

● INVITED REVIEW

Immunomodulators and microRNAs as neurorestorative therapy for ischemic stroke

Bridget Martinez¹, Philip V. Peplow^{2,*}

¹ Department of Molecular and Cellular Biology, University of California, Merced, CA, USA

² Department of Anatomy, University of Otago, Dunedin, New Zealand

How to cite this article: Martinez B, Peplow PV (2017) Immunomodulators and microRNAs as neurorestorative therapy for ischemic stroke. *Neural Regen Res* 12(6):865-874.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Abstract

Most of all strokes are ischemic due to occlusion of a vessel, and comprise two main types, thrombotic and embolic. Inflammation and immune response play an important role in the outcome of ischemic stroke. Pharmaceutical and cell-based therapies with immunomodulatory properties could be of benefit in treating ischemic stroke. Possible changes in microRNAs brought about by immunomodulatory treatments may be important. The pharmaceutical studies described in this review have identified several differentially regulated miRNAs associated with dysregulation of mRNA targets or the upregulation of several neuroprotective genes, thereby highlighting the potential neuroprotective roles of specific miRNAs such as miR-762, -1892, -200a, -145. MiR-124, -711, -145 are the strongly associated miRNAs predicted to mediate anti-inflammatory pathways and microglia/macrophage M2-like activation phenotype. The cell-based therapy studies reviewed have mainly utilized mesenchymal stem cells or human umbilical cord blood cells and shown to improve functional and neurological outcomes in stroke animals. MiR-145 and miR-133b were implicated in nerve cell remodeling and functional recovery after stroke. Human umbilical cord blood cells decreased proinflammatory factors and promoted M2 macrophage polarization in stroke diabetic animals.

Key Words: ischemic stroke; immunomodulators; pharmaceutical therapies; cell-based therapies; microRNAs; animal models

Introduction

Stroke is a potentially fatal or highly debilitating condition with multiple consequences for patients and their families, as well as healthcare providers and society. It is the second most common cause of death worldwide and the leading cause of disability in adults. Approximately 80% of all strokes are ischemic due to occlusion of a vessel, and comprise two main types, thrombotic and embolic. Thrombotic stroke accounts for almost 50% of all strokes. Thrombolysis and/or mechanical thromboectomy may be considered in patients with severe neurological symptoms and early presentation within 3–6 hours of symptom onset. Many stroke patients are not treated by intravenous thrombolysis because of the narrow window of time for treatment. In patients with minor stroke, while the immediate brain damage and associated symptoms are limited, there is a significant risk of a major stroke within 90 days (Emberson, 2014) even when taking aspirin antiplatelet therapy (Wang et al., 2013). Early intervention in stroke is desirable to limit the serious consequence of a major stroke and also to lower the risk of a minor stroke progressing to a seriously disabling stroke. Also extending the window for therapeutic intervention would enable a greater number of stroke patients to be treated.

Evidence indicates that inflammation and immune response play an important role in the outcome of ischemic stroke (Famakin, 2014). Inflammation after stroke involves leukocyte infiltration in brain parenchyma that contributes to cerebral damage. Peripherally derived mononuclear phagocytes, T lymphocytes, natural killer (NK) cells, and polymorphonuclear leukocytes,

which produce and secrete cytokines, can all contribute to central nervous system (CNS) inflammation and gliosis (Brea et al., 2009). Blood-derived leukocytes and resident microglia are the more activated inflammatory cells, accumulating in the brain tissue after cerebral ischemia, leading to inflammatory injury (Akopov et al., 1996). Microglia, the major source of cytokines and other immune molecules of the CNS, are the first non-neuronal cells that respond to CNS injury, becoming phagocytic when fully activated by neuronal death. As cerebral inflammation is one of the earliest events in stroke, early intervention to modify the immune response may have a beneficial effect. We have searched the PubMed database for studies of pharmaceutical and cell-based therapies with immunomodulatory properties that have been used for treating ischemic stroke. We have also examined for possible changes in microRNAs brought about by immunomodulatory treatments on account of a possible role of microRNAs in ischemic stroke (Martinez and Peplow, 2015).

Immunomodulatory Therapies for Ischemic Stroke

Pharmaceutical therapies

The pharmaceutical therapies were with niacin, cytosine-phosphate-guanine (CpG), thymosin beta 4 (Tβ4), lipopolysaccharide (LPS), interleukin-4 (IL-4), MK801 (an NMDA antagonist), and microRNA-146a (miR-146a). These have all been shown to have immunomodulatory properties (niacin: Ganji et al., 2009; CpG: Tan et al., 2009; Tβ4: Badamchian et al., 2003; LPS: Jacobs

*Correspondence to:

Philip V. Peplow,
phil.peplow@otago.ac.nz.

doi: 10.4103/1673-5374.208540

Accepted: 2017-05-08

et al., 1981; IL-4: Splawski et al., 1989; MK801: Esposito et al., 2011; miR-146a: Li et al., 2010). The seven animal studies utilizing these pharmaceutical agents are summarized in **Table 1**.

Niacin treatment of middle cerebral artery occlusion (MCAO) normal rats reduced infarct volume and improved neurological outcomes. It decreased apoptosis, attenuated tumor necrosis factor- α (TNF- α) expression, and increased vascular endothelial growth factor (VEGF), phosphoinositide 3-kinase (PI3K)/Akt activity in the ischemic brain (Shehadah, 2010). Conditioning with CpG prior to stroke in mice differentially regulated several miRNAs that were associated with neuroprotective genes (Vartanian et al., 2015). T β 4 upregulated miR-200a level in the rat ischemic brain and may induce Akt activation and protect brain cells from brain ischemia-mediated apoptosis (Santra et al., 2016). Under LPS stimulation of mouse microglia, miR-155 was the most significantly upregulated miRNA and regulates the signal transducer and activator of transcription 3 signaling pathway enabling the late phase response to M1-skewing by LPS stimulation (Freilich et al., 2013; **Figure 1**). In IL-4 stimulated microglia, miR-145 was the most increased miRNA (Freilich et al., 2013). MiR-145 potentially regulates peripheral monocyte/macrophage differentiation and facilitates the M2 phenotype in microglia/macrophages by IL-4 stimulation (Liu et al., 2016; **Figure 1**). Treatment with MK801 reduced the infarct volume in rat ischemic brain and caused alterations in miRNA profile in brain and blood compared to control rats. MiR-132 was upregulated in MK801-treated rats (Lim et al., 2010). Elevation of miR-146a in mouse oligodendrocyte precursor cells (OPCs) promoted their differentiation, while in neural progenitor cells (NPCs), miR-146a enhanced differentiation of these cells into neuronal and oligodendrocyte lineage cells (Liu et al., 2017).

Cell-based therapies

The cell-based therapies were with mesenchymal stem cells (MSCs), human umbilical cord blood cells (HUCBCs), and endothelial progenitor cells (EPCs). They have all been associated with immunomodulatory properties (MSCs: Gao et al., 2016; Zhao et al., 2016; HUCBCs: Yu et al., 2009; EPCs: Nuzzolo et al., 2014; Bartaula-Brevik et al., 2016). The studies utilizing these cells are summarized in **Table 2**. The MSCs were isolated from bone marrow stroma, while HUCBCs and EPCs were obtained commercially. Human cord blood is a source of MSCs (Roura et al., 2012) and EPCs (Lin et al., 2011; Yoder, 2012).

MSC therapy

Four animal studies and one clinical trial were found. Administration of MSCs had positive benefit after stroke in normal rats (Chen et al., 2001a), and transfecting MSCs with miR-133b increased functional outcomes with exosomes-enriched extracellular particles being released from MSCs and transferred to adjacent astrocytes and neurons (Xin et al., 2013). One study showed that MSC treatment after stroke in type 1 diabetes mellitus (T1DM) rats did not improve functional outcomes (Chen et al., 2011). Interestingly treatment with MSCs from T1DM rats improved functional outcome and promoted neurorestorative effects in stroke T1DM rats (Cui et al., 2016). The neurorestorative effects were decreased by administration of MSCs from T1DM rats transfected with miR-145 (Cui et al., 2016). In a phase 1/2a clinical trial, administration of SB623 cells was shown to be safe and improved clinical outcome at 12 months in patients with stable chronic

stroke (Steinberg et al., 2016).

HUCBC therapy

Five animal studies were found. HUCBC treatment after stroke of normal rats improved functional recovery, with the HUCBCs surviving and migrating after entering the brain (Chen et al., 2001b). Some HUCBCs were reactive for the astrocyte marker glial fibrillary acidic protein and the neuronal markers NeuN and microtubule-associated protein 2 (Chen et al., 2001b). In stroke rats with type 2 diabetes mellitus (T2DM), HUCBC treatment increased white matter and vascular remodeling, decreased proinflammatory factors Toll-like receptor 4 (TLR4), matrix metalloproteinase 9 (MMP-9), and RAGE expression, and promoted M2 macrophage polarization in the ischemic brain (Yan et al., 2015). Interestingly, HUCBC treatment in T2DM mice after stroke increased miR-126 expression in blood serum and ischemic brain tissue, and miR-126 may contribute to HUCBC-induced neurorestorative effects (Chen et al., 2016). Increased functional recovery occurred in T1DM rats treated with HUCBCs post-stroke, with increased white matter and vascular remodeling in the ischemic brain and increased angiopoietin 1 (Ang1) and decreased RAGE expression in the ischemic boundary zone (IBZ) (Yan et al., 2014). Administration of CD34⁺ or CD34⁻ cells to spontaneously hypertensive rats after stroke improved functional and neurological outcomes (Boltze et al., 2008).

EPC therapy

One animal study was found. Administration of EPCs transfected with miR-145 promoted cell proliferation and migration and recanalization of arterial thrombosis in normal mice post-stroke (Chen et al., 2015).

Neurorestorative Effects in Stroke of Pharmaceutical and Cell-Based Therapies with Immunomodulatory Properties

Astrocytes and microglia are immune cells of the brain and elicit an inflammatory response by the production of inflammatory mediators (Ransohoff and Brown, 2012). Proinflammatory cytokines are increased during brain ischemia and lowering the levels of these cytokines has been shown to ameliorate ischemic brain injury (Lin et al., 2016; Shu et al., 2016). Cytokines regulate the expression of brain endothelial miRNAs that either promote or inhibit inflammatory pathways to orchestrate neuroinflammation in the brain (Lopez-Ramirez et al., 2016). Proinflammatory cytokines alter the levels of several important miRNA clusters. Members of the miRNA family have been shown to mediate many biological effects including induced cell proliferation and decreased apoptosis (Lopez-Ramirez et al., 2016). MiRNAs play an important role in the regulation of adult neurogenesis, and form an important class of epigenetic regulators that contribute to chronic inflammation in microglia of the brain causing the progression of neurological diseases such as ischemic stroke. Cytokines are likely to be involved in changes in brain capillaries with age and diabetes, as shown for renal capillaries (Bianchi et al., 2016), and increased risk of stroke.

The pharmaceutical studies described in this review have identified several differentially regulated miRNAs associated with dysregulation of mRNA targets or the upregulation of several neuroprotective genes, and thereby implicating these miRNAs in neuroprotection. These have included miR-762, -1892, -200a,

Table 1 Studies of pharmacological therapies with immunomodulatory properties to treat stroke

Pharmacological therapy, reference	No. of animals, gender, ages, treatment	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Niacin, Shehadah et al. (2010)	Adult Wistar male rats, 270–300 g subjected to right MCAO for 2 hours using intraluminal suture. MCAO rats were treated with niacin 20 mg/kg ($n = 8$), 40 mg/kg ($n = 8$), or 80 mg/kg ($n = 8$) by gavage at 2 and 24 hours after MCAO. Functional tests were performed before MCAO and at 1, 3 and 7 days after MCAO. Rats were sacrificed at 7 days after MCAO.	Control, MCAO rats treated with water by gavage at 2 and 24 hours after MCAO.	MIRNA expression in brain cortical tissue was measured in saline-treated and CpG-preconditioned mice 3, 24 and 72 hours after injection and 3 and 24 hours after stroke. Microarray analysis showed minimal changes in miRNA at the time points before stroke. After stroke, an increased number of miRNAs was altered at 3 and 24 hours in both treatment groups. All miRNAs regulated at 3 hours after MCAO was also regulated at 24 hours after MCAO. The largest number of miRNAs was regulated 24 hours after MCAO and these showed the greatest magnitude of change. 57 miRNAs were regulated in CpG-preconditioned mice compared with 73 miRNAs in saline-treated animals	MCAO rats treated with niacin 40 mg/kg had significantly improved functional recovery compared to MCAO control. Marginally improved functional outcome was detected in MCAO rats treated with Niaspan 20 mg/kg. The only significant decrease in infarct volume was in MCAO rats treated with niacin 40 mg/kg. Niacin 40 mg/kg decreased the number of TUNEL-positive cells and cleaved caspase-3 expression in ischemic brain of MCAO rats. Niacin 40 mg/kg also attenuated TNF- α expression and increased VEGF, PI3K/Akt activity in the ischemic brain.	Niacin treatment at 2 hours after MCAO reduced infarct volume and improved neurological outcomes. The neuroprotective effects of niacin were associated with reduction of apoptosis and attenuation of TNF- α expression and increased VEGF, PI3K/Akt activity in the ischemic brain.
Cytosine-phosphate-guanine (CpG), Vartanian et al. (2015)	Adult C57Bl/6j male mice, 8–12 weeks, injected s.c. with 1.6 mg/kg CpG 72 hours before MCAO, and subjected to MCAO using intraluminal suture for 45 minutes.	Controls received saline 72 hours before MCAO.	MIRNA expression in brain cortical tissue was measured in saline-treated and CpG-preconditioned mice 3, 24 and 72 hours after injection and 3 and 24 hours after stroke. Microarray analysis showed minimal changes in miRNA at the time points before stroke. After stroke, an increased number of miRNAs was altered at 3 and 24 hours in both treatment groups. All miRNAs regulated at 3 hours after MCAO was also regulated at 24 hours after MCAO. The largest number of miRNAs was regulated 24 hours after MCAO and these showed the greatest magnitude of change. 57 miRNAs were regulated in CpG-preconditioned mice compared with 73 miRNAs in saline-treated animals	40 miRNAs were downregulated 24 hours after stroke in both CpG- and saline-treated animals. 11 miRNAs were upregulated 24 hours after stroke in both CpG- and saline-treated animals. 22 miRNAs were upregulated in saline-treated animals and downregulated in CpG-treated animals. The relationships between the suppressed miRNAs and their inverse target genes indicated that miR-762, -1892, -1224 and -1894-3p all have three or more inversely related targets suggesting that they may highly influence the genomic response to stroke in preconditioned mice. By RT-PCR, a significant decrease was found in miR-762 and miR-1894-3p expression in CpG-preconditioned animals compared with saline-treated animals. MiR-1892 showed a trend for suppressed expression in CpG-preconditioned animals compared with saline-treated animals while miR-1224 showed no difference between the treatment groups. Nupr1 was targeted by three suppressed miRNAs, including miR-762 and miR-1892. Using RT-PCR, Nupr1 was significantly increased in CpG- preconditioned versus saline-treated animals 24 hours after stroke.	CpG preconditioning did not alter miRNA expression before stroke. After stroke, differentially regulated miRNAs between CpG- and saline-treated animals were associated with the upregulation of several neuroprotective genes, implicating these miRNAs in genomic reprogramming increase neuroprotection.
Thymosin beta 4 (T β 4), Santra et al. (2016)	Adult Wistar male rats 300–400 g subjected to right MCAO using intraluminal suture. Neuroprogenitor cells were obtained by surgical dissection of the subventricular zone (SVZ) of adult male Wistar rats. SVZ cells were cultured in neurosphere growth medium. Primary embryonic oligodendrocyte progenitor cells (OPCs) were isolated on embryonic day 17 from embryos collected from a pregnant Wistar rat. The cells ($10^7/cm^2$) were treated with 0, 25, 50 or 100 ng/ml T β 4 for 7 days. For <i>in vivo</i> treatment, T β 4 (12 mg/kg) was injected i.p. 24 hours after MCAO. The injection was continued every 3 days for four additional doses (12 mg/kg).	Controls for <i>in vitro</i> studies 0 ng/mL T β 4 for 7 days. For <i>in vivo</i> studies, MCAO with 0 mg/kg T β 4 (<i>i.e.</i> , vehicle only) injected i.p. 24 hours after MCAO.	T β 4 treatment markedly upregulated miR-200a, -200b and -429 <i>in vitro</i> in a dose-dependent manner, but rarely affected miR-200c and miR-141. These data were consistent with <i>in vivo</i> upregulation of miR-200a level in brain tissue of rats subjected to MCAO after T β 4 treatment. In contrast, T β 4 treatment had no effect on the expression level of miR-200b, -200c, -429 and -141. T β 4 treatment not only upregulated miR-200a expression, but also downregulated <i>in vivo</i> miRNA expression of Mig-6 target. MCAO had no effect on Mig-6 expression. T β 4 treatment inhibited apoptosis of SVZ-neurospheres and primary OPCs exposed to oxygen-glucose deprivation (OGD) <i>in vitro</i> for 4 hours.	RT-PCR and western blot revealed that T β 4 treatment significantly upregulated EGFR protein levels in a dose-dependent manner. EGFR signaling is required for myelin basic protein (MBP) synthesis and OPC differentiation into mature oligodendrocytes. T β 4 downregulated Mig-6 expression in SVZ progenitor cells and OPCs. Mig-6 is a negative regulator of EGFR. <i>In vivo</i> upregulation of miR-200a may activate EGFR signaling.	T β 4 treatment caused significant downregulation <i>in vivo</i> and <i>in vitro</i> of miR-200 targets <i>e.g.</i> , GRB2, FOG2 and Pten. T β 4 treatment may induce Akt activation and protect brain cells from MCAO-mediated apoptosis.

Table 1 Continued

Pharmacological therapy, reference	No. of animals, gender, ages, treatment	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Lipopolysaccharide (LPS), interleukin-4 (IL-4), Freilich et al. (2013)	Pregnant CD-1 female mice. Primary microglia isolated from brain tissue of P0 or P1 pups. Magnetic bead separation performed to increase the purity of the microglia. The cultured primary microglia were placed in serum-free media overnight and then stimulated with 100 ng/mL LPS or 20 ng/mL IL-4 for 4 hours.	Controls were cells treated with PBS for 4 hours.	LPS (M1 polarization)-treated primary microglia differentially regulated a total of 4,275 genes, with the IL-6 gene the most upregulated gene and the expression of many other canonical M1-marker genes in peripheral macrophages <i>e.g.</i> , IL-12 α , NOS2, IL-1 β are highly enhanced by LPS stimulation of microglia. The expression of TNF- α gene was also induced. IL-4 (M2 polarization)-treated primary microglia differentially regulated a total of 1,606 genes. The chinese-3 like 3 (CHI3L3) gene, a known M2-marker in peripheral macrophages, was the most upregulated gene, and enhanced expression of other M2-markers including RETNLA (FIZZ1), and arginase 1 (ARG) also observed. During LPS stimulation, the expression of 47 out of 722 miRNAs was significantly changed, 12 were significantly increased and 35 were significantly reduced. IL-4 stimulation significantly changed the expression of 44 miRNAs, 16 were increased and 28 were decreased.	Under LPS stimulation of microglia, miR-155 was the most significantly upregulated miRNA (9.69-fold increase vs. control). Other significantly increased miRNAs in M1 polarized microglia include miR-297b-5p, -302c, -191*, -10b, -105, -495, -7a, -670, -1934, -201, -200c*, -214*, -673, and -141*. There was a significant reduction in the expression of miR-1928, -3474, -383, -192, -1939, -466b-3p, -2132, -1901, -762, -689, -128*, -542, -700, -219, and -705. In IL-4 stimulated microglia, miR-145 was the most increased miRNA (2.66-fold), together with miR-297b-5p and miR-214 (all > 2.0-fold increase). There was significant reduction of expression of miR-1939, -711, -124, -200a*, -762, -2138, -2861, -1971, -133a, -2132, -2135, -2133, -124, -2137, and -325.	MiR-155, the most strongly up-regulated miRNA, regulates the signal transducer and activator of transcription 3 signaling pathway enabling the late phase response to M1-skewing by LPS stimulation. Reduced expression in miR-689 and miR-124 are associated with dis-inhibition of many canonical inflammatory pathways. MiR-124, miR-711, miR-145 are the strongly associated miRNAs predicted to mediate anti-inflammatory pathways and M2-like activation phenotype. Reductions in miR-711 and miR-124 may regulate inflammatory signaling pathways and peroxisome proliferator-activated receptor-gamma pathway. MiR-145 potentially regulates peripheral monocyte/macrophage differentiation and facilitates the M2-skewing phenotype by IL-4 stimulation.
IL-4, Liu et al. (2016)	Wild-type (WT) or IL-4 knockout (KO) male C57/BL6 mice, 8–10 weeks, 25–30 g, were subjected to transient MCAO for 60 minutes. WT MCAO mice received intracerebral infusion of IL-4 (60 ng/dl, 0.5 μ L/hour) starting 6 hours after MCAO and continued to 7 days after MCAO. Neurobehavioral tests performed during both acute and recovery stages after MCAO. Animals sacrificed at 35 days after MCAO.	Controls, sham operated animals without MCAO received vehicle or IL-4. WT MCAO mice received intracerebral infusion of PBS.	IL-4 in the mouse brain increased slightly but not significantly between 1 and 7 days after MCAO and then decreased on day 14. Although the sensorimotor outcomes were comparable between IL-4 KO and WT mice early after MCAO, the IL-4 KO mice exhibited much slower recovery over time, resulting in significant deterioration of behavioral performance at late stages after stroke. IL-4 KO mice exhibited greater infarct volumes than WT mice at 5 days after MCAO as measured by loss of microtubule-associated protein 2 (MAP2) and NeuN. The neuronal tissue loss in IL-4 KO mice was not significantly different from WT mice at 14 and 21 days after MCAO. In IL-4 KO mice, there was an enhancement in M1 polarization and cerebral inflammation that extended into the late stages after MCAO. IL-4 KO mice exhibited reduced expression of M2-like markers in the ischemic brain at both 5 and 14 days after MCAO. IL-4 administration to WT MCAO mice did not improve neurobehavioral performance 1 to 7 days after MCAO, but improved performance at late stages (14–35 days) after MCAO. No significant effect of IL-4 treatment on neuronal tissue loss at 14 and 35 days after MCAO was found.	IL-4 improved long-term functional recovery after MCAO, perhaps through M2 phenotype induction in microglia/macrophages.	

Table 1 Continued

Pharmacological therapy, reference	No. of animals, gender, ages, treatment	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
NMDA antagonist MK801, Lim et al. (2010)	Adult male Sprague-Dawley rats 200–300 g. Transient MCAO was induced by intraluminal filament occlusion of left MCA for 1 hour. MK801 was injected i.p. in each rat in the MK801 treatment group. MCAO rats were sacrificed at 0 (at time of reperfusion), 1, 3, 6, 12, 18, 24 and 48 hours upon reperfusion and brain samples collected. To determine changes in miRNA pattern with MK801 treatment, MK801 was administered i.p. in three doses 2.5 µg/g body weight 30 minutes prior to MCAO followed by 1.25 µg/g body weight at 6 and 14 hours post-occlusion.	Control for effect of MK801 treatment on infarct volume was MCAO rats sacrificed at 0 hour. Control for effect of MK801 treatment on miRNA profile was MCAO rats after 24 hours of reperfusion.	Infarct volume gradually increased to a maximum at 24 hours and then decreased at 48 hours. The 24 hours reperfusion time point was chosen for MK801 treatment. Infarct volume of MK801 treated rats was reduced compared to that of untreated rats at 24 hours after MCAO. The miRNA profile at the 24 hours reperfusion time point changed considerably upon MK801 treatment. 156 miRNAs were differentially regulated in rat brain tissue between MCAO and MCAO + MK801 treatment. Among them, 44 miRNAs were upregulated and 47 miRNAs were downregulated in MK801-treated rats compared to MCAO. In blood, 100 and 133 miRNAs were significantly expressed (vs. normal) in MCAO and MCAO+MK801 rats. Among these, 119 miRNAs were differentially regulated in MCAO + MK801 compared to MCAO.	To identify miRNAs that could possibly lead to a reduction in infarct volume, the profile of miRNAs that were downregulated after 48 hours of reperfusion was analyzed. Only miR-137, -136, -153, -190, -196a, -301a, -322*, -33, -347, -378*, -411, -487b, -76a and -879 were downregulated in ipsilateral brain tissue after 48 hours of reperfusion. When compared to 24 hours time point, miR-190, -196a, -322*, -33, -411 and -487b were highly downregulated (27-, 52-, 35-, 195-, 42- and 12-fold, respectively) at 48 hours time point. Upon MK801 treatment, the angiogenic miRNAs, miR-126, -15b, -16, -221, -222, -27b, -92a, showed a mixed expression. MiR-143 and miR-145 that are closely integrated in vascular homeostasis and maintenance of smooth muscle showed opposing expression in MK801-treated rats (vs. MCAO). MiR-181b&d (SLR -0.71 and -0.89) and miR-132 (SLR 0.12) were inversely regulated in MK801 treatment. MiR-181b promotes matrix metalloproteinases MMP2 and MMP9 activity, and induces growth by inhibiting TIMP3, while miR-132 targets MMP-9. A significant number of miRNAs found in the blood of MCAO and MCAO+MK801 rats appeared to have originated from the brain. Stress-associated miRNAs such as miR-195 and miR-122 were upregulated while miR-92a that inhibits angiogenesis and miR-375 that inhibits exocytosis were downregulated upon MK801 treatment (vs. MCAO).	Changes in miRNA expression occurred quite immediately following brain ischemic injury. Treatment with NMDA antagonist, MK801, reduced the infarct volume in MCAO rats and caused alterations in miRNA profile in brain and blood compared to MCAO rats. MiR-132 was upregulated in MK801-treated rats (vs. MCAO). It is possible that treatment with MK801 may have induced the expression of glutamate receptors in neurons for additional synaptic plasticity and dendrite formation. Brain derived neurotrophic factor (BDNF) that significantly upregulates miR-132 is known to promote the expression of the glutamate receptors in neurons.
miR-146a, Liu et al. (2017)	Neural progenitor cells (NPCs) dissociated from the SVZ of the lateral ventricle of adult mice. Cells were cultured in growth medium, and the generated neurospheres (primary spheres) passaged by mechanical dissociation and reseeded as single cells. SVZ cells from ischemic brain extracted 7 days after MCAO of male Wistar rats, 3–4 months. MCAO was performed by placing an embolus at the origin of the right MCA. Primary OPCs isolated from embryonic day 18 embryos from pregnant Wistar rats. The miRNA or synthetic small interference RNA (siRNA) was introduced into OPCs by the N-Ter nanoparticle transfection system. To knock down the interleukin-1 receptor-associated kinase 1 (IRAK1) expression, a siRNA against IRAK1 was introduced and a scrambled siRNA as a negative control, which was mixed with N-Ter buffer as the miRNA mimics. MiRNA or siRNA were delivered into SVZ NPCs using nucleofector electroporation.	Stroke significantly upregulated miR-146a in cells in the ipsilateral SVZ and corpus callosum compared to that in homologous regions of the contralateral hemisphere. Transfection of OPCs with miR-146a mimics considerably elevated miR-146a levels compared to OPCs transfected with mimic control, cell-miR-67.	Immunocytochemistry showed that elevation of miR-146a in OPCs resulted in a significant increase in the number of myelin basic protein (MBP)-positive oligodendrocytes. Attenuation of endogenous miR-146a expression in OPCs by miR-146a hairpin inhibitors blocked OPCs from differentiating into mature oligodendrocytes. Transfection of NPCs with miR-146a mimics considerably increased Tuj1-positive neuroblasts and O4-positive OPCs but did not significantly alter GFAP-positive astrocytes. MiR-146a mimics substantially reduced proliferating NPCs. IRAK1 was shown to mediate miR-146a-promotion of oligodendrocyte differentiation.	Elevation of miR-146a in OPCs promotes their differentiation, while in NPCs, miR-146a enhances differentiation of NPCs into neuronal and oligodendrocyte lineage cells.	

EGFR: Epidermal growth factor receptor; GFAP: glial fibrillary acidic protein; GRB2: growth factor receptor-bound protein-2; MCAO: middle cerebral artery occlusion; NMDA: N-methyl-D-aspartate; NOS2: nitric oxide synthase 2; PI3K: phosphoinositide 3-kinase; Pren: phosphatase and tensin homolog; RT-PCR: reverse transcription polymerase chain reaction; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor.

Table 2 Studies of cell-based therapies with immunomodulatory properties to treat stroke

Cell-based therapy; reference	No. of animals or patients, gender, ages, treatment	Comparison	Changes in miRNAs in animals or patients	Functional outcomes	Conclusion
Mesenchymal stem (MSC) cells					
Chen et al. (2001a)	Adult Wistar male rats, 270–300 g, subjected to transient middle cerebral artery occlusion (MCAO) 2 hours. Rats received MSCs (1×10^6) injected i.v. at 24 hours after MCAO (group 2, $n = 6$), MSCs (3×10^6) at 24 hours after MCAO (group 3, $n = 7$). Rats in groups 2 and 3 sacrificed at 14 days after MCAO. Rats received i.v. infusion of MSCs (3×10^6) at 7 days after MCAO (group 5, $n = 7$), sacrificed at 35 days after MCAO. Behavioral tests performed before and at 1, 7, 14, 21, 28 and 35 days after MCAO.	Control rats, MCAO alone with no donor cell administration (group 1, $n = 6$), sacrificed at 14 days after MCAO; MCAO alone (group 4, $n = 6$) sacrificed at 35 days after MCAO.	Significant recovery of somatosensory and Neurological Severity Score (NSS) in animals infused with 3×10^6 MSCs at 1 or 7 days compared with control animals. MSCs were found to survive and be localized in the ipsilateral ischemic hemisphere, and a few cells expressed protein marker phenotypic neural cells. No significant reduction of volume of ischemic damage was found in rats with donor MSCs compared to control rats with MCAO alone.	MSCs delivered by i.v. route can provide therapeutic benefit after stroke.	
Chen et al. (2011)	Adult Wistar male rats, 225–250 g, were injected i.p. streptozotocin (60 mg/kg) to induce type 1 diabetes mellitus (T1DM). Animals were used 2 weeks after diabetes induction. Right MCAO was induced by intraluminal filament, and after 2 hours the filament was withdrawn. At 24 hours after surgery, T1DM MCAO injected i.v. with MSCs (3×10^6) ($n = 12$). Functional outcome tests were performed before and at 1, 7 and 14 days after MCAO.	Controls, nondiabetic MCAO, phosphate buffer saline (PBS) ($n = 8$); nondiabetic MCAO, MSCs (3×10^6) ($n = 8$); T1DM MCAO, PBS ($n = 13$); nondiabetic sham, PBS ($n = 4$); T1DM sham, PBS ($n = 4$)	No functional benefit was found in T1DM MCAO rats treated with MSC compared to T1DM MCAO controls. MSC treatment in T1DM MCAO rats had increased mortality, blood-brain barrier (BBB) leakage, brain hemorrhage, and angiogenesis. Internal carotid artery neointimal formation and cerebral arteriole narrowing/occlusion were also observed in T1DM MCAO + MSCs rats compared with T1DM MCAO controls, but not in nondiabetic stroke rats. The expression of angiogenin (an angiogenic factor) and ED1 (a marker for macrophages) was significantly increased in the T1DM MCAO + MSC rats in the ischemic brain and internal carotid artery compared with nontreated T1DM MCAO rats, but not in nondiabetic stroke rats.	MSC treatment initiated 24 hours after MCAO in T1DM rats increased brain hemorrhage, BBB leakage, and accelerated cerebral arteriosclerosis and did not improve functional outcome.	
Cui et al. (2016)	Adult male Wistar rats were injected i.p. with streptozotocin (60 mg/kg). At 2 weeks later, diabetic (T1DM) rats were subjected to 2 hours of MCAO via intraluminal vascular occlusion. Rats ($n = 8$ /group) were treated 24 hours after stroke with i.v. injection of (c) MSCs from T1DM rats (DM-MSCs), (d) DM-MSCs with miR-145 overexpression (miR145+/+ DM-MSCs), (e) Nor-MSCs with miR-145 knockdown (miR145-/- Nor-MSCs). Functional tests were performed before MCAO and on days 1, 7 and 14 after MCAO. Rats were sacrificed at 14 days after MCAO.	Controls, T1DM MCAO rats treated i.v. 24 hours after stroke with (a) PBS, (b) MSCs from normal nondiabetic rats (Nor-MSCs).	In T1DM MCAO rats, DM-MSC treatment significantly improved functional outcome, increased vascular and white matter remodeling compared with Nor-MSCs or PBS treatment. MiR-145+/+DM-MSCs significantly increased serum miR-145 expression and decreased brain ABCA1 and IGFRI expression, as well as attenuated DM-MSC-induced neurorestorative effects in T1DM MCAO rats.	In T1DM MCAO rats, DM-MSC treatment improved functional outcome and promoted neurorestorative effects. The miR-145/ABCA1/IGFRI pathway may contribute to the enhanced DM-MSC functional and neurorestorative effects in T1DM stroke rats.	
Xin et al. (2013)	Adult male Wistar rats, 270–300 g, were subjected to right MCAO via intraluminal vascular occlusion. Reperfusion was performed after 2 hours by withdrawing the suture. At 24 hours post stroke, rats ($n = 6$ /group) received i.v. naive MSCs, miR-133b+MSC, or miR-133b-MSC. Behavioral tests were performed at day 1 post MCAO immediately prior to the treatment and at days 3, 7 and 14 after MCAO. Rats were sacrificed at 14 days after MCAO and brains removed. Cerebrospinal fluid (CSF) samples were collected immediately prior to sacrifice.	Controls, MCAO rats receiving i.v. PBS, miR-133b ^{-CON} MSC, miR-133b ^{-CON} MSC	The miR-133b level in exosomes collected from the CSF was significantly increased after miR-133b+MSC treatment, and was significantly decreased after miR-133b-MSC treatment. Tagging exosomes with green fluorescent protein demonstrated that exosomes-enriched extracellular particles were released from MSCs and transferred to adjacent astrocytes and neurons. The expression of selective targets for miR-133b, connective tissue growth factor and ras homolog gene family member A, were significantly decreased in the IBZ after miR-133b+MSC treatment, while their expression remained at similar elevated levels after miR-133b-MSC treatment, compared to naive MSC treatment.	Exosomes from MSCs may mediate the miR-133b transfer to astrocytes and neurons, which regulate gene expression, subsequently benefit neurite remodeling and functional recovery after stroke.	

Table 2 Continued

Cell-based therapy, reference	No. of animals or patients, gender, ages, treatment	Comparison	Changes in miRNAs in animals or patients	Functional outcomes	Conclusion
SB623 cells, Steinberg et al. (2016)	18 patients (7M/11F), mean age 61 years, with stable chronic stroke received single doses of 2.5×10^6 ($n = 6$), 5.0×10^6 ($n = 6$) or 10×10^6 ($n = 6$) SB623 cells. MRI was used to define the target area surrounding the residual stroke volume. At baseline, the mean poststroke interval was 22 months. Five 20 μ L cell deposits were made in the peri-infarct area. Patients were assessed for acute and long-term outcomes.			All patients experienced at least 1 treatment-emergent adverse event. All serious treatment-emergent adverse events resolved without sequelae. Sixteen patients completed 12 months of follow-up. Significant improvement from baseline was reported for European Stroke Scale, National Institutes of Health Stroke Scale, Fugl-Meyer total score, Fugl-Meyer motor function total score. No changes occurred in modified Rankin Scale. The area of magnetic resonance T2 fluid-attenuated inversion recovery signal change in the ipsilateral cortex 1 week after implantation of SB623 cells significantly correlated with clinical improvement at 12 months on European Stroke Scale.	SB623 cells were safe and improved clinical outcome at 12 months.
Human umbilical cord blood (HUCBC) cells					
Yan et al. (2015)	T2DM rats were subjected to 2 hours MCAO <i>via</i> intraluminal vascular occlusion. At 3 days after MCAO, rats injected i.v. with HUCBCs (5×10^6 , $n = 10$). Functional tests were performed before MCAO and after MCAO on days, 7, 14, 21 and 28. Rats were sacrificed 28 days after MCAO.	Controls, T2DM MCAO rats treated with PBS at 3 days after MCAO ($n = 10$)		HUCBC treatment of T2DM MCAO rats did not significantly decrease BBB leakage and brain lesion volume but significantly improved long-term functional outcome and decreased brain hemorrhage when compared with the PBS-treated T2DM MCAO rats. HUCBC treatment significantly promoted white matter remodeling in the IBZ. HUCBC promoted vascular remodeling and significantly increased arterial and vascular density. HUCBC treatment of T2DM MCAO rats significantly increased M2 macrophage polarization in the ischemic brain compared to PBS-treated T2DM MCAO rats. HUCBC treatment of T2DM MCAO rats significantly decreased expression levels of neuroinflammatory factors Toll-like receptor 4 (TLR4), matrix metalloproteinase 9 (MMP9), and RAGE compared to PBS-treated T2DM MCAO rats.	HUCBC treatment increased white matter and vascular remodeling in the ischemic brain of T2DM MCAO rats, as well as decreased proinflammatory factors TLR4, MMP9, and RAGE expression and promoted M2 macrophage polarization.
Chen et al. (2001b)	Adult male Wistar rats, 270–300 g, were subjected to MCAO <i>via</i> intraluminal occlusion. Repfusion was performed 2 hours after MCAO by withdrawing the suture. MCAO rats injected i.v. with HUCBCs (2.3×10^6) at 24 hours after MCAO (group 2, $n = 6$), and MCAO rats injected i.v. with HUCBCs (5.3×10^6) at 7 days after MCAO (group 5, $n = 5$). Rats in groups 2 and 5 were sacrificed at 14 and 35 days after MCAO, respectively. Behavioral tests were performed before MCAO and at 1, 7, 14, 21, 28 and 35 days after MCAO.	Controls, MCAO alone (group 1, $n = 5$), MCAO alone (group 3, $n = 5$), MCAO injected with PBS at 24 hours after MCAO (group 4, $n = 8$). Rats in groups 1, 3 and 4 were sacrificed at 14 and 35 days after MCAO, respectively.		HUCBC treatment of MCAO rats at 24 hours after MCAO significantly improved functional recovery. HUCBC treatment of MCAO rats at 7 days after MCAO significantly improved function only on the Modified Neurological Severity Score (mNSS) test. Within the brain tissue, HUCBCs survived and were distributed throughout the damaged brain of recipient rats, with the vast majority localized to the IBZ. Significantly more HUCBCs were found in the ipsilateral hemisphere than in the contralateral hemisphere. Some HUCBCs were reactive for the astrocyte marker glial fibrillary acidic protein and the neuronal markers NeuN and microtubule-associated protein 2. A significant increase in HUCBC migration activity was found in the presence of ischemic brain tissue collected at 24 hours after MCAO.	HUCBCs injected i.v. entered the brain, survived, migrated, and improved functional recovery after MCAO.
Yan et al. (2014)	Adult male Wistar rats, 225–250 g, were injected i.p. with streptozotocin (60 mg/kg). After 2 weeks, T1DM rats underwent transient 2 hours MCAO <i>via</i> intraluminal vascular occlusion. T1DM MCAO rats injected i.v. with HUCBCs (5×10^6) at 24 hours after MCAO ($n = 6$). Functional tests were performed before MCAO, at 1 day after MCAO before treatment, and at 7 and 14 days after MCAO. Rats were sacrificed at 14 days after MCAO.	Controls, T1DM MCAO injected with PBS ($n = 9$), T1DM sham controls (intraluminal suture not inserted) injected with PBS ($n = 4$) or injected i.v. with HUCBCs (5×10^6) at 24 hours after sham surgery ($n = 4$).		HUCBC treatment significantly improved functional recovery without reduction of lesion volume, and increased white matter and vascular remodeling in the ischemic brain of T1DM MCAO rats compared to T1DM MCAO control. HUCBC treatment significantly increased angiopoietin 1 (Ang1) and decreased RAGE expression in IBZ of T1DM MCAO rats compared to T1DM MCAO control. <i>In vitro</i> , HUCBCs and Ang1 treatment significantly increased brain endothelial capillary tube formation and decreased inflammatory factor RAGE and TLR2 expression.	HUCBC treatment increased Ang1 and induced white matter and vascular remodeling in T1DM MCAO rats.

Table 2 Continued

Cell-based therapy, reference	No. of animals or patients, gender, ages, treatment	Comparison	Changes in miRNAs in animals or patients	Functional outcomes	Conclusion
CD34 ⁺ or CD34 ⁻ cells, Boltze et al. (2008)	Male spontaneously hypertensive (SH) rats, 220–275 g, were subjected to permanent MCAO. At 8 to 10 hours after MCAO, SH rats were injected i.v. with viable CD34 ⁺ or CD34 ⁻ cells (1 × 10 ⁶). All rats received a 3-week functional behavioral testing before MCAO, and together with mNSS until day 29. Animals were sacrificed on day 29.	Controls, SH MCAO rats received PBS at 8 to 10 hours after MCAO.		SH MCAO rats treated with HUCBC of both populations showed a remarkable reduction of functional and neurological deficits. On comparing CD34 ⁺ with CD34 ⁻ cells, no significant difference was found in regard to the beneficial effect of both populations. After administration of fluorescently labeled CD34 ⁺ as well as CD34 ⁻ cells, labeled cells were found that accumulated in the border zone between central necrosis of the ischemic lesion and functional brain tissue. Immunohistology indicated an accumulation of human and rat monocytes in the border zone of the lesion while neuronal cells of human origin could not be detected in the brains of the treated SH MCAO rats.	Administration of CD34 ⁺ or CD34 ⁻ cells was successful for treatment of brain ischemic injury after permanent MCAO in SH rats.
Chen et al. (2016)	Adult male BKS.Cg-m+/+Leprdb/J (db/db, T2DM) mice, 12–14 weeks, were subjected to right permanent MCAO by coagulating the main branch of the MCA with a small heater probe, and the vessel then transected. At 3 days after MCAO, mice were injected i.v. with naive-HUCBC (1 × 10 ⁶) (n = 9/group) or miR-126 knockdown HUCBCs (miR-126 ^{-/-} HUCBC, n = 9/group). Functional tests were performed 3 days (before treatment) and 7 and 14 days after MCAO. Mice were sacrificed at 14 days after MCAO.	Controls, db/+ (non-DM) mice, 12–14 weeks subjected to right permanent MCAO. At 3 days after MCAO, mice injected i.v. with PBS.	T2DM MCAO mice exhibited significantly decreased miR-126 expression in blood serum, ischemic brain tissue in the IBZ compared to non-DM MCAO mice. HUCBC treatment in T2DM MCAO mice or coculture T2DM-brain endothelial cells (BECs) with HUCBCs significantly increased miR-126 expression in blood serum and ischemic brain tissue compared to T2DM MCAO mice or T2DM-BECs alone. MiR-126 knockdown in BECs exhibited significantly increased miR-126 expression and capillary tube formation compared to knock-in control and naive-BECs, respectively. MiR-126 ^{-/-} -HUCBC significantly decreased miR-126 expression in HUCBC compared to miR-126 ^{-/-} -Con-HUCBC and naive-HUCBC.	HUCBC treatment significantly improved functional outcome in T2DM MCAO mice. HUCBC treatment significantly decreased brain hemorrhage and BBB leakage, and increased vascular and white matter remodeling and M2 macrophage polarization in T2DM MCAO mice. HUCBC treatment decreased miR-126 target gene VCAM-1 and MCP-1 expression in T2DM MCAO mice.	HUCBC treatment increased vascular and white matter remodeling and anti-inflammatory effect in T2DM MCAO mice. MiR-126 may contribute to HUCBC-induced neurorestorative effects in T2DM MCAO mice.
Endothelial progenitor cells (EPCs)					
Chen et al. (2015)	Mice subjected to cerebral infarction and injected i.v. with mouse EPCs transfected with miR-145.	Controls, EPCs not transfected and EPCs transfected with nonsense microRNA (miR control).		Treatment with EPCs transfected with miR-145 potentiated cell proliferation and migration and recanalization of arterial thrombosis in cerebral infarction mice. MiR-145 activated c-Jun N-terminal kinase (JNK) pathway in EPCs.	MiR-145 increased proliferation and migration of EPCs and recanalization of arterial thrombosis in cerebral infarction mice.

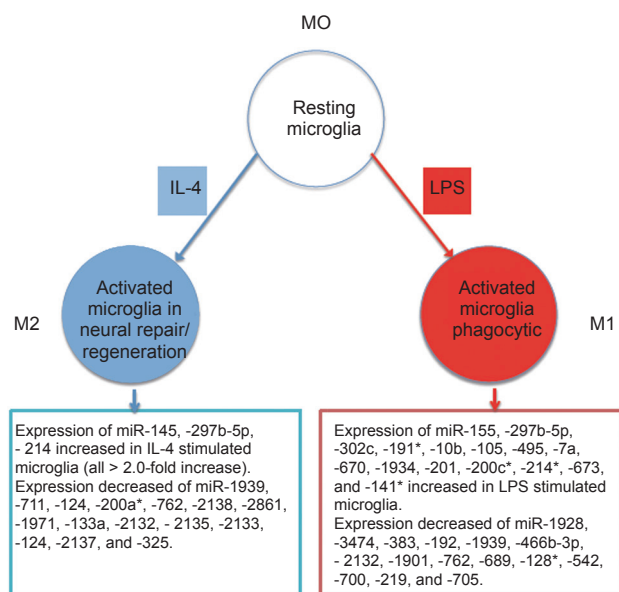


Figure 1 M1- and M2-like activation phenotypes induced by stimulating murine microglia with LPS and IL-4, respectively, and altered miRNA expressions (Freilich et al., 2013).

IL-4: Interleukin-4; LPS: lipopolysaccharide.

-145 (Table 1). IL-4 induced M2 polarization in microglia/macrophages (Figure 1). MiR-124, -711, -145 are the strongly associated miRNAs predicted to mediate anti-inflammatory pathways and M2-like activation phenotype. Interestingly, niacin and T β 4 treatment protected brain cells from stroke-mediated apoptosis, while niacin and the NMDA antagonist MK801 reduced the infarct volume in stroke rats. Niacin treatment attenuated the proinflammatory cytokine TNF- α and increased VEGF, PI3K/Akt activity in the ischemic brain. Transfection of neuroprogenitor cells with miR-146a enhanced their differentiation into neuronal and oligodendrocyte lineage cells. The cell-based therapy studies reviewed have mainly utilized MSCs or HUCBCs and shown to improve functional and neurological outcomes in stroke animals. MiR-145 and miR-133b were implicated in nerve cell remodeling and functional recovery after stroke. HUCBCs decreased proinflammatory factors and promoted M2 macrophage polarization in stroke diabetic animals (Table 2).

Future Perspectives

The *in vivo* stroke studies were mostly performed with young adult male animals. Future studies need to be made in female animals, and also in aged animals. The possible reasons for MSC therapy not improving functional outcomes in stroke type 1 diabetic rats require scientific clarification. Also the role of miRNAs in pharmaceutical and cell-based therapies in improving functional and neurological outcomes in ischemic stroke needs to be further investigated. Preconditioning of MSCs *ex vivo* by hypoxia, inflammatory stimuli, or other factors/conditions prior to their use in therapy as an adaptive strategy to prepare such cells to survive in the harsh environment at the site of tissue injury/inflammation should be examined (Saparov et al., 2016). This would also apply to HUCBCs. At the present time, the lack of a complete understanding of the mechanism of action mediating the observed therapeutic benefits of adult stem cell therapy in stroke is a critical limitation (Bang et al., 2016) and restricts their use in clinical studies. Also the role of specific microRNAs in inducing angio-

genesis, neurogenesis and oligodendrogenesis could be examined by injection of a vector carrying the microRNA into the brain of normal and stroke animals. MicroRNAs with anti-inflammatory properties include miR-146a, -122, -let-7c (Alexander et al., 2015; Roy et al., 2015; Yu et al., 2016) and these should be trialed.

MiRNAs are major molecular regulators and appear to have pivotal roles in cell-based and possibly pharmacological restorative therapies for stroke. Targeting specific miRNAs may provide major restorative therapies for stroke. This will still hold many challenges due to possible delivery and potential off-target effects.

Author contributions: BM, PVP drafted, reviewed and finalized the paper. Both authors contributed equally to this paper.

Conflicts of interest: None declared.

Open access statement: This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

Contributor agreement: A statement of "Publishing Agreement" has been signed by an authorized author on behalf of all authors prior to publication.

Plagiarism check: This paper has been checked twice with duplication-checking software iThenticate.

Peer review: A double-blind and stringent peer review process has been performed to ensure the integrity, quality and significance of this paper.

Open peer review report:

Reviewer: Pio Conti, University of Chieti, Italy.

Comments to author: Astrocytes are immune cells of the brain that elicit an immune and inflammatory response with production of cytokines, during brain damage. Cytokines regulate the expression of brain endothelial microRNAs that either promote or inhibit inflammatory pathways to orchestrate neuroinflammation in the brain. Proinflammatory cytokines alter the levels of several important microRNA clusters. Members of microRNA family have been shown to mediate many biological effects, including induced cell proliferation and decreased apoptosis, by targeting pro-apoptotic genes. miRNAs play an important role in the regulation of adult neurogenesis. An important class of epigenetic regulators are microRNAs (miRNAs) that contribute to chronic inflammation of microglia in the brain causing the progression of neurological diseases such as ischemic stroke. However, miRNAs are major molecular regulators and appear to have pivotal roles in cell-based, and possibly, pharmacological restorative therapies for stroke. Using miRNAs as therapeutic targets still hold many challenges, due to possible delivery and potential off-target effects, since miRNAs are major molecular regulators and appear to have pivotal roles in cell-based, and possibly, pharmacological restorative therapies for stroke. Targeting microRNA therapeutic strategies to ameliorate CNS disorders and dysfunctions are welcome. See details in the additional file.

Additional file: Open peer review report 1.

References

- Akopov SE, Simonian NA, Grigorian GS (1996) Dynamics of polymorphonuclear leukocyte accumulation in acute cerebral infarction and their correlation with brain tissue damage. *Stroke* 27:1739-1743.
- Alexander M, Hu R, Runtsch MC, Kagele DA, Mosbrugger TL, Tolmachova T, Seabra MC, Round JL, Ward DM, O'Connell RM (2015) Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. *Nat Commun* 6:7321.
- Badamchian M, Fagarasan MO, Danner RL, Suffredini AF, Damavandy H, Goldstein AL (2003) Thymosin beta(4) reduces lethality and down-regulates inflammatory mediators in endotoxin-induced septic shock. *Int Immunopharmacol* 3:1225-1233.
- Bang OY, Kim EH, Cha JM, Moon GJ (2016) Adult stem cell therapy for stroke: challenges and progress. *J Stroke* 18:256-266.
- Bartaula-Brevik, Pederson TO, Finne-Wistrand A, Bolstad AI, Mustafa K (2016) Angiogenic and immunomodulatory properties of endothelial and mesenchymal stem cells. *Tissue Eng Part A* 22:244-252.
- Bianchi E, Ripandelli G, Taurone S, Feher J, Plateroti R, Kovacs I, Magliulo G, Orlando MP, Micera A, Battaglione E, Artico M (2016) Age and diabetes related changes of the renal capillaries: An ultrastructural and immunohistochemical study. *Int J Immunopath Pharmacol* 29:40-53.

- Boltze J, Kowalski I, Geiger K, Reich D, Gunter A, Buhrie C, Egger D, Kamprad M, Emmrich F (2008) Experimental treatment of stroke in spontaneously hypertensive rats by CD34⁺ and CD34⁻ cord blood cells. *Ger Med Sci* 3:Doc09.
- Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M (2001a) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 32:1005-1011.
- Chen J, Sanberg PR, Li Y, Wang L, Liu M, Willing AE, Sanchez-Ramos J, Chopp M (2001b) Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. *Stroke* 32:2682-2688.
- Chen J, Ye X, Yan T, Zhang C, Yang XP, Cui X, Cui Y, Zacharek A, Roberts C, Liu X, Dai X, Lu M, Chopp M (2011) Adverse effects of bone marrow stromal cell treatment of stroke in diabetic rats. *Stroke* 42:3551-3558.
- Chen J, Venkat P, Zacharek A, Chopp M (2014) Neurorestorative therapy for stroke. *Front Hum Neurosci* 8:382.
- Chen J, Ning R, Zacharek A, Cui C, Cui X, Yan T, Venkat P, Zhang Y, Chopp M (2016) MiR-126 contributes to human umbilical cord blood cell induced neurorestorative effects after stroke in type-2 diabetic mice. *Stem Cells* 34:102-113.
- Chen R, Chen S, Liao J, Chen X, Xu X (2015) MiR-145 facilitates proliferation and migration of endothelial progenitor cells and recanalization of arterial thrombus in cerebral infarction mice via JNK signal pathway. *Int J Clin Exp Pathol* 8:13770-13776.
- Cui C, Ye X, Chopp M, Venkat P, Zacharek A, Yan T, Ning R, Yu P, Cui G, Chen J (2016) miR-145 regulates diabetes-bone marrow stromal cell-induced neurorestorative effects in diabetes stroke rats. *Stem Cells Trans Med* 5:1656-1667.
- Emberson J, Lees KR, Lyden P, Blackwell L, Albers G, Bluhmki E, Brott T, Cohen G, Davis S, Donnan G, Grotta J, Howard G, Kaste M, Koga M, von Kummer R, Lansberg M, Lindley RI, Murray G, Olivot JM, Parsons M, et al. (2014) Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet* 284:1929-1935.
- Esposito E, Paterniti I, Mazzon E, Genovese T, Galuppo M, Meli R, Bramanti P, Cuzzocrea S (2011) MK801 attenuates secondary injury in a mouse experimental compression model of spinal cord trauma. *BMC Neurosci* 12:31.
- Famakin BM (2014) The immune response to acute focal cerebral ischemia and associated post-stroke immunodepression: a focused review. *Aging Dis* 5:307-326.
- Freilich RW, Woodbury ME, Ikezu T (2013) Integrated expression profiles of mRNA and miRNA in polarized primary murine microglia. *PLoS One* 8:e79416.
- Ganji SH, Qin S, Zhang L, Kamanna VS, Kashyap ML (2009) Niacin inhibits vascular oxidative stress, redox-sensitive genes, and monocyte adhesion to human aortic endothelial cells. *Atherosclerosis* 202:68-75.
- Gao F, Chiu SM, Motan DA, Zhang Z, Chen L, Ji HL, Tse HF, Fu QL, Lian Q (2016) Mesenchymal stem cells and immunomodulation: current status and future prospects. *Cell Death Dis* 7:e2062.
- Jacobs DM (1981) Immunomodulatory effects of bacterial lipopolysaccharide. *J Immunopharmacol* 3:119-132.
- Li L, Chen XP, Li YJ (2010) MicroRNA-146a and human disease. *Scand J Immunol* 71:227-231.
- Lim KY, Chua JH, Tan JR, Swaminathan P, Sepramaniam S, Armugam A, Wong PT, Jeyaseelan K (2010) MicroRNAs in cerebral ischemia. *Transl Stroke Res* 1:287-303.
- Lin J, Pang L, Liu XL, Xing J (2016) Role of vitacamphore in improving cerebral central pro-inflammatory cytokines following transient global ischemia. *J Biol Regul Homeost Agents* 30:1091-1098.
- Lin Z, Dreyzin A, Aamodt K, Dudley AC, Melero-Martin JM (2011) Functional endothelial progenitor cells from cryopreserved umbilical cord blood. *Cell Transplant* 20:515-522.
- Liu X, Liu J, Zhao S, Zhang H, Cai W, Cai M, Ji X, Leak RK, Gao Y, Chen J, Hu X (2016) Interleukin-4 is essential for microglia/macrophage M2 polarization and long-term recovery after cerebral ischemia. *Stroke* 47:498-504.
- Liu XS, Chopp M, Pan WL, Wang XL, Fan BY, Zhang Y, Kassis H, Zhang RL, Zhang XM, Zhang ZG (2017) MicroRNA-146a promotes oligodendrogenesis in stroke. *Mol Neurobiol* 54:227-237.
- Lopez-Ramirez MA, Reijerkerk A, de Vries HE, Romero IA (2016) Regulation of brain endothelial barrier function by microRNAs in health and neuroinflammation. *FASEB J* 10:2662-2672.
- Martinez B, Peplow PV (2015) Blood microRNAs as potential diagnostic and prognostic markers in cerebral ischemic injury. *Neural Regen Res* 11:1375-1378.
- Nuzzolo ER, Capodimonti S, Martini M, Iachininoto MG, Bianchi M, Cocomazzi A, Zini G, Leone G, Larocca LM, Teofili L (2014) Adult and cord blood endothelial progenitor cells have different gene expression profiles and immunogenic potential. *Blood Transfus* 12 Suppl 1:s367-s374.
- Ransohoff RM, Brown MA (2012) Innate immunity in the central nervous system. *J Clin Invest* 122:1164-1171.
- Roura S, Bago JR, Soler-Botija C, Pujal JM, Galvez-Monton C, Prat-Vidal C, Lucia-Valideperas A, Blanco J, Bayes-Genis A (2012) Human umbilical cord blood-derived mesenchymal stem cells promote vascular growth in vivo. *PLoS One* 7:e49447.
- Roy S, Benz F, Luedde T, Roderburg C (2015) The role of miRNAs in the regulation of inflammatory processes during hepatofibrogenesis. *Hepatobiliary Surg Nutr* 4:24-33.
- Santra M, Chopp M, Santra S, Nallani A, Vyas S, Zhang ZG, Morris DC (2016) Thymosin beta 4 up-regulates miR-200a expression and induces differentiation and survival of rat brain progenitor cells. *J Neurochem* 136:118-132.
- Saparov A, Ogay V, Nuzgzhin T, Jumabay M, Chen WC (2016) Preconditioning of human stem cells to enhance their regulation of the immune response. *Stem Cells Internat* 2016:3924858.
- Shehadah A, Chen J, Zacharek A, Cui Y, Ion M, Roberts C, Kapke A, Chopp M (2010) Niaspan treatment induces neuroprotection after stroke. *Neurobiol Dis* 40:277-283.
- Shu Y, Li Z, Han B (2016) Penhexylidene hydrochloride postconditioning ameliorates cerebral ischemia-reperfusion injury: critical role of mitochondrial ATP sensitive potassium channel. *J Biol Regul Homeost Agents* 30:41-53.
- Splawski JB, Jelinek DF, Lipsky PE (1989) Immunomodulatory role of IL-4 on the secretion of Ig by human B cells. *J Immunol* 142:1569-1575.
- Steinberg GK, Kondziolka D, Wechsler LR, Lunsford LD, Coburn ML, Billigen JB, Kim AS, Johnson JN, Bates D, King B, Case C, McGrogan M, Yankee EW, Schwartz NE (2016) Clinical outcomes of transplanted modified bone marrow-derived mesenchymal stem cells in stroke. A phase 1/2a study. *Stroke* 47:1817-1824.
- Tan L, Rogers TJ, Hatzirodos N, Baker LM, Ooi E, Wormald PJ (2009) Immunomodulatory effect of cytosine-phosphate-guanosine (CpG)-oligonucleotides in nonasthmatic chronic rhinosinusitis: an ex-plant model. *Am J Rhinol Allergy* 23:123-129.
- Vartanian KB, Mitchell HD, Stevens SL, Conrad VK, McDermott JE, Stenzel-Poore MP (2015) CpG preconditioning regulates miRNA expression that modulates genomic reprogramming associated with neuroprotection against ischemic injury. *J Cereb Blood Flow Metab* 35:257-266.
- Wang Y, Wang Y, Zhao X, Liu L, Wang D, Wang C, Wang C, Li H, Meng X, Cui L, Jia J, Dong Q, Xu A, Zeng J, Li Y, Wang Z, Xia H, Johnston SC; CHANCE Investigators (2013) Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med* 369:11-19.
- Xin H, Li Y, Liu Z, Wang X, Shang X, Cui Y, Zhang ZG, Chopp M (2013) MiR-133b promotes neural plasticity and functional recovery after treatment of stroke with multipotent mesenchymal stromal cells in rats via transfer of exosome-enriched extracellular particles. *Stem Cells* 31:2737-2746.
- Yan T, Venkat P, Ye X, Chopp M, Zacharek A, Ning R, Cui Y, Roberts C, Kuzmin-Nichols N, Sanberg CD, Chen J (2014) HUCBCs increase angiopoietin 1 and induce neurorestorative effects after stroke in T1DM rats. *CNS Neurosci Ther* 20:935-944.
- Yan T, Venkat P, Chopp M, Zacharek A, Ning R, Cui Y, Roberts C, Kuzmin-Nichols N, Sanberg CD, Chen J (2015) Neurorestorative therapy of stroke in type 2 diabetes mellitus rats treated with umbilical cord blood cells. *Stroke* 46:2599-2606.
- Yoder MC (2012) Human endothelial progenitor cells. *Cold Spring Harb Perspect Med* 2:a006692.
- Yu G, Borlongan CV, Stahl CE, Hess DC, Ou Y, Kaneko Y, Yu SJ, Yang T, Fang L, Xie X (2009) Systemic delivery of umbilical cord blood cells for stroke therapy: a review. *Restor Neurol Neurosci* 27:41-54.
- Yu JH, Long L, Lao ZX, Li LM, You JR (2016) Anti-inflammatory role of microRNA let-7c in LPS treated alveolar macrophages by targeting STAT3. *Asian Pacific J Trop Med* 9:72-75.
- Zhao Q, Ren H, Han Z (2016) Mesenchymal stem cells: immunomodulatory capability and clinical potential in immune diseases. *J Cell Immunother* 2:3-20.