Neurol Sci (2016) 37:1765–1771
DOI 10.1007/s10072-016-2643-5

REVIEW ARTICLE

The interplay of microRNAs and post-ischemic glutamate excitotoxicity: an emergent research field in stroke medicine

Alireza Majdi[1] · Javad Mahmoudi[1] · Saeed Sadigh-Eteghad[1] · Mehdi Farhoudi[1] · Siamak Sandoghchian Shotorbani[2]

Received: 6 March 2016 / Accepted: 20 June 2016 / Published online: 27 June 2016

Abstract Stroke is the second leading cause of death and the most common cause of adult disabilities among elderlies. It involves a complex series of mechanisms among which, excitotoxicity is of great importance. Also, miRNAs appear to play role in post-stroke excitotoxicity, and changes in their transcriptome occur right after cerebral ischemia. Recent data indicate that specific miRNAs such as miRNA-223, miRNA-181, miRNA-125a, miRNA-125b, miRNA-1000, miRNA-132 and miRNA-124a regulate glutamate neurotransmission and excitotoxicity during stroke. However, limitations such as poor in vivo stability, side effects and inappropriate biodistribution in miRNA-based therapies still exist and should be overcome before clinical application. Thence, investigation of the effect of application of these miRNAs after the onset of ischemia is a pivotal step for manipulating these miRNAs in clinical use. Given this, present review concentrates on miRNAs roles in post-ischemic stroke excitotoxicity.

Keywords Ischemic stroke · Glutamate excitotoxicity · MicroRNA · NMDA receptors · AMPA receptors

Introduction

Stroke is the second leading cause of death, the second most common cause of dementia and the most common cause of adult disabilities among elderlies [1–3]. Globally, it is responsible for nearly 5.5 million deaths every year, with 44 million disability-adjusted life-years lost which will increase up to 61 million in 2020 [2, 4]. Of this, ischemic stroke accounts for approximately 73 to 86 %, whereas hemorrhagic stroke is responsible for 8 to 18 % of the cases [5].

Brain ischemia initiates a cascade of pathological events that finally lead to irreversible neuronal damage [6]. It involves a complex series of biological and molecular mechanisms such as excitotoxicity, oxidative stress, inflammation, ionic imbalance, blood–brain barrier disruption and apoptosis [7, 8]. Among these mechanisms, excitotoxicity is notable. It is mainly a glutamate-mediated specific type of neurotoxicity and is the overlooked link between ischemic stroke and neuronal damage and cell death [9]. It stems from excessive accumulation of excitatory amino acids such as glutamate and leads to toxic increases in intracellular calcium [7, 10] which mediates neuronal damage in stroke [11].

miRNAs are small (~19 to 23 nucleotides) noncoding endogenous molecules that bind messenger RNAs (mRNA) and promote their degradation or repression of translation [12]. They have been found to be responsible for neuronal differentiation and development [6] and their role as mediators of silencing of post-transcriptional gene in pathological aspects of ischemic stroke has been proved [13]. Changes in the microRNAs (miRNA) transcriptome occur right after focal cerebral ischemia which indicates miRNAs role in the pathological cascades of ischemic stroke [13–15]. Alteration in miRNAs level after brain ischemic injury was first noticed in focal brain and forebrain ischemia and recent studies have assessed the significance of local changes in miRNA expression level [12, 16]. Furthermore, microRNAs aberrant, both up and down, regulation has been reported during cerebral

✉ Javad Mahmoudi
javadmahmoudi1992@yahoo.com

[1] Neurosciences Research Center (NSRC), Tabriz University of Medical Sciences, P.O. Box: 5166614756, Tabriz, Iran

[2] Department of Immunology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

ischemia and/or reperfusion [6, 17]. Also, it has been shown that in the aging brain, miRNAs play a neuroprotective role and are effective in controlling synaptic function and plasticity [18].

Although accumulating evidence shows that trafficking and phosphorylation of receptors have an important role in glutamate receptor's regulation during and after stroke, the exact mechanisms behind remain poorly understood. Recent data indicate that specific miRNAs regulate glutamate receptors expression level [11, 19, 20] and excitotoxicity during stroke [21]. Among these miRNAs, miR-223, miR-181, miR-125a, miR-125b, miR-1000, miR-132 and miR-124a seem to play a role in glutamate receptors and their associated proteins regulation in neurons and astrocytes after an episode of stroke [11, 18, 19, 22, 23] and thus can influence post-stroke excitotoxicity [21]. Apart from excitotoxicity, changes in the miRNA transcriptome right after cerebral ischemia would affect acute phase molecular mechanisms linked to oxidative stress, inflammation, blood–brain barrier breakdown, cytoskeletal remodeling, metabolic changes, mitochondrial dysfunction, and apoptosis all of which are associated with post-stroke damage [13, 17].

Among these changes, excitotoxicity is an important trigger of tissue damage in both focal experimental, and clinical ischemia. Furthermore, glutamate receptors are the key mediators of death during excitotoxic injury. Besides as noted above, microRNAs play an important role in the etiology and pathophysiology of post-stroke excitotoxicity and [23, 24] these small regulatory RNAs will allow the assessment of their application as promising therapeutic targets to reduce the ischemic penumbra volume, post-ischemic brain damage and neurological dysfunction [25, 26]. As shown in Table 1, this review focuses on miRNAs function and their roles in post-ischemic stroke excitotoxicity.

miRNAs: a novel therapeutic target in stroke medicine and the problems ahead

miRNAs could be considered a major therapeutic breakthrough in stroke medicine [6, 27] for two main reasons: (1) a single miRNA can control and influence many target genes, and (2) they can be inhibited both in vitro and in vivo [15].

In the CNS miRNAs can be manipulated by several methods [28]. Systemic delivery of modified oligonucleotides through either intravenous injection or cerebrospinal fluid infusion is one of the methods of miRNA manipulation. Sense, antisense miRNAs and artificial miRNAs have been used in mammalians with different effects on targets [29]. Lately, miRNA sponge, aimed to a specific miRNA, has been manipulated as an alternative to antisense oligonucleotides. These sponges could be delivered using viral vectors to allow for stable inhibition of specific, disease-related miRNAs [30]. Blood brain barrier (BBB) is a major problem to any therapy which involves systemic delivery of oligonucleotides. However, rabies-virus peptide solves this problem and helps oligonucleotides to cross the BBB and target neurons [29]. Viral methods also exist and are more target specific and avoid the problems of systemic delivery of oligonucleotides, however, toxicity-related problems and immunogenicity limit their clinical applications [28].

The use of miRNA technology to treat excitotoxic neuronal injury is also being pursued. Because a large number of miRNAs change between 3 h and 3 days after ischemic insult to the brain, expression of miRNAs has been also observed to change with reperfusion the early use of miRNA-based therapies could change the course of the disease [29, 31]. Thence, limitations such as poor in vivo stability, side effects including toxicity and immunogenicity and inappropriate biodistribution in miRNA-based therapies still exist and should be overcome before clinical application [28, 32].

Also, approved and appropriate animal models are important to validate miRNA drugs' efficacy and unfortunately most animal models cannot completely recapitulate some aspects of human diseases such as stroke which limits the clinical usage of these models [33].

Role of miRNAs in glutamate-induced excitotoxicity in ischemic stroke

Excitotoxicity is the central missing link between ischemic insult in stroke and neuronal death [34–37]. Glutamate receptors have been hypothesized to play a major role in excitotoxicity-induced neuronal death in neurodegeneration and stroke [37, 38]. Following the activation of glutamate receptors during an ischemic insult, intracellular Ca^{2+} load increases, which in turn, activates Ca^{2+}-dependent enzymes such as proteases, lipases, phosphatases and endonucleases [34], increases reactive nitrogen and oxygen species, disrupts cell membrane and mitochondria, induces fragmentation of DNA and initiates cell death cascades [34, 39].

Following cerebral ischemic episode, changes in the miRNA transcriptome indicate miRNAs role in the pathological events such as excitotoxicity which ensue the episode and might affect the disease outcome [12, 13, 17, 21, 31]. miRNAs affect post-transcriptional gene expression by interaction, repression or degradation, with their target mRNAs [40]. Although many studies have reported the suppressive role of miRNAs, some have also

Table 1 Summary of miRNAs and their relevant targets in post-ischemia excitotoxicity

miRNA	Target element(s)	Effects on target element(s)	miRNA's post-stroke expression level	Possible influence in post-stroke excitotoxicity	References
miR-223	NR2B of NMDA and GluR2 of AMPA receptors	Downregulation	Upregulation	Decrease	Dharap et al. [31], Harraz et al. [11], Jeyaseelan et al. [48], Tan et al. [49]
miR-1000	VGlut receptor	Downregulation	Not assessed	Decrease	Verma et al. [18]
miR-125b	NR2A of NMDA receptor	Upregulation	Upregulation	Increase	Sepramaniam et al. [46], von Engelhardt et al. [55]
miR-124	Astroglial GLT1/EAAT2	Upregulation	Upregulation	Decrease	Morel et al. [58], Ouyang et al. [12], Sun et al. [62]
miR-181a	Astroglial GLT1/EAAT2	Prevents downregulation	In hippocampus and ischemic core: upregulation	Decrease	Moon et al. [69], Ouyang et al. [12]
	GluA2/GluR2 Of AMPA receptors	Downregulation	In dentate gyrus and penumbra: downregulation	Not assessed	Saba et al. [20]
miR-132	NR2A and NR2B of NMDA and GluR1 of AMPA receptors	Upregulation	Upregulation	Increase	Kaur et al. [23], Kawashima et al. [81]

suggested their inductive role in post-translational level of gene expression [40–42].

miRNAs are also thought to be neuroprotective in stroke [43–45] and among them, glutamate receptors-specific miRNAs such as miR-223, miR-1000 and miR-125b are interesting targets for this purpose [11, 18, 22, 46].

Neuroprotective role of miR-223 in ischemic stroke by targeting glutamate receptors

Due to its various functions in the cell, miR-223 is important [47]. It is also expressed in central nervous system (CNS) and has been shown to be neuroprotective by targeting α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunit GluR2 and *N*-methyl-D-aspartate (NMDA) receptor subunit NR2B. Indeed, miR-223-mediated change in NMDA and AMPA function and composition controls neuronal excitatory response to glutamate in hippocampus [11]. Evidence shows that miR-223 is upregulated following the middle cerebral artery occlusion in a rat model of stroke [31, 48, 49]. Moreover, miR-223 expression is increased in circulating blood samples of patients with acute ischemic stroke and the severity and volume of infarct were lesser in patients who had more expression of miR-223 [21, 31]. Evidence shows that overexpression of miR-223 attenuates neuronal loss after excitotoxic insult [11] and its silencing is paralleled with increasing in GluR2 and NR2B subunits which subsequently increases miniature excitatory post-synaptic currents (mEPSCs) amplitude and decay time and enhances NMDA-mediated Ca^{2+} influx and results in nitric oxide production and excitotoxicity. This finally increases neuronal cell death following middle cerebral artery stroke in hippocampus or global brain ischemia (Table 1) [11, 22, 50]. Therefore, it appears that miR-223 direct delivery to the neuronal cell could be a novel therapeutic strategy to reduce excitotoxicity and neuronal cell death after ischemic stroke [51].

miR-1000-mediated neuroprotection by limiting post-stroke glutamate excitotoxicity

There is very limited information about miR-1000 and its role in post-stroke excitotoxicity. The glutamate transporter expression in vesicles (VGlut) is controlled presynaptically by miR-1000 in an activity-dependent fashion. This transporter retains glutamate in synaptic vesicles. It has been shown that genetically knocked down miR-1000 results in glutamate excitotoxicity and early-onset neuronal damage and death (Table 1) [18]. So, misregulation of miR-1000 after ischemic stroke may worsen excitotoxic damage in the damaged brain tissue of the affected patients. Whether the expression of this miRNA changes during ischemic stroke is not yet well understood and more studies are needed to evaluate its role in post-ischemic stroke excitotoxicity.

miR-125b exacerbates post-stroke excitotoxicity

miR-125 family includes 125a, 125b1 and 125b2 members and their overexpression has been found to have a proliferative effect on cells and diminish the apoptosis rate by

proapoptotic genes downregulation [52]. Among these miRNAs, miR-125b is of increasing importance in the CNS. Although miR-125b is commonly expressed in brain and is upregulated during neurogenesis and neuronal differentiation [53], evidence shows that miR-125b overexpression in neurons leads to thinner spines and decreases mEPSCs amplitude [54]. Studies also suggest that NMDA receptors (NR2A) are the direct targets of this miRNA in hippocampal neurons and its overexpression or knock down results in NR2A up and down regulation, respectively (Table 1) [11, 22, 54]. On the other hand, the analysis of stroke patients shows that miR-125b manifests its maximum expression level within the acute phase of stroke in humans and it can be of diagnostic value [46]. So, it can be inferred from the studies that overexpression of miR-125b which occurs after ischemic stroke can upregulate NR2ARs, which promote cell death in mature cultures [55], and exacerbate post-stroke excitotoxicity. Further studies are needed to elucidate miR-125b diagnostic and therapeutic values in post-ischemic stroke excitotoxicity.

miR-124 effects on astroglial glutamate receptors control post-ischemic stroke excitotoxicity

miR-124 is selectively expressed in the CNS and its concentration is one hundred times higher in the CNS than other body tissues and is considered the most abundant CNS miRNA [56–58]. miR-124 has a key role in neuronal differentiation and function [6] and its aberrant expression contributes to pathological conditions of the CNS [59].

Evidence shows that neuronal exosomal miR-124a can control astroglial glutamate type I transporter/excitatory amino acid transporters 2 (GLT1/EAAT2) expression in astrocytes. It has been shown that transfer of this miRNA to astrocytes from neuronal exosomes increases GLT1/EAAT2 expression by altering the translation of GLT1 mRNA to its protein. This process is likely to be indirect is mediated through other factors in astrocytes. GLT1/EAAT2 is one of the important mechanisms of astrocyte protection against excitotoxicity which is mediated by rapid removal of excess glutamate from synaptic sites [58, 60].

Although findings about the role of miR-124 in stroke is controversial [61–63], some studies show that the expression level of miR-124 increases in ischemic penumbra in comparison to non-ischemic territory of middle cerebral artery occlusion model of mice and this miRNA is protective in neurons against the brain injury in experimental stroke models (Table 1) [59, 62, 64]. Also, it has been demonstrated that miR-124 could be detected in plasma after stroke and could be of diagnostic value [17, 65]. Therefore, it is highly plausible that miR-124 upregulation in penumbra can increase GLT1/EAAT2 expression in astrocytes and decrease glutamate levels in synapse and excitotoxicity and thus can be considered as a potential novel target for cerebral ischemia neuronal injuries.

miR-181 affects post-ischemic stroke excitotoxicity through astrocytes

The miR-181 family includes four members (miR-181a, miR-181b, miR-181c, and miR-181d) [60]. These family members, specifically miR-181a and miR-181b, are abundantly found in the brain [66] and their uncontrolled expression has been found to be responsible for some of important brain diseases [60]. miR-181 is one of the miRNAs whose level changes in the ischemic brain [67]. Evidence shows that following both global and focal ischemia miR-181a expression is upregulated and decreases in the penumbra and hippocampal dentate gyrus which are ischemia-resistant areas of the brain (Table 1) [16, 60, 67, 68]. These up and down regulations are associated with neuronal cell death in hippocampal CA1 and neuronal survival in dentate gyrus after ischemic injury, respectively [60]. Besides, anti-miR-181a diminishes infarct size in focal and hippocampal CA1 neuronal loss in global cerebral ischemia [60, 67, 69]. Thus, it seems to be a key mediator in the evolution of stroke-induced injury and its outcome [70].

It has been shown that direct delivery of anti-miR-181a to astrocytes is associated with increased Bcl-2, an important anti-apoptotic protein level which decreases oxidative stress and preserves GLT-1 receptors on astrocytes. This increases astrocytic glutamate uptake and limits excitotoxicity (Table 1) [69]. These changes have been found be cell-specific and do not happen in neurons after ischemia-like injuries in animal models [60].

Interestingly, studies have shown that GluA2/GluR2 subunit of AMPA receptors is also miR-181a target [23, 71, 72]. Upon miR-181a expression in neurons GluA2 surface expression and mEPSC frequency significantly decrease and neurons that overexpress this miRNA have fewer and smaller spines which proves miR-181a functional role in the synapse [20]. There are no data that show the relationship of miR-181a-related changes of AMPA receptors and excitotoxicity. Also, due to the different effects of miR-181 in neurons vs. astrocytes, future studies should assess the possible role of miR-181 in cell type specific responses and whether this miRNA influences post-stroke excitotoxicity by affecting glutamate receptors or not. Furthermore, although pretreatment with anti-miR-181a has been tried in some studies, investigation of the effect of application of this astrocyte-enriched miRNA after the onset of ischemia is a pivotal step for manipulating this idea in clinical use.

miR-132's controversial role in post-ischemic stroke excitotoxic damage

MiR132 is a neuron-specific miRNA and its expression is enriched in the brain [73, 74]. It is mandatory for the proper development, morphogenesis and function of neurons and its dysregulation results in several neurological disorders. It has been lately classified as a 'neurimmiR', which acts within and between the neural and immune systems in which via targeting acetylcholinesterase, it reduces brain inflammation and increases acetylcholine [75–78]. This miRNA is specifically expressed in active synapses and its knock down causes impaired synapse formation. So, it plays a role in the long term activation of synapse and participates in memorization processes [79, 80].

The role of miR-132 in neuronal death has not yet well understood and some studies indicated that brain-derived neurotrophic factor overexpression during cerebral ischemic attacks, upregulates expression of miR-132 through the MAPK/ERK1/2 pathway in cultured cortical neurons, which in turn induces the expression of NR2A and NR2B subunits of NMDA and GluR1 subunit of AMPA receptors. Hence, the use of miR-132 antagomir in this case may have neuroprotective effects by suppressing glutamate receptor expression, thereby reducing excitotoxicity (Table 1) [23, 81].

Conclusion

miRNAs have emerged as mediators of post-transcriptional silencing of genes in ischemic stroke pathogenesis and pathology. These components exert their effects through changing the transcriptional level of glutamate receptors which are important in post-ischemic stroke excitotoxicity. A growing body of evidence shows that specific miRNAs such as miR-223, miR-181, miR-125a, miR-125b, miR-1000, miR-132 and miR-124a regulate glutamate receptors expression level and excitotoxicity during stroke.

Thus, these miRNAs may also be applied to positively affect stroke outcomes through modulating excitotoxicity. This suggests novel strategies in the treatment of ischemic stroke pathological cascade. However, until now, none of the presented miRNAs and their targets has been proved in the context of ischemic stroke treatment. Besides, the lack of the standardized technology used in different experimental platforms of ischemic stroke mandates the need for more studies in the future.

Acknowledgments We would like to extend our sincerest thanks and appreciation to the director of Neurosciences Research Center (NSRC) Professor Mehdi Farhoudi who helped us accomplish this study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ovbiagele B, Nguyen-Huynh MN (2011) Stroke epidemiology: advancing our understanding of disease mechanism and therapy. Neurotherapeutics 8(3):319–329
2. Feigin V, Krishnamurthi R (2014) Epidemiology of stroke. Oxford textbook of stroke and cerebrovascular disease, pp 1–289
3. Farhoudi M, Mehrvar K, Sadigh-Eteghad S, Majdi A, Mahmoudi J (2014) A review on molecular mechanisms of reocclusion following thrombolytic therapy in ischemic stroke patients. J Exp Clin Neurosci (JECNS) 1:1
4. Mukherjee D, Patil CG (2011) Epidemiology and the global burden of stroke. World Neurosurg 76(6):S85–S90
5. Shiber JR, Fontane E, Adewale A (2010) Stroke registry: hemorrhagic vs ischemic strokes. Am J Emerg Med 28(3):331–333
6. Wu P, Zuo X, Ji A (2012) Stroke-induced microRNAs: the potential therapeutic role for stroke (review). Exp Ther Med 3(4):571–576
7. Guo Y, Li P, Guo Q, Shang K, Yan D, Du S, Lu Y (2014) Pathophysiology and biomarkers in acute ischemic stroke—a review. Trop J Pharm Res 12(6):1097–1105
8. Shaafi S, Mahmoudi J, Pashapour A, Farhoudi M, Sadigh-Eteghad S, Akbari H (2014) Ketogenic diet provides neuroprotective effects against ischemic stroke neuronal damages. Adv Pharm Bull 4(Suppl 2):479–481. doi:10.5681/apb.2014.071
9. Lai TW, Zhang S, Wang YT (2014) Excitotoxicity and stroke: identifying novel targets for neuroprotection. Prog Neurobiol 115:157–188
10. Mehta SL, Manhas N, Raghubir R (2007) Molecular targets in cerebral ischemia for developing novel therapeutics. Brain Res Rev 54(1):34–66
11. Harraz MM, Eacker SM, Wang X, Dawson TM, Dawson VL (2012) MicroRNA-223 is neuroprotective by targeting glutamate receptors. Proc Natl Acad Sci 109(46):18962–18967
12. Ouyang Y-B, Xu L, Yue S, Liu S, Giffard RG (2014) Neuroprotection by astrocytes in brain ischemia: importance of microRNAs. Neurosci Lett 565:53–58
13. Rink C, Khanna S (2011) MicroRNA in ischemic stroke etiology and pathology. Physiol Genomics 43(10):521–528
14. Liu D-Z, Tian Y, Ander BP, Xu H, Stamova BS, Zhan X, Turner RJ, Jickling G, Sharp FR (2010) Brain and blood microRNA expression profiling of ischemic stroke, intracerebral hemorrhage, and kainate seizures. J Cereb Blood Flow Metab 30(1):92–101
15. Fasanaro P, Greco S, Ivan M, Capogrossi MC, Martelli F (2010) MicroRNA: emerging therapeutic targets in acute ischemic diseases. Pharmacol Ther 125(1):92–104
16. Yuan Y, Wang JY, Xu LY, Cai R, Chen Z, Luo BY (2010) MicroRNA expression changes in the hippocampi of rats subjected to global ischemia. J Clin Neurosci 17(6):774–778
17. Lim K-Y, Chua J-H, Tan J-R, Swaminathan P, Sepramaniam S, Armugam A, Wong PT-H, Jeyaseelan K (2010) MicroRNAs in cerebral ischemia. Transl Stroke Res 1(4):287–303
18. Verma P, Augustine GJ, Ammar M-R, Tashiro A, Cohen SM (2015) A neuroprotective role for microRNA miR-1000 mediated by limiting glutamate excitotoxicity. Nat Neurosci 18:379–385
19. Karr J, Vagin V, Chen K, Ganesan S, Olenkina O, Gvozdev V, Featherstone DE (2009) Regulation of glutamate receptor subunit availability by microRNAs. J Cell Biol 185(4):685–697

20. Saba R, Störchel PH, Aksoy-Aksel A, Kepura F, Lippi G, Plant TD, Schratt GM (2012) Dopamine-regulated microRNA MiR-181a controls GluA2 surface expression in hippocampal neurons. Mol Cell Biol 32(3):619–632
21. Wang Y, Zhang Y, Huang J, Chen X, Gu X, Wang Y, Zeng L, Yang G-Y (2014) Increase of circulating miR-223 and insulin-like growth factor-1 is associated with the pathogenesis of acute ischemic stroke in patients. BMC Neurol 14(1):77
22. Eacker SM, Dawson TM, Dawson VL (2013) The interplay of microRNA and neuronal activity in health and disease. Front Cell Neurosci 7:1–9
23. Kaur P, Liu F, Tan JR, Lim KY, Sepramaniam S, Karolina DS, Armugam A, Jeyaseelan K (2013) Non-coding RNAs as potential neuroprotectants against ischemic brain injury. Brain Sci 3(1):360–395
24. Godwin JG, Ge X, Stephan K, Jurisch A, Tullius SG, Iacomini J (2010) Identification of a microRNA signature of renal ischemia reperfusion injury. Proc Natl Acad Sci 107(32):14339–14344
25. Dhiraj DK, Chrysanthou E, Mallucci GR, Bushell M (2013) miRNAs-19b,-29b-2* and-339-5p show an early and sustained up-regulation in ischemic models of stroke. PLoS One 8(12):e83717. doi:10.1371/journal.pone.0083717
26. Dharap A, Vemuganti R (2010) Ischemic pre-conditioning alters cerebral microRNAs that are upstream to neuroprotective signaling pathways. J Neurochem 113(6):1685–1691
27. Selvamani A, Sathyan P, Miranda RC, Sohrabji F (2012) An antagomir to microRNA Let7f promotes neuroprotection in an ischemic stroke model. PLoS One 7(2):e32662
28. Zhang Y, Wang Z, Gemeinhart RA (2013) Progress in microRNA delivery. J Control Release 172(3):962–974
29. Hutchison ER, Okun E, Mattson MP (2009) The therapeutic potential of microRNAs in nervous system damage, degeneration, and repair. Neuromol Med 11(3):153–161
30. Kumar P, Wu H, McBride JL, Jung K-E, Kim MH, Davidson BL, Lee SK, Shankar P, Manjunath N (2007) Transvascular delivery of small interfering RNA to the central nervous system. Nature 448(7149):39–43
31. Dharap A, Bowen K, Place R, Li L-C, Vemuganti R (2009) Transient focal ischemia induces extensive temporal changes in rat cerebral microRNAome. J Cereb Blood Flow Metab 29(4):675–687
32. Corey DR (2007) Chemical modification: the key to clinical application of RNA interference? J Clin Investig 117(12):3615
33. Campbell K, Booth SA (2015) MicroRNA in neurodegenerative drug discovery: the way forward? Expert Opin Drug Discov 10(1):9–16
34. Bretón RR, Rodríguez JCG (2012) Excitotoxicity and oxidative stress in acute ischemic stroke. Stroke 8:9
35. Arundine M, Tymianski M (2004) Molecular mechanisms of glutamate-dependent neurodegeneration in ischemia and traumatic brain injury. Cell Mol Life Sci 61(6):657–668
36. X-x Dong, Wang Y, Z-h Qin (2009) Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. Acta Pharmacol Sin 30(4):379–387
37. Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma P (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. Eur J Pharmacol 698(1):6–18
38. Besancon E, Guo S, Lok J, Tymianski M, Lo EH (2008) Beyond NMDA and AMPA glutamate receptors: emerging mechanisms for ionic imbalance and cell death in stroke. Trends Pharmacol Sci 29(5):268–275
39. Prentice H, Modi JP, Wu J-Y (2015) Mechanisms of neuronal protection against excitotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction in stroke and neurodegenerative diseases. Oxid Med Cell Longev 2015:964518. doi:10.1155/2015/964518
40. Ouyang Y-B, Stary CM, Yang G-Y, Giffard R (2013) MicroRNAs: innovative targets for cerebral ischemia and stroke. Curr Drug Targets 14(1):90
41. Ørom UA, Nielsen FC, Lund AH (2008) MicroRNA-10a binds the 5′ UTR of ribosomal protein mRNAs and enhances their translation. Mol Cell 30(4):460–471
42. Vasudevan S, Tong Y, Steitz JA (2007) Switching from repression to activation: microRNAs can up-regulate translation. Science 318(5858):1931–1934
43. Vemuganti R (2010) The microRNAs and stroke: no need to be coded to be counted. Transl Stroke Res 1(3):158–160
44. Nelson PT, Wang WX, Rajeev BW (2008) MicroRNAs (miRNAs) in neurodegenerative diseases. Brain Pathol 18(1):130–138
45. Lee S-T, Chu K, Jung K-H, Yoon H-J, Jeon D, Kang K-M, Park K-H, Bae E-K, Kim M, Lee SK (2010) MicroRNAs induced during ischemic preconditioning. Stroke 41(8):1646–1651
46. Sepramaniam S, Tan J-R, Tan K-S, DeSilva DA, Tavintharan S, Woon F-P, Wang C-W, Yong F-L, Karolina D-S, Kaur P (2014) Circulating microRNAs as biomarkers of acute stroke. Int J Mol Sci 15(1):1418–1432
47. Haneklaus M, Gerlic M, O'Neill L, Masters S (2013) miR-223: infection, inflammation and cancer. J Intern Med 274(3):215–226
48. Jeyaseelan K, Lim KY, Armugam A (2008) MicroRNA expression in the blood and brain of rats subjected to transient focal ischemia by middle cerebral artery occlusion. Stroke 39:959–966
49. Tan KS, Armugam A, Sepramaniam S, Lim KY, Setyowati KD, Wang CW, Jeyaseelan K (2009) Expression profile of microRNAs in young stroke patients. PLoS One 4(11):e7689
50. Martel M-A, Ryan TJ, Bell KF, Fowler JH, McMahon A, Al-Mubarak B, Komiyama NH, Horsburgh K, Kind PC, Grant SG (2012) The subtype of GluN2 C-terminal domain determines the response to excitotoxic insults. Neuron 74(3):543–556
51. Majid A (2014) Neuroprotection in stroke: past, present, and future. ISRN Neurol 2014:515716. doi:10.1155/2014/515716
52. Wojtowicz EE, Walasek MA, Broekhuis MJ, Weersing E, Ritsema M, Ausema A, Bystrykh LV, de Haan G (2014) MicroRNA-125 family members exert a similar role in the regulation of murine hematopoiesis. Exp Hematol 42(10):909–918 **(e901)**
53. Le MT, Xie H, Zhou B, Chia PH, Rizk P, Um M, Udolph G, Yang H, Lim B, Lodish HF (2009) MicroRNA-125b promotes neuronal differentiation in human cells by repressing multiple targets. Mol Cell Biol 29(19):5290–5305
54. Edbauer D, Neilson JR, Foster KA, Wang C-F, Seeburg DP, Batterton MN, Tada T, Dolan BM, Sharp PA, Sheng M (2010) Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron 65(3):373–384
55. von Engelhardt J, Coserea I, Pawlak V, Fuchs EC, Köhr G, Seeburg PH, Monyer H (2007) Excitotoxicity in vitro by NR2A- and NR2B-containing NMDA receptors. Neuropharmacology 53(1):10–17
56. Mishima T, Mizuguchi Y, Kawahigashi Y, Takizawa T, Takizawa T (2007) RT-PCR-based analysis of microRNA (miR-1 and -124) expression in mouse CNS. Brain Res 1131:37–43
57. Cao X, Pfaff SL, Gage FH (2007) A functional study of miR-124 in the developing neural tube. Genes Dev 21(5):531–536
58. Morel L, Regan M, Higashimori H, Ng SK, Esau C, Vidensky S, Rothstein J, Yang Y (2013) Neuronal exosomal miRNA-dependent translational regulation of astroglial glutamate transporter GLT1. J Biol Chem 288(10):7105–7116
59. Sun Y, Luo Z-M, Guo X-M, Su D-F, Liu X (2015) An updated role of microRNA-124 in central nervous system disorders: a review. Front Cell Neurosci 9:193
60. Ouyang Y-B, Xu L, Liu S, Giffard RG (2014) Role of astrocytes in delayed neuronal death: GLT-1 and its novel regulation by microRNAs. Glutamate and ATP at the interface of metabolism and signaling in the brain. Springer, Switzerland, pp 171–188

61. Zhu F, Liu J-L, Li J-P, Xiao F, Zhang Z-X, Zhang L (2014) MicroRNA-124 (miR-124) regulates Ku70 expression and is correlated with neuronal death induced by ischemia/reperfusion. J Mol Neurosci 52(1):148–155
62. Sun Y, Gui H, Li Q, Luo ZM, Zheng MJ, Duan JL, Liu X (2013) MicroRNA-124 protects neurons against apoptosis in cerebral ischemic stroke. CNS Neurosci Ther 19(10):813–819
63. Doeppner TR, Doehring M, Bretschneider E, Zechariah A, Kaltwasser B, Müller B, Koch JC, Bähr M, Hermann DM, Michel U (2013) MicroRNA-124 protects against focal cerebral ischemia via mechanisms involving Usp14-dependent REST degradation. Acta Neuropathol 126(2):251–265
64. Di Y, Lei Y, Yu F, Changfeng F, Song W, Xuming M (2014) MicroRNAs expression and function in cerebral ischemia reperfusion injury. J Mol Neurosci 53(2):242–250
65. Weng H, Shen C, Hirokawa G, Ji X, Takahashi R, Shimada K, Kishimoto C, Iwai N (2011) Plasma miR-124 as a biomarker for cerebral infarction. Biomed Res 32(2):135–141
66. Miska EA, Alvarez-Saavedra E, Townsend M, Yoshii A, Šestan N, Rakic P, Constantine-Paton M, Horvitz HR (2004) Microarray analysis of microRNA expression in the developing mammalian brain. Genome Biol 5(9):R68
67. Ouyang Y-B, Lu Y, Yue S, Xu L-J, Xiong X-X, White RE, Sun X, Giffard RG (2012) miR-181 regulates GRP78 and influences outcome from cerebral ischemia in vitro and in vivo. Neurobiol Dis 45(1):555–563
68. Hutchison ER, Kawamoto EM, Taub DD, Lal A, Abdelmohsen K, Zhang Y, Wood WH, Lehrmann E, Camandola S, Becker KG (2013) Evidence for miR-181 involvement in neuroinflammatory responses of astrocytes. Glia 61(7):1018–1028
69. Moon J-m XuL, Giffard RG (2013) Inhibition of microRNA-181 reduces forebrain ischemia-induced neuronal loss. J Cereb Blood Flow Metab 33(12):1976–1982
70. Stary CM, Giffard RG (2015) Advances in astrocyte-targeted approaches for stroke therapy: an emerging role for mitochondria and microRNAS. Neurochem Res 40(2):301–307
71. Follert P, Cremer H, Béclin C (2014) MicroRNAs in brain development and function: a matter of flexibility and stability. Front Mol Neurosci 7:1–8
72. Zhao L, Sun C, Xiong L, Yang Y, Gao Y, Wang L, Zuo H, Xu X, Dong J, Zhou H (2014) MicroRNAs: novel mechanism involved in the pathogenesis of microwave exposure on rats' hippocampus. J Mol Neurosci 53(2):222–230
73. Tognini P, Pizzorusso T (2012) MicroRNA212/132 family: molecular transducer of neuronal function and plasticity. Int J Biochem Cell Biol 44(1):6–10
74. Conaco C, Otto S, Han J-J, Mandel G (2006) Reciprocal actions of REST and a microRNA promote neuronal identity. Proc Natl Acad Sci USA 103(7):2422–2427
75. Wanet A, Tacheny A, Arnould T, Renard P (2012) miR-212/132 expression and functions: within and beyond the neuronal compartment. Nucleic Acids Res 40(11):4742–4753
76. O'Neill LA (2009) Boosting the brain's ability to block inflammation via microRNA-132. Immunity 31(6):854–855
77. Shaked I, Meerson A, Wolf Y, Avni R, Greenberg D, Gilboa-Geffen A, Soreq H (2009) MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase. Immunity 31(6):965–973
78. Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T, Goodman RH, Impey S (2005) A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. Proc Natl Acad Sci USA 102(45):16426–16431
79. Nudelman AS, DiRocco DP, Lambert TJ, Garelick MG, Le J, Nathanson NM, Storm DR (2010) Neuronal activity rapidly induces transcription of the CREB-regulated microRNA-132, in vivo. Hippocampus 20(4):492–498
80. Luikart BW, Bensen AL, Washburn EK, Perederiy JV, Su KG, Li Y, Kernie SG, Parada LF, Westbrook GL (2011) miR-132 mediates the integration of newborn neurons into the adult dentate gyrus. PLoS One 6(5):e19077–e19077
81. Kawashima H, Numakawa T, Kumamaru E, Adachi N, Mizuno H, Ninomiya M, Kunugi H, Hashido K (2010) Glucocorticoid attenuates brain-derived neurotrophic factor-dependent upregulation of glutamate receptors via the suppression of microRNA-132 expression. Neuroscience 165(4):1301–1311