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Small non-coding RNAs as important players, biomarkers and therapeutic targets in multiple sclerosis: A comprehensive overview

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ABSTRACT

Multiple sclerosis (MS) is a leading cause of progressive disability among young adults caused by inflammation, demyelination and axonal loss in the central nervous system. Small non-coding RNAs (sncRNAs) are important regulators of various biological processes and could therefore play important roles in MS. Over the past decade, a large number of studies investigated sncRNAs in MS patients, focusing primarily on microRNAs (miRNAs). Overwhelming 500 miRNAs have been reported as dysregulated in MS. Nevertheless, owing to a large heterogeneity between studies it is challenging to evaluate the reproducibility of findings, in turn hampering our knowledge about the functional roles of miRNAs in disease. We systematically searched main databases and evaluated results from all studies that examined sncRNAs in MS to date (n = 61) and provided a detailed overview of experimental design and findings of these studies. We focused on the mechanisms of the most dysregulated sncRNAs and used predicted targets of the most dysregulated sncRNAs as input for functional enrichment analysis to highlight affected pathways. The prime affected pathway was TGF-β signaling. This multifunctional cytokine is important in the differentiation and function of T helper type 17 (Th17) and regulatory T (Treg) cells, with opposing functions in the disease. Recent studies demonstrate the importance of miRNAs in controlling the balance between Th17/Th1 cells and Tregs and, importantly, the potential to exploit this paradigm for therapeutic purposes. Additionally, some of the discussed miRNAs could potentially serve as biomarkers of disease. In order to assist researchers in evaluating the evidence of a particular sncRNA in the pathogenesis of MS, we provide a detailed overview of experimental design and findings of these studies to date.

1. Introduction

Multiple sclerosis (MS) is the most common cause of non-traumatic lifelong disability in young adults, affecting women almost three times as often as men [1]. It is a chronic inflammatory and neurodegenerative disease characterized by infiltration of immune cells into the central nervous system (CNS) with subsequent demyelination, axonal damage and neuronal death. Such damage is represented by lesions at diverse locations which correlate with the heterogeneous nature of symptoms consisting of fatigue, motor and balance disturbances, sensory and visual impairments and cognitive deficits [1]. Approximately 85% of MS patients initially present with clinically isolated syndrome (CIS), the first clinical manifestation of MS. In the course of time most of the CIS patients convert to MS [2]. Of these MS patients, the vast majority (85–90%) is diagnosed with relapsing-remitting form of MS (RRMS), marked by bouts of active disease followed by clinical inactivity. Eventually, most of RRMS patients develop secondary progressive disease (SPMS) characterized by a continuous worsening with or without overlaid clinical relapses. The remaining fraction (10–15%) of MS patients presents with primary progressive form (PPMS), which is distinguished by a gradual worsening of symptoms from onset without the presence of relapses [1].

Despite the revolution in therapeutic options for MS care over the past decade, these treatments primarily act on the inflammatory component and are therefore mostly effective in patients with early RRMS. In addition, therapies are either moderately beneficial or connected to rare adverse effects and none completely halt disease or prevent progression [3]. A growing body of evidence suggests that early treatment with potent immunomodulatory drugs can postpone progressive stage of disease [4]. However, it is noteworthy that many MS patients remain undiagnosed until later stages, notably because definite diagnosis of MS involves a battery of clinical assessments including neurological examination, cerebrospinal fluid (CSF) analysis, magnetic resonance imaging (MRI) and electrophysiological tests [5]. Overall, the lack of robust diagnostic, prognostic and predictive biomarkers in MS hinders efficient care of patients. There are no clinically validated biomarkers

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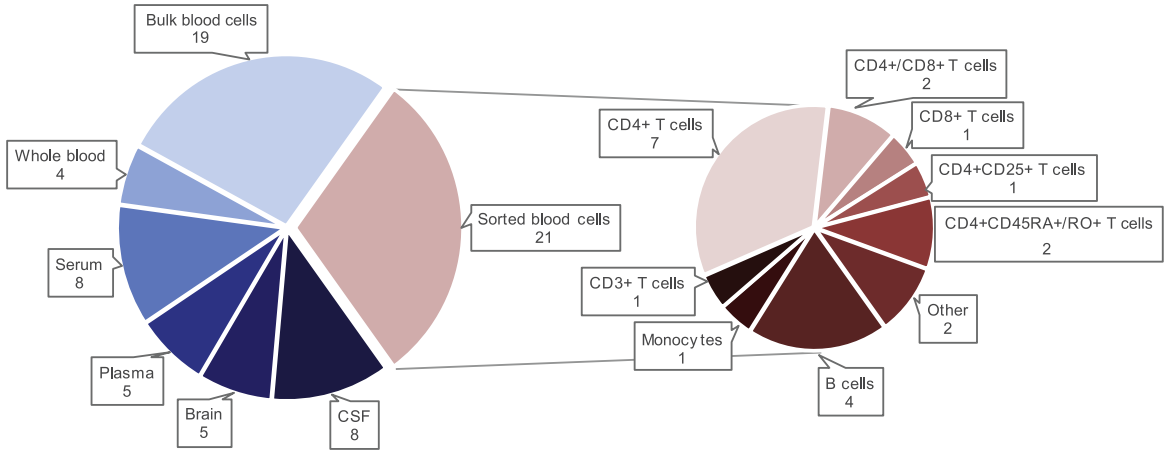

Fig. 1. Overview of sncRNAs analyses in multiple sclerosis. The total number of independent investigations of a specific compartment (i.e. tissue, cell type or biofluid) across 61 independent studies is provided.

that can differentiate MS from alternative diagnoses early on, distinguish between MS subtypes, and objectively monitor or predict disease progression and treatment response [3,6].

MS arises from a complex interplay between genetic and environmental factors, however, the exact causes and molecular pathways that lead to the disease development are still unknown. Over the past years, non-coding RNAs (ncRNAs) appeared as key regulators of different biological processes including cell growth, differentiation, immunity and inflammation and were suggested to play a significant role in mechanisms underlying MS pathogenesis. NcRNAs are functional RNA molecules that are not translated into proteins, exerting their actions as regulatory nucleic acids. In the last decades, many studies have demonstrated the importance of ncRNAs in regulating most biological functions at the transcriptional and post-transcriptional level and during RNA processing [7]. The most studied class of ncRNAs are microRNAs (miRNAs) that belong to the category of small ncRNAs (sncRNAs). MiRNAs target specific messenger RNAs (mRNAs) based on sequence complementarity to the 3′ untranslated region (UTR) of mRNA leading to translational inhibition or degradation. Interestingly, miRNAs are able to regulate the expression of not only a single mRNA but multiple downstream targets and therefore often modulate the activity of pathways [8]. Among other described species of sncRNAs, small interfering RNAs (siRNAs) cleave complementary mRNAs, similarly to miRNAs, and have been implicated in transposon silencing. Transfer RNAs (tRNAs) are required for protein synthesis, transferring corresponding amino acids into the ribosomes. Small nucleolar RNAs (snoRNAs) and small Cajal body RNAs (scaRNAs) have important roles in the processing and modification of precursor ribosomal RNA, whereas small nuclear RNAs (snRNAs) engage in pre-mRNA processing. Both tRNAs and snoRNAs give rise to smaller RNA fragments, transfer RNA fragments (tRFs) and snoRNA-derived RNAs (sdRNAs), respectively. These fragments have different functions from their precursors: tRFs are involved in translational regulation and gene silencing [9] and sdRNAs show miRNA-like functions [10,11]. SncRNAs-mediated modulation of biological processes may provide new therapeutic strategies for MS care, with the possibility to 'correct' dysregulated sncRNAs, e.g. supplement downregulated sncRNAs or eliminate upregulated sncRNAs, using specific oligonucleotides. Additionally, sncRNAs, and in particular miRNAs, are considered as novel type of highly suitable biomarkers. Given the complexity of MS, the development of precise and reliable diagnostic and prognostic tools, which will likely rely on the combination of several biomarkers, could considerably benefit from miRNA-based panels.

The study of sncRNAs, miRNAs in particular, has gained tremendous and rapid interest over the past few years. An exponentially growing number of studies has investigated miRNAs in different MS stages and under various treatments reporting a large number of dysregulated miRNAs. However, most findings lack support from independent studies hampering mechanistic studies and clinical utility of miRNAs. In this review, we systematically searched PubMed, Web of Science and Google Scholar for relevant studies up to December 15, 2018 with the inclusion criteria of (i) human cohorts comprising MS patients and (ii) data including sncRNA/miRNA expression. We evaluated and summarized results from all studies (n = 61) and discuss their potential to provide insights into disease mechanisms, as MS biomarkers and therapeutic targets. In addition, predicted targets of the most dysregulated miRNAs were retrieved using mirDIP and used as input for functional enrichment analysis in Ingenuity Pathway Analysis to determine key affected pathways.

2. Heterogeneity of miRNA studies in multiple sclerosis

Using a systemic literature search we have retrieved and compared all significantly different sncRNAs, as reported by the original studies. Most of the studies examined miRNAs in immune tissues in case-control cohorts. An overview of different tissues and sorted cell types investigated in the studies is presented in Fig. 1.

We first focused on comparing differentially expressed sncRNAs, predominantly miRNAs, reported by different studies. We summarized details of study designs as well as the reported findings in Fig. 2 (the most replicated miRNAs) and Supplementary Table 1 (complete overview of all reported sncRNAs studies in MS). Given the multicellular nature of the tissue and high RNA yields, it is not surprising that most differentially expressed miRNAs have been reported in complex tissues such as whole blood, peripheral blood mononuclear cells (PBMCs) and CNS compared to sorted cells and biofluids (Supplementary Table 1). Out of the reported 650 differentially expressed sncRNAs in MS patients, 27.5% (179/650) miRNAs were found dysregulated with the same directionality of change in at least two independent studies. Using more stringent criteria, only 9% (58/650) of miRNAs were found dysregulated with the same direction in a minimum of three independent studies.

The lack of replication between the studies reflected by the poor overlap between identified miRNAs has often been discussed and is likely a consequence of heterogeneity between the samples, cohorts and methodologies used. Importantly, miRNAs are regulated in a highly tissue- and state-specific manner [12] and therefore, the major source of heterogeneity comes from differences in cellular source of miRNAs between studies. Even when the same cellular source has been used, there is a likely cohort heterogeneity between studies, considering the high clinical heterogeneity of MS, i.e. different MS subtypes as well as the varying treatments and types of controls (for details see

Fig. 2. Most dysregulated microRNAs in multiple sclerosis. Overview of the most replicated microRNAs (miRNAs) in multiple sclerosis (MS) in a minimum of 4 independent studies. MiRNAs are arranged from left to right according to the predominant directionality of change, from miRNAs that are mainly downregulated (left) to miRNAs that are mainly upregulated (right) in MS patients. To increase evidence miRNAs from the same family or cluster were converged and the individual miRNAs are listed below the table. Circulating miRNAs with a good biomarker potential are marked with a star (*), with further description in section 7. Blue - downregulated, red - upregulated, dark blue and red - multiple miRNAs in family or cluster downregulated or upregulated, dotted blue - upregulated after treatment, dotted red - downregulated after treatment. CNS - central nervous system, NA - not available, PBMCs -peripheral blood mononuclear cells, PBLs - peripheral blood leukocytes, Hippocampus-d - demyelinated hippocampus, Lesions-a - active lesions, Lesions-i - inactive lesions, CSF - cerebrospinal fluid, NAWM - normal-appearing white matter, RR - relapsing-remitting MS, SP - secondary progressive MS, PP - primary progressive MS, HC - healthy controls, C - controls, CIS - clinically isolated syndrome, NINDC - non-inflammatory neurological disease control, AHSCT - autologous hemopoietic stem cell transplantation, IFNb - interferon-beta, GA - glatiramer acetate.

Supplementary Table 1). For instance, miRNA patterns in MS were occasionally inconsistent in a whole blood study where natalizumab-treated patients were included [13]. Considering the fact that natalizumab leads to significant changes in the blood cell composition, this might explain discrepancies with other studies [14]. Further complexity arises from additional clinical parameters. In that regard, it has been suggested that even within one MS subtype, miRNA expression might be affected by certain clinical parameters such as Expanded Disability Status Scale (EDSS), disease duration and flare-up frequency [15–17]. This might explain why statistical significance is not always reached for dysregulated sncRNAs between the discovery and validation cohorts, even though the same trend is usually confirmed [18,19]. Other factors influencing miRNA profiles are sex and age [20–22]. In accordance with this, a set of miRNAs was found to be differently expressed in a sex-dependent manner between relapse and remission [23,24] and with age [25]. Furthermore, several studies have suggested that genetic polymorphisms associated with MS might influence the expression of certain miRNAs, indicating that the different genetic background between the cohorts could also be a cause of discordant results across studies [26,27]. A third source of heterogeneity relies on the various methodologies employed by studies, with the use of different isolation techniques, detection technologies and the lack of standardized analytical pipelines (Supplementary Table 1). For instance, the variability in sample composition, such as the differences in protein levels, between samples could cause bias during the RNA isolation procedure [28]. Additionally, various techniques for RNA isolation may lead to a different level of contaminants affecting downstream reactions. Moreover, the use of a variety of microarray panels with different miRNA sets, levels of sensitivity, intrinsic controls and normalization strategies will undoubtedly introduce issues when comparing results between various studies [29].

3. Most commonly dysregulated miRNAs in multiple sclerosis

MiRNAs that display strong association with MS in independent studies have been listed in Fig. 2, where evidence has been summarized for miRNA families considering high functional overlap between the different members of one family [30]. MiRNAs that have consistently been found to be upregulated in MS patients, across the immune and CNS compartments and in a minimum of four independent studies, are miR-142-3p, miR-146a/b, miR-145, miR-155, miR-22, miR223/-3p, miR-326 and miR-584 (Fig. 2). Overexpression of these miRNAs suggest their implication in either the pathogenic inflammation observed in MS patients or instead in anti-inflammatory processes attempting to reduce inflammation. The most consistently reported miRNA in MS, i.e. miR-155, was found upregulated in eight independent studies across immune compartments and CNS tissue. MiR-155 has previously been associated with other inflammatory disorders and a variety of functions

such as immune cell activation, dysregulation of the blood-brain barrier and neurodegeneration [31]. Interestingly, upregulation of miR-155 and miR-142-3p in T cells and PBMCs from MS patients was significantly reduced by treatments such as autologous hematopoietic stem cell transplantation and glatiramer acetate (GA), supporting their potential role in pro-inflammatory processes [32,33]. Members of the well-known miRNA family associated with MS, i.e. miR-146, have also been found consistently upregulated, particularly in PBMCs and whole blood but also in CNS lesions of MS patients. Similar to miR-155 and miR-142-3p, GA was also found to downregulate miR-146a in PBMCs of treated MS patients [33].

While evidence from levels detected in biofluids, i.e. plasma and serum, was mirroring the majority of upregulated intracellular miRNAs, two independent studies demonstrated reduced levels of miR-223 in the serum of MS patients [17,27]. This myeloid-enriched anti-inflammatory miRNA that suppresses canonical NF-κB signaling has been implicated in cell-to-cell communication and thus levels between the intracellular and extracellular compartment might differ [34]. Likewise, members of the miR-17 and miR-320 family, as well as miR-21 and miR-150, exhibit different patterns across the compartments with predominant downregulation in the immune tissue and upregulation in the CNS of MS patients (Fig. 2). This might suggest different modes of regulation and roles between the compartments, however, other mechanisms may also operate. For example, miR-150 shows an interesting pattern where it is downregulated intracellularly in T cells, B cells and PBMCs and upregulated in biofluids, serum and CSF, of MS patients. This implies that circulating miR-150, known to be actively secreted by monocytes in other conditions [35,36], might play a role in MS by mediating cell-to-cell communications. An additional, mutually non-exclusive, explanation is that immune cells actively sequester out miR-150 to release functions needed for MS pathogenesis. Similarly, miR-181c/d also shows an opposite intracellular/extracellular pattern with downregulation in CNS tissue and upregulation in CSF of MS patients. In addition, this is the only miRNA that is predominantly detected in the CNS compartment.

Another miRNA that is commonly detected but does not display a consistent directionality of the change is miR-21. This miRNA exhibits both pro- and anti-inflammatory functions [37] and seems to be upregulated during active disease and downregulated in remission and SPMS.

On the other hand, members of the miR-103, miR-15, miR-548 and let-7 families and miR-140 display predominant downregulation in MS patients (Fig. 2). Of them, let-7 and miR-548 family members have been detected to be dysregulated exclusively in the immune compartment and while let-7a/b/d behave similarly other members of the family do not display a distinct pattern (Supplementary Table 1). The miR-103 family and miR-15a/b are downregulated in all cellular compartments apart from Tregs, which could be due to their anti-inflammatory function compared to disease-promoting cell types.

4. Functions of most commonly dysregulated miRNAs in multiple sclerosis

In order to get more insight into the cellular impact of miRNAs dysregulation in MS patients, we performed functional analysis of the most consistently reported differentially expressed miRNAs from all studies. Since miRNAs typically target several mRNAs in a biologically relevant pathway [8], we conducted pathway analysis (Ingenuity Pathway Analysis, Qiagen) on predicted targets (mirDIP) of MS-associated miRNAs to indicate their tentative functions (Supplementary Table 2). Significantly enriched pathways implicate, among others, functions involved in: (i) immune cell activation and differentiation, (ii) adhesion and gap junction formation, (iii) neuronal development and function, and (iv) regulation of cell cycle and proliferation (Fig. 3). Most of the pathways were targeted by both up- and downregulated miRNAs, confirming their well-known function in fine-tuning biological processes. However, stronger enrichment could be found for targets of downregulated miRNAs suggesting that lower levels of MS-associated miRNAs might be directly involved in releasing the break from the pro-inflammatory activation in MS. Remarkably, cell cycle and proliferation pathways were almost exclusively significant for targets of downregulated miRNAs suggesting their failure to control proliferation of immune cells in MS patients.

The most significant pathway affected by both up- and downregulated miRNAs is TGF-β signaling (Fig. 3). Interestingly, TGF-β has previously been shown to induce expression of a subset of miRNAs [38] including many of the most dysregulated miRNAs in MS (let-7a, -7b, -7d and miR-103, -107, -140, -181c, -18a, -18b, -20a, -20b, -21, -142, -145, -223). Conversely, miRNAs such as miR-155 have been shown to indirectly modulate this pathway by targeting *SMAD2* and *SMAD5* engaged in the TGF-β signaling pathway [39,40]. This highlights TGF-β signaling pathway as an important player in MS, both by regulating miRNAs and by being fine-tuned by miRNAs. TGF-β is a pleiotropic growth factor and a pivotal cytokine for the differentiation and function of T cells, particularly Treg and T helper 17 (Th17) cells, among many other functions [41,42]. Treg cells are formally known as immunosuppressor T cells maintaining homeostatic peripheral tolerance, and failure in their suppressive capacity could contribute to MS pathogenesis [43]. In that context, dysregulated miRNAs targeting the TGF-β signaling pathway have been proposed to be one of the underlying causes of the defective Tregs in MS [44]. MiRNAs reported upregulated in MS, such as miR-142, miR-145 and miR-210, have all been shown to suppress Treg cell differentiation and function through regulation of key players of Treg phenotype. For instance, miR-142-3p targets adenylate cyclase *ADCY9* gene resulting in low and likely insufficient levels of cAMP to promote Treg function [45]. Both miR-210 and miR-145, which display low constitutive levels in Treg cells, have been shown to target *FOXP3*, an essential transcription factor for Treg function [46] and *CTLA-4*, a checkpoint inhibitor playing important roles in the regulatory function of Treg cells [46], respectively. Conversely, miR-21 mainly found downregulated in MS immune compartments, has been found to positively regulate the expression of *FOXP3*, *TGF-β* and *IL-10* genes [47,48]. These findings collectively support that impairment of Treg cell differentiation and function in MS is mediated, at least partly, by miRNAs.

The T helper cell imbalance observed in MS patients is further supported by an increased activity of pathogenic Th17 cell compartment. Elevated frequency of Th17 cells as well as levels of the Th17-cytokine IL-17 have been detected in MS plaques and CSF [49]. Many of the miRNAs summarized in Fig. 2, such as miR-155, miR-223 and miR-326 found upregulated in MS tissues, have been implicated in the regulation of Th17 commitment. The most documented miRNA, miR-155, has been functionally explored *in-vivo* in experimental autoimmune encephalomyelitis (EAE), a well-established animal model of MS [50,51]. MiR-155 was upregulated in $CD4^+$ T cells and promoted the development of inflammatory Th1 and Th17 cell subsets during induction of disease [51,52]. MiR-155 effect on Th17 cell differentiation was mediated by its target *SOCS1* [50]. Consistent with these results, deficiency of miR-155 alleviated EAE symptoms [52]. Similarly, miR-223 and miR-326, the latter being upregulated in EAE as well, have also been involved in Th17 cell differentiation [53–55]. Knock-out of miR-223 ameliorated EAE by increasing spleen myeloid-derived suppressor cells [56] and reducing CNS infiltrating myeloid dendritic cells, Th1 and Th17 cells [57,58]. The mechanisms underlying inhibition of cell infiltration involved an impaired function of the myeloid dendritic cells and a diminished capability to induce Th1 and Th17 cells [57,58]. Knock-down of miR-326 in EAE animals resulted in milder disease accompanied by a reduced amount of Th17 cells. The effect seemed to be mediated by a miR-326 target, *ETS-1*, a negative regulator of Th17 differentiation [54].

Among the miRNAs that were downregulated in MS, miR-15b, -18a, -20b and -103 also showed reduced expression in EAE, with

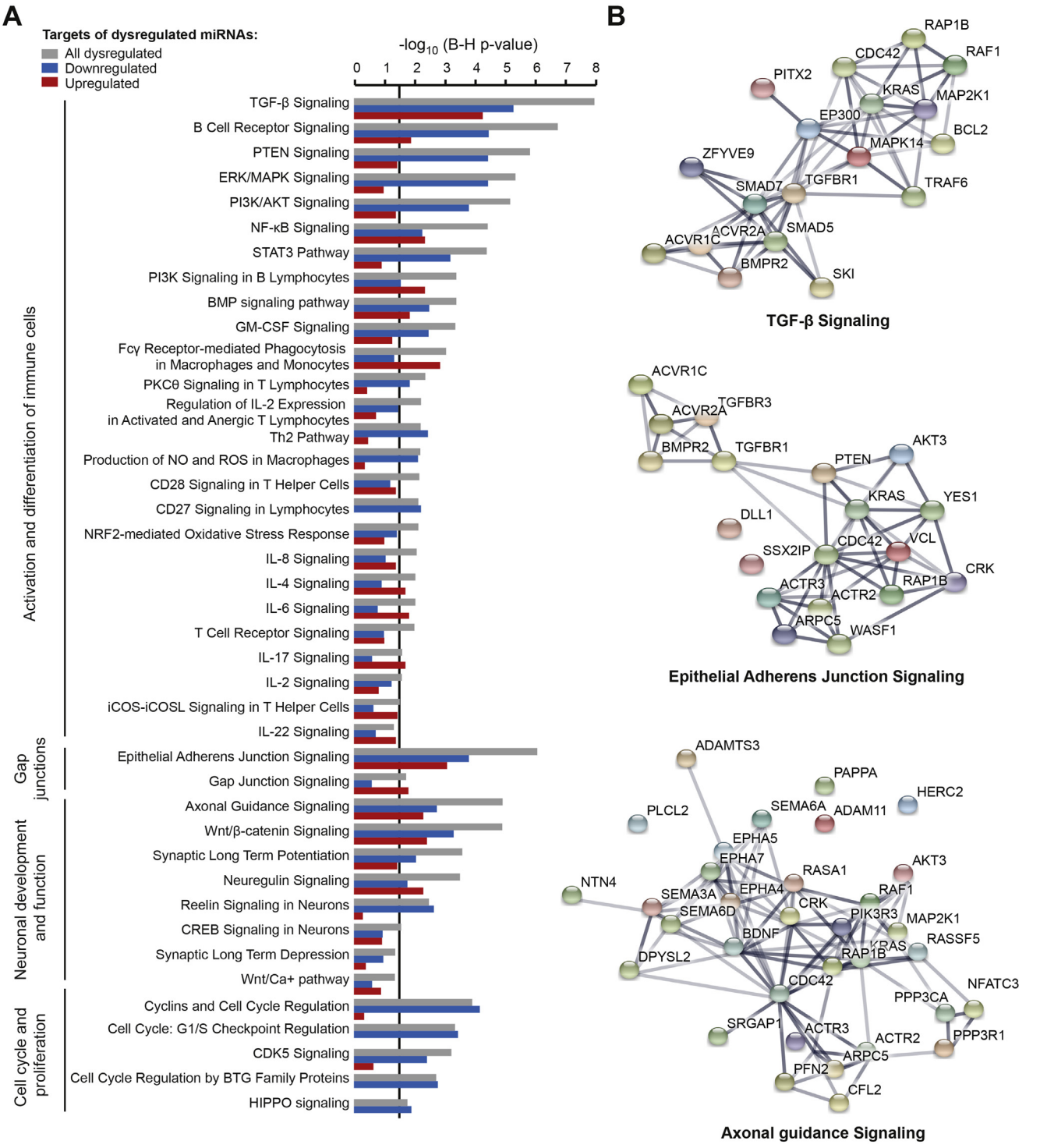

Fig. 3. Functional exploration of dysregulated microRNAs in multiple sclerosis. **a.** Top enriched canonical pathways associated with the target genes of the most consistently dysregulated microRNAs (miRNAs) in multiple sclerosis (MS), with grey, blue and red colors depicting all, down- and upregulated miRNAs in MS, respectively. Significance is represented as $-\log_{10}$ Benjamini-Hochberg adjusted P-value with the black line indicating the threshold of P-value < 0.05. **b.** Representation of the genes networks from the top enriched pathways using STRING analysis. Grey line gradient indicates the strength of data support (darker grey representing stronger confidence). . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

overexpression of miR-15b alleviating EAE symptoms [13,59]. Interestingly, these miRNAs have been associated with Th17 lineage as well. MiR-15b inhibited Th17 differentiation via targeting of *OGT* and subsequent repression of NF-κB dependent genes such as the Th17-defining transcription factor *RORγT*, among others [59]. Translation of this finding to MS is supported by the negative correlation between miR-15b and *OGT* expression observed in whole blood and T cells of RRMS patients [59]. Whereas deletion of the miR-106a ~ 363 cluster, which contains miR-20b, led to an aggravated EAE course, overexpression of miR-20b specifically led to upregulation of established miR-20b targets including *RORC* and *STAT3*, both important in Th17 differentiation [13].

Due to the majority of studies being performed in the immune compartment, there is likely a bias which results in the largest part of

the most reproducible miRNAs to be implicated in peripheral immune processes. However, miR-181c/d expression has been consistently associated with the CNS tissue. MiR-181c has previously been associated with other neurodegenerative diseases where it has been found downregulated in frontal cortex of Alzheimer's disease patients [60] as well as in plasma of Parkinson's disease patients [61]. Moreover, it is interesting that neuronal pathways appear among the most significantly enriched pathways in our pathway analysis. This suggests that dysregulated miRNAs have a capacity to modulate CNS intrinsic processes. Given that a specific miRNA function on a cellular level will be dependent on the presence of specific target gene(s), it would not be surprising that miRNAs with immune functions in MS might also regulate pathways connected to functioning and survival of CNS cell types. One of the first studies profiling miRNAs in active and inactive MS brain lesions identified several dysregulated miRNAs [62]. The well-known immune-related miRNAs miR-155 and miR-326 were found to be upregulated in active compared to inactive lesions, which may indicate that they originate from the infiltrating immune cells. However, miR-155 displayed increased expression in normal appearing white matter (NAWM) compared to controls. As NAWM is typically devoid of immune infiltrates, this supports miR-155 involvement in pathological processes occurring in the CNS cells without or prior to focal damage of the tissue [63]. In line with this, miR-155 along with miR-326 have been involved in promoting myelin phagocytosis by targeting CD47 [62]. Likewise, the aforementioned miR-142-3p, which was found to be increased in the CSF of MS patients as well as EAE brains, was proposed to cause increased glutamatergic transmission and glutamate excitotoxicity through IL-1β-dependent synaptic dysfunction [64].

Since the functions of miRNAs are highly cell-type specific, additional studies are warranted to disentangle the cellular mechanisms underpinning miRNA dysregulation in MS.

5. Other emerging classes of dysregulated sncRNAs in multiple sclerosis

While the majority of studies examining sncRNAs in MS are restricted to miRNAs, the recent development of technology has enabled the investigation of other classes of sncRNAs, including snoRNAs, scaRNAs and sdRNAs in MS [23,65–67]. Amongst the studies summarized in this review merely 6 studies investigated these other classes of sncRNAs. Dysregulation of SNORA40 was found in T cells from MS patients and in PBMCs of MS females during relapse [67,68]. However, the lack of information on the directionality of change impedes proper interpretation of these findings. Nevertheless, by combining sncRNA profiling with genome-wide mRNA analysis the later study proposed SNORA40, SNORD23 and SNORA5C as central regulators of gene expression in MS [67]. While these emerging studies suggest a role of snoRNAs in MS pathogenesis, their contribution to MS disease is difficult to appreciate due to our limited knowledge about specific snoRNA functions.

Besides human sncRNAs, 3 studies detected viral-derived miRNAs in B cells, PBMCs and serum. These viral miRNAs were derived from Epstein-Barr virus (EBV; herpes virus), Marek's disease virus (MDV; avian herpes virus) and Simian virus 40 (SV40; Polyoma virus). EBV is one of the most established environmental risk factor for MS [69] and both MDV and SV40 have been associated with MS to a lesser extent [70–72]. Functional studies have demonstrated a role of some of these viral miRNAs in altering the host immune processes. One of the EBV miRNAs found in MS, MiR-BHRF1-2, has been previously shown to inhibit Th1 differentiation of naïve $CD4^+$ T cells by preventing lysosomal protein degradation and expression of HLA class II and co-stimulatory molecules [73]. Another EBV miRNA, miR-BART3, was suggested to hinder expression of immune co-receptors and adhesion molecules on $CD4^+$ T cells [73]. SV40-miR-S1-5p detected in MS, negatively regulates the expression of viral T antigens, thereby reducing host attack [74]. Reversely, several human miRNAs, such as miR-155, demonstrate the ability to regulate viruses by limiting their replication [75]. It is known that in latent phases of viral infection, viruses almost exclusively express miRNAs to escape host recognition while still being able to control their own and their hosts' cellular processes. These viral miRNAs are known to maintain the viral latency and promote survival of infected cells [76]. Remarkably all of the intracellularly detected viral miRNAs were downregulated in MS, while the extracellular viral miRNAs levels were increased. Whether viral miRNAs are actively secreted by the host cells and participate in a cell-to-cell communication in MS is still to be explored. In that regard, upregulation of viral miRNAs that have been linked to the lytic phase of viruses have also been associated with MS relapses [77]. Moreover, the association with relapses is further reinforced by the fact that these viral miRNAs target important players in immune homeostasis. However, more understanding is needed on the mechanisms underlying the contribution of viral miRNAs to the pathogenesis of MS.

6. Promising therapeutic potential of miRNAs

The ability of miRNAs to fine-tune entire pathways has made them attractive targets for novel RNA-based therapies. Dysregulation of miRNAs can be tackled by therapies aiming at miRNA replacement or inhibition of miRNA function. So far, several approaches have been developed for miRNAs inhibition including the use of antisense oligonucleotide inhibitors (antimiR) [78], peptide nucleic acids or miRNA sponges [79]. On the other hand, restoring levels of downregulated miRNAs can be achieved by delivery of a vector overexpressing the desirable miRNA [80] or double-stranded synthetic miRNAs (miRNA mimics) [81]. The therapeutic potential of these strategies has been explored in EAE, with the prospect of targeting pro-inflammatory miRNAs. Despite the imperfect parallelism between EAE and MS disease, EAE pathogenesis recapitulates important aspects of the human disease and therefore provides a robust experimental model for functional and therapeutic investigations [82].

Among the most promising candidates, miR-155 has been a target of a choice due to its unequivocal upregulation in MS and important role in EAE [26,32,33,62,63,83,84]. Accordingly, miR-155 inhibition with an antimiR reduced the numbers of Th1 and Th17 cells and resulted in milder EAE disease [51]. Inhibition of miR-326 in EAE both with an antimiR or a miRNA sponge led to suppression of Th17 differentiation and alleviated disease symptoms [54]. On the other hand, downregulated miRNAs in MS patients, such as miR-20a and miR-20b [13,85,86], were shown to modulate T cell activation genes [86] and their overexpression in EAE animals led to disease suppression through inhibition of Th17 differentiation [87].

Currently, challenges of miRNA-based therapeutics concern their stability, safety and potential off-target effects. Emerging fields in advanced delivery systems such as nanocells, nanoparticles, liposomes and different polymers present with the advantage to increase cargo stability by avoiding degradation by nucleases and endosomal escape, minimize the toxicity and improve specific delivery. Some of these therapies have now been translated into promising clinical trials for hepatitis C infection, malignant pleural mesothelioma, non-small cell lung cancer, scleroderma, cutaneous T cell lymphoma and non-alcoholic fatty liver disease [78]. Undoubtedly, the clinical heterogeneity together with the poor accessibility of the target organ in MS hinders specific clinical translations. Considering the efficacy of complementary therapeutic approaches thus appears instrumental for an efficient care of MS patients. In that regard, miRNA-based therapies show great potential for personalized and precision medicine approach of MS patients, with the possibility to modulate dysfunctional pathways instead of a single target.

7. Circulating miRNAs as a new class of biomarkers

In addition to their intracellular localization, miRNAs can also be

packaged and exported into the extracellular environment and can therefore be detected in biofluids, such as urine, blood and CSF [88]. Circulating miRNAs are secreted in vesicles such as exosomes, microvesicles and apoptotic bodies or bound to protein complexes such as AGO and high-density lipoprotein. These carrier systems are thought to contribute to remarkable stability of miRNAs in biofluids, even under unfavorable conditions [89]. The study of circulating miRNAs could be not only instrumental in understanding disease mechanisms that involve cell-to-cell communication, but would also offer vast possibilities as biomarkers. They are currently at the forefront of biomarkers for a broad range of diseases, including MS. Their high stability, cost-effective screening due to easy detection and possibility to multiplex add to favorable biomarker potential.

Circulating miRNA profiles have been explored in different cell-free compartments including plasma, serum and CSF from MS patients. Among the most replicated circulating miRNAs, miR-150 was found to be upregulated in plasma and serum from MS patients but its dysregulation was mostly replicated in CSF [51,90,91]. MiR-150 levels in CSF show promising biomarker potential as they could aid in discriminating RRMS from controls [90,91], as well as between CIS patients that would later convert to MS compared with those that would not [90]. Interestingly, concomitant to being enriched in cell-free compartments, miR-150 was found downregulated in PBMCs and T cells of MS patients [66,92]. Activated monocytes, T and B cells have been shown to actively package and secrete miR-150 into the extracellular environment. Moreover treatment studies showed extracellular expression of miR-150 correlated with the location of immune cells [90]. Altogether, these data suggest immune cells as the putative cellular source of circulating miR-150. Likewise, miR-181c has been consistently detected in CSF at different stages of disease. High miR-181c levels were detected in CSF from CIS patients that would later on convert to MS compared to those that would not [93] as well as in CSF from RRMS compared to SPMS patients [94]. Conversely, miR-181c displayed reduced levels in MS lesions [62] and in PBMCs from SPMS [92], implying that miR-181c levels might be associated with early and active stages of diseases. Thus, levels of circulating miRNAs, such as miR-150 and miR-181c, differ between MS groups and specific differences between CIS-converters and non-converters can be further exploited for an early MS diagnosis.

Circulating miRNAs have also been associated with a number of disease parameters. For instance, the profiles of several circulating miRNAs (i.e. miR-15b, -23a, -92a, -135a, -145, -223, -337-3p, -454, -500, -574-3p and -648a) detected in plasma, serum and CSF could not only differentiate RRMS from healthy controls and from SPMS, but additionally correlated with EDSS score, disease duration, remission time and frequency of relapses [15–17]. For example, serum miR-337-3p displayed significant negative correlation with EDSS in three independent cohorts [95]. Other studies have found high CSF levels of miR-142-3p and miR-125a-3p to associate with active inflammatory brain lesions [64,96]. Knowing that prediction of disease progression remains one of the biggest challenges in managing MS patients, the potential of miRNAs to reflect disease activity could have considerable clinical relevance for the monitoring and prognosis of MS patients.

The potential of miRNAs as biomarkers of treatment response has also been explored by studies examining both intracellular and circulating miRNAs during the course of treatment. They have reported miRNA expression changes in immune cells, e.g. whole blood, PBMCs, B cells and CD4 T cells, after treatment with natalizumab [13,97,98], interferon beta [18,65] and GA [33]. Among them, miR-150 levels were affected both in CSF and plasma after fingolimod and natalizumab treatment of RRMS patients [90]. Interestingly, whole blood miR-320, miR-320b and miR-629 levels were suggested as possible biomarkers to assess individual's risk of developing progressive multifocal leukoencephalopathy associated with natalizumab treatment. In conclusion, the sensitivity of miRNA to therapeutic intervention makes them promising biomarkers in monitoring treatments and possible predicting treatment effect.

Altogether, patterns of circulating miRNAs could be valuable not only in understanding pathogenic mechanisms in MS but may also serve as diagnostic and prognostic biomarkers as well as biomarkers of treatment response or treatment-associated side effects. More importantly, with emerging technologies it has become easier to assess a whole profile of sncRNAs, which will significantly improve their predictive power [90,99].

8. Concluding remarks

Our comprehensive review of the 61 existing studies of sncRNAs in MS reveals noticeable discrepancies between the findings, which likely arise from the heterogeneity on the level of cohorts, samples and methodologies used. Nevertheless, consistent findings point to dysregulation of both intracellular and circulating miRNAs and highlight opportunities that miRNAs may offer for the care of MS patients. Regulation of miRNAs is often context- and cell type-dependent and unravels important roles in the immune and CNS tissue of MS patients. From a clinical perspective, further investigations of sncRNAs/miRNAs have the potential to aid our understanding of the phenotypic diversity related to disease course, subtype or clinical parameters as well as to elucidate mechanisms underpinning MS disease. Moreover, sncRNA/miRNA profiling could provide robust biomarkers for an improved diagnosis and prognosis and prediction of response. Long-term, the use of targeted RNA-based therapies could serve as a promising tool for personalized medicine. Future work appears instrumental in translating findings from sncRNA research into a clinical practice for MS patients.

Declaration of interests

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.04.002.

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