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MicroRNAs mediating CNS inflammation: Small regulators with powerful potential

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Abstract

MicroRNAs (miRNAs) are a family of small non-coding RNAs (~22 nucleotides) that fine-tune protein expression by either silencing mRNA translation or directly targeting gene transcripts for degradation. In the central nervous system (CNS), neuroinflammation plays a critical role in brain injury and neurodegeneration. Increasing evidence supports the involvement of miRNAs as key regulators of neuroinflammation. Altered expression or function of particular miRNAs has been identified in various CNS pathological conditions, including neuroinflammation, neurodegeneration, and autoimmune diseases. Several miRNAs have been shown to play a critical role in the microglia-mediated inflammatory response including miR-155 and miR-146a. In this review, we summarize recent advances in the field of miRNAs associated with CNS inflammation, including our studies of unique inflammatory pathways involving miR-155 and miR-146a. We discuss how specific miRNAs influence microglia activation states in response to inflammatory stimuli, and describe the potential of miRNAs as both biomarkers of inflammation and therapeutic tools for the modulation of microglia behavior.

Keywords

microRNA; miR-155; miR-146a; neuroinflammation; p53; c-Maf; presenilin 2

Microglia

Microglia are the specialized resident innate immune cells of the central nervous system (CNS) that play essential roles in development, plasticity and immune surveillance. While innate immune cell function is generally studied in response to injury or pathogen exposure, microglia have been demonstrated to participate in a variety of homeostatic roles in the developing and adult CNS. During homeostasis, microglia may act as sensors of environmental change and perform essential functions including monitoring synaptic activity, promoting neuronal apoptosis, clearing apoptotic debris and synaptic

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pruning (Marín-Teva et al., 2004; Paolicelli et al., 2011; Sierra et al., 2010; Tremblay et al., 2010; Wake et al., 2009). Under basal conditions, microglia have ramified morphology with long and thin processes. These processes are highly motile and continuously survey the surrounding microenvironment (Norden and Godbout, 2013). Continuous sampling of the microenvironment enables microglia to quickly respond to any disorder in homeostasis (Nimmerjahn et al., 2005).

Microglia are the first to respond and propagate inflammatory signals initiated by brain injury and other stressors. Microglia can generate a variety of inflammatory responses, and over time, will transition from initial host response behaviors involved in inflammatory activation to subsequent participation in the resolution of inflammation and promotion of tissue repair. In response to inflammatory stimuli, such as interferon- γ (INF- γ) and/or toll like receptor (TLR) ligands, microglia initially respond by generating pro-inflammatory cytokines and inducing expression of enzymes involved in the generation of reactive oxygen species (ROS) (Yang et al., 2007). This pattern of behavior resembles macrophage “classical” pro-inflammatory activation that promotes the continuation and amplification of an inflammatory response (Figure 1). Classically activated myeloid cells perform functions important for pathogen suppression, such as generation of ROS and secretion of pro-inflammatory mediators. At the time of brain injury, microglia rapidly transform their morphology, release pro-inflammatory cytokines and increase expression of immunomodulatory surface antigens (Weinstein et al., 2010). In contrast, microglia that are exposed to anti-inflammatory signals such as interleukins (IL)-4, -10 or -13, immune complexes, transforming growth factor (TGF)- β and glucocorticoids suppress pro-inflammatory activities and promote behaviours involved tissue remodelling (Kigerl et al., 2009). While microglia are distinct from circulating macrophages in both ontogeny and experience, their expression of most markers of activation differs quantitatively but not qualitatively from that observed in peripheral macrophages (Ponomarev et al., 2013).

The majority of *in vitro* studies utilize rodent neonatal microglial cultures. Although these cells have proliferative potential and have been useful to study several signal transduction and transcriptional response systems, cultured neonatal microglia have a unique morphology and are likely to be functionally distinct from adult microglial cells. Analysis of microglia isolated from different postnatal ages revealed a developmental reorganization of the gene expression profile and varied cellular responses to TLR4 stimulation (Scheffel et al., 2012). Moreover, neonatal microglia exhibit a partially activated phenotype *in vitro*, as indicated by an intermediate expression level of MHC class II and co-stimulatory molecules that is not observed in adult microglia *in situ* (Carson et al., 1998). Gene expression signatures observed in microglia isolated directly from the adult mouse brain are distinct from the global gene expression pattern of cultured neonatal microglia (Butovsky et al., 2014; Hickman et al., 2013). Taken together, these findings suggest that experiments employing cultured neonatal microglia may not accurately depict the response of functionally mature microglia in disease models and confirmation of miRNA functions should be made using acutely isolated adult microglia whenever possible.

MicroRNA

MicroRNAs (miRNAs) are a growing class of small non-coding RNAs (~22 nucleotides) that regulate gene expression post-transcriptionally by targeting the 3' untranslated region (3'UTR) of messenger RNAs (mRNAs). The nomenclature and classification schemes for miRNAs have not yet been finalized, however it is generally considered that miRNAs with identical sequences at nucleotides 2–8 of the mature miRNA belong to the same 'miRNA family' (Bartel, 2009). Although first discovered in the early 1990s as molecules that control the development of nematodes *Caenorhabditis elegans* (Lee et al., 1993), miRNAs were not systematically studied until after the discovery of RNA interference (RNAi). The majority of canonical human miRNAs are encoded by introns of noncoding or coding transcripts, while some miRNAs are encoded by exonic regions. The biogenesis and function of miRNAs are tightly regulated by many transcriptional and posttranscriptional factors, and their dysregulation is often associated with human diseases. MiRNAs are transcribed from primary transcripts (pri-miRNA) from intragenic or intergenic regions by RNA polymerase II or RNA polymerase III (the canonical pathway). Pre-miRNAs are actively transported from the nucleus, after initial processing by nuclear microprocessor complex comprised with Drosha and DGCR8, to the cytoplasm by exportin 5 (EXP5) in complex with Ran-GTP. In the cytoplasm, the pre-miRNAs are processed into mature miRNAs by the Dicer RNase and its cofactors and finally loaded onto the AGO protein to form the effector complex: RNA-induced silencing complex (RISC) (Ha and Kim, 2014). Transcriptional factors, such as p53 and MYC, can regulate miRNA expression positively or negatively (Ha and Kim, 2014). Epigenetic mechanisms, such as DNA methylation and histone modification, also contribute to miRNA gene regulation. Mature miRNAs contribute to the epigenetic regulation of gene expression involved in both normal physiological processes and pathologies (Ponomarev et al., 2011). miRNAs can influence expression of many genes by forming imperfect base pairing with sequences in the 3' UTR of a target mRNA, concurrently interrupting mRNA translation and causing degradation of the targeted mRNA (Bartel, 2009). Since each individual miRNA has multiple targets, a single miRNA has the capacity to concurrently modulate a large number of proteins and thus may exert profound influence on a gene expression network involved in determining a specific pattern of cellular behavior.

MicroRNAs in the CNS

To date, miRNAs are the smallest identified ribonucleic acid carriers of highly specific, genetic regulatory information. They are the most abundant extracellular, highly soluble nucleic acids present in multiple human circulatory fluids and serum, and are capable of spreading genetic signaling information, both homeostatic and pathogenic, among neighboring CNS cells and tissues. Compared with other organs, the brain has a particularly high percentage of tissue-specific and tissue-enriched miRNAs (Kim et al., 2004; Lagos-Quintana et al., 2002). Each of the major cell types within the brain exhibits specific patterns of miRNA expression, with some miRNAs promoting neuronal differentiation, while others clearly support glial differentiation patterns (Jovicic et al., 2013). For example, neuron-enriched miRNAs, miR-376a and miR-434, are also critical for driving neural stem cell differentiation towards neurons, while some glia-enriched miRNAs, miR-223, miR-146a, miR-19, and miR-32 exert an opposite effect (Jovicic et al., 2013). Additionally, microglia

isolated from the adult mouse brain specifically express miRs-99a, -125b-5p and -342-3p. These three miRNAs were not expressed by tissue macrophages from other organs and interestingly were expressed at widely varying levels in microglia populations isolated from different regions of the CNS (Butovsky et al., 2014). These studies suggest that miRNAs are key potential regulators of cell type specific behaviors in the CNS and may be regulated by signals from the surrounding tissue environment and differentially influence the behavior of microglia in a region specific manner.

miRNA regulation of CNS inflammation

In addition to their well-studied roles in CNS cell fate determination, several studies have shown that miRNAs regulate both innate and adaptive immune responses (Baltimore et al., 2008). miRNAs play significant roles in inflammatory activation and the resolution of the phasic pro-inflammatory response as diagramed in Figure 1. Some well-studied miRNA modulators of inflammation have been evaluated in cultured microglia and have been identified as either promoting pro-inflammatory behaviors or associated with deactivation of the pro-inflammatory response. Microarray expression profiling and bioinformatics experiments using cultured murine microglia revealed several miRNAs that may participate in the inflammatory activation of microglia in CNS (Freilich et al., 2013). After exposure to a pro-inflammatory signal (LPS), cultured microglia demonstrate increased expression of miR-155, but decreased expression of miR-689 and miR-124. Treatment with IL-4, a cytokine known to promote alternative activation induced expression of miR-145 and suppressed expression of miR-124 and miR-711. Since miR-124 was suppressed by both types of activation signals, it has been suggested the miR-124 acts to promote microglia quiescence (Caldeira et al., 2014; Ponomarev et al., 2011). Ponomarev et. al. showed that miR-124 is downregulated in microglia in experimental autoimmune encephalomyelitis (EAE). Conversely, knock-down of miR-124 in microglia and macrophages resulted in pro-inflammatory activation *in vitro* and *in vivo* (Bird, 2011; Ponomarev et al., 2011). Overexpression of miR-124 led to downregulation of pro-inflammatory-mediators, such as IL-6, tumor necrosis factor alpha (TNF- α), and inducible nitric oxide synthase (iNOS), as well as increased expression of several proteins associated with the resolution of inflammation and/or tissue repair (Ponomarev et al., 2011). Thus, miR-124 plays an important role in regulating microglia behavior in the CNS.

miR-146a is another well studied regulator of innate immune responses. Pro-inflammatory signaling through the NF- κ B transcriptional pathway leads to increased expression of miR-146a, which subsequently suppresses NF- κ B transcriptional activity (Li et al., 2011), serving as a negative feedback mediator of pro-inflammatory responses. However the impact of these molecules in CNS inflammation is less well understood. To address this issue, several authors have recently published studies profiling miRNA expression specifically in microglia cells and in CNS pathology models (Cardoso et al., 2012a; Jayadev et al., 2013; Lynch, 2009; Su et al., 2014). These studies have converged with others employing a candidate approach to determine if miRNAs that regulate inflammation in peripheral macrophages are also involved modulating microglia behaviors. We have recently evaluated the impact of several miRNAs with known roles in innate immune signaling on microglia behaviors (Jayadev et al., 2013) (Su et al., 2014). These efforts have focused on miRNAs

induced by pro-inflammatory activation of p53 mediated transcription: miR-155, miR-145 and miR34a as well as on how presenlin-2 function influences expression of miR-146a (Table I).

miR-155

miR-155 was identified as a B cell integration cluster (*bic*), which induces B cell leukemia in chickens following activation through viral promoter insertion (Eis et al., 2005). Subsequent studies have shown that transgenic overexpression of miR-155 in B cells generated lymphoma, suggesting that miR-155 is oncogenic (Mashima, 2015). In addition, miR-155 was shown to be upregulated in macrophages, monocytes, and microglia in response to several pro-inflammatory stimuli, such as LPS, IFN γ , and TNF α (Arora et al., 2013; Bala et al., 2011; Wang et al., 2010), suggesting a role for miRNAs as modulators of inflammatory activation in both microglia and macrophages (Table I). miR-155 promotes tissue inflammation by enhancing the production of Th17 cells, and is highly upregulated during macrophage inflammatory responses and in multiple sclerosis lesions (Murugaiyan et al., 2011). Furthermore, miR-155 has been implicated in increasing pro-inflammatory cytokine secretion by targeting SOCS1 mRNA.

We identified a novel pathway which involves miR-155 as a component of the p53-mediated pro-inflammatory network in microglia (Jayadev et al., 2011; Su et al., 2014). Previously we demonstrated that p53 played a role in regulating microglia activation in response to pro-inflammatory cytokines *in vitro* and ischemia *in vivo* (Jayadev et al., 2011). We observed that during recovery from the middle cerebral artery occlusion (MCAO) stroke model, p53 knock out (p53^{-/-}) mice demonstrate an increased number of microglia expressing markers of alternatively activated macrophages. Cultured microglia from p53^{-/-} mice also have increased expression of genes associated with alternatively activated macrophages and demonstrate a blunted pro-inflammatory response to treatment with interferon gamma (IFN γ). We discovered that one media of the p53 effect in microglia was c-Maf, a transcription factor known to promote functional differentiation and anti-inflammatory responses in both lymphocytes and myeloid cells (Su et al., 2014). The Maf family of transcription factors influence cellular differentiation and c-Maf specifically suppresses expression of pro-inflammatory cytokines while promoting expression of anti-inflammatory cytokines (Motohashi et al., 1997). Since the pro-inflammatory miRNA miR-155 has been shown to negatively regulate c-Maf expression in T-cells (Rodriguez et al., 2007), we examined miR-155 expression in p53^{-/-} microglia. We observed that induction of miR-155 by IFN γ treatment was significantly suppressed in cultured p53^{-/-} microglia compared to p53 expressing microglia (Su et al., 2014). We also observed that miR-155 knockout recapitulates a portion of the p53^{-/-} phenotype in microglia both *in vivo* and *in vitro*, suggesting that p53 may act through miR-155 to negatively regulate c-Maf expression (Figure 2A). Meanwhile, c-Maf expression is also transcriptionally regulated by Twist-2, a basic helix-loop-helix (bHLH) transcription factor (Sharabi et al., 2008). We observed that Twist-2 was also upregulated in cultured p53^{-/-} microglia, and hypothesized that p53-dependent suppression of Twist-2 expression also involved a miRNA. We identified two additional p53-dependent miRNAs, miR-34a and miR-145, that act as negative regulators of

Twist-2, and demonstrated that miR-34a and miR-145 mimics can alter both Twist-2 and c-Maf expression in the RAW macrophage cell line (Su et al., 2014).

While we demonstrated that p53 influences c-Maf expression in several paradigms including cultured microglia, murine macrophage cell line (RAW cells) and microglia extracted from normal adult mouse brain, the question remains whether or not these miRNAs modulate inflammatory gene expression *in vivo* during neuroinflammation. To address this, we induced neuroinflammation by brief (15 min.) MCAO which induces an inflammatory response and ischemic preconditioning, but does not cause infarction (Weinstein et al., 2010). We observed that 15 minute ischemia lead to a significant induction of microglia p53 transcriptional activity as early as 3 days following this injury paradigm and sustained for as long as 7 days. To determine if this model of neuroinflammation involves p53-mediated upregulation of miR-155, miR-145 and/or miR-34a in microglia, we obtained forebrain microglia by *ex vivo* flow cytometry 3 and 7 days after 15 minute MCAO. We observed an increase in the level of miR-155 at both 3 and 7 days (Figure 2B and 2D). MiR-145 was not induced but miR-34a was activated at the 7-day time point (Figure 2D). Increased expression of p53 dependent pro-inflammatory miRNAs was accompanied by increased expression of other pro-inflammatory marker genes including IL-1 β and MARCO. In addition, c-Maf mRNA was decreased in microglia from the ischemic side of the brain at both time points (Figure 2C). In summary, these data suggest that neuroinflammation *in vivo* is associated with induction of p53-dependent miRNAs in microglia that suppress c-Maf.

Taken together, we proposed a p53-dependent pro-inflammatory pathway requiring the induction of p53-dependent miRNAs in microglia as illustrated in Figure 2E. In the absence of p53, microglia fail to induce expression of miR-155, a miRNA that directly targets c-Maf mRNA for degradation. Thus, when p53 is activated in microglia by ROS, spontaneous DNA damage, or cellular stress associated with CNS disease and injury, p53 dependent miRNAs suppress c-Maf, thereby suppressing microglia anti-inflammatory and tissue repair behaviors and making it more likely for microglia to adopt pro-inflammatory behaviors that can be injurious to surrounding neural cells.

miR-145

miR-145 was first recognized as a tumor suppressor miRNA that is transcriptionally regulated by p53 and is often co-expressed with miR-143 (Chen et al., 2010). miR-143 and miR-145 form a bicistronic cluster in 5q33.1 region and these two miRNAs have been extensively studied for their role in neoplastic pathways in epithelial cell malignancies (Kent et al., 2014). miR-143/miR-145 was involved in the phenotypic switch of vascular smooth muscle cells and has been associated with atherosclerosis. In the CNS, miR-145 was found to have a significant increase after transient cerebral ischemia in rat (Dharap et al., 2009), while miR-145 level was usually downregulated in cancers. Antagomir-mediated prevention of miR-145 activity led to an increased protein expression of superoxide dismutase-2 (SOD2) in the brain, indicating that miR-145 plays a role in antioxidant defense of the brain (Dharap et al., 2009). In addition, miR-145 was found directly target interferon-beta (IFN β) in the CNS, highlighting its role in IFN β regulated innate immune responses (Witwer et al., 2010). Profiling studies of miRNAs induced by inflammatory activation of microglia

demonstrated increased expression of miR-145 after treatment with IL-4 (Freilich et al., 2013) and treatment of astrocytes with the TLR-4 ligand lipopolysaccharide (LPS) leads to suppression of miR-145 expression (Wang et al., 2015). These studies have been interpreted as suggesting that miR-145 may have anti-inflammatory function. However, they do not specifically demonstrate miR-145 function and do not rule out the possibility that miR-145 expression may be induced as part of a negative feedback loop. Data from other cell types support a pro-inflammatory role for miR-145. For example, miR-145 suppresses SMAD-3, an activator of the anti-inflammatory mediator transforming growth factor-beta (TGF β) (Megiorni et al., 2013) and over-expression of miR-145 in adipocytes promotes secretion of the pro-inflammatory mediator TNF α (Lorente-Cebrián et al., 2014). We observed that miR-145 in microglia negatively regulates Twist2, a c-Maf transcriptional activator (Figure 2). Transfection of miR-145 mimics into the RAW macrophage cell line reduced the level of both Twist2 and c-Maf proteins (Su et al., 2014). However, we did not observe induction of microglial miR-145 *in vivo* up to 7 days following ischemia/reperfusion (Figure 2B and 2D) suggesting that the induction of miR-145 may require a higher threshold of activation or a longer time frame than miR-155.

miR-146

The miR-146 miRNA family consists of two evolutionary conserved miRNA genes: miR-146a and miR-146b (Boldin et al., 2011). miR-146a is an inducible, NF- κ B-regulated miRNA ubiquitously expressed in microglia and astrocytes of the brain and retina (Alexandrov et al., 2014; Boldin et al., 2011; Li et al., 2011). The 5' regulatory region of the miR-146a gene harbors NF- κ B binding sites and its expression is upregulated by pro-inflammatory signals (Lukiw et al., 2008).

Alterations in expression of miR-146a have been observed in several neurodegenerative disorders including Alzheimer's disease (AD), age-related macular degeneration (AMD), and prion disease (PrD) (Alexandrov et al., 2014). It was recently shown in AD mouse models that miR-146a targets several key inflammatory regulators, including the 155 kDa sialic-acid containing glycoprotein immune repressor complement factor H (CFH), the membrane spanning beta-amyloid precursor protein (β APP)-associated TSPAN12, and the inflammation mediator interleukin receptor-associated kinase IRAK-1 (Cui et al., 2010). In A β and TNF- α stressed human microglia cells, miR-146a induction was inversely correlated with the level of inflammation-related proteins, including CFH and IRAK-1 (Li et al., 2011) (Table I).

Differential expression of miR-146a has been reported in several AD studies, suggesting this miRNA as a candidate biomarker and potential therapeutic target. AD occurs in both sporadic and familial forms, and familial AD can be caused by autosomal dominant mutations in amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2). Presenilins are a family of transmembrane proteins that function as the catalytic sub-unit of the γ -secretase intramembrane protease complex that processes β -amyloid (A β) peptide from APP (De Strooper et al., 2012). Defects in the APP processing machinery may promote extracellular A β accumulation into plaques, the hallmark pathological feature of AD. However, most of the identified AD causing mutations in PS1 and PS2 do not clearly

enhance A β generation, suggesting that alternate roles for these proteins may contribute to AD pathogenesis. Interestingly, both PS1 and PS2 appear to have roles in regulating inflammatory responses. We have reported that loss of normal PS2 function is associated with exaggerated microglia pro-inflammatory responses *in vitro* (Jayadev et al., 2010).

A wide variety of substrate molecules are regulated by γ -secretase mediated proteolytic cleavage and could participate in regulation of inflammatory behaviors. To identify pathways by which PS2 regulates microglia pro-inflammatory responses and determine how PS2 dysfunction may lead to altered microglia function, an unbiased miRNA expression array analysis was performed on RNA extracted from murine PS2 knock out (PS2^{-/-}) and wild-type microglia. One candidate identified as differentially expressed in this study was miR-146a. MiR-146a was significantly downregulated in PS2^{-/-} microglia yielding higher levels of its target protein IL-1 receptor associated kinase-1 (IRAK-1) and increased NF κ B transcriptional activity (Jayadev et al., 2013). Altered transcriptional regulation of inflammatory cytokine production by NF- κ B in PS2^{-/-} microglia suggests alterations of inflammatory signaling upstream of cytokine transcription. Therefore, PS2 dysfunction caused by aging or mutations may contribute to neurodegeneration by influencing microglia pro-inflammatory behaviors through misregulation of the anti-inflammatory miRNA, miR-146a.

Interactions between miR-155 and miR-146 may contribute to microglia activation in disease

Emerging evidence shows that miRNAs can work together and play critical regulatory roles that affect neuroimmune functions. miR-155 and miR-146a are commonly shown to act together in modulation of different stages of the innate immune response during inflammation and infection (Elton et al., 2013; O'Connell et al., 2010). Both miR-155 and miR-146a seem to play a fundamental role in the microglial inflammatory profile. While miR-146a acts as a negative regulator of inflammation by suppressing NF κ B transcriptional activity, miR-155 usually acts to potentiate the microglia-mediated pro-inflammatory responses. Monocytes that overexpress miR-146a showed a dampened inflammatory response, and TRAF6 and IRAK1 are the major miR-146a targets that mediate this effect (Boldin et al., 2011). In addition, miR-146a was also shown to negatively regulate expression of pro-inflammatory cytokines, like IL-6 and TNF- α . In contrast, miR-155 enhances pro-inflammatory responses by suppressing expression of anti-inflammatory proteins and may act as a negative feedback regulator of miR-146a. Taken together, by targeting the 3'UTR of mRNAs that encode specific inflammatory mediators, small RNAs like miR-155 and miR-146a significantly impact the magnitude of the subsequent inflammatory response of microglia.

miRNAs regulating inflammatory responses are involved in behavior and disease

The role of miRNAs in neurological disease has been extensively studied. Altered expression of miRNAs has been shown to be not only responsible for gene expression

changes, but also for inducing disease phenotypes including cancer, metabolic disorders and neurological abnormalities. Therefore, use of miRNAs as a disease biomarker and potential therapeutic targets has been strongly advocated.

One recent example of a miRNA biomarker was employed in a study of post-stroke depression (PSD) (Zeng et al., 2011). Clinically significant mood disturbance after stroke, PSD, is one of the most common co-morbidities arising in the period of stroke recovery. PSD is characterized by behavioral changes including mood abnormalities, self-blaming, and sadness and can have a major influence on post-stroke recovery. Expression of miR-210, a miRNA regulated by hypoxia, was downregulated in the plasma of stroke patients and miR-210 levels predicted clinical outcomes including PSD in stroke patients (Zeng et al., 2011).

Another disorder where miRNA biomarkers have been explored is postpartum psychosis (PP), which is thought to belong to the spectrum of bipolar disorders (Weigelt et al., 2013). In this study miRNA expression was profiled in monocytes from PP patients and healthy postpartum women. miR-146a and miR-212 expression were significantly down-regulated in the monocytes of PP patients compared to controls. Furthermore, a correlation between miRNA downregulation and regulatory T cell function was also observed in PP patients. This study demonstrated that changes in miR-146a and miR-212 correlate with the behavioral outcomes of PP, suggesting the hypothesis that the pathophysiology of PP may involve dysregulation of inflammatory responses as well as identifying miRNAs involved in inflammatory regulation as potential disease biomarkers.

Several other miRNAs have been studied in CNS disease models. One example is miR-124, which is predominantly expressed in neurons of the developing and adult brain, spinal cord, and retina, but has also been demonstrated to promote microglia quiescence. In the experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis, miR-124 modulates the activation of macrophages and monocytes in the periphery as well as microglia responses (Ponomarev et al., 2011). In addition, miR-124 dysregulation has been observed in experimental models of chronic pain including rat trigeminal ganglion during inflammatory muscle pain (Kusuda et al., 2011) and in the spinal cord after spinal cord injury (Kynast et al., 2013).

Patients with familial and sporadic forms of amyotrophic lateral sclerosis (ALS) demonstrate increased pro-inflammatory responses both in the peripheral circulation and in affected regions of the CNS. Both profiling and hypothesis driven studies have identified increased expression of miR-155 in ALS patients and in the mutant superoxide dismutase type 1 (SOD1) genetic mouse model of ALS. To determine if this finding was involved in disease pathogenesis, Butovsky and colleagues crossed mice expressing the familial ALS associated SOD1 mutation with mice deficient in miR-155. They observed that several proteins dysregulated in human ALS spinal cord were normalized in SOD1^{mut}/miR-155 knock-out mice compared to SOD1^{mut} mice expressing miR-155. They observed that microglial molecular signatures (P2ry12, Tmem119, Olfml3), transcription factors (Egr1, Atf3, Jun, Fos, Maf), and the upstream regulators (Csf1r, TGFb1, and Tgfbr1), which are essential for microglial survival, were lost in SOD1 mice, and expression of miR-155 was

increased. Genetic depletion of miR-155 also restored the normal microglia and monocyte molecular signatures, delayed disease progression and significantly prolonged survival of SOD1 mice (Butovsky et al., 2015). These findings suggest that altering expression of a single miRNA may have a profound effect on disease phenotype and that regulation of miR-155 is a potential therapeutic target for ALS.

Conclusions and future directions

The above-mentioned results highlight the prominent role of miRNAs in the regulation of inflammatory responses in the CNS and suggest new possibilities for development of anti-inflammatory therapies. Although the precise contribution of microglia to CNS inflammation and neurodegeneration remains to be fully elucidated, targeting the behavior of microglia has been suggested as a potential novel therapeutic strategy for a wide variety of CNS disorders. Microglia function could be directed toward removing amyloidogenic proteins, suppressing autoimmune attacks on the CNS, fighting CNS viral infection, or promoting tissue repair functions rather than neurotoxic inflammation in response to acute CNS injury or neurodegenerative diseases. Given the important roles for miRNAs in regulating gene expression during inflammation, we hypothesize that the power of miRNAs could be harnessed to influence microglia activity and modulate the neuroinflammatory response in CNS disease. Using appropriate gene therapy tools, miRNA modulation could be an interesting and promising strategy to fine-tune the immune response, skewing microglia behavior according to the specific requirements for each disease setting. We further suggest that miR-155 and miR-146a are two specific miRNA molecules with demonstrated roles in microglia that are likely to serve as important future biomarkers of disease and potential therapeutic targets in several CNS disorders.

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Highlights

1. Microglia behavior is influenced by microRNAs.
2. MiR-155 is a major regulator of pro-inflammatory responses in microglia.
3. Activation of p53 in microglia induces expression of pro-inflammatory miRNAs.
4. Presenilin 2 influences basal expression of miR-146a in microglia.

Figure 1. MicroRNAs involved in microglia inflammatory responses

Microglia function as the innate immune cells of the CNS, responding to tissue injury and pathogens with distinct phases of pro-inflammatory behavior followed by deactivation and tissue repair phases. Under physiological conditions microglia exist in a homeostatic state characterized by a small cell body and thin highly ramified processes that continuously monitor the microenvironment. When an insult (ischemia, trauma, pathogens, or DNA damage) is detected, microglia initially transform their morphology and molecular signature, leading to the initiation of the pro-inflammatory response. Pro-inflammatory microglia demonstrate increased miR-155 and decreased miR-124 and miR-146a. These miRNA changes promote NF κ B transcriptional activity and expression of pro-inflammatory molecules. After induction of pro-inflammatory mediators, miRNA expression shifts to downregulation of miR-155 and upregulation of miR-146a and miR-124. This promotes a transitional inflammatory state where expression of anti-inflammatory molecules (IL-10, TGF- β , c-Maf) ensues. Upregulation of anti-inflammatory mediators promotes microglia tissue repair functions and miRNA-mediated reduction of proinflammatory transcriptional regulators and upregulation of molecules involved in tissue repair.

Figure 2. p53-dependent miRNA expression Modulates c-Maf Expression in Microglia

(A) p53 is activated in microglia by ROS, spontaneous DNA damage, or cellular stress associated with CNS disease and injury. Once activated, p53 promotes expression of the BIC gene that codes for miR-155, which targets c-Maf for degradation, promoting microglia pro-inflammatory functions. In the absence of p53, microglia fail to induce expression of miR-155. (B) Expression of miR-155, miR-145, and miR-34a in murine adult microglia isolated by *ex vivo* flow cytometry 3 days after a 15 minute transient MCAO. MiRNA levels were normalized to small-RNA controls, Sno202 and Sno234 RNAs (from Su et al., 2014).

(C) Expression of the anti-inflammatory gene c-Maf relative to housekeeping gene expression in microglia extracted 3days following 15 minute transient MCAO (from Su et al., 2014). (D) Expression levels of miR-155, miR-145, and miR-34a in murine microglia extracted by *ex vivo* flow cytometry 7 days after 15 minute transient MCAO. MiRNA levels were normalized as in (B) (* $p < 0.01$ by two-way ANOVA). (E) Our proposed model for p53 modulation of c-Maf expression. p53-mediated transcriptional activity is required for the induction of miR-155, miR-145 and miR-34a. While miR-155 directly targets c-Maf mRNA for degradation, miR-145 and miR-34a target the transcriptional regulator Twist2, for suppression. Both miRNA mediated regulatory pathways (direct and indirect) downregulate the expression of the anti-inflammatory transcription factor c-Maf. Therefore, when p53 is activated in microglia, the effectors upregulated by p-53 transcriptional regulation together to suppress the expression of c-Maf, suppressing the anti-inflammation and tissue repair behaviors of microglia and making it more likely for microglia to adopt pro-inflammatory behaviors that can be injurious to surrounding neural cells.

Figure 3. Presenilin 2 (PS2) signaling in microglia modulates miR-146a levels

PS2 influences NF κ B transcriptional activity in microglia through miR-146a(A) Functional PS2 expression in microglia maintains miR-146a levels, and suppresses NF κ B transcriptional activity, enabling proper function of this negative feedback mechanism. (B) PS2 deficient microglia show decreased miR-146a expression levels, increased IRAK1 mRNA and protein levels, increased NF κ B transcriptional activity and increased pro-inflammatory cytokine expression.

Table I

MiRNAs with demonstrated function in microglia innate immune signaling.

	Mature sequence & miRBase ID (Mus musculus)	Functions in CNS and periphery	Target genes	References
miR-155	5'UUAAGCUGAAUUGUGAUAGGGU3' (MI0000177)	Hematopoiesis	SPI1(PU.1)	(Hu et al., 2010)
		Microglia Differentiation	IRF8	(Pareek et al., 2014)
		Innate Immune response	SOCS1 c-Maf	(Cardoso et al., 2012b) (Su et al., 2014)
miR-146	5'UGAGAACUGAAUCCAUGGGU3' (MI0000170)	Hematopoiesis	NF- κ B IL-6	(Zhao et al., 2013)
		Cell fate determination	TRAF6 STAT1	(Baumjohann and Ansel, 2013)
		Innate Immune response	IRAK1/2 CFH TSPAN IL-1 β	(Li et al., 2011) (Jayadev et al., 2013) (Cui et al., 2010) (Iyer et al., 2012)
miR-145	5'GUCCAGUUUCCAGGAAUCCU3' (MI0000169)	Tumor suppressor	BNIP3	(Chen et al., 2010)
		Antioxidant defense in CNS	SOD2	(Dharap et al., 2009)
		Innate Immune response	IFN β Twist2	(Witwer et al., 2010) (Su et al., 2014)
miR-34a	5'UGGCAGUGUCUAGCUGGUUGU3' (MI0000584)	Innate Immune response	Twist2	(Su et al., 2014)
		Tumor suppressor	AKT/mTOR	(Rathod et al., 2014)