See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

MicroRNAs change the games in central nervous system pharmacology

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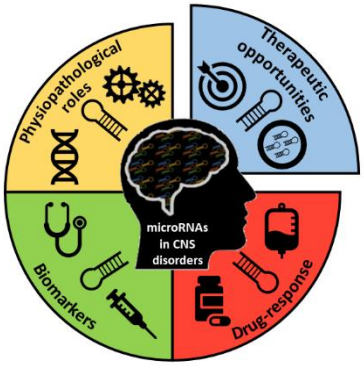
Abstract

MicroRNAs (miRNAs) represent a class of important post-transcriptional regulators of gene expression, enabling cells to follow their intrinsic developmental program. By directly binding to their targets, miRNAs can both promote transcriptional patterns in crucial steps of cell growth, and act as powerful buffering system that titrate protein content in case of aberrant gene expression. The literature of the last decade showed that the presence of tissue-enriched miRNAs in body fluids could be reminiscent of disease state. This is particularly relevant in neurodegenerative disorders, in which peripheral biomarkers could be helpful means to detect disease onset. However, dysregulation of miRNAs is not merely a consequence of disease, but directly contributes to pathological outcomes. On this basis, increasing interest is growing in the development of pharmacological agents targeting specific miRNAs. Actually, this apparently futuristic approach is already part of the current therapies. In fact, several drugs approved for CNS disorders, such as L-Dopa or valproic acid, were also demonstrated to restore some miRNAs. Moreover, ongoing clinical trials suggest that miRNA-based drugs are effective against tumors, fostering the concept of miRNAs as a promising class of therapeutic molecules. However, several issues still need to be addressed, particularly in case of CNS diseases, in which stability and delivery are crucial aspects of the therapy. In this commentary, we highlighted potential advantages and limitations of miRNAs as next generation targets in CNS pharmacology, focusing on multiple sclerosis, a chronic demyelinating disease lacking specific therapeutic targets and bona-fide biomarkers.

See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

Graphical abstract



1. Introduction

MicroRNAs (miRNAs) are small, highly conserved non-coding RNA molecules involved in post-transcriptional regulation of approximately 60% of all coding-genes [1]. MiRNAs mainly act by directly interacting with their target transcripts by either promoting their degradation, recruiting cytoplasmic exonucleases or inhibiting the conversion into protein, preventing the initiation or elongation step of translation [1]. MiRNAs do not silence their target mRNAs; in many cases, the effect of a single miRNA maybe subtle and leads to a less than 2-fold reduction in target protein levels [2]. The biological outcome of miRNA-mRNA interaction largely depends on their binding strength; 7-base matches are enough to induce mild post-transcriptional regulation. This apparently reduces miRNA target specificity, but, on the other hand, it enables the simultaneous regulation of a wide array of target transcripts [3]. Moreover, several papers reported that miRNA-target interactions may involve multiple sites and that different miRNAs may cooperatively co-target a given mRNA [4], leading to a cumulative effect. MiRNAs can also modulate biological processes by targeting multiple genes in shared pathways and by forming negative/positive feedback loops with transcription factors [2]. MiRNA-mediated targeting of molecular pathways is not the result of random associations and the correlation between the levels of a specific miRNA and the activity of the targeted pathway is disease-relevant and can be useful for patient stratification [5].

Many miRNAs are finely regulated during development in a tissue-specific manner [6]. Whole genome analysis revealed that approximately 50% of the miRNAs are located within the intron or the exon of protein-coding genes, transcribed together with their host gene and, thus, under the control of its transcriptional

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regulation. Other miRNAs originate from non-protein-coding transcripts, have their own promoter and are likely transcribed as independent transcription units. In physiological condition, cytokines, growth factors and other signaling molecules regulate their expression as happens for all the other genes, via transcriptional (e.g., transcription factors recruitment), post-transcriptional (e.g., changes in pri-miRNA processing) or epigenetic (e.g., promoter hypermethylation) regulation [7]. Interestingly, emerging evidence have shown that aberrant miRNA expression profiles have been associated to several human diseases, even if the precise molecular mechanisms underlying this regulation are largely overlooked. Indeed, most of the studies in the literature focused on the identification of miRNA targets and on-disease related miRNA alterations in cells and body fluids as potential biomarkers [8, 9]. However, miRNAs in biofluids are not only a mere consequence of plasma membrane breakdown; virtually all the cells also release miRNAs in a paracrine fashion in response to extracellular stimuli and contribute to post-transcriptional regulation of recipient cells. Recent studies showed that part of the therapeutic action of several drugs is due to their capability to restore altered miRNA levels [10], suggesting that miRNAs may be drug targets exactly as receptors or enzymes. This fascinating discovery opens both opportunities and challenges in pharmacology. How can we translate the current knowledge on miRNAs into clinical practice? As homeostatic regulators of entire pathways, miRNAs can change the games during disease; are we ready to exploit miRNAs as pharmacological targets? How can we link the altered levels of a miRNA in body fluids to altered functions in a complex system like the central nervous system (CNS)?

In this commentary, we have analyzed some miRNAs involved in fundamental processes in the CNS, their molecular mechanism of action, and the pathological consequences of their dysregulation to explore possible therapeutic approaches targeting miRNAs. The cited studies concern neurodegeneration and demyelination, with a focus on multiple sclerosis (MS).

2. Pathophysiological roles of miRNAs in the central nervous system

MiRNAs have been shown to play a role in the modulation of almost every physiological process investigated so far, including CNS development; as a consequence, dysregulation of their expression levels has been linked to the onset and/or pathogenesis of several CNS-related disorders. Thus, restoring altered miRNA levels may

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be exploited as a therapeutic strategy for neurodegenerative diseases. In the following paragraphs, we will discuss some examples of miRNA-mediated regulation of key neurobiological processes like neurogenesis and myelination and their implication for diseases characterized by neurodegeneration and demyelination.

2.1. Neuronal survival, neurogenesis and neurodegeneration

In the CNS, miRNAs play important roles in neurogenesis, a finely regulated process during which neural stem cells (NSCs) proliferate, migrate and differentiate into mature neurons, allowing the development of neural circuits. Alterations in adult neurogenesis and in the proper formation of neuronal networks appear to be a common hallmark in different neurodegenerative (i.e Alzheimer, AD, and Parkinson disease, PD, and amyotrophic lateral sclerosis, ALS) and cognitive disorders (i.e. epilepsy and schizophrenia) [11]. Several studies have addressed the role of miRNAs in the regulation of these processes. For example, miR-34 and miR-25, important regulators of the Notch signaling, specifically drive NSC proliferation. It has been demonstrated that miR-34a stimulates symmetric division of progenitor cells by targeting the Notch inhibitor Numbl [12], whereas its knock-down decreases Notch signaling and in turn increases the expression of the pro-neural genes NeuroD1 and Mash1, downstream targets of Notch signaling. MiR-34c over-expression has been associated to impaired neuronal differentiation, reduced dendritic complexity and spine length in the hippocampus of AD patients, in the APPPS1-21 transgenic mouse model of AD and in aged mice. In the same experimental models, miR-34c inhibition improved memory performance and cognitive functions [13]. Interestingly, both miR-34a and miR-34c were found up-regulated in chronic stage of epileptogenesis in rodent models [14], suggesting that their alteration may be closely associated to cognitive impairment.

MiRNAs also play an essential role in the transition between proliferation and differentiation of NSCs. Two miRNAs, namely miR-9 and let-7b, can strongly induce neuronal differentiation of NSCs by targeting TLX [15], an activator of the Wnt-β-catenin pathway, which is crucial to maintain NSC self-renewal. Over-expression of miR-9 can also induce a neurogenic program in non-neuronal cells [16]. MiR-9 expression levels were found decreased in neurodegenerative diseases such as Huntington, AD and ALS [17]. Moreover, inhibition of miR-9 impairs cognitive functions and synaptic plasticity in the hippocampus [18]. Interestingly, let-7b exerts a double repressive effect on TLX signaling by also directly regulating its downstream effector Cyclin D1. This

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mechanism is particularly relevant in glioblastoma multiforme (GBM), where down-regulation of let-7b was shown to increase Cyclin D1 expression and tumor malignancy [19].

Of note, the brain enriched miRNAs miR-124 and miR-125b, important players in the transition of NSCs from proliferation to differentiation stages, are up-regulated during neurogenesis. MiR-124 alteration may contribute to several neurodegenerative mechanisms. In AD patients, miR-124 shows a reverse correlation with BACE1, a secretase involved in amyloid cleavage [20]. Exogenous administration of miR-124 reduces brain injury and functional impairment in mice 8 weeks after stroke induced by middle cerebral artery occlusion [21]. Instead, over-expression of miR-125b increases tau phosphorylation, impairs associative memory and affects synaptic morphology [22] and was found up-regulated in AD patients [23].

2.2. Myelination, de-myelination and re-myelination

MiRNAs are also involved in myelination, an essential process during which oligodendrocytes enwrap axons with multilamellar extensions of their plasma membrane, thus allowing a fast conduction of electrical impulses. To produce myelin, oligodendroglial cells follow a multi-step differentiation program, finely regulated by both extrinsic and intrinsic factors, including miRNAs [24]. Oligodendrocytes require a precise expression pattern of miRNAs during their development. As depicted in Figure 1, aberrant miRNA levels (green and red boxes) can be responsible of impaired myelination, contribute to demyelination during diseases such as MS, or delay remyelination.

In oligodendrocyte precursor cells (OPCs), conditional ablation of Dicer, the major ribonuclease involved in miRNA processing, leads to an initial increase in the proliferation, followed by maturation failure [4, 25], suggesting the existence of miRNAs that specifically promote OPC terminal maturation. Comparative microarray analysis of mature oligodendrocytes and OPCs allowed the identification of miRNA specifically expressed at high levels by mature cells, such as miR-219, miR-338 and miR-138. Loss of function experiments *in vivo* demonstrated that miR-219 potently activates oligodendrocyte maturation [26]. Moreover, its exogenous administration rescues myelination defects in Dicer-null mice [25] and promotes in vivo re-myelination in the lysolecithin-induced demyelination model [26], by repressing Pdgfr-α, Zfp238, Sox6, Foxj3, Lingo1 and Etv5, factors that physiologically inhibits OPC maturation. A recent study demonstrated that miR-

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219 is able to rapidly transform mouse embryonic stem cells into oligodendrocytes, and that the transplantation of miR-219-over-expressing OPCs stimulates remyelination, improves cognitive function and promotes the proliferation of endogenous neural precursor cells (NPCs) in the cuprizone-induced chronic demyelination [27].

Similarly to miR-219, miR-338 inhibition decreases, whereas its over-expression promotes, the differentiation of OPC into mature oligodendrocytes, directly repressing Hes5 and Sox6 [4]. Despite miR-338 knockout mice did not show alterations in oligodendrocyte development, miR-338 deletion exacerbated the dysmyelinating phenotype in miR-219 KO mice [26]. Moreover, the implantation of a hydrogel fiber scaffold loaded with miR-219 and miR-338 mimics in the injury site enhanced remyelination in a rat model of spinal cord injury [28], suggesting these miRNAs cooperate to regulate the entire oligodendrocyte maturation process and might be exploited to foster endogenous remyelination.

Gene expression profile analysis in oligodendrocytes allowed the identification of the miR-17~92 cluster, previously categorized as a group of onco-miRs, as an important player in myelination. Conditional ablation of the miR-17~92 cluster in OPCs decreased, whereas its over-expression increased the number of oligodendrocytes [29]. Further evidence revealed that this miRNA cluster controls OPC proliferation by targeting PTEN, whose inactivation leads to increased proliferation rate through the Akt signaling [30]. The repression of PTEN is also sustained by miR-23a, a known activator of myelin gene expression through direct interaction with laminin B1 [31]. Nevertheless, further studies also demonstrated that miR-23a over-expression in CNPase-expressing oligodendrocytes results in hind-limb paralysis and loss of muscle tone, due to hyper-myelination [32], suggesting that specific miRNAs may act as buffering system to maintain the proper gene expression.

Also demyelinating injury can trigger the expression of specific miRNAs that act as potent inhibitors of oligodendrocyte maturation and myelination. For example, miR-125a-3p was found up-regulated in corpus callosum after white matter (WM) demyelination and inside WM lesions in multiple sclerosis patients. Interestingly, it has been shown that miR-125a-3p over-expression blocks, whereas its silencing promotes OPC maturation and remyelination [33]. As summarized in Table 1, these pre-clinical results highlight miRNAs

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as modulators of different neurodegenerative diseases and underline that miRNAs can be therapeutically manipulated *in vivo* and may have clinical applications as drug targets.

3. Circulating miRNAs in neurodegenerative diseases: biomarkers and second messengers

As very stable, independent, small entities enriched in brain cell cytoplasm, cerebrospinal fluid and in peripheral blood, miRNAs may serve as useful biomarkers for the early diagnosis of human pathologies. More than other disorders, CNS chronic diseases necessarily need peripheral biomarkers that should be reliable and disease-specific. For example, in MS, the identification of potential biomarkers would greatly advance the chances of early diagnosis and early pharmacological intervention. Furthermore, biomarkers could help the stratification of MS patients, monitor disease progression and identify patients who will better respond to a specific drug.

From the beginning of miRNA discovery, the research was aimed at identifying miRNA alterations inside brain lesions of MS patients, to unveil new molecular mechanisms potentially involved in disease pathogenesis. Junker and colleagues analyzed the expression of 365 miRNAs in 16 active and 5 inactive white matter MS brain lesions compared to 9 control white matter specimens. Some miRNAs known to be expressed by astrocytes, such as miR-155, miR-326 and miR-24a, were specifically up-regulated in MS active lesions, and have been proposed to play a role in the regulation of macrophage activity [34]. How can this discovery be translated into a diagnosis of MS? MiRNAs are very stable also when released in body fluids. Is there a connection between miRNA alteration inside the lesions and miRNA levels in body fluids? In the last years, several studies have tried to address this issue. CSF analysis on a cohort of 53 MS patients compared to 39 subjects with other neurological diseases revealed that miR-922, miR-181c, and miR-633 were differentially expressed in the two groups, and that miR-181c and miR-633 differentiated relapsing-remitting MS (RRMS) from secondary progressive MS (SPMS) with 82% specificity and 69% sensitivity [35].

Recently, MS diagnosis has been strongly associated to undetectable levels of miR-219 in the CSF; this miRNA, essential for oligodendrocyte maturation (see also above), is virtually absent in MS lesions [36, 37]. On the contrary, independent studies demonstrated that miR-150 is enriched both in MS lesions and in the CSF of MS patients [38, 39], suggesting that alterations of miRNA levels in the CSF might reflect pathological events

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of the CNS. Of note, higher levels of miR-150 were also found in patients with clinically isolated syndrome (CIS) who converted to MS compared to those who never converted [38]. CSF clearly represents the most reliable biofluid because of its proximity to CNS and this may reflect brain damage better than other body fluids. However, in the last years several studies were aimed at profiling miRNAs in blood samples (i.e. PBMC, plasma or serum), that represent a more convenient sampling method. For example, miR-20a-5p, a miRNA involved in the regulation of T cell activity, was reported to be down-regulated, whereas miR-22-5p was up-regulated in blood samples of MS patients compared to healthy controls [40, 41]. The plasma levels of 29 miRNAs, including miR-150, were found up-regulated in RRMS subjects compared to SPMS and healthy controls [8]. The miRNA pattern in plasma can also discriminate between MS and other neurodegenerative diseases such as ALS. MiR-92a resulted useful to distinguish RRMS from SPMS and controls and showed association with EDSS (Expanded Disability Status Scale) and disease duration [8]. Instead, let-7a differentiated SPMS from RRMS subjects and controls. Interestingly, these two miRNAs can be also useful to distinguish RRMS from ALS, but no change were found between SPMS and ALS, suggesting some common pathological features.

However, the link between miRNA alterations in brain lesions, CSF and blood samples is not obvious and subjected to several confounding factors. Dysregulated levels of miRNAs in blood can be related to immunologic components. This does not diminish the clinical relevance as potential disease indicator per se, but it is an important issue when comparing results from different studies.

It is worth to mention that, in biological fluids, extracellular miRNAs are associated with Argonaute proteins, packed in high- and low- density lipoproteins or in extracellular vesicles (EVs), that prevent their endonuclease-mediated degradation and provide higher stability [42]. EVs are small membrane-enclosed particles released by virtually all cells. Both microvesicles, directly originated from the cell plasma membrane by budding, and exosomes, generated by membrane invagination of the late endosomes, play a crucial role in cell-to-cell communication. Their cargo is highly heterogeneous, but similar to the cells of origin and include proteins (signaling molecules, receptors, cytokines and integrins), bioactive lipids, organelles and nucleic acids such as miRNAs, through which they can influence recipient cells [43]. In many neuroinflammatory diseases, there is a significant increase in concentration of circulating EVs [44]. Since their

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content specifically reflects ongoing conditions, the miRNAs stored in EVs are progressively gaining attention and may represent promising candidate biomarkers of disease. A recent study analyzed the expression profile of exosome-associated microRNAs in serum samples from MS patients and healthy controls and they identified a group of nine differentially expressed exosomal miRNAs that distinguished RRMS from SPSM [45]. A similar analysis was performed in the CSF of AD patients and identified 15 differentially expressed exosomal miRNAs. In particular, a strong positive association was found between undetectable CSF level of miR-9-5p and miR-598 and AD diagnosis [46]. A recent study aimed to identify miRNAs differentially expressed after acute ischemic stroke has shown that miR-9 and miR-124, two brain-specific miRNAs that are highly expressed in the developing and adult brain, are significantly up-regulated in serum exosomes. Their levels also correlated with the infarct volume and the over-expression of inflammatory cytokines, reflecting the degree of damage caused by the ischemic injury [47].

It is clear that miRNAs, will play a very important role in clinical practice in the future as biomarkers in CNS pathophysiology, in particular in MS, in which the presence of oligoclonal bands in the CSF is the only available biological analysis to confirm a diagnosis so far, albeit not univocal. However, more efforts are needed in the standardization of measurement methods, to allow the comparison of the results from multiple studies.

All the studies reported above reason that circulating miRNAs have a great potential to become bona-fide biomarkers for the diagnosis and prognosis of human CNS-related diseases, but, what is the significance of these alterations? Are they merely a consequence of biological processes taking place in the CNS (i.e. passive leakage from dead cells) or do they represent paracrine messengers? Several studies demonstrated that miRNAs associated to shedding vesicles can be taken by other cells and modulate target transcripts in the recipient cells [48]. Of course, these mechanisms are extremely relevant in disease, where extracellular miRNA levels may contribute to disease etiology and pathogenesis. In the experimental autoimmune encephalomyelitis (EAE) a murine model of MS, it has been shown that the inhibition of either miR-326 or miR-155, both up-regulated in human MS lesions and plasma, increased EAE severity, through mechanisms that modulate Th17 cell differentiation. Considering that Th17 cells release exosomes that can be accepted by other T cells, alteration in extracellular levels of these miRNAs may represent a non cell-autonomous

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mechanism that enhance MS pathogenesis [49]. It has been shown that extracellular miRNAs may also act in a non-canonical way. For example, let-7b can act as signaling molecule and activate the TLR7 pathway, leading to neurodegeneration. Accordingly, administration of let-7b in the CSF of mice resulted in increased neuronal damage. Interestingly, increased amount of let-7b was also found in the CSF of AD patients [50], suggesting that this alteration may contribute to AD pathogenesis.

Further studies are needed to shed light on the functional contribution of circulating miRNAs, a crucial point for the translation of the current research to applied pharmacology.

4. Pharmacological therapy can modulate miRNA expression

Drugs are known to modulate important signaling pathways through their interaction with receptors, enzymes, and/or by directly or indirectly modulating gene expression. It is not surprising that also miRNA expression can be changed by pharmacological intervention. The identification of miRNAs modulated following drug administration is becoming an attractive research interest also in neuropharmacology. Indeed, increasing evidence showed that some drugs can restore expression levels of miRNA in several neurological disorders [10]. This complicates the already complex molecular mechanisms of drugs, but also opens new opportunities in pharmacology, since levels of circulating miRNAs could be monitored as clinical outcomes of pharmacological treatments. Here, we have reported some representative studies in which the relationship between drugs and miRNAs has been investigated in neurodegenerative diseases.

In a mouse model of AD, high levels of miR-206 were demonstrated to contribute to the pathogenesis by repressing BDNF release in the brain [51]. In the same model, the treatment with donepezil, an acetylcholinesterase inhibitor approved for AD therapy, was able to restore its normal levels [52]. Interestingly, infusion of miR-206-3p mimic counteracted the beneficial anti-dementia effect of donepezil, consolidating its involvement in the mechanisms of action of this drug [52].

Recently, the treatment with simvastatin, a lipid-lowering drug also discovered to increase BDNF release in the hippocampus by activating the PPARα-CREB pathway, has been shown to ameliorate memory deficits both in AD patients and animal models of AD. Simvastatin also reduced the expression of inflammatory mediators, prevented NSC apoptosis and lowered protein levels of APP and BACE1, two proteins involved in

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the formation of pathogenic β-amyloid aggregates, by down-regulating miR-106b, a pro-apoptotic miRNA enriched in murine models of AD [53].

In a preliminary study on PD patients, treatment with Levodopa (L-DOPA) caused increased levels of miR-29a-3p, miR-30b-5p and miR-103a-3p in peripheral blood, while no difference between untreated patients and healthy controls was found. An integrated *in silico* analysis revealed several genes related to neurodegeneration and PD, such as akt1, mex3b and bcl2, as putative targets of these three miRNAs, thus supporting the hypothesis that over-expression of such miRNAs may almost partially underlie the mechanism of action of L-Dopa [54]. Moreover, a clinical study demonstrated that L-Dopa was able to restore the levels of miR-155-5p, another important marker of neuroinflammation found upregulated in PBMCs derived from PD patients [55].

MiRNA alterations have been also involved in epileptogenesis. In particular, miR-134 is strongly up-regulated in plasma samples of epilepsy patients compared to healthy controls [56, 57]. Consistently, the intracerebroventricular injection of antago-miR-134 in rodent models of status epilepticus significantly reduced both evoked and spontaneous seizures [56]. MiR-134 directly interacts with Tulp1, a protein involved in the regulation of neuronal susceptibility to glutamate excitotoxicity, and Limk1, a kinase involved in dendritic spine growth [57], both downregulated in pathological conditions. Interestingly, the treatment with the widely used anti-epileptic drug valproate was able to restore the homeostasis of both miR-134 and its downstream targets [57].

On the contrary, miR-128 was found down-regulated in both rat models of epilepsy and patients [58]. Interestingly, the administration of miR-128 mimic protected hippocampal neurons from apoptosis, while the inhibition of miR-128 worsened neuronal damage after seizures in rats [59]. Of note, the mGluR2/3 agonist 2R,4R-APDC was able to counteract neuronal apoptosis in the pilocarpine model of epilepsy by up-regulating this miRNA in the hippocampus, and simultaneous administration of miR-128 inhibitor counteracted this effect [59].

Taken together, these results suggest that already marketed drugs may also act through unexpected miRNA-mediated mechanisms. Virtually, this consideration may be relevant for all the drugs and for all the diseases, but the research in this field is still in its infancy.

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4.1 Pharmacological modulation of miRNA expression in MS: active mechanism or response to therapy?

An increasing number of studies demonstrated that most of the approved immunomodulatory drugs for MS, such as interferon-β, glatiramer acetate, fingolimod and natalizumab are able to restore the physiological levels of miRNAs pathologically altered during the disease. These findings also suggest that miRNAs can be actively involved in the therapeutic mechanism of drugs (Table 2).

In a longitudinal study analyzing miRNA expression changes induced by IFN-β treatment, which acts by both reducing immune cell activation and shifting the pro-inflammatory Th1/Th17 cells to a Th2 phenotype, it has been suggested that miRNA-mediated regulation of MS-related inflammation and apoptotic processes could be at the basis of the beneficial effects of this drug [60]. In this respect, it has been shown that aberrant blood levels of miR-145 and miR-20a-5p, typical of MS patients, were normalized after IFN-β treatment. Molecular signaling pathway enrichment analysis unveiled several genes involved in the MAP kinases cascade as targets of these two miRNAs, suggesting a key role of this pathway in mediating the pleiotropic effects of IFN-β [61]. Furthermore, it has been demonstrated that RRMS patients responding to IFN-β therapy show reduced levels of miR-26a, together with up-regulation of its putative target SLC1A1, a glutamate transporter, probably counteracting excitotoxicity [62].

Another study in PBMCs from RRMS patients reported the up-regulation of four miRNAs previously discussed for being involved in Th17 differentiation, regulation of immune tolerance and innate immunity, namely miR-326, miR-155, miR-146a and miR-142-3p. Interestingly, the expression levels of these miRNAs were not affected by IFN-β treatment, while Glatiramer Acetate (GA) administration was able to restore normal levels of miR-146a and miR-142-3p, suggesting that they may be specifically involved in the mechanism of action of the latter [63].

Also fingolimod, a “biased” agonist of the sphingosine 1 phosphate receptor S1P1 that prevents immune cell migration into the brain, was shown to promote its beneficial action by restoring blood levels of some miRNAs down-regulated in MS patients. These include miR-23a, a key regulator of myelination described in paragraph 2.1, miR-15b, a suppressor of Th17 differentiation, and miR-223, involved in inflammatory processes by targeting STAT5 [64]. In addition, it has been shown that 10 miRNAs are differentially expressed in B

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lymphocytes obtained from RRMS patients treated with natalizumab (NTZ), a monoclonal antibody able to prevent immune cell infiltration into the CNS [65]. In particular, NTZ significantly up-regulated the miR-17~92 cluster, demonstrated to play a key role in remyelination, and miR-106b~25, a cluster involved in the regulation of NSC proliferation (see also paragraph 2). Both of them were found down-regulated in RRMS patients compared to healthy controls and NTZ treatment was able to restore their levels [65]. In a different study, 1-year treatment with NTZ increased the blood levels of miR-18a, miR-20b, miR-29a and miR-103 in RRMS patients, all miRNAs expressed by $CD4^+$ T-cells that were originally down-regulated in RRMS patients compared to controls and, on this basis, probably involved in MS pathogenesis as well as in NTZ therapeutic action [66]. In particular, the authors found that genetic ablation of the miR-106a~363 cluster, which includes miR-20b, exacerbates EAE progression and, in parallel, up-regulates the expression of inflammatory targets of miR-20b, such as Rorγt, Stat3 and Vegf-α. These data suggest an active role for miR-20b in the pathogenesis of MS and provide a new insight in the mechanisms underlying NTZ beneficial effect [66].

Overall, these findings suggest that the therapeutic effect of pharmacological treatments may be due, at least in part, to the active restoration of miRNA levels that are dysregulated in the CNS or in the immune system during disease progression. However, it remains to be clarified when miRNA restoring is only an indirect consequence of improved clinical conditions. Concerning this, new exciting evidence suggest that miRNA profiling in the blood or CSF of MS patients may be used as possible prognostic tool for monitoring response to therapy. For example, the treatment with rituximab, a monoclonal antibody approved for MS and other autoimmune diseases, was able to restore aberrant levels of 10 miRNAs detected in whole blood samples from patients with neuromyelitis optica; these miRNA include six brain-specific or brain-enriched miRNAs, such as miR-125b, miR-760, miR-135a, miR-134, miR-138 and miR-135b, that has been proposed as potential biomarkers for disease progression and response to therapy [9]. Moreover, CSF levels of miR-150, a putative biomarker for MS diagnosis (also described in paragraph 3), were found increased in MS patients initiating NTZ therapy, and decreased after treatment, suggesting this miRNA as a potential biomarker also for monitoring MS patient's response to therapy [38]. Furthermore, NTZ treatment was able to restore also miR-26a and miR-155 levels, which were found strongly up-regulated in PBMCs of MS patients compared to

controls [67]. MiR-155 plays a central role in many processes involved in the pathogenesis of MS, such as immune cell activation, neurodegeneration and permeabilization of the BBB, as extensively reviewed by McCoy [68]. Interestingly, down-regulation of miR-155 was also found in circulating monocytes of patients treated after treatment with fingolimod or dimethyl fumarate (DMF) [69], suggesting that miR-155 levels can be used as a biomarker of neuroinflammation. The up-regulation of the pro-inflammatory miR-155-5p was also found in urine exosomes, as well as in plasma and spinal cord samples from EAE mice at pre-onset stage of the disease [70]. Interestingly, in the same study, the authors have found that GA treatment was able to modulate not only the levels of miR-155-5p, but also miR-27a-3p, miR-9-5p and miR-350-5p, in plasma and urine exosomes, proposing them as putative biomarkers of drug response [70]. In line with these studies, a recent work profiled the expression of exosome-associated miRNAs in serum of naive and IFN-β–treated groups of MS patients [71]. Of note, 16 circulating miRNAs in serum exosomes were found differentially expressed in IFN-β–treated MS patients compared to naive MS patients. The screening revealed that 2 of the 16 miRNAs, miR-22-3p and miR-660-5p, were up-regulated, and 14 of the 16 filtered miRNAs, including regulators of myelination (miR-23a-3p), NSC proliferation (miR-let-7b-5p), response to excitotoxicity (miR-26a-5p) and inflammation (miR-15b-3p; miR-223-3p) discussed also in the previous paragraphs, were down-regulated. Of interest, the majority of these exosome-associated miRNAs have been previously described as circulating biomarkers in MS [71].

Globally, these data suggest that a longitudinal analysis of circulating miRNAs obtained from different sources (e.g. urine or serum exosomes, PBMCs, whole blood; see also Table 3) from MS patients could represent a reliable approach for monitoring the response to therapy [72]. This is an important means, considering that MS patients require life-long chronic therapies and a continuous follow-up. However, further investigations are required to introduce exosome-associated miRNAs as diagnostic and prognostic biomarkers in clinical practice.

4.2. Involvement of miRNAs in drug resistance

An important issue in pharmacological therapies is the existence of patients not adequately responding to drug treatments [72]. Evidence arising from neuro-oncology, where this phenomenon is well-studied, suggest

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that altered expression of several miRNAs could interfere with patient's response to therapy. It has been demonstrated that down-regulation of miR-203, which may occasionally occur both in patients affected by GBM and in GBM cell lines, leads to chemoresistance against imatinib treatment [73]. The authors suggested that in this phenomenon miR-203 could play a role by blocking epithelial-mesenchymal transition, thus reducing cell motility, invasion, proliferation, and resistance to apoptosis, via inhibition of its direct target SNAI2, a zinc finger transcriptional repressor. On this basis, low levels of miR-203 may serve as an important biomarker for drug-resistant in GBM patients, while high expression of miR-203 may prefigure a better prognosis [73]. Furthermore, a recent study showed that over-expression of miR-423-5p, frequently found in clinical samples from GBM patients, enhanced cancer cell proliferation, angiogenesis and invasion and it was responsible for chemoresistance to temozolomide [74].

Treatment of non-responder patients is a critical issue also in the clinical management of MS [75] and, unfortunately, it remains a widely unexplored field. The first idea of miRNA involvement in drug-resistance comes from very a recent paper, showing different miRNA patterns in MS patients responding to fingolimod compared to non-responders [76]. MiR-34a-5p and miR-211-5p were found down-regulated in non-responder patients compared to responders, while miR-204-5p was up-regulated. However, due to the preliminary nature of the study, none of them could be a suitable biomarker for distinguishing patients responding to fingolimod therapy from non-responders [76].

Despite the molecular mechanisms linking aberrant miRNA levels to therapeutic failure remain to be elucidated, these preliminary studies suggest that, in the near future, miRNAs levels might become good indicators to address more personalized therapies.

5. MiRNA-based therapeutic opportunities

In case that miRNA dysregulation contributes to the pathogenesis of a disease, restoring its expression apparently represents the best choice. As described above, drugs can modulate the expression of miRNAs, but the most direct way to achieve this goal is to use miRNA mimics or miRNA inhibitors. Mimic is a synthetic molecule able to enhance the endogenous expression of a miRNA, whereas miRNA inhibitor (or antagomiR), is the complementary sequence able to bind an endogenous miRNA and to abolish its activity.

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As extensively reviewed in [77] and [78], several clinical trials exploring the therapeutic opportunities of miRNAs are already ongoing for different diseases (www.clinicaltrials.gov). The first miRNA-based drug was miravirsen, a locked nucleic acid (LNA)-modified antisense oligonucleotide able to inhibit miR-122 and designed for the treatment of Hepatitis C Virus (HCV) infection. MiR-122, produced by hepatic cells, is indeed essential for Hepatitis C Virus (HCV) replication [79]. From 2008 to date miravirsen entered in several phase I-II clinical trials, showing that its subcutaneous injections was well-tolerated and demonstrating a significant and sustained reduction of HCV RNA levels in subjects with chronic HCV [80]. Ongoing clinical trials aim to propose miravirsen as an alternative treatment for null responder patients to standard PEGylated interferon alpha plus ribavirin treatment.

In 2013, a new clinical trial evaluated the effect of the restoration of miR-34a levels in solid tumors. Indeed, miR-34a is frequently lost or downregulated in a broad range of human cancer types and this alteration closely correlate with reduced survival [81]. Based on this evidence, a liposomal miRNA mimic, namely MRX34, was developed to restore the functionality of the tumor suppressor miR-34a. In the clinical study, 47 adult patients with solid tumors refractory to standard treatments were treated intravenously twice weekly for three weeks in 4-week cycles. Despite 5 patients experienced severe immune-related adverse events, the study showed that the treatment was associated with acceptable safety and highlighted evidence of antitumor activity in a subset of patients [82].

Another phase I clinical trial evaluated the effect of MRG-201, a miRNA drug designed to mimic the activity of miR-29, in induced cutaneous fibrosis. The trial showed a good safety and a reduction in fibroplasia, a histopathological marker of scar tissue deposition (ClinicalTrials.gov Identifier: NCT02603224). MiR-29 is indeed involved in the regulation of collagen deposition and formation of fibrous scar tissue [83].

Recently, a phase I clinical trial (ClinicalTrials.gov Identifier: NCT02580552) evaluated safety, efficacy and pharmacokinetics of cobomarsen, a locked nucleic acid-modified oligonucleotide inhibitor of miR-155, in patients with the mycosis fungoides form of cutaneous T-cell lymphoma (CTCL). In this study 92% of subjects treated with cobomarsen by systemic administration showed improvement in mSWAT score, a measurement of the severity of skin disease. Cobomarsen has been generally well-tolerated at the range of doses tested and no serious adverse events have been attributed to its administration. These positive results prompted a

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phase II clinical trial to study the effects of cobomarsen in comparison to vorinostat, an already approved drug for the treatment of CTCL (SOLAR; ClinicalTrials.gov Identifier: NCT03713320).

In 2018, a phase I clinical trial evaluated safety, tolerability, pharmacokinetic and pharmacodynamic of MRG-110 (ClinicalTrials.gov Identifier: NCT03603431), a miRNA-targeting drug developed for the treatment of chronic ischemic disorders. MRG-110 is a LNA modified oligonucleotide inhibitor of miR-92, that has shown pro-angiogenic effects in pre-clinical studies [84].

Interestingly, some of the miRNA-based drugs described here act on miRNAs (i.e., miR-34, miR-155, miR-92) whose involvement in neurodegeneration is consolidated. Despite no miRNA-based clinical trials are ongoing for the treatment of neurodegenerative diseases, these studies can be considered a "proof of principle", showing that miRNA-targeting drugs can be effective for the treatment of human diseases, including CNS-related disorders. Of course, these next-generation drugs, as standard drugs, require specific delivery systems to easily cross the BBB, reach the CNS compartment and alter the dynamics of the recipient cells, also to minimize systemic off-target effects.

5.1 Delivery of miRNA-based drugs in the CNS

In CNS-related disorders, delivery systems should be aimed at improving the pharmacokinetics of miRNA agents. In the last years, a variety of both viral and non-viral approaches have been developed and applied for CNS miRNA delivery [85]. Viral vectors such as adeno-associated virus (AAV), adenovirus, retrovirus and lentivirus have been extensively explored to deliver gene therapy *in vivo*, but only selected classes are suitable for CNS applications. In particular, AAV are considered powerful delivery vectors to transfer miRNA in CNS due to their abilities to transduce mitotic and post-mitotic cells, tropism for neurons and glial cells, absence of pathogenicity or cytotoxicity, and low immunogenicity [86]. A successful example of recombinant AAV-delivered miRNA therapy was described in a preclinical study in which rAAV9 was used to deliver miR-196a to treat spinal bulbar muscular atrophy (SBMA) [87]. SBMA, popularly known as Kennedy disease, is a progressive debilitating neurodegenerative disorder caused by the expansion of the polyglutamine (polyQ) tract of the androgen receptor (AR-polyQ), resulting in muscle cramps and progressive weakness due to loss of motor neurons. Early intervention of miR-196a delivered by an AAV vector was found to ameliorate the

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SBMA phenotype in a mouse model of the disease [87]. However, large scale production of viral vectors represents a potential limitation to their use; furthermore, the high gene transfer efficiency is often associated to brain toxicity and potential tumorigenic and immunogenic effects that limit clinical translation [85].

Non-viral approaches show less efficiency, but on the other hand they have lower toxicity and immunogenicity. Among them, nanoparticles (NPs) present some advantages such as good stability and cellular uptake. Various classes of nanoparticles, including metallic, polymeric and lipid nanoparticles, have been shown to cross the BBB and enter the brain through endocytotic mechanisms (extensively reviewed in [88]).

Recently, a significant number of studies highlighted extracellular vesicles (EVs) as paracrine mediators responsible for the therapeutic potential of mesenchymal stem cells, other adult stem cells or immune cells in experimental models of neurodegenerative diseases including multiple sclerosis [89] or brain injury [90]. These observations have generated interest on the use of EVs in clinical applications for regenerative medicine purpose as novel paradigm for cell-free therapy; furthermore, their intrinsic abilities to be exported out of the parental cells, to deliver along the circulation system, to cross BBB and to target specific cells, make them a promising tool for the delivery of therapeutic bioactive molecules, including miRNA, to the CNS. In this respect, researchers are trying diverse strategies to manipulate EVs content [91] and improve target specificity introducing special membrane proteins/peptides in order to reduce risks associated to the possible uptake of miRNA agents by non-target tissues and cells [92].

Two main approaches for loading miRNA have been investigated: i) pre-loading or endogenous method, which is based on genetic engineering of exosome-producing cells, such as mesenchymal stem cells (MSCs) [93] and ii) post-loading or exogenous method, in which miRNAs are loaded into previously purified EVs [94]. Many recent preclinical studies have employed miRNAs or antago-miRNAs enclosed into EVs as active agents against various types of tumors (recently reviewed in [95]). Results from these studies suggest that EVs can be efficient drug-delivery tools and their use for the application of miRNA therapeutic strategies for neurodegenerative diseases is under active investigation. For example, EVs derived from neuronal cell exogenously transfected with miR-124a caused an increase of the glutamate transporter GLT1, that is

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selectively lost in animal model of ALS, suggesting their therapeutic potential for ALS [96]. Other studies have utilized EVs engineered with brain homing peptides or brain-specific antibodies in order to enhance targeting efficiency. For example, exosomes derived from dendritic cells, previously loaded with siRNA for the beta secretase enzyme BACE-1, were shown to cross the BBB after engineering dendritic cells to express Lamp2b, an exosomal membrane protein, fused to the central nervous system-specific rabies viral glycoprotein (RVG) peptide that binds to the acetylcholine receptor [97]. Intravenously injection of RVG-targeted exosomes, previously loaded with BACE-1 siRNA (or GAPDH siRNA as control) by electroporation, resulted in a brain-specific gene knockdown, without causing an immune response. Similarly, the systemic administration of engineered EVs with an exogenous siRNA for alpha-synuclein significantly decreased the level of a pro-aggregating human form of this protein in a transgenic PD mouse model [98]. Although attempts to modify EV surface to target distinct cells and tissue are still in their infancy, these exciting findings suggest that EVs can be genetically engineered to be promising potent therapeutics for neurodegenerative disease.

Nevertheless, several issues need to be further explored before using EVs as therapeutic tools [94]. The most critical problems are related to the identification of the most proficient cellular source of EVs suitable for clinical application and to the optimization of the methods for obtaining high yields of pure EVs [94]. Additional specific issues arise for the clinical translation of EVs-mediated miRNA delivery for neurological disorders. Globally, a better understanding of the molecular mechanisms by which miRNAs are sorted into EVs and subsequently released by different cell types is needed. Moreover, methods for miRNA/antago-miRNA loading into EVs and defining the yield of the loaded cargo should be optimized [42]. For therapeutic intervention, EVs loaded with miRNAs/antago-miRs can be delivered systemically, although exogenously systemic administration of EVs have been shown to be rapidly cleared by the macrophages [99]. Intranasal administration of EVs may increase brain accumulation and reduce the risks of dissemination, off-target effects and toxicity, an approach that has been successfully used in a rat model of Parkinson's disease [100]. Understanding biodistribution and pharmacokinetics profiles of EVs administered by different routes and modalities might hold the potential for clinical translation. Although the mechanisms involved in the cellular uptake of EVs are not completely elucidated and much remains to be done to standardize the use of EVs as

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therapeutic tools in CNS diseases, it is expected that EV-mediated miRNA therapeutics will be an important class of therapeutic molecule in the future.

6. Future directions

Although the discovery of miRNAs has progressively revolutionized the landscape of cell and molecular biology, their exact role in the pathophysiology of disease is far from being understood. MiRNAs are often studied as single entities in gain-/loss-of-function experiments to examine their functions more in detail, but they always act in concert with other miRNAs. Synergy and redundancy represent the strength of their regulatory mechanism, and this should always be taken into account to avoid data misinterpretation. Moreover, the ability to observe minimal fluctuations of a single circulating miRNA is limited by the sensitivity of the detection systems. Although many progresses have been made in addressing the technical issues, more work is needed to translate the current knowledge into clinical practice. MiRNAs are, indeed, entitled to become the biomarkers of the future. However, biomarkers are molecules universally accepted as indicators of normal or pathological processes, and, to date, miRNAs are only “potential” biomarkers, because of the variability observed in different studies, often due to the lack of a common normalization framework. Moreover, their validation is even more complicated in neurodegenerative disorders due to high complexity in terms of disease progression rates, in addition to the age and sex dependency. Large cohorts of patients should be analyzed, and patient stratification, sampling, processing, data analysis and normalization should be homogeneously performed to ensure a correct interpretation of results. Based on recent evidence, we expect that a set of miRNAs will become a more reliable diagnostic and prognostic biomarker than single miRNAs.

The existence of miRNAs changed the games also in pharmacology. One of the most challenging opportunities is to exploit them as targets for cutting-edge pharmacological agents to restore or enhance their expression. However, considering that miRNAs are able to influence not only a single gene, but entire cellular pathways and processes, the off-target effects of mimics or antagomiRs still remain a major concern. For this reason, as any other conventional therapeutics they require a careful evaluation of toxicity and side effects. MiRNA-targeting agents might promote beneficial effects in pathological conditions, by fine-tuning

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several players in the same pathway, as described for endogenous miRNAs, highlighting the power of this approach. Mimic and antagomiR administration is feasible, and may take advantage of EVs; however, the strategies to deliver specific miRNAs into the CNS still require further investigation. Of note, also conventional drugs modulate miRNA expression levels and this could be part of their therapeutic potential. A more detailed study of the "miRNA pharmacology" might also explain some side effects and the non-response to therapy, paving the way to personalized medicine for the treatment of neurodegenerative diseases.

Funding: this work was supported by Fondazione Cariplo grant no. 2014-1207 to DL and grant no. 2015-0910 to MF, by Università degli Studi di Milano, piano di sostegno alla Ricerca 2015/2017, linea 2 to MF and to DL, and by the "Department of Excellence" grant program from the Italian Ministry of Research (MIUR).

Conflicts of Interest: the authors have no conflicts of interest.

References

[1] R.C. Friedman, K.K. Farh, C.B. Burge, D.P. Bartel, Most mammalian mRNAs are conserved targets of microRNAs, Genome research 19(1) (2009) 92-105.
[2] M.S. Ebert, P.A. Sharp, Roles for microRNAs in conferring robustness to biological processes, Cell 149(3) (2012) 515-24.
[3] A. Valinezhad Orang, R. Safaralizadeh, M. Kazemzadeh-Bavili, Mechanisms of miRNA-Mediated Gene Regulation from Common Downregulation to mRNA-Specific Upregulation, International journal of genomics 2014 (2014) 970607.
[4] X. Zhao, X. He, X. Han, Y. Yu, F. Ye, Y. Chen, T. Hoang, X. Xu, Q.S. Mi, M. Xin, F. Wang, B. Appel, Q.R. Lu, MicroRNA-mediated control of oligodendrocyte differentiation, Neuron 65(5) (2010) 612-26.
[5] R. Ben-Hamo, S. Efroni, MicroRNA regulation of molecular pathways as a generic mechanism and as a core disease phenotype, Oncotarget 6(3) (2015) 1594-604.
[6] Z. Wang, H. Yao, S. Lin, X. Zhu, Z. Shen, G. Lu, W.S. Poon, D. Xie, M.C. Lin, H.F. Kung, Transcriptional and epigenetic regulation of human microRNAs, Cancer letters 331(1) (2013) 1-10.
[7] G. Michlewski, J.F. Caceres, Post-transcriptional control of miRNA biogenesis, Rna 25(1) (2019) 1-16.
[8] R. Gandhi, B. Healy, T. Gholipour, S. Egorova, A. Musallam, M.S. Hussain, P. Nejad, B. Patel, H. Hei, S. Khoury, F. Quintana, P. Kivisakk, T. Chitnis, H.L. Weiner, Circulating microRNAs as biomarkers for disease staging in multiple sclerosis, Annals of neurology 73(6) (2013) 729-40.
[9] A. Vaknin-Dembinsky, H. Charbit, L. Brill, O. Abramsky, D. Gur-Wahnon, I.Z. Ben-Dov, I. Lavon, Circulating microRNAs as biomarkers for rituximab therapy, in neuromyelitis optica (NMO), Journal of neuroinflammation 13(1) (2016) 179.
[10] W. Zhang, M.E. Dolan, The emerging role of microRNAs in drug responses, Current opinion in molecular therapeutics 12(6) (2010) 695-702.
[11] B. Winner, J. Winkler, Adult neurogenesis in neurodegenerative diseases, Cold Spring Harbor perspectives in biology 7(4) (2015) a021287.

See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

[12] S.K. Fineberg, P. Datta, C.S. Stein, B.L. Davidson, MiR-34a represses Numbl in murine neural progenitor cells and antagonizes neuronal differentiation, PloS one 7(6) (2012) e38562.
[13] A. Zovoilis, H.Y. Agbemenyah, R.C. Agis-Balboa, R.M. Stilling, D. Edbauer, P. Rao, L. Farinelli, I. Delalle, A. Schmitt, P. Falkai, S. Bahari-Javan, S. Burkhardt, F. Sananbenesi, A. Fischer, microRNA-34c is a novel target to treat dementias, The EMBO journal 30(20) (2011) 4299-308.
[14] J.A. Gorter, A. Iyer, I. White, A. Colzi, E.A. van Vliet, S. Sisodiya, E. Aronica, Hippocampal subregion-specific microRNA expression during epileptogenesis in experimental temporal lobe epilepsy, Neurobiology of disease 62 (2014) 508-20.
[15] C. Zhao, G. Sun, S. Li, Y. Shi, A feedback regulatory loop involving microRNA-9 and nuclear receptor TLX in neural stem cell fate determination, Nature structural & molecular biology 16(4) (2009) 365-71.
[16] A.S. Yoo, A.X. Sun, L. Li, A. Shcheglovitov, T. Portmann, Y. Li, C. Lee-Messer, R.E. Dolmetsch, R.W. Tsien, G.R. Crabtree, MicroRNA-mediated conversion of human fibroblasts to neurons, Nature 476(7359) (2011) 228-31.
[17] S. Sharma, H.C. Lu, microRNAs in Neurodegeneration: Current Findings and Potential Impacts, Journal of Alzheimer's disease & Parkinsonism 8(1) (2018).
[18] S.E. Sim, C.S. Lim, J.I. Kim, D. Seo, H. Chun, N.K. Yu, J. Lee, S.J. Kang, H.G. Ko, J.H. Choi, T. Kim, E.H. Jang, J. Han, M.S. Bak, J.E. Park, D.J. Jang, D. Baek, Y.S. Lee, B.K. Kaang, The Brain-Enriched MicroRNA miR-9-3p Regulates Synaptic Plasticity and Memory, The Journal of neuroscience : the official journal of the Society for Neuroscience 36(33) (2016) 8641-52.
[19] Y. Guo, K. Yan, J. Fang, Q. Qu, M. Zhou, F. Chen, Let-7b expression determines response to chemotherapy through the regulation of cyclin D1 in glioblastoma, Journal of experimental & clinical cancer research : CR 32 (2013) 41.
[20] F. An, G. Gong, Y. Wang, M. Bian, L. Yu, C. Wei, MiR-124 acts as a target for Alzheimer's disease by regulating BACE1, Oncotarget 8(69) (2017) 114065-114071.
[21] T.R. Doeppner, M. Doehring, E. Bretschneider, A. Zechariah, B. Kaltwasser, B. Muller, J.C. Koch, M. Bahr, D.M. Hermann, U. Michel, MicroRNA-124 protects against focal cerebral ischemia via mechanisms involving Usp14-dependent REST degradation, Acta neuropathologica 126(2) (2013) 251-65.
[22] D. Edbauer, J.R. Neilson, K.A. Foster, C.F. Wang, D.P. Seeburg, M.N. Batterton, T. Tada, B.M. Dolan, P.A. Sharp, M. Sheng, Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132, Neuron 65(3) (2010) 373-84.
[23] P.N. Alexandrov, P. Dua, J.M. Hill, S. Bhattacharjee, Y. Zhao, W.J. Lukiw, microRNA (miRNA) speciation in Alzheimer's disease (AD) cerebrospinal fluid (CSF) and extracellular fluid (ECF), International journal of biochemistry and molecular biology 3(4) (2012) 365-73.
[24] B. Emery, Q.R. Lu, Transcriptional and Epigenetic Regulation of Oligodendrocyte Development and Myelination in the Central Nervous System, Cold Spring Harbor perspectives in biology 7(9) (2015) a020461.
[25] J.C. Dugas, T.L. Cuellar, A. Scholze, B. Ason, A. Ibrahim, B. Emery, J.L. Zamanian, L.C. Foo, M.T. McManus, B.A. Barres, Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination, Neuron 65(5) (2010) 597-611.
[26] H. Wang, A.L. Moyano, Z. Ma, Y. Deng, Y. Lin, C. Zhao, L. Zhang, M. Jiang, X. He, Z. Ma, F. Lu, M. Xin, W. Zhou, S.O. Yoon, E.R. Bongarzone, Q.R. Lu, miR-219 Cooperates with miR-338 in Myelination and Promotes Myelin Repair in the CNS, Developmental cell 40(6) (2017) 566-582 e5.
[27] H.B. Fan, L.X. Chen, X.B. Qu, C.L. Ren, X.X. Wu, F.X. Dong, B.L. Zhang, D.S. Gao, R.Q. Yao, Transplanted miR-219-overexpressing oligodendrocyte precursor cells promoted remyelination and improved functional recovery in a chronic demyelinated model, Scientific reports 7 (2017) 41407.
[28] U. Milbreta, J. Lin, C. Pinese, W. Ong, J.S. Chin, H. Shirahama, R. Mi, A. Williams, M.E. Bechler, J. Wang, C. Ffrench-Constant, A. Hoke, S.Y. Chew, Scaffold-Mediated Sustained, Non-viral Delivery of miR-219/miR-338 Promotes CNS Remyelination, Molecular therapy : the journal of the American Society of Gene Therapy 27(2) (2019) 411-423.
[29] H. Budde, S. Schmitt, D. Fitzner, L. Opitz, G. Salinas-Riester, M. Simons, Control of oligodendroglial cell number by the miR-17-92 cluster, Development 137(13) (2010) 2127-32.
[30] E.P. Harrington, C. Zhao, S.P. Fancy, S. Kaing, R.J. Franklin, D.H. Rowitch, Oligodendrocyte PTEN is required for myelin and axonal integrity, not remyelination, Annals of neurology 68(5) (2010) 703-16.

See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

[31] S.T. Lin, Y.H. Fu, miR-23 regulation of lamin B1 is crucial for oligodendrocyte development and myelination, Disease models & mechanisms 2(3-4) (2009) 178-88.
[32] S.T. Lin, Y. Huang, L. Zhang, M.Y. Heng, L.J. Ptacek, Y.H. Fu, MicroRNA-23a promotes myelination in the central nervous system, Proceedings of the National Academy of Sciences of the United States of America 110(43) (2013) 17468-73.
[33] D. Lecca, D. Marangon, G.T. Coppolino, A.M. Mendez, A. Finardi, G.D. Costa, V. Martinelli, R. Furlan, M.P. Abbracchio, MiR-125a-3p timely inhibits oligodendroglial maturation and is pathologically up-regulated in human multiple sclerosis, Scientific reports 6 (2016) 34503.
[34] A. Junker, M. Krumbholz, S. Eisele, H. Mohan, F. Augstein, R. Bittner, H. Lassmann, H. Wekerle, R. Hohlfeld, E. Meinl, MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47, Brain : a journal of neurology 132(Pt 12) (2009) 3342-52.
[35] A. Haghikia, A. Haghikia, K. Hellwig, A. Baraniskin, A. Holzmann, B.F. Decard, T. Thum, R. Gold, Regulated microRNAs in the CSF of patients with multiple sclerosis: a case-control study, Neurology 79(22) (2012) 2166-70.
[36] A. Junker, R. Hohlfeld, E. Meinl, The emerging role of microRNAs in multiple sclerosis, Nature reviews. Neurology 7(1) (2011) 56-9.
[37] I.B. Bruinsma, M. van Dijk, C. Bridel, T. van de Lisdonk, S.Q. Haverkort, T.F. Runia, L. Steinman, R.Q. Hintzen, J. Killestein, M.M. Verbeek, C.E. Teunissen, B.A. de Jong, Regulator of oligodendrocyte maturation, miR-219, a potential biomarker for MS, Journal of neuroinflammation 14(1) (2017) 235.
[38] P. Bergman, E. Piket, M. Khademi, T. James, L. Brundin, T. Olsson, F. Piehl, M. Jagodic, Circulating miR-150 in CSF is a novel candidate biomarker for multiple sclerosis, Neurology(R) neuroimmunology & neuroinflammation 3(3) (2016) e219.
[39] E. Quintana, F.J. Ortega, R. Robles-Cedeno, M.L. Villar, M. Buxo, J.M. Mercader, J.C. Alvarez-Cermeno, N. Pueyo, H. Perkal, J.M. Fernandez-Real, L. Ramio-Torrenta, miRNAs in cerebrospinal fluid identify patients with MS and specifically those with lipid-specific oligoclonal IgM bands, Multiple sclerosis 23(13) (2017) 1716-1726.
[40] M.B. Cox, M.J. Cairns, K.S. Gandhi, A.P. Carroll, S. Moscovis, G.J. Stewart, S. Broadley, R.J. Scott, D.R. Booth, J. Lechner-Scott, A.N.M.S.G. Consortium, MicroRNAs miR-17 and miR-20a inhibit T cell activation genes and are under-expressed in MS whole blood, PloS one 5(8) (2010) e12132.
[41] S.R. Siegel, J. Mackenzie, G. Chaplin, N.G. Jablonski, L. Griffiths, Circulating microRNAs involved in multiple sclerosis, Molecular biology reports 39(5) (2012) 6219-25.
[42] B. Mateescu, E.J. Kowal, B.W. van Balkom, S. Bartel, S.N. Bhattacharyya, E.I. Buzas, A.H. Buck, P. de Candia, F.W. Chow, S. Das, T.A. Driedonks, L. Fernandez-Messina, F. Haderk, A.F. Hill, J.C. Jones, K.R. Van Keuren-Jensen, C.P. Lai, C. Lasser, I.D. Liegro, T.R. Lunavat, M.J. Lorenowicz, S.L. Maas, I. Mager, M. Mittelbrunn, S. Momma, K. Mukherjee, M. Nawaz, D.M. Pegtel, M.W. Pfaffl, R.M. Schiffelers, H. Tahara, C. Thery, J.P. Tosar, M.H. Wauben, K.W. Witwer, E.N. Nolte-'t Hoen, Obstacles and opportunities in the functional analysis of extracellular vesicle RNA - an ISEV position paper, Journal of extracellular vesicles 6(1) (2017) 1286095.
[43] C. Verderio, Extracellular membrane microvesicles and nanotubes in the brain: understanding their nature, their function in cell-to-cell communication, their role in transcellular spreading of pathological agents and their therapeutic potential, Frontiers in physiology 4 (2013) 163.
[44] C. Verderio, L. Muzio, E. Turola, A. Bergami, L. Novellino, F. Ruffini, L. Riganti, I. Corradini, M. Francolini, L. Garzetti, C. Maiorino, F. Servida, A. Vercelli, M. Rocca, D. Dalla Libera, V. Martinelli, G. Comi, G. Martino, M. Matteoli, R. Furlan, Myeloid microvesicles are a marker and therapeutic target for neuroinflammation, Annals of neurology 72(4) (2012) 610-24.
[45] S. Ebrahimkhani, F. Vafaee, P.E. Young, S.S.J. Hur, S. Hawke, E. Devenney, H. Beadnall, M.H. Barnett, C.M. Suter, M.E. Buckland, Exosomal microRNA signatures in multiple sclerosis reflect disease status, Scientific reports 7(1) (2017) 14293.
[46] J. Riancho, J.L. Vazquez-Higuera, A. Pozueta, C. Lage, M. Kazimierczak, M. Bravo, M. Calero, A. Gonalezalez, E. Rodriguez, A. Lleo, P. Sanchez-Juan, MicroRNA Profile in Patients with Alzheimer's Disease: Analysis of miR-9-5p and miR-598 in Raw and Exosome Enriched Cerebrospinal Fluid Samples, Journal of Alzheimer's disease : JAD 57(2) (2017) 483-491.

See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

[47] Q. Ji, Y. Ji, J. Peng, X. Zhou, X. Chen, H. Zhao, T. Xu, L. Chen, Y. Xu, Increased Brain-Specific MiR-9 and MiR-124 in the Serum Exosomes of Acute Ischemic Stroke Patients, PloS one 11(9) (2016) e0163645.
[48] I. Prada, M. Gabrielli, E. Turola, A. Iorio, G. D'Arrigo, R. Parolisi, M. De Luca, M. Pacifici, M. Bastoni, M. Lombardi, G. Legname, D. Cojoc, A. Buffo, R. Furlan, F. Peruzzi, C. Verderio, Glia-to-neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations, Acta neuropathologica 135(4) (2018) 529-550.
[49] F. Jagot, N. Davoust, Is It worth Considering Circulating microRNAs in Multiple Sclerosis?, Frontiers in immunology 7 (2016) 129.
[50] S.M. Lehmann, C. Kruger, B. Park, K. Derkow, K. Rosenberger, J. Baumgart, T. Trimbuch, G. Eom, M. Hinz, D. Kaul, P. Habbel, R. Kalin, E. Franzoni, A. Rybak, D. Nguyen, R. Veh, O. Ninnemann, O. Peters, R. Nitsch, F.L. Heppner, D. Golenbock, E. Schott, H.L. Ploegh, F.G. Wulczyn, S. Lehnardt, An unconventional role for miRNA: let-7 activates Toll-like receptor 7 and causes neurodegeneration, Nature neuroscience 15(6) (2012) 827-35.
[51] N. Tian, Z. Cao, Y. Zhang, MiR-206 decreases brain-derived neurotrophic factor levels in a transgenic mouse model of Alzheimer's disease, Neuroscience bulletin 30(2) (2014) 191-7.
[52] C.N. Wang, Y.J. Wang, H. Wang, L. Song, Y. Chen, J.L. Wang, Y. Ye, B. Jiang, The Anti-dementia Effects of Donepezil Involve miR-206-3p in the Hippocampus and Cortex, Biological & pharmaceutical bulletin 40(4) (2017) 465-472.
[53] W. Huang, Z. Li, L. Zhao, W. Zhao, Simvastatin ameliorate memory deficits and inflammation in clinical and mouse model of Alzheimer's disease via modulating the expression of miR-106b, Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 92 (2017) 46-57.
[54] A. Serafin, L. Foco, S. Zanigni, H. Blankenburg, A. Picard, A. Zanon, G. Giannini, I. Pichler, M.F. Facheris, P. Cortelli, P.P. Pramstaller, A.A. Hicks, F.S. Domingues, C. Schwienbacher, Overexpression of blood microRNAs 103a, 30b, and 29a in L-dopa-treated patients with PD, Neurology 84(7) (2015) 645-53.
[55] E. Caggiu, K. Paulus, G. Mameli, G. Arru, G.P. Sechi, L.A. Sechi, Differential expression of miRNA 155 and miRNA 146a in Parkinson's disease patients, eNeurologicalSci 13 (2018) 1-4.
[56] C.R. Reschke, L.F. Silva, B.A. Norwood, K. Senthilkumar, G. Morris, A. Sanz-Rodriguez, R.M. Conroy, L. Costard, V. Neubert, S. Bauer, M.A. Farrell, D.F. O'Brien, N. Delanty, S. Schorge, R.J. Pasterkamp, F. Rosenow, D.C. Henshall, Potent Anti-seizure Effects of Locked Nucleic Acid Antagomirs Targeting miR-134 in Multiple Mouse and Rat Models of Epilepsy, Molecular therapy. Nucleic acids 6 (2017) 45-56.
[57] X. Wang, Y. Luo, S. Liu, L. Tan, S. Wang, R. Man, MicroRNA-134 plasma levels before and after treatment with valproic acid for epilepsy patients, Oncotarget 8(42) (2017) 72748-72754.
[58] W. Alsharafi, B. Xiao, Dynamic Expression of MicroRNAs (183, 135a, 125b, 128, 30c and 27a) in the Rat Pilocarpine Model and Temporal Lobe Epilepsy Patients, CNS & neurological disorders drug targets 14(8) (2015) 1096-102.
[59] Y.B. Feng, Y.T. Lin, Y.X. Han, Y.J. Pang, J.J. Xu, Y. Xue, H. Yao, 2R,4R-APDC, a Metabotropic Glutamate Receptor Agonist, Reduced Neuronal Apoptosis by Upregulating MicroRNA-128 in a Rat Model After Seizures, Neurochemical research 43(3) (2018) 591-599.
[60] M. Hecker, M. Thamilarasan, D. Koczan, I. Schroder, K. Flechtner, S. Freiesleben, G. Fullen, H.J. Thiesen, U.K. Zettl, MicroRNA expression changes during interferon-beta treatment in the peripheral blood of multiple sclerosis patients, International journal of molecular sciences 14(8) (2013) 16087-110.
[61] N. Ehtesham, F. Khorvash, M. Kheirollahi, miR-145 and miR20a-5p Potentially Mediate Pleiotropic Effects of Interferon-Beta Through Mitogen-Activated Protein Kinase Signaling Pathway in Multiple Sclerosis Patients, Journal of molecular neuroscience : MN 61(1) (2017) 16-24.
[62] N. Potenza, N. Mosca, P. Mondola, S. Damiano, A. Russo, B. De Felice, Human miR-26a-5p regulates the glutamate transporter SLC1A1 (EAAT3) expression. Relevance in multiple sclerosis, Biochimica et biophysica acta. Molecular basis of disease 1864(1) (2018) 317-323.
[63] A. Waschbisch, M. Atiya, R.A. Linker, S. Potapov, S. Schwab, T. Derfuss, Glatiramer acetate treatment normalizes deregulated microRNA expression in relapsing remitting multiple sclerosis, PloS one 6(9) (2011) e24604.
[64] C. Fenoglio, M. De Riz, A.M. Pietroboni, A. Calvi, M. Serpente, S.M. Cioffi, M. Arcaro, E. Oldoni, E. Scarpini, D. Galimberti, Effect of fingolimod treatment on circulating miR-15b, miR23a and miR-223 levels in patients with multiple sclerosis, Journal of neuroimmunology 299 (2016) 81-83.

See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

[65] C. Sievers, M. Meira, F. Hoffmann, P. Fontoura, L. Kappos, R.L. Lindberg, Altered microRNA expression in B lymphocytes in multiple sclerosis: towards a better understanding of treatment effects, Clinical immunology 144(1) (2012) 70-9.
[66] J. Ingwersen, T. Menge, B. Wingerath, D. Kaya, J. Graf, T. Prozorovski, A. Keller, C. Backes, M. Beier, M. Scheffler, T. Dehmel, B.C. Kieseier, H.P. Hartung, P. Kury, O. Aktas, Natalizumab restores aberrant miRNA expression profile in multiple sclerosis and reveals a critical role for miR-20b, Annals of clinical and translational neurology 2(1) (2015) 43-55.
[67] G. Mameli, G. Arru, E. Caggiu, M. Niegowska, S. Leoni, G. Madeddu, S. Babudieri, G.P. Sechi, L.A. Sechi, Natalizumab Therapy Modulates miR-155, miR-26a and Proinflammatory Cytokine Expression in MS Patients, PloS one 11(6) (2016) e0157153.
[68] C.E. McCoy, miR-155 Dysregulation and Therapeutic Intervention in Multiple Sclerosis, Advances in experimental medicine and biology 1024 (2017) 111-131.
[69] M.A. Michell-Robinson, C.S. Moore, L.M. Healy, L.A. Osso, N. Zorko, V. Grouza, H. Touil, L. Poliquin-Lasnier, A.M. Trudelle, P.S. Giacomini, A. Bar-Or, J.P. Antel, Effects of fumarates on circulating and CNS myeloid cells in multiple sclerosis, Annals of clinical and translational neurology 3(1) (2016) 27-41.
[70] J. Singh, M. Deshpande, H. Suhail, R. Rattan, S. Giri, Targeted Stage-Specific Inflammatory microRNA Profiling in Urine During Disease Progression in Experimental Autoimmune Encephalomyelitis: Markers of Disease Progression and Drug Response, Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology 11(1) (2016) 84-97.
[71] I. Manna, E. Iaccino, V. Dattilo, S. Barone, E. Vecchio, S. Mimmi, E. Filippelli, G. Demonte, S. Polidoro, A. Granata, S. Scannapieco, I. Quinto, P. Valentino, A. Quattrone, Exosome-associated miRNA profile as a prognostic tool for therapy response monitoring in multiple sclerosis patients, FASEB journal : official publication of the Federation of American Societies for Experimental Biology 32(8) (2018) 4241-4246.
[72] J. Rio, M. Comabella, X. Montalban, Predicting responders to therapies for multiple sclerosis, Nature reviews. Neurology 5(10) (2009) 553-60.
[73] H. Liao, Y. Bai, S. Qiu, L. Zheng, L. Huang, T. Liu, X. Wang, Y. Liu, N. Xu, X. Yan, H. Guo, MiR-203 downregulation is responsible for chemoresistance in human glioblastoma by promoting epithelial-mesenchymal transition via SNAI2, Oncotarget 6(11) (2015) 8914-28.
[74] S. Li, A. Zeng, Q. Hu, W. Yan, Y. Liu, Y. You, miR-423-5p contributes to a malignant phenotype and temozolomide chemoresistance in glioblastomas, Neuro-oncology 19(1) (2017) 55-65.
[75] A. Bertolotto, F. Gilli, Interferon-beta responders and non-responders. A biological approach, Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology 29 Suppl 2 (2008) S216-7.
[76] M.M. Eftekharian, A. Komaki, M. Mazdeh, S. Arsang-Jang, M. Taheri, S. Ghafouri-Fard, Expression Profile of Selected MicroRNAs in the Peripheral Blood of Multiple Sclerosis Patients: a Multivariate Statistical Analysis with ROC Curve to Find New Biomarkers for Fingolimod, Journal of molecular neuroscience : MN 68(1) (2019) 153-161.
[77] R. Rupaimoole, F.J. Slack, MicroRNA therapeutics: towards a new era for the management of cancer and other diseases, Nature reviews. Drug discovery 16(3) (2017) 203-222.
[78] P. Sun, D.Z. Liu, G.C. Jickling, F.R. Sharp, K.J. Yin, MicroRNA-based therapeutics in central nervous system injuries, Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 38(7) (2018) 1125-1148.
[79] C.L. Jopling, M. Yi, A.M. Lancaster, S.M. Lemon, P. Sarnow, Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA, Science 309(5740) (2005) 1577-81.
[80] H.L. Janssen, S. Kauppinen, M.R. Hodges, HCV infection and miravirsen, The New England journal of medicine 369(9) (2013) 878.
[81] J. Wang, G. Dan, J. Zhao, Y. Ding, F. Ye, H. Sun, F. Jiang, J. Cheng, F. Yuan, Z. Zou, The predictive effect of overexpressed miR-34a on good survival of cancer patients: a systematic review and meta-analysis, OncoTargets and therapy 8 (2015) 2709-19.
[82] M.S. Beg, A.J. Brenner, J. Sachdev, M. Borad, Y.K. Kang, J. Stoudemire, S. Smith, A.G. Bader, S. Kim, D.S. Hong, Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors, Investigational new drugs 35(2) (2017) 180-188.

See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

[83] J. Guo, Q. Lin, Y. Shao, L. Rong, D. Zhang, miR-29b promotes skin wound healing and reduces excessive scar formation by inhibition of the TGF-beta1/Smad/CTGF signaling pathway, Canadian journal of physiology and pharmacology 95(4) (2017) 437-442.
[84] C.L. Gallant-Behm, J. Piper, B.A. Dickinson, C.M. Dalby, L.A. Pestano, A.L. Jackson, A synthetic microRNA-92a inhibitor (MRG-110) accelerates angiogenesis and wound healing in diabetic and nondiabetic wounds, Wound repair and regeneration : official publication of the Wound Healing Society [and] the European Tissue Repair Society 26(4) (2018) 311-323.
[85] P. Pereira, J.A. Queiroz, A. Figueiras, F. Sousa, Current progress on microRNAs-based therapeutics in neurodegenerative diseases, Wiley interdisciplinary reviews. RNA 8(3) (2017).
[86] S.R. Choudhury, E. Hudry, C.A. Maguire, M. Sena-Esteves, X.O. Breakefield, P. Grandi, Viral vectors for therapy of neurologic diseases, Neuropharmacology 120 (2017) 63-80.
[87] Y. Miyazaki, H. Adachi, M. Katsuno, M. Minamiyama, Y.M. Jiang, Z. Huang, H. Doi, S. Matsumoto, N. Kondo, M. Iida, G. Tohnai, F. Tanaka, S. Muramatsu, G. Sobue, Viral delivery of miR-196a ameliorates the SBMA phenotype via the silencing of CELF2, Nature medicine 18(7) (2012) 1136-41.
[88] D.S. Hersh, A.S. Wadajkar, N. Roberts, J.G. Perez, N.P. Connolly, V. Frenkel, J.A. Winkles, G.F. Woodworth, A.J. Kim, Evolving Drug Delivery Strategies to Overcome the Blood Brain Barrier, Current pharmaceutical design 22(9) (2016) 1177-1193.
[89] F. Laso-Garcia, J. Ramos-Cejudo, F.J. Carrillo-Salinas, L. Otero-Ortega, A. Feliu, M. Gomez-de Frutos, M. Mecha, E. Diez-Tejedor, C. Guaza, M. Gutierrez-Fernandez, Therapeutic potential of extracellular vesicles derived from human mesenchymal stem cells in a model of progressive multiple sclerosis, PloS one 13(9) (2018) e0202590.
[90] H. Xin, Y. Li, Z. Liu, X. Wang, X. Shang, Y. Cui, Z.G. Zhang, M. Chopp, MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles, Stem cells 31(12) (2013) 2737-46.
[91] M.E. Hung, J.N. Leonard, A platform for actively loading cargo RNA to elucidate limiting steps in EV-mediated delivery, Journal of extracellular vesicles 5 (2016) 31027.
[92] D.E. Murphy, O.G. de Jong, M. Brouwer, M.J. Wood, G. Lavieu, R.M. Schiffelers, P. Vader, Extracellular vesicle-based therapeutics: natural versus engineered targeting and trafficking, Experimental & molecular medicine 51(3) (2019) 32.
[93] R. Kim, S. Lee, J. Lee, M. Kim, W.J. Kim, H.W. Lee, M.Y. Lee, J. Kim, W. Chang, Exosomes derived from microRNA-584 transfected mesenchymal stem cells: novel alternative therapeutic vehicles for cancer therapy, BMB reports 51(8) (2018) 406-411.
[94] T. Lener, M. Gimona, L. Aigner, V. Borger, E. Buzas, G. Camussi, N. Chaput, D. Chatterjee, F.A. Court, H.A. Del Portillo, L. O'Driscoll, S. Fais, J.M. Falcon-Perez, U. Felderhoff-Mueser, L. Fraile, Y.S. Gho, A. Gorgens, R.C. Gupta, A. Hendrix, D.M. Hermann, A.F. Hill, F. Hochberg, P.A. Horn, D. de Kleijn, L. Kordelas, B.W. Kramer, E.M. Kramer-Albers, S. Laner-Plamberger, S. Laitinen, T. Leonardi, M.J. Lorenowicz, S.K. Lim, J. Lotvall, C.A. Maguire, A. Marcilla, I. Nazarenko, T. Ochiya, T. Patel, S. Pedersen, G. Pocsfalvi, S. Pluchino, P. Quesenberry, I.G. Reischl, F.J. Rivera, R. Sanzenbacher, K. Schallmoser, I. Slaper-Cortenbach, D. Strunk, T. Tonn, P. Vader, B.W. van Balkom, M. Wauben, S.E. Andaloussi, C. Thery, E. Rohde, B. Giebel, Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper, Journal of extracellular vesicles 4 (2015) 30087.
[95] G. Di Rocco, S. Baldari, G. Toietta, Exosomes and other extracellular vesicles-mediated microRNA delivery for cancer therapy, Translational Cancer Research 6(8) (2017).
[96] L. Morel, M. Regan, H. Higashimori, S.K. Ng, C. Esau, S. Vidensky, J. Rothstein, Y. Yang, Neuronal exosomal miRNA-dependent translational regulation of astroglial glutamate transporter GLT1, The Journal of biological chemistry 288(10) (2013) 7105-16.
[97] L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal, M.J. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, Nature biotechnology 29(4) (2011) 341-5.
[98] J.M. Cooper, P.B. Wiklander, J.Z. Nordin, R. Al-Shawi, M.J. Wood, M. Vithlani, A.H. Schapira, J.P. Simons, S. El-Andaloussi, L. Alvarez-Erviti, Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice, Movement disorders : official journal of the Movement Disorder Society 29(12) (2014) 1476-85.

See published version at: https://doi.org/10.1016/j.bcp.2019.06.019

[99] Y. Takahashi, M. Nishikawa, H. Shinotsuka, Y. Matsui, S. Ohara, T. Imai, Y. Takakura, Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection, Journal of biotechnology 165(2) (2013) 77-84.
[100] K. Narbute, V. Pilipenko, J. Pupure, Z. Dzirkale, U. Jonavice, V. Tunaitis, K. Kriauciunaite, A. Jarmalaviciute, B. Jansone, V. Klusa, A. Pivoriunas, Intranasal Administration of Extracellular Vesicles Derived from Human Teeth Stem Cells Improves Motor Symptoms and Normalizes Tyrosine Hydroxylase Expression in the Substantia Nigra and Striatum of the 6-Hydroxydopamine-Treated Rats, Stem cells translational medicine 8(5) (2019) 490-499.

Figures

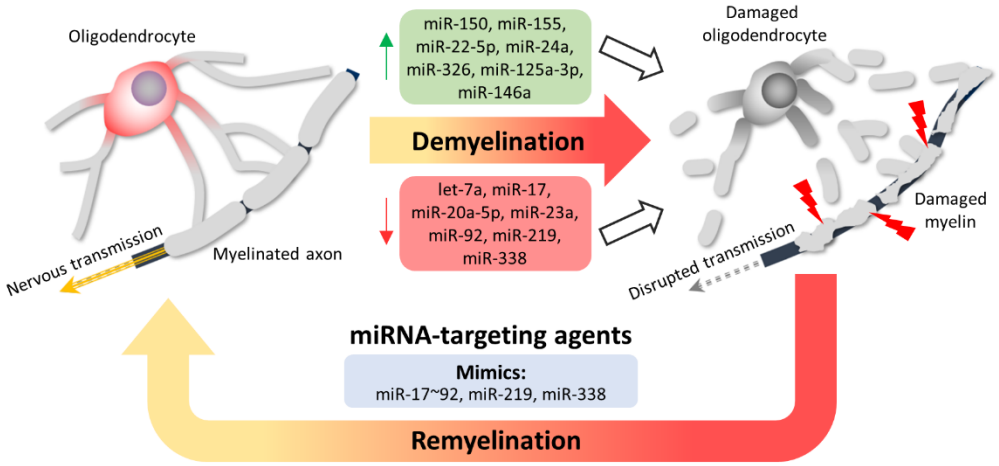

Fig.1. Connections between miRNA alteration and demyelination. Demyelination alters the expression of several miRNAs that can contribute to the onset or development of the disease and impair the recovery. Up-regulated and down-regulated miRNAs are reported in the green and red boxes, respectively. MiRNA-targeting agents able to restore the proper levels of these miRNAs (light blue box), were reported to promote endogenous remyelination.

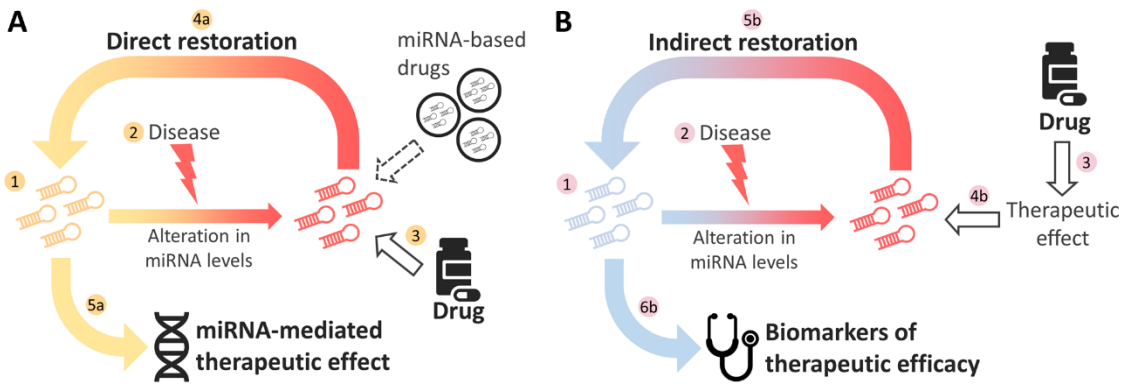

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Fig. 2 Pharmacological therapies can modulate the expression of miRNAs altered during disease. In physiological conditions, miRNAs contribute to cell homeostasis (1). Pathological events can induce alteration in miRNA levels, which, in turn, can participate to disease pathogenesis (2). Drugs can restore the expression levels of altered miRNAs (3). In panel A, miRNA restoration is directly induced by the drug (4a) and contributes, at least in part, to its therapeutic effect (5a). Alternatively, miRNA-based drugs may also be used to directly restore the physiological miRNA expression. Instead, as depicted in panel B, miRNA restoration could also be an indirect consequence of the therapeutic effect of the drug (4b, 5b), thus representing a useful biomarker for monitoring drug efficacy (6b).

miRNA	Physiological regulation	Pathological alteration	miRNA modulation	Mechanism
miR-34	Symmetric division of NPCs [12]	Over-expressed in AD and Epilepsy [13, 14]	miR-34 inhibition improved memory performance and cognitive functions [13]	Activation of Notch signalling
miR-9	neuronal differentiation of NSC [15]	Down-regulated in neurodegenerative diseases [17]	miR-9 inhibition impaired cognitive functions and synaptic plasticity [18]	Inhibition of Wnt-B-catenin pathway
miR-124	neuronal specification [20]	Down-regulated in AD [20]	Exogenous administration of miR-124 reduces brain injury after MCAO [21]	Regulation of Beta-Amyloid production
miR-125b	differentiation of neuronal cells [22]	Up-regulated in AD [23]	Over-expression of miR-125b impaired synaptic plasticity [22]	Activation of neurogenic pathways
miR-219	OPC differentiation [4]	Down-regulated in MS lesions [36]	Exogenous administration of miR-219 promoted in vivo re-myelination [26, 27]	Inhibition of multiple inhibitors of OPC maturation
miR-338	OPC differentiation [4]	Down-regulated in MS lesions [36]	Exogenous administration of miR-338 promoted remyelination after SCI [28]	Inhibition of multiple inhibitors of OPC maturation
miR-17~92 cluster	OPC proliferation [29]	Down-regulated in peripheral blood of MS patients [40]	Over-expression of miR-17-92 cluster led to increased numbers of OLs in vitro [29]	Activation of AKT signaling
miR-23a	myelin formation [32]	Down-regulated in plasma of MS patients [64]	miR-23a over-expression induced hind-limb paralysis due to hypermyelination [32]	Regulation of AKT and IGF signaling
miR-125a-3p	OPC maturation [33]	Over-expressed in CSF of MS patients [33]	miR-125a-3p inhibition promoted OPC maturation [33]	Inhibition of multiple pro-myelin genes

Table 1. Role of selected miRNAs in the pathophysiology of neurodegenerative and demyelinating diseases, and their mechanism of action.

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Drug	miRNA	Change	Source
Interferon-β [61]	miR-145	↓	Whole blood
	miR-20a-5p	↑	
Interferon-β [62]	miR-26a-5p	↓	Platelets
Glatiramer acetate [63]	miR-142-3p	↓	PBMC
	miR-146a	↓	
Fingolimod [64]	miR-23a	↑	Plasma
	miR-15b	↑	
	miR-223	↑	
Natalizumab [65]	miR-17~92	↑	B cell
	miR-106b~25	↑	
Natalizumab [66]	miR-18a	↑	Whole blood
	miR-20b	↑	
	miR-29a	↑	
	miR-103	↑	

Table 2 List of miRNAs potentially involved in the therapeutic mechanism of drugs approved for MS. The arrows indicate up- or down-regulation of miRNAs following treatment. PBMC: peripheral blood mononuclear cells; CSF: cerebrospinal fluid.

Source	Change	miRNA	Drug
Whole blood	↓	miR-125b	Rituximab [9]
	↓	miR-760	
	↓	miR-134	
	↓	miR-135a	
	↓	miR-135b	
	↓	miR-138	
CSF	↓	miR-150	Natalizumab [38]
PBMC	↓	miR-155	Natalizumab [67]
	↓	miR-26a	
PBMC	↓	miR-155	Dimethylfumarate [69] Fingolimod [69]
Exosomes from plasma and urine	↓	miR-155-5p	Glatiramer acetate [70]
	↓	miR-27a-3p	
	↓	miR-9-5p	
	↓	miR-350-5p	
Exosomes from serum	↑	miR-22-3p	Interferon-β [71]
	↑	miR-660-5p	

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↓	miR-486-5p
↓	miR-451a
↓	let-7b-5p
↓	miR-320b
↓	miR-122-5p
↓	miR-215-5p
↓	miR-320d
↓	miR-19b-3p
↓	miR-142-3p
↓	miR-146a-5p
↓	miR-15b-3p
↓	miR-23a-3p
↓	miR-223-3p

Table 3 List of circulating miRNAs described as indicators of the therapeutic efficacy of drugs approved for MS. The arrows indicate up- or down-regulation of miRNAs following pharmacological treatment. PBMC: peripheral blood mononuclear cells; CSF: cerebrospinal fluid.