

# NeurimmiRs: microRNAs in the neuroimmune interface

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**Recent reports of microRNA (miR) modulators of both neuronal and immune processes (here termed NeurimmiRs) predict therapeutic potential for manipulating NeurimmiR levels in diseases affecting both the immune system and higher brain functions, such as Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS) and anxiety-related disorders. In our opinion, NeurimmiRs that function within both the nervous and the immune systems, such as miR-132 and miR-124, may act as 'negotiators' between these two interacting compartments. We suggest that NeurimmiRs primarily target transcriptional or other regulatory genes, which enables modulation of both immune and cognitive processes through direct or indirect alterations of neuron–glia and/or brain-to-body signaling. Thus, manipulating NeurimmiR control over the immune contributions to cognitive pathways may offer new therapeutic targets.**

'A healthy body is the guest-chamber of the soul; a sick, its prison.'

Francis Bacon

## MicroRNAs can modulate both neuronal and immune functions

MicroRNAs (miRs) are increasingly appreciated as being involved in all aspects of cellular functioning in a wide range of eukaryotic organisms [1]. However, although most miRs have multiple predicted targets, the majority of current miR studies focus on experimental validation of only one target. To the best of our knowledge, no hierarchy of miR–target interactions has yet been published for any miR. Furthermore, numerous mRNAs carry multiple putative binding sites for many miRs, but the experimental validation of miR–target relationships is rather laborious. Therefore, most studies have validated only one of those miRs experimentally. Also, whether or not any of those miR–target interactions override the others, and the underlying principles for such putative competition, remain unknown (Box 1).

The brain modulates immune functions using neurotransmitters, neuropeptides and hormones, senses peripheral inflammation by detecting inflammatory agents (such as proinflammatory cytokines and other immune regulators), and can potentiate or counteract inflammation in a 'top-down' effect [2]. Reciprocally, the immune system can

affect the brain in a 'bottom-up' manner, and converging lines of evidence highlight the role of neuroinflammation in both psychiatric and neurodegenerative diseases [3]. Of particular interest are microglia, myeloid cells of the monocyte/macrophage lineage that reside within the central nervous system (CNS) and thus are prime candidates for mediating communication between the two systems (Box 2). Furthermore, 'classic' immune proteins, such as MHC class I and complement factors, are considered to play new roles within the nervous system [4], suggesting that these interacting compartments might also share common miRs.

In the mammalian immune system, miRs control differentiation as well as innate and adaptive immune responses [5]. In the central nervous system, miRs are involved in diverse functions, including neuronal development, plasticity and physiological reactions [6]. A subset of these miRs, here designated NeurimmiRs, notably affects both immune and neuronal functions. Given that the validation of many miR targets is far from complete, appreciating the full neuroimmune impact of NeurimmiRs is in its infancy. Nevertheless, outlining the existence and assessing the scope of NeurimmiRs involved in neuroinflammatory processes can assist in evaluating the therapeutic potential of manipulating their levels in 'classic' neuroimmune disorders, neurodegenerative diseases and psychiatric syndromes. Below, we suggest several putative candidates for this new class of miRs and focus on one of them, miR-132, as an exemplar NeurimmiR with far-reaching implications.

We suggest that the co-occurrence of NeurimmiRs in the brain and peripheral organs has a function in the crosstalk between these two compartments, both locally (i.e. brain inflammation) or in a broad context (regulation of inflammation via the autonomous nervous system). Many miRs have a broad affect within a certain tissue owing to their regulation of transcription factors and other regulatory elements. Therefore, various scenarios might exist in which the expression of a miRNA 'shared' by two tissues is altered. This in turn might alter gene expression in either one or both of these compartments. Correspondingly, impaired miR levels were found in many nervous system diseases [7]. Also, miRs are involved in hematopoiesis [5] and in the immune components of diverse neural diseases such as epilepsy or Alzheimer's disease (AD) [8,9]. We also propose that immune insults change the levels of NeurimmiRs. Owing to the regulatory roles of these miRs, such changes often alter inflammation-responsive transcripts within the brain [e.g. multiple sclerosis (MS)] [10], or in peripheral

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### Box 1. The biogenesis and functions of miRs

MiRs are short non-protein-coding RNAs with both conserved and species-specific members. MiRs regulate transcription, mRNA stability and translation and are critical for the expression and epigenetic control of at least one-half to two-thirds of all known genes and biochemical pathways [34]. miR precursors (stem-and-loop molecules generated from a primary transcript) are cleaved to mature 22-base pair double-stranded molecules, with one strand usually guiding the complex to a partially complementary sequence often found in the 3'-untranslated region (3'-UTR) of target genes. The miR 5'-end 'seed' region determines target specificity and dictates mRNA degradation and translational repression, whereas mRNA cleavage is dependent on perfect complementary base-pairing at positions 10/11 as well as on target sites located at the center of the miR [56]. Classically, every miR can bind to its mRNA targets by incomplete base pairing, with only the short, approximately seven nucleotides long 'seed' region displaying full complementation; therefore, each miR can potentially target hundreds of transcripts and achieve network-level regulation of gene expression [61]. MiR regulation takes place post-transcription-

ally, and is thought to perform rapid fine-tuning rather than total silencing or induction of targets ('on' and 'off' switching). Furthermore, repeated binding motifs enable one miR to target several mRNAs involved in a particular pathway and achieve gene-network-level regulation. Although a particular miR often affects only a few targets in specific cell types, the potential multiplicity of gene targeting makes assessing and proving that a specific gene or pathway is indeed a target of a particular miR an elusive and nontrivial task. A major disadvantage in miR research is the difficulty in validating putative target mRNAs identified for a specific miR; a variety of online algorithms that utilize different approaches to predict miR-mRNA interactions exist, but the predicted targets for each miR might be dissimilar and largely non-overlapping and often exclude many experimentally proven and validated targets. Thus, current bioinformatics tools, as well as the understanding of miR biology itself, are still in their infancy. In many cases, a target-based search for complementary miRs [35] might be more useful than a miR screen for targets.

leukocytes. In the periphery, this would alter complement factors, adhesion molecules, reactive oxygen species and proinflammatory cytokines, facilitate crossing of the blood-brain-barrier (BBB) and influence a broad spectrum of neuronal processes such as neurotransmission, synapse morphology and cognition [11].

### NeurimmiR-modulated processes link immune and neuronal functions

Several NeurimmiR-related processes involve measurable developmental or deterioration changes of neuronal and hematopoietic pathways. Figure 1 schematically presents how NeurimmiR functioning might be viewed as operating within and between the neural and immune worlds, with each interaction affecting both cognitive and immune features in a multi-layered fashion. A prominent example is the brain-enriched miR-124, which enhances neuronal maturation by targeting the transcription factor *sox9* [12], inhibits the neuronal transcription regulator complex REST involved in the neurodevelopment disorder Rett syndrome [13], modulates serotonin-dependent long-term

potentiation (LTP) in sensory-motor neurons [14] and is involved in regulating hippocampal-induced plasticity under chronic cocaine intake [15]. In the hematopoietic compartment, miR-124 is exclusively expressed in microglia and CNS macrophages where it supposedly targets the transcription factor C/EBP- $\alpha$  and therefore also its downstream gene, the myeloid cell-associated transcription factor Pu.1. A recent study reports a miR-124-mediated microglial phenotype switch from an inflammatory (CD45<sup>hi</sup>MHCII<sup>+</sup>) to a quiescent state (CD45<sup>lo</sup>MHCII<sup>-</sup>). This successfully inhibits the onset of experimental autoimmune encephalomyelitis (EAE), a rodent model of MS [16]. Furthermore, miR-124 targets glucocorticoid receptors, an important component of stress responses and thus might contribute to controlling stress disorders [17]. As is often the case, none of these studies addresses the miR-124 targets studied by others, so that comparative analysis of the different processes in which this miR might be involved is virtually impossible at the present time.

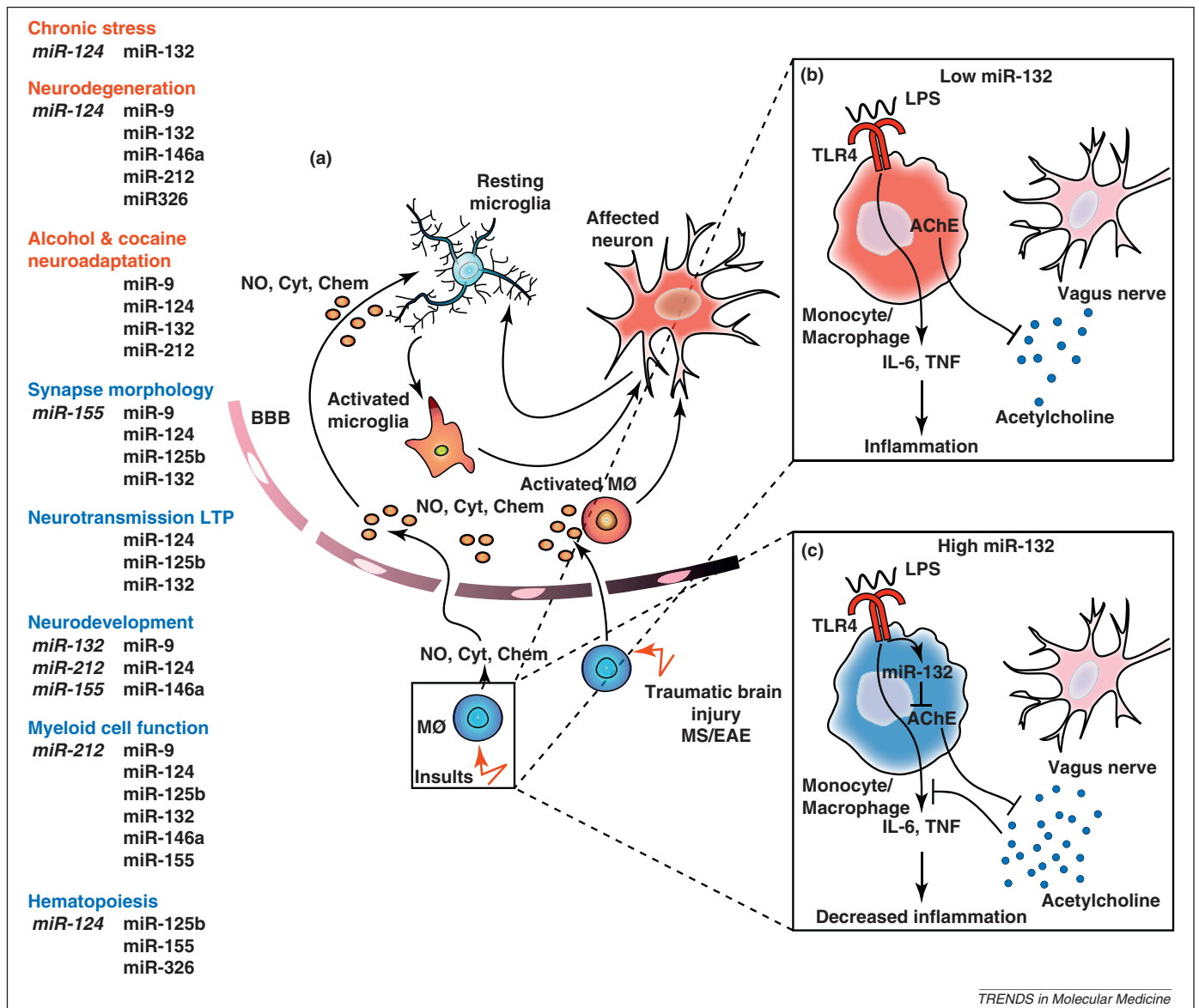
Another brain-enriched REST-targeting NeurimmiR, miR-9, shows decreases in Huntington's disease [18]. By

### Box 2. Neuroinflammation, cognition and the anti-inflammatory reflex

Neuroinflammation is an immune response initiated by the innate immunity arm of the CNS, and sometimes mediated by the adaptive arm, which involves increases in proinflammatory cytokines, chemokines, complement factors, reactive oxygen species and other agents, secreted by either resident or infiltrating leukocytes. In human patients, neuroinflammation is triggered by injury and aging, and peripheral immune challenges emerge as being involved in a wide range of CNS diseases such as AD, MS, PD and ALS [3]. Proinflammatory cytokines affect hippocampal LTP, neuronal differentiation and neurogenesis. Elevated levels of such cytokines have been found in the CNS of aged and post-traumatic stress disorder (PTSD) patients, particularly in brain regions associated with learning, memory and cognition. Compatible with this, inflammation affects emotions and mood, causing a depression-like state. Moreover, systemic inflammation increases cytokine production, most importantly IL-1 $\beta$ , which is capable of modulating the HPA axis, thereby enhancing anxiety-initiating body-to-brain signals [2]. The brain's immune system consists of various subsets of macrophage-associated cells, most notably the microglia [62]. Experimental exposure to proinflammatory cytokines such as IL-1 $\beta$ , protein aggregates such as A $\beta$  or self antigens such as the MOG peptide induces activation of astrocytes and microglia, leading to further expression of cytokines and leukocyte recruitment in a feed-

forward loop. Following peripheral LPS injection, such increases are accompanied by impaired cognitive functions (e.g. hippocampus-dependent learning tasks) and altered neuronal differentiation. Brain-infiltrating leukocytes secrete proinflammatory cytokines, reactive oxygen species and others that might both be detrimental to neurons and activate surrounding resting microglia, suggesting involvement in disease initiation and progression by the interlocked neuroimmune communication pathways.

The ascending fibers of the vagus nerve can signal the presence of peripheral inflammation to the brain, through cytokine receptors expressed by parasympathetic ganglia cells [2]. Reciprocally, macrophages and dendritic cells, T cells and B cells can react to ACh released from the descending fibers of the vagus nerve or from internal or peripheral sources, such as the splenic nerve. In cells exposed to inflammatory insults, NF- $\kappa$ -B translocates into the nucleus, and downstream inflammatory products are expressed and secreted. Cholinergic activation of the nicotinic  $\alpha$ 7 receptor interferes with this translocation and limits the secretion of proinflammatory cytokines. Overall, ACh has an anti-inflammatory effect, dubbed 'the cholinergic anti-inflammatory reflex' [2]. By contrast, extensive ACh hydrolysis by AChE exerts a proinflammatory output; thus, counteracting AChE activity by miR-132 exerts a net anti-inflammatory effect [35].



**Figure 1.** NeurimmiRs as mediators of brain immune-modulation. **(a)** Peripheral immune insults activate macrophages and induce the expression of blood-borne proinflammatory agents, which enhance leukocyte activation. Insults such as traumatic brain injury increase blood–brain barrier (BBB) penetration of both cytokines and cytokine secreting leukocytes, with direct effects on neurons or neighboring resting microglia. Consequent changes in the expression of NeurimmiRs alter both neuron–glia interactions and neuronal functions, affecting plasticity, neurotransmission, neurodegeneration and neurodevelopment. Other events such as drug consumption and mental stress can affect neuroinflammation as well as neuronal functions, thus NeurimmiRs might also participate in the modulation of these neuroimmune processes. Those miRs shown in *italic* are predicted to have functionality based on their corresponding mRNA targets. **(b)** Following rapid activation of toll-like receptors (TLRs), such as TLR4, downstream proinflammatory cytokines are secreted. During this time, miR-132 levels are low, which means that acetylcholinesterase (AChE) is expressed and hydrolyzes acetylcholine (ACh) secreted from the vagus nerve, perpetuating inflammation. **(c)** When TLR signaling elevates miR-132 levels, AChE activity is suppressed, potentiating cholinergic anti-inflammatory transmission [60]. Abbreviations: MΦ, macrophages; Cyt, cytokine; Chem, chemokine; NO, nitric oxide; MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis. Pathological events and processes are shown in red, and homeostatic or maintenance-associated events and processes are shown in blue.

regulating the transcription factor FoxP1, miR-9 promotes spinal columnar organization [19]. Correspondingly, miR-9 is downregulated in an animal model of spinal muscular atrophy (SMA) [20]. By destabilizing a splice variant of the large-conductance calcium- and voltage-activated potassium channel (BK) [21] miR-9 was proposed to modulate neuroadaptation to alcohol. In myeloid cells, miR-9 targets the vital inflammatory transcription factor NF-κB1 and is upregulated following exposure to activators of the innate immune toll-like receptors (TLRs) or proinflammatory cytokines [22]. Similarly bifunctional is miR-125b, which modulates the morphology and excitability of neurons through its Fragile X mental retardation protein (FMRP)

target [23], and was recently shown, in an exceptionally wide-scope study, to promote neurite growth through its capacity to target multiple transcripts [24]. MiR-125b also modulates lymphoid cell differentiation and targets the mRNA of the critical proinflammatory cytokine TNF-α, suggesting a significant role in the regulation and resolution of inflammation [25].

Following peripheral or central immune insults, immune cell changes in NeurimmiR levels might influence neuronal functions, whether directly by suppressing genes within neurons or indirectly by influencing the functioning of immune or supporting glial cells. For example, miR-155 regulates T cell and B cell differentiation as well as

dendritic cell function by targeting the SHIP-1 inositol phosphatase and controlling IL-1 signaling [26]. In a human-derived neuronal cell line, the mature MiR-155 can also target the methyl-CpG binding protein 2 (MeCP2), a co-activator of REST, and is deregulated in Down and Rett's syndrome and other neurodevelopmental diseases [27]. Mice that are null for *miR155* (*miR155*<sup>-/-</sup>) show significantly reduced numbers of encephalogenic CD4<sup>+</sup> Th17 cells, an inflammatory T cell subset known to have a significant role in MS and react to the neuroinflammatory stimulus of the MOG peptide by increased proliferation of CD4<sup>+</sup> Th17 cells. Also, *miR155*<sup>-/-</sup> CD4<sup>+</sup> T cells failed to passively induce the EAE experimental model of MS when transferred to lymphocyte-deficient animals [26]. In addition, impaired regulation of SHIP-1 and the suppressor of cytokine signaling 1 (SOCS1) in *miR155*<sup>-/-</sup> dendritic cells reduces the Th17 regulating cytokines IL-6 and IL-23. A similar role was proposed for miR-326, which regulates the Th17 cells by targeting the transcription factor Est-1 [28]. Both miRs were over-expressed in astrocytes derived from MS lesions in human patients and were suggested to modulate phagocytosis of neighboring neurons by regulating the macrophage/microglial SIRP $\alpha$  protein [29]. By deregulating Th17 and myeloid cell functions, miR-155 and miR-326 emerge as MS-associated NeurimmiRs.

Other NeurimmiR examples include the NF- $\kappa$ -B-dependent miR-146a, which is upregulated in the brain of AD patients and might enhance inflammation by targeting the inflammation-repressing complement factor H (CFH) [9]. MiR-146a is also upregulated in the hippocampus of both temporal lobe epilepsy patients and a rat epilepsy model [8]. Under HIV-1 infection, miR-146a elevation in human microglia supports the fine-tuning of immune functions by targeting the chemokine CCL8/MCP-2 [30]. Within the TLR innate immune pathway, miR146a suppresses the TRAF6 and IRAK1/2 proteins, potentially enhancing RIG-I mediated interferon-1 (IFN-1) production [5] and suggesting multiple routes contributing to microglial function.

Of note, different NeurimmiRs show overlapping patterns of immune-related changes: LPS-exposed human monocytes show upregulation of both miR-146a and miR-155, whereas TNF- $\alpha$  exposure upregulates miR-155 and miR-125b [25]. Similar to miR-155, miR-146a is also associated with MeCP2 because this miR is downregulated in the Rett syndrome model (*MeCP2*<sup>-/-</sup> animals) [31]. Furthermore, MeCP2 regulates the expression of miRs [32] and miR-146a can downregulate the IL-1 signaling protein IRAK1 both in neurons and in myeloid cells, suggesting its involvement in the neuroimmune crosstalk by IL-1. The evidence accumulated so far is compatible with the theory that a single NeurimmiR affects a pathway of inter-related transcripts, all involved in a cellular process, rather than single proteins. Because of the tendency of miR studies to focus on one protein–one miR interaction, the global miR effect on different pathways is often overlooked. One such example is miR-1, which was shown to coregulate several cholinergic-signaling related proteins in the neuromuscular junction of *Caenorhabditis elegans* [33]. Others proposed that the capacity of several miRs to target key regulatory transcripts associated with the same process ensures tight

regulation [34]. Thus, the complete scope of NeurimmiRs functioning in the neuroimmune interface appears to be central.

### Inflammation controlling NeurimmiRs are implicated in multiple syndromes

Inflammation is primarily aimed at fighting invading pathogens and is terminated by reciprocal brain-to-body signals. Most elderly individuals show excessive inflammation, suggesting failure of their natural control mechanisms [2]. In the mammalian brain, aging is associated with miR changes which have been associated with increases in inflammatory markers in astrocytes, microglia and neurons, as well as with reduced neurogenesis, increased neuron death and neurodegenerative processes [3,7]. The causes for the heightened inflammatory state in advanced age or the impact of inflammation on aging in general are not well understood, but NeurimmiRs that target transcripts involved in the neuroimmune interface (e.g. miR-125b, miR-132 and miR-146a) are probably involved, which makes them promising targets for interference with the hope of reducing age-associated inflammation.

Recent findings demonstrate that small RNAs functioning within the neuroimmune interface play a significant role in controlling the bidirectional communication line between neuronal circuits and inflammatory processes [35]. For example, in Parkinson's disease (PD), where neuroinflammation is a central component, mutated LRRK2 globally interferes with miR-mediated processes [36], whereas Let-7 targets the PD hallmark fibril-forming protein  $\alpha$ -synuclein [37]. In amyotrophic lateral sclerosis (ALS), upregulating miR-206 was shown to reinnervate damaged neuromuscular junctions by targeting histone deacetylase 4 [38]. Specifically, NeurimmiR alterations under peripheral or central inflammation are compatible with the hypothesis that these changes contribute to the emergence of several neurodegenerative and neuropsychiatric conditions (Table 1). Peripheral immune insults and/or BBB promiscuity [11] induce the secretion and brain penetration of inflammatory agents and leukocytes, which would consequently modify NeurimmiRs expression in the CNS. Although the direct association of such miR changes with neuronal function and malfunction is yet to be confirmed, NeurimmiRs are primarily adapted for such body-to-brain signaling in the rapidly growing field of neuroimmunity. For exploring the relevance of this phenomenon, we can turn to the well-investigated brain-enriched miR-132, which was implicated with body-to-brain communication [35], as a case study.

MiR-132 transcription is controlled both by the cyclic-AMP response element binding protein (CREB) [35], a transcription factor classically involved in learning and memory as well as in neural growth, and by the neuronal growth factor BDNF that is associated with cholinergic functioning. MiR-132 transfection suppresses the Rho family GTPase activating protein p250GAP while enhancing neurite sprouting in cortical neurons, compatible with miR-132 upregulation during postnatal development and with the attenuation of neurite growth by miR-132-targeted antisense oligonucleotides [39]. MiR-132 was also predicted to target several ion channels, and thus affect cell



**Table 1. Relevance of NeurimmiRs for various neuropathologies**

miRNA	Neuropathology	Direction of expression change	Validated target	Physiological outcome	Refs.
miR-9	Huntington's disease	Downregulation	REST	Disrupted regulation of neuronal gene expression	[18]
	SMA	Downregulation	Heavy neurofilament subunit	Disrupted axonal cytoskeleton	[20]
	AD	Downregulation	?	?	[49]
miR-124	EAE	Downregulation	C/EBP- $\alpha$	Over-activation of microglia and infiltrating macrophages	[16]
miR-125b	AD	Upregulated	?	?	[49]
miR-132	Huntington's disease	Upregulation	?	?	[18]
	AD	Downregulation	?	?	[49]
	Cerebral ischemia	Downregulation (following ischemic preconditioning)	MeCP2	Neuroprotection against subsequent ischemic insults	[54]
	Schizophrenia/bipolar disorder	Upregulation	?	?	[52]
miR-146a	AD	Upregulation	CFH	Enhanced inflammatory response in neocortex and hippocampus of AD patients	[9]
	Epilepsy	Upregulation	?	?	[8]
	HIV-encephalitis	Upregulation	MCP-2	Regulation of excessive inflammatory response during reaction to viral infection	[30]
	Rett syndrome	Downregulation	IRAK-1	?	[31]
miR-155	Down's syndrome	Multiple copies <sup>a</sup>	MeCP2	Broad disruption of gene expression as a result of aberrant expression of transcription factors downstream of MeCP2 <sup>b</sup>	[27]
	MS/EAE	Upregulation	CD47, SHIP1	Aberrant 'don't eat me signal' to macrophages/microglia; aberrant regulation of Th17 subset via dendritic cell signaling	[26,29]
miR-212	Schizophrenia/bipolar disorder	Upregulation	?	?	[52]
	AD	Downregulation	?	?	[49]
miR-326	MS/EAE	Upregulation	CD47, Est-1	Aberrant 'don't eat me signal' to macrophages/microglia; deregulated shift of Th0 cells to Th17 cells	[28,29]

<sup>a</sup>MiR-155 resides within chromosome 21, which is tripled in Down's syndrome.

<sup>b</sup>MeCP2 is also relevant for Rett's syndrome, although the miRNA is not yet reported to be directly involved in the disease.

excitability; correspondingly, overexpressed pri-miR-132 potentiates glutamate, NMDA, or K<sup>+</sup>-mediated depolarization of cultured neurons, suggesting global involvement in regulating neurotransmission and plasticity. However, involvement of other putative miR-132 targets has not been tested in these experiments, which leaves the detailed mechanisms underlying the neuronal growth effects of miR-132 incompletely understood.

In transfected cultured neurons, miR-132 leads to wider and shorter dendritic spines, accompanied by increased miniature excitatory postsynaptic currents [23]. In newborn hippocampal neurons, Cre-lox mediated deletion of miR-132 decreases dendritic length and arborization in adult mice [40], supporting a role for miR-132 in neuronal differentiation, synaptogenesis and maintenance. Conversely, cells transfected with the TNF- $\alpha$ -inducible miR-125b extend long and thin spines and show reduced excitatory postsynaptic currents. Both miR-132 and miR-125b were co-immunoprecipitated with FMRP, and FMRP knockdown abolished the morphological effects of both miR-132 and miR-125b. This suggested that miR-132 and miR-125b balance each other's effects through competitive interaction with FMRP [23]. Compatible with the stress-associated alteration of cholinergic signaling, miR-132 was upregulated in the hippocampus of chronically stressed rats [41]. Another miR-132 target is the light-induced transcription regulatory factor X 4

(RFX4), abundant in the suprachiasmatic nucleus (SCN) of the hypothalamus, which regulates biological clocks and rhythms. MiR-132 levels are higher during the light part of the circadian cycle in the SCN [42]. Infused antisense oligonucleotide against miR-132 (AntagoMir) impaired the resetting of the circadian clock while elevating RFX4. In this case as well, no other possible targets were measured in live mice.

Cultured human monocytes react to LPS by overexpressing the NeurimmiRs miR-146a, miR-155 and miR-132 [5]. Correspondingly, all three miRs are increased in the autoimmune disease rheumatoid arthritis [43]. In human lymphatic endothelial cells infected with Kaposi's sarcoma-associated virus, as well as in viral or bacterial-infected brain tissue, miR-132 induces transcriptional arrest by downregulating the CREB co-activator p300 [44]. This inhibits both inflammatory cytokines and antiviral genes, indicating that stress-induced facilitation of anti-inflammatory protection, which would also support cognitive alertness, might take its toll by compromising anti-viral immune functions. Because p300 is also a coactivator of the developing brain, this process may further intensify the damaging effects of viral infection in neuronal development.

At the body-to-brain communication level, miR-132 regulates cholinergic anti-inflammatory signaling by potentiating the cholinergic anti-inflammatory pathway (Box 2)

**Box 3. Outstanding questions**

- Do NeurimmiRs function similarly within peripheral leukocytes and in CNS resident and infiltrating leukocytes?
- To what degree do changes in NeurimmiRs contribute to the onset and development of neuropathologies?
- Can NeurimmiR levels and functions be manipulated for interfering with neuropathologies with inflammatory components?

[35]. By targeting acetylcholinesterase (AChE), miR-132 lowers acetylcholine (ACh) hydrolysis. Both gain- and loss-of-function experiments validated the miR-132/AChE interaction; miR-132 overexpression by lentivirus infection reduces AChE activity, whereas mutagenesis of the miR-132 binding site in AChE mRNA elevates it. Correspondingly, in vivo antisense suppression of miR-132 levels increases AChE activity in the intestine, spleen and serum. Furthermore, mice expressing an AChE transgene devoid of the miR-132 binding site lose the ability to control inflammation via ACh and show altered thermoregulation under inflammatory insults, despite their intact response to nicotine. These mice present higher miR-132 levels in the inflammation-responding hypothalamus and the bone marrow. This would predictably enhance the cholinergic output, potentiating cholinergic anti-inflammatory signaling [2] and contributing to the resolution of inflammation in both macrophages and intestinal tissues (Figure 1a,b). However, the relative contributions of p300, CREB and AChE suppression to the anti-inflammatory effects of miR-132 await further studies (Box 3).

**Future implications of NeurimmiRs in inherited and acquired diseases**

Studying miR involvement in neurological and neuropsychiatric disorders is still at a pioneering level, but some of the players are highly likely to be NeurimmiRs. A microdeletion in the 22q11.2 region, known to be associated with schizophrenia and autism [45], disrupts DGCR8, a component of the miR biogenesis pathway, which in turn impairs proper miR biogenesis [46]. In the amygdala of stressed rats, miR-134 and miR-183 are both elevated, where MiR-183 targets the stress-inducible splice factor SC35, which modulates the stress-responsive alternative splicing of AChE mRNA [41]. Cholinergic signaling also initiates increases in the hematopoietic miR-181a, which is also present in the CNS [47]. Several other miRs were implicated in a number of neurological, neurodevelopmental and neurodegeneration disorders (Table 1) [7,10,47].

AD is an intriguing target for NeurimmiR manipulations. As yet, no unbiased comprehensive study has definitively identified and validated those sets of miRs that drive the aberrant regulation of neuroimmune processes in the AD-damaged brain regions, so that the selection of NeurimmiR targets for interference with AD, like the scope of the affected processes, remains incomplete. Recent evidence, however, suggests that the neuroimmune components of AD, such as the presence of complement system factors within the CNS, is associated with miR expression and can thus influence downstream neuronal functions [48]. Correspondingly, NeurimmiRs such as miR-132 and miR-9 are downregulated, whereas miR-125b is

upregulated in the hippocampus and medial frontal gyrus of AD patients [49]. An intriguing leukocyte subset for NeurimmiRs screening is the microglia, a population in which only a handful of miRs were described [10,30,50]. Because microglia express functional anti-inflammatory ACh receptors [51], the miR-132/AChE connection is a promising candidate for studying the corresponding NeurimmiR regulatory effects. Several miR-132 target genes, most notably regulators of transcription such as MeCP2 and p300, highlight such neuroimmune functions (Table 1). As miRs can regulate cognitive processes, impairments in their functioning might also be especially risky for psychiatric disorders.

Once produced, miRs are likely to act relatively quickly, due to their post-transcriptional mode of activity. By sharing multiple regulatory targets with many other miRs and being susceptible for numerous regulatory processes, each miR is subject to a tight but flexible network of checks and balances, which in normal circumstances ensure gradual tuning rather than a complete arrest of their target processes. This and the brain-to-body activities of NeurimmiRs as proposed in this review, further points at the challenging yet promising potential of altering miR activities for therapeutic purposes (Box 3). Thus, in syndromes involving AChE suppression, including imbalanced cognition, impaired cholinergic signaling and autoimmune syndromes, oligonucleotide-mediated knockdown of miR-132 should help by elevating AChE [35]. Although approximately 20-mer miR-size oligonucleotides are unlikely to cross the BBB, peripheral administration would change the levels of cytokines, which can penetrate and affect the brain indirectly.

At the genome level, miR-132 is encoded in tandem with miR-212 within 300 base pairs at the same chromosomal location. These two miRs share an identical 'seed' sequence and might share the same transcript, forming the miR-132/miR-212 cluster, which suggests common regulation and mutual targets. The miR-132/miR-212 complex is overexpressed in schizophrenic and bipolar brain tissues [52] and in the dorsal striatum of a rat model for cocaine addiction [53]. However, a recent report singled out miR-132 as the prime partner of this cluster in neuronal differentiation, with little effect attributed to miR-212 [40], suggesting that this dual cluster might not always operate as a single entity.

Of note, several NeurimmiRs associate with different components of the REST/MeCP2 complex: miR-9 directly targets REST, miR-124 targets the REST complex component SCP1, miR-155 targets MeCP2, miR-146a is regulated by MeCP2 and the miR-132/miR-212 cluster directly targets the REST cofactor MeCP2. Recent reports describe other MeCP2-associated miRs to be involved with neuronal differentiation [32], suggesting that these miRs might also act as NeurimmiRs. During the progression of Huntington's disease, miR-132 levels increase [18], REST binding to the regulatory huntingtin protein is impaired and miR-9 levels decrease in a feedback response. Inverse to the excessive susceptibility of *MeCP2*<sup>-/-</sup> mice to ischemic damage, miR-132 levels decrease and MeCP2 levels increase in rapid ischemic preconditioning, which protects the brain from subsequent prolonged ischemia [54].

Biomedical tools that are being developed for miR modulations include, but are not limited to, chemically protected AntagoMir oligonucleotides for suppressing targeted miRs [35], miR mimics for potentiating their action [55] and lentiviral tools for long-term manipulations [44,56]. Further experiments must be undertaken to establish the efficacy of these potential minimally intrusive manipulations for neuroinflammatory conditions. Promising examples involve AD-related downregulation of the miR-29ab-1 cluster [57] and of miR-107 in a double transgenic mouse AD model [58], all associated with  $\beta$ -amyloid precursor protein-converting enzyme (BACE1), which presumably contributes to AD development, and the predicted regulation of BACE1 by miR-298 and miR-328 [59].

### Concluding remarks

The field of miR research has undergone a wide-ranging expansion in the last decade. However, the multiple modes of function of these small RNAs have hampered the progress in understanding the full scope of their effects. This is particularly the case for miRs involved in neuroimmune maladies, which we have named NeurimmiRs. Both peripheral and central immune insults have already been shown to upregulate various NeurimmiRs, either in neurons, surrounding cells (e.g. glia, microglia and infiltrating leukocytes) or in peripheral leukocytes. We propose that miRs are involved in regulating neuroimmune functions both due to central control and by peripheral immune cells sending back messages; such messages alert the central command, leading to a complex set of bidirectional feedback consequences. Owing to their physical properties and multiple roles in the nervous and immune systems, NeurimmiRs may initiate such communication cascades both in health and disease and their manipulation can be therapeutically useful. To progress toward this goal, several open questions remain to be addressed (Box 3). By contributing to the monitoring by the brain of cognition and inflammation alike, as well as to the detrimental effects of systemic and chronic changes in immune functions in neurodegenerative diseases, these miRs might be prime candidates for therapeutic intervention for neuroimmune impairments.

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