up, 8 down); 11 of these 28 miRNAs are also dysregulated in the same way in chronic inactive plaques (CIAP; Table 2). In general, 74 miRNAs have been described as being dysregulated in CIAP (46 up, 28 down). Chronic lesions of the gray matter (GM) revealed 48 dysregulated miRNAs (27 up, 21 down); as there was a great overlap between different subtypes of GML (localization: hippocampal, subpial and leucocortical) (see Table 1) the results were summarized. Interestingly, more than half of the 27 dysregulated miRNAs in chronic GML were dysregulated in the same way as miRNAs in CIAP (Table 2). NAWM harbored 64 dysregulated miRNAs (19 up, 45 down). Notably, there was only one single miRNA that was dysregulated in the same way in CIAP (up: miR-155), whereas there were six miRNAs overlapping between NAWM and AP (Table 2). MiR-155 seems to play a superordinate role, as it is dysregulated in CIAP, AP as well as in NAWM. The figure was generated using CIRCOS software (http://circos.ca).
Impact of miRNAs on inflammatory cells

It is important to keep in mind that miRNAs might appear to be more abundant in MS lesions only because they are expressed in the leucocytes that invade the CNS during inflammation and not because they are upregulated in CNS parenchyma under these conditions. MiRNAs are not even necessarily regulated in these invading cells compared with their counterparts in blood or other organs under non-inflammatory conditions. Nonetheless, if they are altered in leukocytes during inflammation, the function of a miRNA is still of interest for the pathophysiological processes of MS. It is known that miRNAs in lymphocytes activate various signaling pathways, regulate transcription factors, influence differentiation of Th17 cells and have an impact on the development of regulatory T cells (Tregs) [75]. The following paragraph deals with miRNAs that are upregulated in active WML and that are known to exhibit effects on immune cells or microglia/macrophages. Bearing in mind that inflammatory infiltrates are sparse in inactive lesions in white and gray matter as well as in NAWM, it is not assumed that miRNAs upregulated in these areas originate from immune cells.

Among the functions of miR-155 discussed above, it is also involved in the regulation of the immune system. Deficits in miR-155 lead to resistance to the development of active EAE in transgenic mice [76] and it is known that especially the immune response of B lymphocytes and the reaction of Th2 cells are defective [77,78]. B cells require miR-155 to differentiate to plasma cells [77–79]. Furthermore, miR-155 levels are markedly enhanced in CD4+ T cells upon activation, indicating its role in T cell functions [80]. In addition, transcription factors such as c-Maf, cytokines and signal proteins were identified as targets for miR-155 (reviewed in [81]). C-Maf promotes the development of Th2 cells [77], whereas another miR-155 target, SOCS1, is inhibited in both FoxP3+CD4+ Tregs and in FoxP3+CD4+ T cells and therefore has an impact on the functionality of Tregs [82]. miRNA profiles of CD4+CD25+ Tregs and in FoxP3+CD4+ T cells and therefore has an impact on the functionality of Tregs [82]. miRNA profiles of CD4+CD25+ Tregs and naive T cells differed substantially, as several miRNAs, such as miR-21, miR-146a, miR-223, miR-214, miR-125a and miR-155, were upregulated in the former [83]. Expression of most of these miRNAs could be induced by overexpression of the Treg-specific transcription factor FoxP3. Remarkably, miR-21, miR-146a, miR-223 and miR-155 are all upregulated in active MS lesions [5]. Last, but not least, miR-155 [76] and miR-326 [84] influence the development of Th17 cells.

There is significant and reproducible overlap of miRNA profiles between different lesion types

Comparison of miRNA expression between diverse lesion types reveals that several miRNAs are regulated in the same way in at least two different lesions. This overlap is visualized graphically in Figure 3. But can one draw conclusions on pathophysiological processes from this overlap? Are these overlapping miRNAs the ones that can teach us the most about MS pathophysiology, or is the overlap only a coincidence?

To answer these questions, we have to look at the differences and similarities between active and chronic lesions. Active and inactive lesions differ in terms of (1) inflammation, (2) the density of macrophagocytic cells, (3) the phagocytosis of myelin proteins, i.e. active demyelination, (4) the extent of astrogliosis and microgliosis and (5) the activation of endothelial cells. Moreover, WML and GML differ in terms of the absence or presence of neuronal cell bodies, as well as their compositions of glial cells (astrocytes, oligodendrocytes, microglia) and myelin products.

Despite these differences, diverse lesions have many aspects in common, although the extent varies between different lesion stages. They share (1) astrogliosis, (2) microgliosis, (3) axonal transport disturbances, (4) axonal injury, (5) diminished numbers of mature oligodendrocytes and (6) demyelination. It is plausible that changes in these cells share similar molecular alterations and that miRNAs are important regulators of the underlying processes. Therefore, miRNAs being regulated in the same way in diverse lesion types may clarify pathophysiological causalities which are now unknown. The plausibility of this hypothesis will be supported by examples below.

Active and chronic inactive lesions share regulated miRNAs

Some miRNAs that are regulated in the same direction in early active and chronic inactive WML are presumably miRNAs of local cells (i.e. astrocytes, oligodendrocytes or microglial cells). If the miRNAs were particularly abundant in lymphocytes, they would have been upregulated in active but not in chronic inactive lesions. As discussed above, astrogliosis, microgliosis and demyelination are processes that are obvious in all lesion types. As discussed above, astrogliosis, microgliosis and demyelination are processes that are obvious in all lesion types. We found the most abundant miRNAs in active lesions are expressed in human astrocytes in vitro and in situ (after laser capture microdissection) [5]. Of the 11 miRNAs regulated in the same direction in early active and chronic inactive lesions (Table 2), miR-130a-3p, miR-142-3p, miR-155-5p, miR-200c, miR-23a and miR-34a are expressed in astrocytes [5]. Furthermore, miR-155-5p, miR-200c and miR-23a were upregulated in astrocytes after cytokine stimulation in vitro.

The miR-181 family is important for the astrocytic response to inflammatory stimuli [85] and overexpression of miR-181 in astrocytes results in enhanced...
expression of anti-inflammatory cytokines, such as IL-10 [85]. miR-23b has been described to suppress auto-inflammatory responses associated with IL-17 in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), as well as in mouse models of SLE, RA and MS [86].

Although active and chronic inactive WML share 11 miRNAs that are similarly regulated, active WML and GML have only three miRNAs in common (Table 2): miR-181c and miR-328 are downregulated in both lesion types, whereas miR-139 is the only miRNA that is dysregulated in opposite directions. It is important to note that the studied GMLs were all of the chronic inactive subtype; GMLs with active inflammation [16] have not yet been investigated. This sparse overlap of miRNA profiles between these two lesion types indicates that glial alterations as discussed above are no longer obvious in chronic inactive GML. It is possible that glial changes differ between earlier and later disease stages and that chronic inactive GML only show chronic glial changes of later stages of the disease with a certain specificity for the gray matter.

MiRNA profiles of chronic inactive WML and GML exhibit a strong similarity

Chronic inactive WML and GML show the greatest overlap of regulated miRNAs [3,5], sharing a total of 27 miRNAs that are regulated in the same direction, whereas only two miRNAs are regulated in opposite directions (Table 2). Possibly, these miRNAs regulated in parallel represent the correlate of chronic glial alterations. Downregulation of three miRNAs of the miR-181 family (miR-181a, b and c) in GML as well as in WML is worth mentioning in this context. As described above, the miR-181 family is important for the astrocytic response to inflammatory stimuli [85]. In animal models, miR-181 knockdown led to an increased LPS-induced production of proinflammatory cytokines (TNF-α, IL-6, IL-1β, IL-8) and HMGB1 [85]. Thus, reduced levels of miRNAs of the miR-181 family in human GML and WML could also maintain inflammatory cytokines in chronic MS lesions and thus contribute to the chronicization of the disease.

Not only the lesions’ milieu seems to be influenced by miRNAs, as discussed above, but also glial cell apoptosis and regeneration. In chronic GML and WML, miRNAs miR-30d and miR-122 appear to be upregulated [2,3,5]. These miRNAs induce apoptosis in astrocytic cells [87,88] while inhibiting autophagy via beclin-1 [88] and cell proliferation [87].

miRNAs in chronic inactive WML and NAWM are regulated inversely

Thirty-one percent of all regulated miRNAs in NAWM (20 miRNAs) are also regulated in chronic inactive WML, albeit in opposite directions (Table 2 and Figure 4). MiR-155 is the only exception, being upregulated in both areas. This observation is probably of great importance. NAWM shows activated microglial cells and sparse lymphocytic infiltrates, resulting in a subtle balance between inflammation and neuroprotection [89]. It has been shown that changes in NAWM may display preliminary stages of active lesions [90]. Activated microglial cells are probably responsible for axonal injury outside demyelinated lesions [91]. In contrast, activated microglial cells and inflammatory infiltrates are less obvious in chronic demyelinated lesions. Therefore, the question arises whether the complex interplay of pro- and anti-inflammatory factors is modulated by differentially regulated miRNAs.

In line with this hypothesis, levels of the proinflammatory miRNAs miR-155 and miR-181 are enhanced in NAWM. miR-9, which modulates the activity of microglial cells by inhibiting MCPIP1 and activating NF-κB [92], is decreased in NAWM and increased in chronic active WML. Overexpression of miR-let-7c-5p (up in MS in NAWM, down in chronic inactive plaques) in a transgenic mouse model of chronic brain injury, inhibited microglia and macrophage activation, which in turn resulted in decreased inflammation and associated neurological dysfunction [93].

Characteristically, active lesions show blood–brain barrier leakage, which is repaired when inflammation in these lesions decreases. miR-126, which is upregulated in chronic inactive lesions, might participate in this restoration. A recent study found that miR-126-3p reduced blood–brain barrier leakage following intracerebral hemorrhage via regulation of VCAM-1 [94].

A main difference between chronic inactive WML and NAWM is the extent of myelin loss and the capacity for remyelination. As discussed above, miR-219 and miR-338 inhibit Lingo1 and Etv5 and therefore influence myelination in general and remyelination in particular [95]. It is noteworthy that both miRNAs are regulated in opposite directions in chronic inactive WML (both are downregulated) and in NAWM (both are upregulated).

Despite these interesting findings, it is remarkable that miRNA profiles of NAWM differ significantly between studies [3,4,6]. NAWM seems to be heterogeneous in nature and so are their miRNA expression profiles. This might be the result of differences in localization themselves (miRNA profiles might differ between different lobes and in proximity to the ventricular system) and the distance to the nearest neighboring lesions on the one hand, or the extent of microglial and lymphocytic cells on the other.

Six miRNAs in active WML and NAWM are regulated in the same way

MiRNAs upregulated in both active WML and NAWM (miR-142-5p, miR-155, miR-223, miR-320a, miR-487b-3p and miR-656-3p) might also modulate inflammatory processes. MiR-223 seems to be of particular interest. It has been shown to be upregulated in monocytes of MS patients [96] and to influence the polarization of macrophages (switch to M2 phenotype) [96], which itself influences inflammatory and regenerative processes in lesions. Cantoni and colleagues [97] found that miR-223 expression levels are enhanced in myeloid-derived suppressor cells (MDSCs) of patients with...
relapsing MS compared with healthy controls; but despite enhanced miR-223 levels, the numbers of MDSCs are decreased. It is postulated that miR-223 modulates the number of MDSCs and suppresses T cell activity by inhibiting STAT3 and the Arg1 signaling pathway [97].

### Table 2. miRNAs of different MS lesions dysregulated in the same or opposite direction

| miRNAs in early active lesions and chronic inactive lesions | miRNAs in early active lesions and GML | miRNAs in chronic inactive lesions and GML |
|-----------------------------------------------------------|----------------------------------------|---------------------------------------------|
| Regulated in the same direction                          | Regulated in opposite directions       | Regulated in the same direction             |
| Regulated in the opposite directions                     |                                        | Regulated in opposite directions           |
| Upregulated miRNAs:                                      |                                        | Upregulated miRNAs:                        |
| miR-130a-3p                                               |                                        | miR-139                                    |
| miR-142-3p                                               |                                        | miR-204-5p                                 |
| miR-155-5p                                               | Downregulated miRNAs:                  |                                        |
| miR-200c                                                 | miR-181c                               | miR-660                                    |
| miR-214                                                  | miR-328                                 |                                            |
| miR-23a-3p                                               |                                        |                                            |
| miR-34a-5p                                               |                                        |                                            |
| Downregulated miRNAs:                                    |                                        |                                            |
| miR-181c-5p                                              |                                        |                                            |
| miR-181c                                                  |                                        |                                            |
| miR-23b-3p                                               |                                        |                                            |
| miR-328                                                  |                                        |                                            |
| miR-340-5p                                               |                                        |                                            |

| miRNAs in early active lesions and NAWM                  | miRNAs in chronic inactive lesions and NAWM |
|-----------------------------------------------------------|---------------------------------------------|
| Regulated in the same direction                          | Regulated in opposite directions           |
| Regulated in the opposite directions                     | Regulated in the same direction             |
| Regulated in the opposite directions                     | Regulated in opposite directions           |
| Upregulated miRNA:                                       | Upregulated miRNA:                         |
| miR-130a                                                  | miR-139                                    |
| miR-142-5p                                               | miR-133a                                   |
| miR-155                                                  | miR151-5p                                  |
| miR-223                                                  | miR181a-3p                                 |
| miR-320a                                                 | miR181b-5p                                 |
| Downregulated miRNAs:                                    | miR181c                                   |
| miR-487b-3p                                              | miR190a-5p                                 |
| miR-656-3p                                               | miR194-5p                                  |
| Upregulated miRNAs:                                      |                                            |
| miR-130a-3p                                              |                                            |
| miR-142-5p                                               |                                            |
| miR-155                                                  |                                            |
| miR-223                                                  |                                            |
| miR-320a                                                 |                                            |
| Downregulated miRNAs:                                    |                                            |
| miR-487b-3p                                              |                                            |
| miR-656-3p                                               |                                            |

**Overlap of regulated miRNAs between CNS and body fluids**

Some of the regulated miRNAs discussed above are probably derived from leucocytes, which invade the...
CNS parenchyma during active disease phases. Researchers have therefore tried to establish profiles of dysregulated miRNAs in blood (serum, leucocytes and exosomes) and in CSF (for a review see [98]). Notably, the miRNA profiles in blood and CSF described in the various studies are quite heterogeneous. One reason is that the studies determined miRNAs in multiple ‘compartments’, i.e. whole blood [99–101], PBMCs [102–104], CD4⁺ T cells [105–107], CD8⁺ T cells [108,109], B lymphocytes [106,110,111], monocytes [112], exosomes [113–115], serum [116,117], plasma [118–120] and CSF [121–123]. Furthermore, miRNAs have been evaluated in patients with different courses of the disease. For example, samples were taken from patients with relapsing–remitting MS [105,106] or primary progressive MS [124,125], as well as at different time points during the course of the disease, i.e. during relapse or during remission. Moreover, age, gender, comorbidities, pharmaceuticals and, in particular, therapeutic approaches influence the expression of miRNAs [108,111,126]. The lack of systematic and standardized protocols for studying miRNA profiles in body fluids of MS patients diminishes the comparability and, hence, the generalizability of the study results. This renders it more complicated to draw any conclusions from these results.

Despite this heterogeneity, specific miRNAs have been found to be regulated in the same direction in more than four studies in different ‘compartments’ [98]. Comparable regulation has been identified for miR-142-3p, miR-146a/b, miR-155, miR-22, miR-223/−3p, miR-326 and miR-584. Moreover, there seems to be an association of miRNAs and the activity of different signaling pathways, such as the TGF-β signaling pathway [98]. It is especially remarkable that six of these eight miRNAs (miR-142-3p, miR-146a/b, miR-155, miR-22, miR223/−3p, miR-326) are differentially expressed in CNS parenchyma. In fact, all of these miRNAs are strongly regulated in opposite directions in NAWM and CIAP (Table 2). The figure was generated using CIRCOS software (http://circos.ca/). The background shows a chronic inactive MS plaque and adjacent NAWM (Klüver–Barrera staining for myelin; property of A Junker).

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**Figure 4.** miRNAs dysregulated in opposite directions in NAWM and CIAP. Blue rectangles represent upregulated and red rectangles downregulated miRNAs. miRNAs dysregulated in opposite directions are highlighted by gray lines connecting them. Twenty of 64 miRNAs dysregulated in NAWM are dysregulated in opposite directions in CIAP (Table 2). The figure was generated using CIRCOS software (http://circos.ca/). The background shows a chronic inactive MS plaque and adjacent NAWM (Klüver–Barrera staining for myelin; property of A Junker).
upregulated in active WML [5]. It is conceivable that these miRNAs could be detected in blood or CSF during relapse and thus could function as biomarkers for its occurrence.

Determining the amount of miRNAs (miR-15b, miR-23a, miR-92a, miR-135a, miR-145, miR-223, miR-337-3p, miR-454, miR-500, miR-574-3p and miR-648a) in CSF and blood could help to distinguish relapsing remitting MS cases from secondary progressive MS cases, as well as from healthy controls [116,118,127]. Furthermore, there were correlations between levels of miRs and Expanded Disability Status Score, disease duration, remission or frequency of relapses [116,118,127]. A look at miRNA expression levels during or after MS therapy is also probably very worthwhile. Downregulation of miR-142-3p [108,126], miR-146a [126] and miR-155 [108] – all miRs that are upregulated in active lesions – has been found after MS therapy. miRNA levels might therefore represent biomarkers for response to MS therapy. miRNAs of chronic inactive WML or GML have not been found to be either elevated or decreased in blood or CSF. Nonetheless, future studies will have to determine whether downregulated miRNAs of chronic inactive WML/GML or NAWM are also consistently decreased in these peripheral compartments.

However, the clinical use of miRNAs as biomarkers also possesses difficulties. As indicated above, specific miRNAs might well provide evidence of newly developed active lesions or serve as biomarkers for therapy response. Nonetheless, it should be noted that many miRNAs are ubiquitously expressed and alterations of miRNA levels in body fluids are therefore not specific to pathological processes of the brain. miRNAs that appear upregulated in active MS lesions, such as miR-155 or miR-145, are also elevated in serum in other (inflammatory) conditions, such as SLE (miR-155) or RA (miR-145) [128]. Moreover, miRNAs such as miR-155 also show increased serum levels in various neoplasms [129]. Against this background, the specificity of miRNAs as biomarkers (especially in serum) must always be viewed critically. However, unusually increased levels of miRNAs (e.g. in serum) of MS patients over a period of time might well indicate disease activity.

Concluding remarks, unresolved questions and future perspectives

The results of diverse studies have shown the impact of dysregulated miRNAs in CNS lesions and NAWM on pathophysiological processes in MS, including inflammation, cytokine production, activation of glial cells, demyelination and remyelination. A careful consideration of the regulation of miRNAs in different lesion types bears with it the possibility of drawing conclusions about their functional role in MS pathophysiology.

At least some changes of miRNA profiles in blood and CSF are due to inflammatory alterations in CNS parenchyma, underlining the significance of miRNAs as potential biomarkers of disease activity or even of therapeutic success. The question as to whether miRNA profiles distinguish between different subtypes of MS remains unanswered, but there is considerable potential that future studies will yield more insight into this topic. Importantly, miRNA dysregulation in different lesion types inspires concepts for the pathophysiology of MS. The validation of these concepts remains to be carried out. An essential aim is to increase the knowledge of MS pathophysiology by evaluating dysregulated miRNA patterns in the CNS of MS patients.

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Author contributions statement

STH, EM and AJ researched the data for the article, provided substantial contributions to discussions of the content, contributed equally to writing the article, and to reviewing and editing the manuscript before submission. All authors approved the final version of the manuscript.

List of abbreviations

CIAP, chronic inactive plaques; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; GML, gray matter lesion; MDSC, myeloid-derived suppressor cells; miRNA, microRNA; MS, multiple sclerosis; NAWM, normal-appearing white matter; RA, rheumatoid arthritis; SIRP, signal regulatory protein; SLE, systemic lupus erythematosus; Treg, regulatory T cell; WML, white matter lesion.

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**SUPPLEMENTARY MATERIAL ONLINE**

Table S1. Range of methods in studies showing dysregulated miRNAs in MS tissue