Role of microRNAs in the regulation of innate immune cells under neuroinflammatory conditions

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Abstract

MiRNAs are short, evolutionary conserved noncoding RNA molecules with the ability to control the magnitude of inflammation. The immunosuppressive nature of the brain is sustained by miRNA-dependent regulation of microglial cells, which become activated under neuroinflammatory conditions, such as brain injury and neurodegeneration. The pro-inflammatory and suppressive role of the most studied neuroimmune miRNAs, miR-155 and miR-146a, has been recently challenged. Although the molecular targets of these miRNAs remain unchanged across brain diseases, different kinetics of miRNA expression and degradation can produce different immune outcomes and change microglia phenotypes. Here, we discuss current knowledge regarding the implications of disruption of miRNA networks in neuroinflammation and in the pathophysiology of acute and chronic CNS diseases.

1. Introduction

Innate immune response mechanisms are responsible for the first line of defense against brain damage caused by exogenous or endogenous sources. The innate defense program includes a plethora of highly regulated pathways, whose timely activation allows both efficient elimination of the detected threat and repair of damaged tissue.

Multiple layers of gene regulation must work through a highly dynamic and coordinated process to avoid deleterious effects resulting from lengthy, inappropriate or unchecked inflammation. These regulatory mechanisms start at the membrane level, where pattern recognition receptors (PPRs) control the specificity of the innate immune response. This specificity is dependent on the nature of the danger signal, the type of PPRs available to bind to that signal and the presence of other environmental cues, relayed by adjacent cells. The transcriptional switches triggered following PPR activation transform the environmental input received from the extracellular environment into a set of changes in gene expression, which can be adequately modulated for each particular “danger” condition.

In addition to transcriptional regulation, several post-transcriptional mechanisms play an important role regarding the overall strength and duration of the innate immune response (reviewed in [1]). Most genes involved in innate immune response pathways, particularly those originating transient transcripts, exhibit multiple regulatory elements in their 3' untranslated regions (3'UTR), which allow the recruitment of molecular effectors capable of performing rapid adjustments in transcript translation. In this regard, microRNAs (miRNAs) (reviewed in [2]) have carved a particularly important niche as major regulators of gene expression, due to the ability of a single miRNA to bind multiple mRNAs. This molecular property allows the cell to fine tune entire molecular pathways and quickly adapt to changes in the surrounding environment. This has been shown to be of particular importance during immune cell development and differentiation, as well as in the initiation and termination of inflammatory responses (reviewed in [3-5]).

More than 60% of all human protein-coding genes exhibit miRNA binding sites. Failure to achieve a perfect temporal and spatial control of miRNA expression was

shown to play a critical role in human disease, affecting not only the originally diseased cells, but also the surrounding tissue. The discovery of new forms of cell-to-cell communication, such as exosome-mediated miRNA transfer[6], revealed an important mechanism through which miRNAs can exert immunomodulatory effects across the nervous system in both health and disease settings[7].

2. Brain immune cells

Brain is a deeply repressed immune environment. Therefore, uncontrolled inflammation constitutes a major component of central nervous system (CNS) diseases related to development defects, acute and chronic neurodegenerative processes. Brain immunity is sustained by cells from the mononuclear phagocyte system. Although perivascular, choroid plexus and meningeal macrophages can be found in the brain, microglia are the only hematopoietic cells resident in the CNS parenchyma (reviewed in [8]). Nevertheless, their origin and classification of mononuclear phagocytes constitute a subject of constant update. It is believed that circulating monocytes do not substantially contribute to the subpopulations of macrophages within tissues[9,10], unless under certain inflammatory conditions[11]. Instead, embryonic precursors travel to tissues during development and differentiate, *in situ*, under the influence of specific environmental cues, maintaining themselves through self-renewal. In this context, microglia constitute a privileged type of macrophages that populate a very unique environment, which is responsible for shaping their phenotype through activation of tissue-specific genomic super-enhancers near genes, such as *Cx3cr1*[12], essential for brain functions.

In the healthy CNS, the major job of microglia is surveillance. Two photon microscopy studies revealed an always moving and extremely ramified phenotype with long branches and small soma [13], which rapidly changes to a round morphology in response to injury [14]. This response is partially mediated by ATP and the purinergic receptor P2Y₁₂[15]. While several studies suggest that a neuron-microglia inhibitory signaling is responsible for maintaining the immune suppressive brain environment[16], most CNS pathology studies agree that, in cases of stress, injury,

infection or under a chronic inflammatory stimulus, microglia proliferate, produce inflammatory mediators and upregulate myeloid markers. This last feature greatly hinders our understanding of the contribution of *in situ* microglia proliferation and monocyte infiltration to the local immune response, since both cell types might differentiate into a macrophage-like phenotype upon brain injury.

3. Immune-related miRNAs

Inflammation is the normal response of the organism to an abnormal stimulus and, although it comprises different types of response and participants, is initiated by cells from the innate immune system, which include mononuclear phagocytes. The cellular and molecular components of this phenomenon act in concert to fight the harmful event and restore normal activity. The efficiency of this process is determined by the tight regulation of inflammation signaling pathways to which miRNAs play a crucial role by controlling immune cell development and the expression of important transcription factors and inflammatory mediators. Upon an inflammatory stimulus, signals are sent to the nucleus to promote upregulation or downregulation of these proteins and, in between, some miRNAs are upregulated to decrease the expression of inhibitory regulators of inflammation, while others are decreased to allow upregulation of inflammatory proteins. Later on, other miRNAs are upregulated or downregulated to quench the inflammatory cascades, signaling the end of the immune response. Some inflammatory stimuli can also change the expression of miRNA biogenesis players, as is the case of interferons, which repress Dicer's expression [17], affecting the expression of multiple miRNAs.

The role of miRNAs in immune cell development is illustrated by the involvement of miR-155 and the miR-17-92 cluster in mononuclear phagocyte differentiation from granulocyte-macrophage precursors (reviewed in [18]). MiR-124 is also involved in immune cell differentiation by targeting CEBP α [7], a transcription factor that cooperates with PU.1 activity during macrophage differentiation.

The power of miRNAs to regulate inflammation hyperactivity has also been known by early studies on miR-155 upregulation following lipopolysaccharide (LPS)-stimulation in macrophages [19]. Signaling through Toll-like receptors (TLRs), upon

activation by pathogen-associated molecular patterns (PAMPs), involves the action of the adaptor proteins MyD88 and TIR-domain-containing adapter-inducing interferon- β (TRIF). These cascades culminate in activation of transcription factors such as nuclear factor kappa B (NF- κ B) and c-Jun, responsible for the production of inflammatory cytokines and type I interferons. During this process, the expression of miRNAs is also upregulated, miR-155, miR-146a and miR-21 being the most thoroughly investigated. Depending on the molecular targets and role in inflammation, miR-155 is considered a pro-inflammatory miRNA contributing to the propagation of inflammation, while miR-146a and miR-21 control the magnitude of this response. Following TLR activation, miR-155 expression increases to suppress inhibitory proteins of inflammation, such as the suppressor of cytokine signaling 1 (SOCS-1) and Src homology-2 domain-containing inositol 5-phosphatase 1 (SHIP1). These primary targets have been studied in the context of macrophage [20] and microglia [21] activation, and their downregulation was found to be critical for the development of inflammation. A less studied inflammatory pathway in microglia, in which miR-155 plays a primary role, involves p53. This protein is essential for miR-155 upregulation, which negatively regulates c-Maf expression promoting inflammation [22].

Interestingly, being a negative modulator suppressing TLR signaling, miR-146 is also upregulated during immune activation through the increase in NF- κ B activity [23]. Besides inhibiting NF- κ B directly, one of its main functions is to downregulate the expression of two components of the MyD88 signaling, interleukin 1 receptor-associated kinase (IRAK1) and TNFR-associated factor 6 (TRAF6) [23]. As opposed to miR-155, miR-146a controls the propagation of inflammation by targeting these important adaptors [24]. MiR-21 targets the programmed cell death protein 4 (PDCD4), leading to downregulation of NF- κ B and induction of anti-inflammatory proteins, such as interleukin 10 (IL-10), which makes it a negative regulator of the immune response. MiR-21 expression is also induced during TLR activation in a MyD88-dependent manner. The opposite happens for other miRNAs, such as miR-125a and let-7 [25,26], which are downregulated during inflammation by directly targeting cytokines, including tumor necrosis factor alpha (TNF- α) and interleukin 6 (IL-6). MiR-124, which is highly expressed in microglia but not in macrophages, is responsible for maintaining these cells under surveillance by targeting the CEBP α /PU.1 pathway [27].

In addition to their role in the regulation of inflammation, miRNAs may function as markers of immune cell type and activation phenotype. A unique signature of miRNAs was described for microglia, in comparison to other organ-specific macrophages and several other types of immune cells [28], revealing the miR-29 family as one of the most relevant and enriched in microglia.

It has been proposed that macrophage activation phenotypes are not compartmentalized and are expressed as a continuum along different patterns of gene expression. These phenotypes are classified as M1, M2a, M2b and M2c, depending on the environmental stimuli and the observed changes in gene expression, morphology and secreted molecules. While M1 macrophages are specialized in pathogen elimination, M2 activation promotes tissue repair. Although differences between activation phenotypes are very difficult to prove *in vivo*, similar polarizations have been proposed for microglia. In this context, miRNAs have recently emerged as potential markers for the different activation phenotypes. The disruption of the brain homeostatic balance (M1) is accompanied by increased miR-155 expression, as well as reduced miR-124 expression, its reposition (M2) and maintenance (M0) being associated with an increase in miR-145 and miR-124 expression, respectively [29]. These miRNAs may help to identify the activation phenotypes more prevalent in different disease settings and their modulation may allow a proper shift of these phenotypes.

4. MiRNAs in neuroinflammation and disease

Neuroinflammation, mediated by local and systemic immune cells, has recently assumed an important role as one of the major hallmarks of both acute and chronic brain damage. In view of the essential role of miRNAs in inflammation to maintain homeostasis in an injury situation, it is not surprising that miRNA deregulation has been thoroughly implicated in most neurological diseases harboring inflammatory responses. The role played by immune-related miRNAs in the context of brain disease is not unique to the CNS. In fact, the immune system is present in virtually all body compartments and, although the brain is considered to be an immune privileged site, microglia are ontologically similar to peripheral mononuclear phagocytes. Therefore, it

is expected that the cellular pathways driving their function coincide, leading to similar responses. Since the inflammatory mediators, pattern recognition receptors and transcription factors responsible for triggering miRNA expression are similar in the brain and in other tissues, it is expected that the same inflammatory miRNAs have comparable impact on immune responses. In fact, what truly differentiates the consequences of miRNA deregulation in different disease contexts is not the miRNA itself, but the kinetics of expression of both the miRNA and its targets, as well as their availability in the cytoplasm, where the repression occurs. Examples of the connection of immune-related miRNAs with brain disease are described below and summarized in Table 1.

Table 1

Involvement of miRNAs in disease-related neuroinflammation.

Brain disease	miRNA	Functional role / molecular target	Ref
Japanese encephalitis virus	miR-155	Reduction of virus-induced gene expression	[30]
	miR-146a	Evasion of inflammation / TRAF6, IRAK1, IRAK2, STAT1 Enhancement of viral replication / IFIT-1, IFIT-2	[31]
<i>Angiostrongylus cantonensis</i>	miR-146a	Immunosuppression / TRAF6, IRAK1	[32]
	miR-223	Inhibition of inflammation	[33]
	miR-155	Activation of macrophages (protection)	[33]
Ischemic stroke	miR-122 miR-148a let-7i miR-19a mir-320d miR-363 miR-487b	TLR and NF-kB signaling	[34]
	miR-203	Inhibition of NF-kB signaling / MyD88	[35]

Multiple sclerosis/Experimental autoimmune encephalomyelitis	miR-146a	Disease biomarker Dysfunctional inflammation	[36]
	miR-155 miR-326 miR-34a	Dysfunctional inflammation, myelin phagocytosis / CD47	[37,38]
	miR-124	Regulation of microglia activation	[7]
Alzheimer's disease	let-7	TLR7 activation	[39]
	miR-146a	Regulation of inflammation / CHF	[40,41]
	miR-155	Dysfunctional inflammation / SOCS-1	[42]
Parkinson's disease	let-7	Regulation of TLR expression / α -synuclein	[43,44]
Prion disease	miR-146a	Regulation of inflammation	[45]
Amyotrophic lateral sclerosis	miR-155 miR-146b miR-29b let-7a/b mir-21 miR-210	Dysfunctional inflammation	[46,47]

4.1 Brain infection

Although the brain is an immune privileged organ, physically separated from the rest of the organism by the blood-brain barrier (BBB), it is still extremely sensitive to infectious agents, such as bacteria, virus, parasites and fungi.

Exposure to any pathogen leads to significant changes in the expression of specific miRNAs in immune cells, some of which potentiate the immune response, while others are in charge of controlling it, at a later stage. This is the case of miR-155 and miR-146a, which play an important role during infection by the Japanese encephalitis virus (JEV). JEV infection, which is the leading cause of viral encephalitis in south-east Asia, India and China, occurs in both microglia and neuronal cells. Recently, Pareek and colleagues presented evidence that overexpression of miR-155 negatively modulates JEV replication, reducing virus-induced gene expression in host cells[30]. Although

these authors failed to observe any effect of miR-146a overexpression, a more recent study, by Sharma and coworkers, showed that JEV infection elevates the expression of miR-146a, causing downregulation of TRAF6, IRAK1 and 2 and signal transducer and activator of transcription 1 (STAT1), thus suppressing NF- κ B and JAK-STAT activation[31]. This miR-146a-induced suppressive effect downregulated the expression of interferon-stimulated genes (IFIT-1 and IFIT-2) and facilitated viral replication, providing with a clear example of how, despite their valuable role in coordinating the immune response, some miRNAs can be hijacked to benefit viral infection.

An immunosuppressive effect was also directly related with miR-146a upregulation in both rat and mouse models of parasitic infection by *Angiostrongylus cantonensis*, where increased miR-146a levels led to decreased IRAK1 and TRAF6 expression [32]. Another study, performed in the same mouse model, revealed an increase in the expression of miR-511, miR-223, miR-155, miR-206, miR-142 and miR-21 [33]. MiR-223 expression was associated with disease severity and was suggested to inhibit the pro-inflammatory nature of immune cells. On the other hand, miR-155 upregulation resulted in a protective effect, by promoting activation of macrophages necessary to the elimination of this parasite. Although the pro-inflammatory role of miR-155 has proven to be useful in the setting of pathogen infections, this is not always true, particularly when the deleterious stimulus raising havoc in the neuronal tissue is endogenous.

4.2 Acute CNS injury

Ischemic stroke, intracerebral hemorrhage, epileptic seizures and traumatic brain injury constitute different forms of acute CNS injury that share several mechanisms of cell damage, including excitotoxicity, oxidative stress and inflammation. In the past few years, evidence has emerged that these forms of acute damage lead to important modifications in miRNA networks. Significant changes in the levels of several miRNAs, including miR-145, have been reported, both locally and systemically, in a stroke/reperfusion rat model of injury [48-50]. Ischemic stroke patients also display a decrease in the levels of miR-122, miR-148a, let-7i, miR-19a and miR-320d, while miR-363 and miR-487b levels were shown to be increased in peripheral blood mononuclear

cells (PBMCs)[34]. Importantly, all these miRNAs have been predicted to play a role in inflammation-related pathways, including TLR and NF- κ B signaling. The activation of NF- κ B and other immune-related transcription factors, including c-Jun, has also been extensively implicated in neuronal death. These transcription factors lead to the expression of multiple proinflammatory cytokines, reactive oxygen species (ROS) and upregulation of the inducible nitric oxide synthase (iNOS). Upon release to the extracellular medium, these molecules cause direct damage to neuronal membranes and also activate neuronal receptors, inducing the activation of apoptotic signaling pathways.

MiR-203 has been deeply related with microglia activation in the context of brain ischemia. In their recent work, Yang and coworkers presented evidence that miR-203 provides protection from brain injury[35]. Enforced expression of miR-203 resulted in the inhibition of NF- κ B signaling and alleviation of neuronal injury, which were attributed to miR-203-mediated downregulation of MyD88, a protein involved in TLR and interleukin 1 beta (IL-1 β) signaling. Most importantly, intracerebroventricular injection of miR-203 mimics, immediately following the onset of ischemia in mice subjected to Permanent Middle Cerebral Artery Occlusion (pMCAO), led to a significant reduction of infarct size and production of NF- κ B, interleukin 8 (IL-8), TNF- α and MyD88, indicating a possible therapeutic application for this miRNA [35].

4.3 Autoimmune diseases

MiRNAs have been directly implicated in several autoimmune diseases, including rheumatoid arthritis, allergy, psoriasis and, more relevant in the context of brain diseases, multiple sclerosis (MS). Several miRNAs were shown to play a role in MS, including miR-155, miR-326, miR-182-5p, miR-301a and miR-146a, mainly by influencing the differentiation and function of the different T-cell subsets (reviewed in [51]).

MiR-155 and miR-146a, in particular, have also been implicated in MS and experimental autoimmune encephalomyelitis (EAE), a mouse model of MS, from the perspective of innate immunity (reviewed in [52]). Similar to what was initially described for neurodegenerative diseases, miR-146a has been considered a pro-

inflammatory miRNA in MS, whose upregulation can be directly correlated with disease progression. In this regard, Fenoglio and colleagues reported an increase in miR-146a levels in PBMCs from MS patients in the relapsing-remitting stage[36], suggesting that this miRNA could serve as a disease biomarker.

Similarly to miR-146a, the M1 marker miR-155 has been directly associated with the dysfunctional pro-inflammatory response observed in MS. MiR-155 levels have been found to progressively increase in the brain, spleen and lymph nodes of the EAE model[37] and in active MS lesions [38], while miR-155^(-/-) mice exhibit resistance to EAE induction, which translates into an overall reduction in CNS inflammation[37]. In human MS, miR-155, together with miR-326 and miR-34a, were reported to target the cluster of differentiation 47 (CD47), a protein essential for macrophage inhibition, whose downregulation triggers myelin phagocytosis[38].

Recently, evidences have emerged suggesting that miR-155 suppression can have therapeutic impact in MS and EAE models [37]. For example, miR-155 was shown to modulate the endothelial barrier function by interfering with cell-cell complex molecules and adhesion components. As such, its downregulation in EAE and in human cultured brain endothelium, not only avoided pro-inflammatory signaling but also prevented alterations in BBB junctional organization [53,54], avoiding cytokine-induced increase in BBB permeability.

In contrast with miR-146 and miR-155, miR-124 was shown to promote a quiescent state in brain microglia. However, during the onset of EAE, miR-124 levels tend to decrease in activated microglia, reaching the lower levels typical of blood-derived macrophages [7]. These observations suggest that miR-124 overexpression may have a beneficial effect in MS, which is further supported by the results of Ponomarev and coworkers [7], showing that peripheral administration of miR-124 mimics causes systemic deactivation of macrophages, reduced activation of myelin-specific T cells and marked suppression of disease symptoms. Conversely, knockdown of miR-124 in microglia and macrophages exacerbates cell activation *in vitro* and *in vivo*, further promoting disease progression.

4.4 Chronic neurodegenerative diseases

Neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and prion disease display progressive neuronal loss that potentiates a deterioration of brain function. Although the primary causes for these diseases remain mostly undisclosed, neuroinflammatory events were recently recognized as a major player in disease progression[55].

Along this line, overexpression of TLRs[56,57]and unchecked TLR signaling was associated with most neurodegenerative diseases and, as a consequence, miRNA-mediated TLR modulation has attracted the attention of the scientific community. In the context of AD, different TLRs were shown to be overexpressed and are thought to act as receptors for amyloid β ($A\beta$) peptides in microglia, triggering activation of these cells. The let-7 family of miRNAs was recently suggested to play an unconventional role, acting as ligands for TLR7[39]. Let-7 has been shown to be upregulated in AD patients, contributing to neurodegeneration and neuroinflammation [39].

The let-7 family has also been implicated in PD, with an anti-inflammatory role. Let-7 was shown to be downregulated in PD models and to repress α -synuclein (α -syn) expression [43]. Since it has been suggested that α -syn increases the expression of several TLRs[44], which can act as receptors for the α -syn released by dying neurons and promote microglia activation, it has become clear that a delicate balance exist between let-7 family members, TLRs and α -syn in PD.

Other immune-related miRNAs, such as miR-155 and miR-146, have also been found to be upregulated in AD, thus contributing to the chronicity of the inflammatory response in this disease (Figure 1). MiR-146 was one of the first miRNAs reported to be elevated in the brain regions of AD patients most affected by the disease and to be increased in response to pro-inflammatory mediators such as IL-1 β , TNF- α and $A\beta_{1-42}$ [58,59]. This upregulation was associated with decreased levels of the glycoprotein immune repressor complement factor (CFH), which is a major regulator of the complement response[40], thus suggesting that miR-146 upregulation in AD constitutes a nefarious event. However, this conclusion is now believed to be overly simplistic, since miR-146 seems to exert important immunomodulatory effects regarding microglia and macrophage activation.

Jayadev and colleagues demonstrated that presenilin 2 KO mice have low miR-146 levels in microglia, which results in increased NF- κ B transcriptional activity, impacting

microglial response[41]. However, although miR-146a was found to be upregulated in prion disease mouse brain tissues and in microglia cell lines following TLR2 or TLR4 activation, such increase did not follow the kinetics of cytokine increase, high levels of miR-146a being observed even after cytokine expression returned to base-line[45]. A functional genomic analysis performed in the EOC microglia cell line revealed that miR-146 overexpression leads to downregulation of important phagocytosis and oxidative burst genes, such as iNOS and IL-1 β , and to alterations in downstream mediators of the NF- κ B and JAK-STAT signaling pathways [45]. Altogether, these findings suggest that miR-146 upregulation may not always have the purpose of increasing inflammation but, instead, may reflect an attempt of the immune system to keep it “in-check”.

Contrarily to miR-146, the pro-inflammatory nature of miR-155 is well established in neurodegeneration. This miRNA was found to be upregulated in microglia and astrocytes of the triple transgenic (3xTg) AD mouse model [42], contributing to sustain an M1 phenotype in these cells. MiR-155 upregulation has been related with the activation of both NF- κ B and c-Jun transcription factors and with an increase in several pro-inflammatory mediators, through the downregulation of its target SOCS-1[42], a protein responsible for restricting activation of the NF- κ B and JAK-STAT pathways.

Figure 1

MiR-155 and miR-146a neuroinflammatory mechanisms in Alzheimer's disease (AD). The interaction of amyloid beta (Aβ) fibrils with different receptor complexes at the cell membrane of microglia and astrocytes leads to both cellular internalization and phagocytosis, as well as activation of standard MyD88-mediated inflammatory pathways, culminating in the activation of nuclear factor kappa B (NF-κB) and c-Jun. While c-Jun activity upregulates the expression of miR-155 contributing to suppressor of cytokine signaling 1 (SOCS-1) inhibition and consequent propagation of inflammation, NF-κB-derived miR-146a upregulation can generate an immunomodulatory effect.

Together with other immune-related miRNAs, such as miR-146b, miR-29b, let-7a/b, miR-21 and miR-210, miR-155 was found to be upregulated in ALS mouse models and human patients[46,47], probably as a consequence of TLR2 and TLR4 overactivation. Butovsky and coworkers demonstrated that SOD1 mice display dysfunctional microglia due to downregulation of important transcription factors, upstream regulators and effector proteins[60]. Disease severity in this model was related with miR-155 expression and early upregulation of inflammatory genes. Importantly, these authors showed that treatment with anti-miR-155 oligonucleotides, through intraventricular injection, derepressed microglia homeostatic genes and

prolonged animal survival [60], providing us with one of the first examples of a miRNA-based therapeutic strategy designed to target unchecked inflammation in the brain.

5. Conclusions

Strong evidence supporting the contribution of miRNAs to the regulation of crucial pathways governing immune cell differentiation and activation led to the undeniable conclusion that miRNAs participate in the fine-tuning of innate immune responses. Rapid changes in miRNA expression levels allow immune cells, such as microglia, monocytes and macrophages, to control the strength and duration of these responses, in order to quickly eliminate any threat, while avoiding collateral damage to the healthy tissue.

The strong regulatory role of miRNAs also makes them important players in neurological diseases. MiRNA dysfunction has been linked with neuroinflammation in acute brain injury, infection and neuroimmune and neurodegenerative diseases. Several immune-related miRNAs, including miR-146 and miR-155, exhibit important changes in ischemic stroke, MS, AD, PD and other diseases, which render them promising biomarkers to follow inflammatory processes, both locally and systemically. Similarly to what has been reported for CNS malignancies [61], miRNAs are being increasingly explored as cerebrospinal fluid and blood biomarkers [62]. We have recently observed significant changes in several neuroimmune miRNAs in monocyte populations of AD and Mild Cognitive Impairment (MCI) patients (unpublished results). Interestingly, some of those miRNAs are differently expressed in different stages of dementia, hinting on a possible application of such inflammatory miRNAs in the clinical staging of disease. However, target networks analysis needs to be further improved to fully understand the implications of miRNA deregulations and standardized methods are still required for miRNA expression measurement in a clinical setting.

New miRNA modulation strategies are also currently under development. These strategies aim to keep neuroinflammation under control, potentiating the neuroprotective phenotype of immune cells and reducing their more deleterious features. *In vitro*, a possible beneficial effect of the modulation of inflammatory miRNAs, such as miR-155, has been previously reported [21], illustrating that this kind

of strategy is able to protect neurons from microglia-derived neurotoxicity. Moreover, the modulation of miRNAs to control inflammation in neuronal-associated syndromes, such as retinal endothelial cells hyperglycemia has also been evaluated[63]. Importantly, *in vivo*, the modulation of miR-21 in glioblastoma has shown promising therapeutic outcomes[64] and, although in this study the neuroinflammatory component was not assessed, we believe that, due to the immune-related role of the miRNA, this strategy could also impact the neuroinflammatory milieu. Therefore, although the use of miRNAs as molecular targets and/or therapeutic molecules is still in its early stages, it holds great potential to help control neuroinflammation and change the course of disease.

Acknowledgements

This work was funded by FEDER funds through the Operational Programme Competitiveness Factors - COMPETE - and national funds by FCT - Foundation for Science and Technology - under the project PTDC/BIM-MEC/0651/2012 and strategic project UID/NEU/04539/2013. Joana Guedes is the recipient of a PhD scholarship from FCT (SFRH/BD/51677/2011).

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