

miR-134 Regulates Ischemia/Reperfusion Injury-Induced Neuronal Cell Death by Regulating CREB Signaling

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Abstract microRNA-134 (miR-134) has been reported to be a brain-specific miRNA and is differently expressed in brain tissues subjected to ischemic injury. However, the underlying mechanism of miR-134 in regulating cerebral ischemic injury remains poorly understood. The current study was designed to delineate the molecular basis of miR-134 in regulating cerebral ischemic injury. Using the oxygen-glucose deprivation (OGD) model of hippocampal neuron ischemia in vitro, we found that the overexpression of miR-134 mediated by recombinant adeno-associated virus (AAV) vector infection significantly promoted neuron death induced by OGD/reoxygenation, whereas the inhibition of miR-134 provided protective effects against OGD/reoxygenation-induced cell death. Moreover, cyclic AMP (cAMP) response element-binding protein (CREB) as a putative target of miR-134 was downregulated and upregulated by miR-134 overexpression or inhibition, respectively. The direct interaction between miR-134 and the 3'-untranslated region (UTR) of CREB mRNA was further confirmed by dual-luciferase reporter assay. Overexpression of miR-134 also inhibited the expression of the downstream gene of CREB, including brain-derived neurotrophic factor (BDNF) and the anti-apoptotic gene Bcl-2, whereas the inhibition of miR-134 upregulated the expression of BDNF and Bcl-2 in neurons after OGD/reoxygenation. Notably, the knockdown of CREB by CREB siRNA apparently abrogated the protective effect of anti-miR-134 on OGD/reoxygenation-induced cell death. Taken together, our study suggests that downregulation of miR-134 alleviates ischemic injury through enhancing CREB expression and downstream

genes, providing a promising and potential therapeutic target for cerebral ischemic injury.

Keywords Cerebral ischemic injury · Oxygen-glucose deprivation · Cyclic AMP (cAMP) response element-binding protein · MicroRNA

Abbreviations

miR-134	MicroRNA-134
OGD	Oxygen-glucose deprivation
AAV	Adeno-associated virus
CREB	Cyclic AMP (cAMP) response element-binding protein
UTR	Untranslated region
BDNF	Brain-derived neurotrophic factor
LDH	Lactate dehydrogenase
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

Introduction

Ischemic stroke is one of the death-related diseases with high morbidity and high mortality in adults worldwide (Donnan et al. 2008). Ischemic brain injury is caused by insufficient blood flow to the brain, characterized by oxidative stress, hypoxia, inflammation, and glutamate excitotoxicity, eventually leading to cell death (Dirnagl et al. 1999; Lo et al. 2003). Unexpectedly, the blood flow reperfusion and reoxygenation exacerbates tissue injury (Yellon and Hausenloy 2007). Although clinical trials have revealed that there are opportunities to prevent and treat acute stroke (Marsh and Keyrouz 2010), ischemic stroke remains one of the leading causes of disability and mortality, and numerous surviving stroke victims suffer from disability for the rest of their lives. However,

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the precise mechanism of ischemia-induced neuronal death remains poorly understood. Therefore, understanding of the pathological mechanism of ischemia-related cell death is of great importance for developing effective therapies for acute stroke.

Nowadays, the discovery of microRNAs (miRNAs) has brought novel insights into the regulation of gene expression which may be involved in various diseases (Bartel 2004). miRNAs, a type of small, noncoding RNA with a length of ~24 nucleotides, regulate protein translation through interaction with the 3'-untranslated region (UTR) of messenger RNA (mRNA) of the target gene, leading to destabilization and degradation of mRNA (Bartel 2004; Mendell and Olson 2012; Winter et al. 2009). The involvement of miRNA in regulating various cellular processes, including cell proliferation, apoptosis, and differentiation, provides novel insights in disease research, treatment, diagnosis, and prognosis (Mendell and Olson 2012). Compelling evidences have revealed that a variety of miRNAs were involved and functional in cerebral ischemia reperfusion injury (Di et al. 2014). Of these miRNAs, miR-134 has been demonstrated to be differentially expressed in the brains and peri-infarct region of middle cerebral artery occlusion animal models (Liu et al. 2012). miR-134 has been suggested to be a brain-specific gene that is associated with dendritic and synaptic development (Schratt et al. 2006). Thereby, miR-134 may be extensively implicated in ischemia reperfusion-induced brain injury. However, the molecular mechanism remains to be elucidated.

The transcription factor cyclic AMP (cAMP) response element-binding protein (CREB) has been revealed in the neuronal responses to ischemia (Finkbeiner 2000; Jin et al. 2001; Mayr et al. 2001). CREB is a type of leucine zipper transcription factor and is activated upon Ser-133 phosphorylation (Shaywitz and Greenberg 1999). The pre-survival kinase Akt has been reported to contribute to Ser-133 phosphorylation and promote survival in a CREB-dependent manner (Du and Montminy 1998). The activation of CREB has been demonstrated to be associated with neuronal survival (Walton et al. 1999). Neurotrophic factors such as brain-derived neurotrophic factor (BDNF) (Tao et al. 1998) and anti-apoptotic genes such as Bcl-2 (Riccio et al. 1999) are downstream genes of CREB, which protect neurons from ischemia-induced injury (Han et al. 2000; Martinou et al. 1994). Sublethal ischemic preconditioning has been demonstrated to induce CREB expression exhibiting ischemic tolerance against subsequent severe ischemic injury (Kitagawa 2007; Mabuchi et al. 2001). These studies implied that CREB is a promising molecular target for the prevention and treatment of cerebral ischemia reperfusion injury.

In this study, using the oxygen-glucose deprivation (OGD) model of cell ischemia in vitro, we investigated the role of miR-134 in regulating OGD-induced neuron death and the underlying mechanism. Using the overexpression and knock-down of miR-134, we found that miR-134 plays an important role in OGD-induced neuron death. Furthermore, miR-134 was identified and characterized to interact with 3'-UTR of CREB; thereby, the inhibition of miR-134 expression was capable of upregulating CREB expression and activating the expression of downstream genes that might protect neurons from ischemia-induced cell death.

Materials and Methods

Cell Culture

Human embryonic kidney 293 (HEK293) cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and grown in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Carlsbad, CA, USA) containing 10 % fetal bovine serum (FBS; Sigma, St. Louis, MO, USA). Primary hippocampal neuron cultures were prepared from neonatal SD rats, as previously described (Krohn et al. 1998) with modifications. Briefly, the hippocampi tissues were dissected and dissociated in trypsin-EDTA (0.25 %) and primary hippocampal neurons were maintained in neurobasal media (Life Technologies, Carlsbad, CA, USA) in supplement with GlutaMAX and B27 plus glucose (4.5 g/l) for 7 days. Then, cells were cultured in a medium containing 5 % horse serum (Sigma) and 5 % FBS (Sigma) supplemented with 15 mM glucose for 14 days. All cells were cultured in an incubator with a humidified atmosphere of 5 % CO₂ at 37 °C. Animal procedures followed official government guidelines of the Institutional Animal Care and Use Committee of Shannxi Provincial People's Hospital.

Recombinant Adeno-Associated Virus Vector Construction

Recombinant adeno-associated virus (AAV) vectors were constructed by Biowit Technologies (Biowit, Shenzhen, China). Briefly, the expression sequences encoding pre-miR-134 or anti-134 were subcloned into AAV-shuffer plasmid pSSHG which were then co-transfected with two helper packaging plasmid pAAV/Ad and adenoviral plasmid PFG140 to HEK293 cells via the calcium phosphate precipitate method. The recombinant AAV were harvested and concentrated by CsCl gradient centrifugation. The titers were determined by dot blot analysis before AAV vectors were used.

Oxygen-Glucose Deprivation of Hippocampal Neurons

The culture medium were replaced with glucose-free DMEM, and cells were cultured in hypoxic conditions (1 % O₂/94 % N₂/5 % CO₂) at 37 °C for 3 h. Thereafter, the media were discarded and normal DMEM containing glucose was added and continued to culture for 0–48 h of reoxygenation under normoxic condition (95 % air/5 % CO₂). Cells cultured in growth culture medium under normoxic condition were used as control.

RT-qPCR

Total RNA was extracted from TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For miRNA detection, reverse transcription was performed using the one-step primescript miRNA complementary DNA (cDNA) synthesis kit (TaKaRa, Dalian, China). For mRNA detection, cDNA was generated by using M-MLV reverse transcriptase (Clontech, Palo Alto, CA, USA). To analyze the gene expression, the RT-qPCR mixture system containing cDNA templates, primers, and SYBR Green qPCR Master Mix were subjected to RT-qPCR quantification according to standard methods. β -Actin and U6 SnRNA were used as internal control of mRNA or miRNA, respectively. Relative gene expression was quantified by $2^{-\Delta\Delta C_t}$ method.

Western Blot Analysis

A total of 25 μ g proteins extracted from cells were loaded and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then electrotransferred to nitrocellulose membranes (Amersham, Little Chalfont, UK). The membranes were then blocked in 2.5 % nonfat milk for 1 h at 37 °C. After washed with Tris-buffered saline with Tween, the membranes were incubated with primary antibodies against CREB, BDNF, Bcl-2, β -actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and p-CREB (Cell Signaling Technology, Danvers, MA, USA) at 4 °C overnight. Then, peroxidase-conjugated secondary antibody (Boster Corporation, Wuhan, Hubei, China) diluted in 1:1,000 were added and incubated for 1 h at room temperature. The immunoreactive protein bands were visualized using an enhanced chemiluminescence detection system (Amersham, Little Chalfont, UK).

MTT Assay

Cell viability was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

assay. Briefly, cells were seeded in 96-well plates infected with AAV vectors and subjected to OGD treatments for indicated times. Thereafter, the old media were discarded and fresh medium containing MTT (5 mg/ml MTT in PBS; Sangon, Shanghai, China) was added prior to incubation for an additional 4 h. Dimethyl sulfoxide was used to dissolve the formazan, and the absorbance at 490 nm was measured using an ELISA reader (Bio-tek, Winooski, VT, USA).

LDH Assay

Cell death was detected by the lactate dehydrogenase (LDH) assay using the Cytotoxicity Detection Kit (Roche Applied Science, Indianapolis, IN, USA) according to the supplier's instructions. Briefly, cells were lysed with 0.2 % Triton X-100. After centrifugation at 10,000 \times g for 2 min at 4 °C, the supernatants were harvested and incubated with pyruvate and NADH for 15 min at 37 °C. Then, NaOH (0.4 M) was added to finish the reaction. The absorbance at 530 nm was examined, and the activity was calculated by the equation: [(OD530 (treated)–OD530 (control))/(OD530 (Triton X-100-treated)–OD530 (control))] \times 100 %.

Dual-Luciferase Reporter Assay

The fragment from the 3'-UTR of CREB mRNA containing the predicted miR-134-binding sequences was amplified and subcloned into pGL3 luciferase promoter vector (Promega, Madison, WI, USA). The pGL3 vector containing 3'-UTR of CREB mRNA or mutated forms was co-transfected with AAV-pre-miR-134 or controls into HEK293 cells and incubated for 48 h. Then, cells were harvested and lysed, and the luciferase activity was detected using the dual-luciferase reporter assay kit (Promega, Madison, WI, USA) as per standard protocols. The relative luciferase activities were normalized to that of the control cells.

Cell Transfection

Cells were infected with AAV-anti-miR-134 vectors, and CREB small interfering RNA (siRNA) and nonspecific siRNA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and incubated for 12 h. Then, the transfected cells were subjected to OGD treatments followed by reoxygenation, and then the cells were harvested for further analysis.

Statistical Analysis

The quantitative data were expressed as mean±SD. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Bonferroni test for multiple groups or Student *t* test between two groups. Differences with a *p* value less than 0.05 were regarded as statistically significant.

Results

miR-134 Was Downregulated in OGD/Reoxygenation-Treated Neurons

To determine the expression profiles of miR-134 in OGD/reoxygenation-treated neurons, we firstly detected the expression levels of miR-134 in hippocampal neurons subjected to OGD/reoxygenation by RT-qPCR. As shown in Fig. 1a, the expression level of miR-134 was significantly decreased in neurons after OGD and continued to be downregulated during 12–48 h reoxygenation post-OGD when compared with the normoxic group. In addition, the total protein expression levels of CREB OGD/reoxygenation-treated neurons were determined by using Western blot analysis (Fig. 1b). The quantitative data exhibited that CREB expression was significantly increased following 12–48 h of reoxygenation post-OGD (Fig. 1c). These results suggested a critical role of miR-134 and CREB in ischemic injury.

miR-134 Is Involved in Regulating OGD-Induced Cell Death

To demonstrate the contribution of miR-134 in OGD-induced ischemic injury, neurons were infected with AAV vector expression pre-miR-134 and anti-miR-134. As shown in Fig. 2a, miR-134 expression levels were significantly upregulated or downregulated in hippocampal neurons after 48-h infection of AAV-pre-miR-134 or AAV-anti-miR-134, respectively. Moreover, we found that the overexpression of miR-134 accelerated the OGD/reoxygenation-induced cell death, where the knockdown of miR-134 effectively inhibited OGD/reoxygenation-induced cell death as compared with that of control or normoxia groups (Fig. 2b, c).

miR-134 Directly Regulates CREB Expression

To explore the potential mechanism of miR-134 in regulating OGD-induced cell death, we assessed the biological effect of miR-134 on CREB expression in neurons during OGD/reoxygenation. The results showed

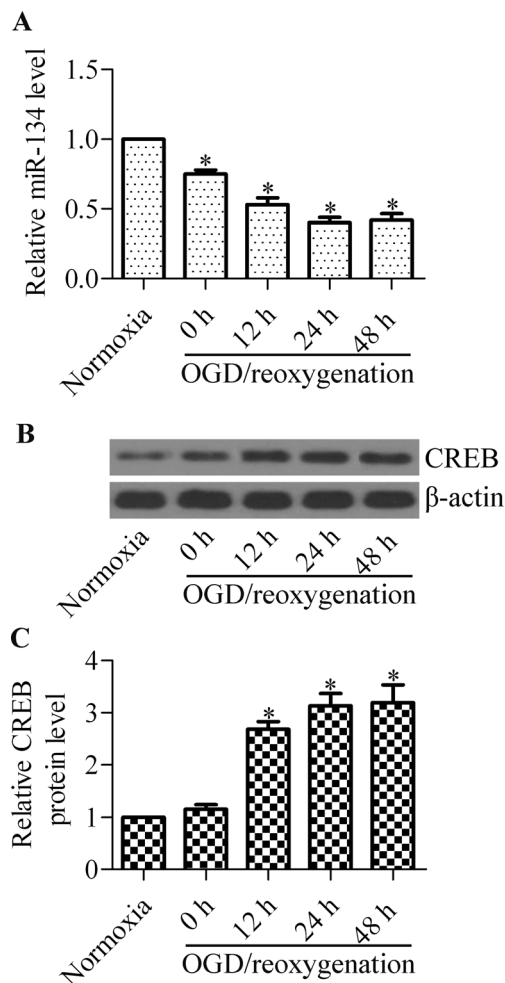


Fig. 1 Changes of miR-134 and CREB expression levels in hippocampal neurons after OGD-induced ischemic injury. **a** RT-qPCR analysis of miR-134 expression levels in neurons during 12–48 h of reoxygenation post-3 h OGD. *N*=3, **p*<0.05 vs. Normoxia. **b** Western blot analysis of total CREB protein expression levels in neurons during 12–48 h of reoxygenation post-3 h OGD. **c** Quantitative analysis of relative CREB protein level using Image-Pro Plus 6.0 software and normalized to β -actin. *N*=3, **p*<0.05 vs. Normoxia

that the mRNA expression level of CREB was significantly decreased by miR-134 overexpression during OGD/reoxygenation, whereas the knockdown of miR-134 markedly increased CREB mRNA expression level (Fig. 3a). Consistently, Western blot results (Fig. 3b) showed that total protein level (Fig. 3c) and phosphorylation level (Fig. 3d) of CREB were both significantly downregulated by miR-134 overexpression and upregulated by miR-134 knockdown. To further delineate whether miR-134 regulates CREB expression directly by targeting the 3'-UTR of CREB mRNA, a dual-luciferase reporter assay was performed. Bioinformatics analysis demonstrated that CREB was a putative target gene of miR-134 (Fig. 4a). Co-transfection of pGL3-CREB-3'-UTR with pre-miR-134 in HEK293 cells significantly downregulated the relative luciferase activity

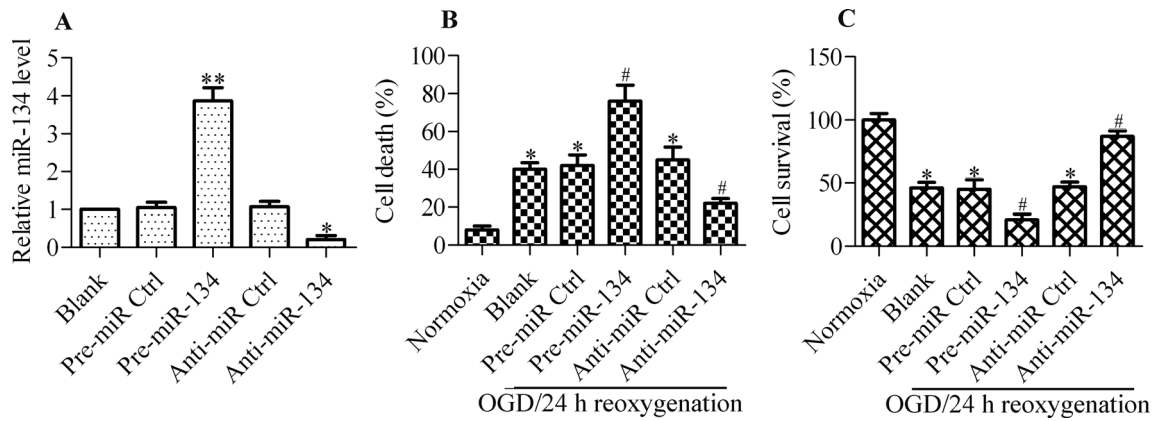


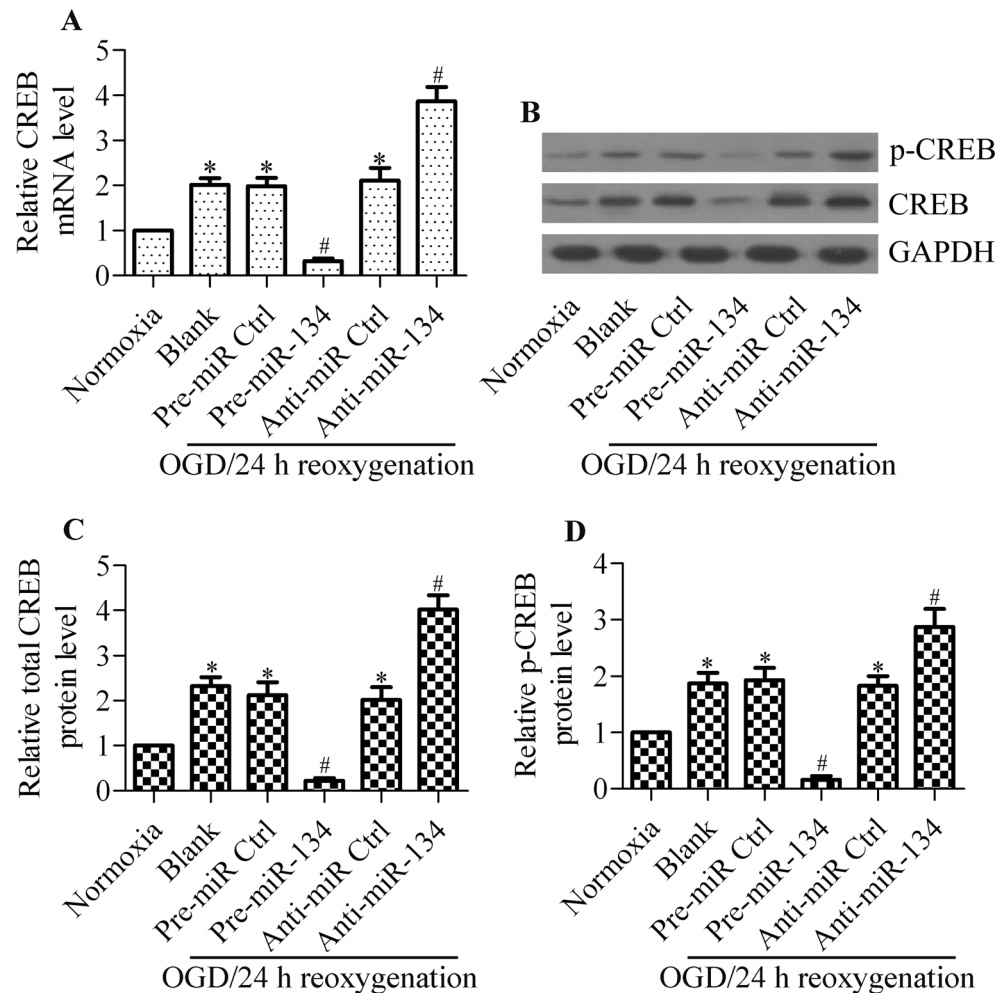
Fig. 2 Effect of miR-134 on OGD-induced cell death. **a** RT-qPCR analysis of miR-134 expression level in different treated groups: *Blank* cells without infection, *Pre-miR Ctrl* cells infected with AAV-control pre-miRNAs, *Pre-miR-134* cells infected with AAV-pre-miR-134, *Anti-miR Ctrl* cells infected with AAV-control anti-miRNAs, *Anti-miR-134* cells

infected with AAV-anti-miR-134. After transfection for 48 h, cells were harvested for analysis. $N=3$, $*p<0.05$ and $**p<0.01$ vs. Normoxia. Detection of cell death (**b**) and cell survival (**c**) by LDH and MTT assays after 24 h of reoxygenation post-OGD. $N=5$, $*p<0.05$ vs. Normoxia; $#p<0.05$ vs. blank or control

in comparison with the control group, whereas cells that co-transfected pre-miR-134 with of pGL3-CREB-Mut-3'-UTR containing mutations in the predicted consensus

sequences for miR-134 had no apparent effect on luciferase activity (Fig. 4b). These findings suggested that CREB represents a direct target gene of miR-134.

Fig. 3 Effect of miR-134 on CREB expression. **a** RT-qPCR analysis of CREB mRNA level in different groups. **b** Western blot analysis of CREB total protein and phosphorylated protein levels in different groups. Different treated cells subjected to 24 h of reoxygenation post-OGD were harvested for analysis. Quantitative analysis of relative protein levels of total CREB (**c**) and phosphorylated CREB (**d**) using Image-Pro Plus 6.0 software and normalized to β -actin. $N=3$, $*p<0.05$ vs. Normoxia; $#p<0.05$ vs. blank or control



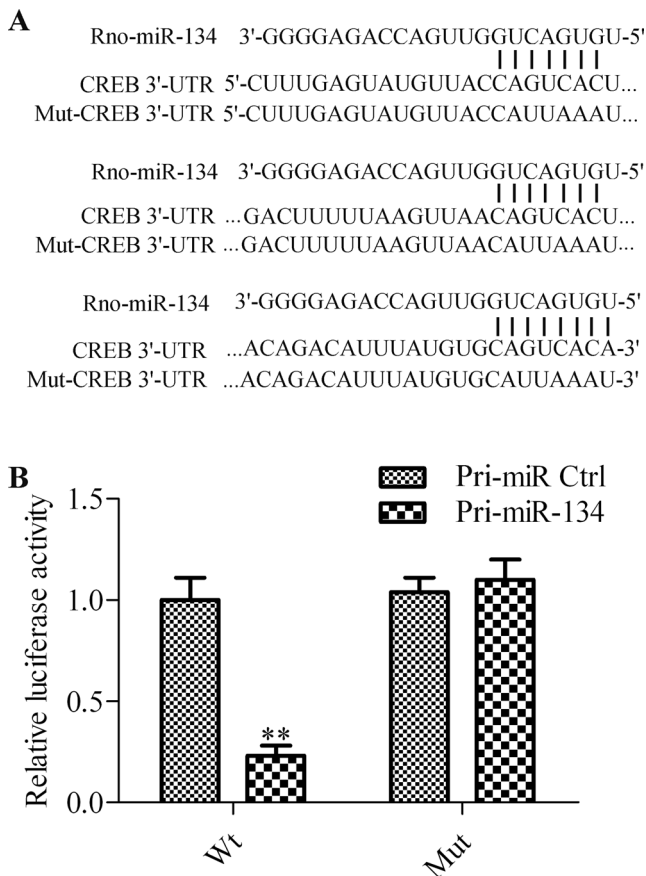


Fig. 4 CREB is a direct target gene of miR-134. **a** The putative binding sequences of 3'-UTR of CREB mRNA with miR-134. **b** Luciferase activity assay was performed to detect the interaction between miR-134 and 3'-UTR of CREB mRNA. The wild-type (Wt) or mutant (Mut) 3'-UTR of CREB mRNA was co-transfected with pre-miR-134 or pre-control for 48 h before being harvested for analysis. $N=5$, ** $p<0.01$ vs. control

miR-134 Is Involved in Regulating the Expression of BDNF and Bcl-2

To further validate the regulatory effect of miR-134 on CREB, the expression levels of downstream genes of CREB, including BDNF and Bcl-2 (Kitagawa 2007), were examined by Western blot analysis (Fig. 5a). The results showed that miR-134 overexpression significantly decreased the protein expression level of BDNF (Fig. 5b) and Bcl-2 (Fig. 5c) as compared with control groups in neurons during OGD/reoxygenation. In contrast, knockdown of miR-134 significantly increased the expression levels of BDNF (Fig. 5b) and Bcl-2 (Fig. 5c) in comparison with control groups.

Knockdown of CREB Abrogates the Neuroprotective Effect of Anti-miR-134 in OGD-Induced Ischemic Injury

To further confirm the contribution of CREB to the biological effects of miR-134 in OGD-induced cell injury, CREB siRNA

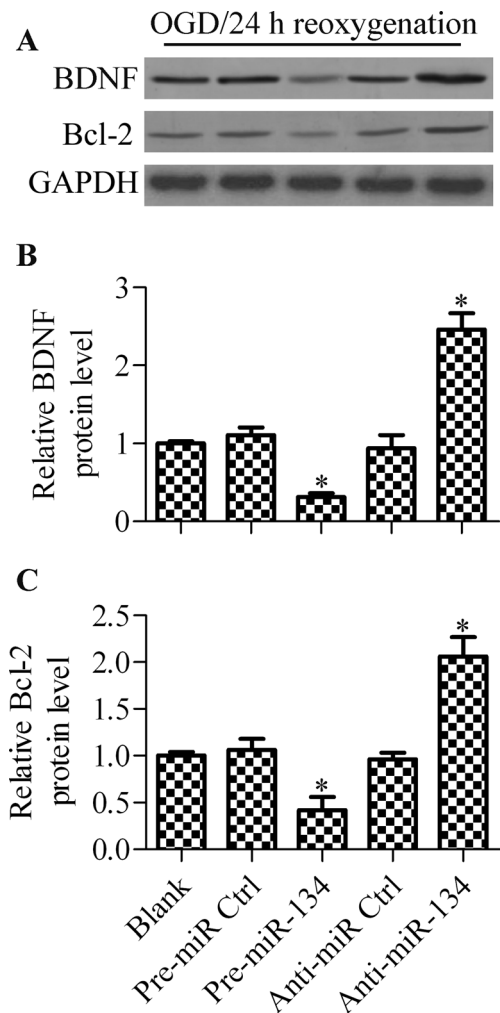


Fig. 5 Effect of miR-134 on the expression of BDNF and Bcl-2. **a** Western blot analysis of the protein expression levels of BDNF and Bcl-2 in different treated groups after 24 h of reoxygenation post-OGD. Quantitative analysis of relative protein levels of total BDNF (**b**) and Bcl-2 (**c**) using Image-Pro Plus 6.0 software and normalized to β -actin. $N=3$, * $p<0.05$ vs. blank or control

and anti-miR-134 were co-transfected in neurons. Western blot results showed that the stimulation effects of anti-miR-134 on the expression levels of CREB, BDNF, and Bcl-2 were apparently blocked by CREB siRNA (Fig. 6a–d). Also, the cell survival improved by anti-miR-134 was markedly inhibited by CREB knockdown (Fig. 6e, f) in neurons OGD/24 h reoxygenation. These results indicated that inhibition of CREB by siRNA abrogated anti-miR-134-induced neuroprotective effect in OGD/reoxygenation-treated neurons.

Discussion

It has reported that miR-134, as a brain-specific miRNA, is decreased in the brain tissues of mice subjected to middle

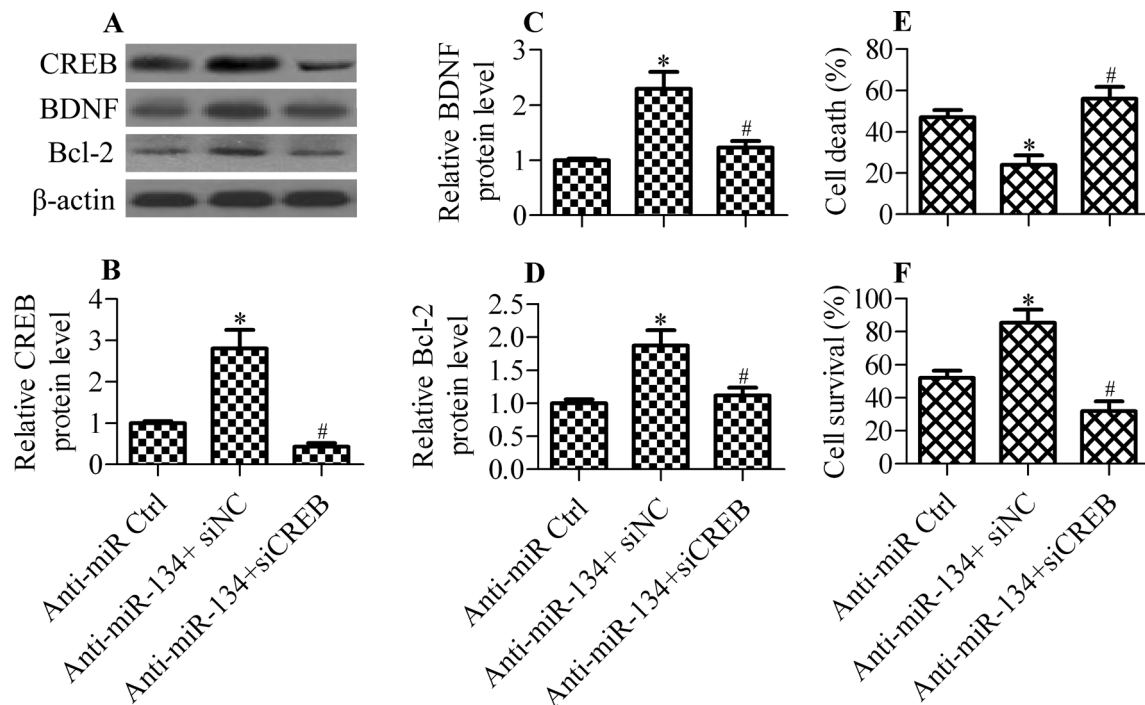


Fig. 6 Effect of CREB siRNA on anti-miR-134-induced neuroprotective effect in OGD/reoxygenation-treated neurons. **a** Western blot analysis of CREB, BDNF, and Bcl-2 protein expression levels in different groups. *Anti-Ctrl* cells treated with control anti-mRNAs, *Anti-miR-134+siNC* cells treated with anti-miR-134 and nonspecific siRNA, *Anti-miR-134+siCREB* cells treated with anti-miR-134 and CREB siRNA. Quantitative

analysis of relative protein levels of total CREB (**b**), BDNF (**c**), and Bcl-2 (**d**) using Image-Pro Plus 6.0 software and normalized to β -actin. $N=3$, $*p<0.05$ vs. control; $\#p<0.05$ vs. siNC. Detection the effect of cotransfection of anti-miR-134 and CREB siRNA on cell death (**e**) and cell survival (**f**) by LDH and MTT assays after 24 h of reoxygenation post-OGD. $N=5$, $*p<0.05$ vs. control; $\#p<0.05$ vs. siNC

cerebral artery occlusion (Liu et al. 2012). Most recently, Chi et al. demonstrated that miR-134 is downregulated in the cerebral ischemic cortex of mice and further downregulation of miR-134 by miR-134 inhibitor provided protective effects against cerebral ischemic injury (Chi et al. 2014). Consistently, we found that miR-134 was downregulated in primary hippocampal neurons subjected to OGD/reoxygenation in vitro in the present study. Also, overexpression of miR-134 accelerated cell death induced by OGD/reoxygenation, whereas knockdown of miR-134 provided protective effects against ischemic injury. Our study further confirmed the importance of miR-134 in regulating cerebral ischemic injury that might contribute to the development of intervention therapy for cerebral ischemic stroke.

Currently, increasing studies have demonstrated that miRNAs play a critical role in regulating neuronal cell death, which is the key factor of stroke pathophysiology (Barca-Mayo and De Pietri Tonelli 2014). Downregulation of miR-30a inhibited cerebral ischemic injury through upregulation of Beclin-1-mediated autophagy (Wang et al. 2014). Peng et al. have reported that inhibition of miR-18b provides a protective effect against ischemia injury by directly regulating the expression of ubiquitin carboxyl-terminal hydrolase isozyme L1 and heat shock protein A5 (Peng et al. 2013). miR-124, another reported brain-specific miRNA, has been demonstrated to be downregulated in rats subjected to focal cerebral

ischemia, and the inhibition of miR-124 reduced neuron death, infarct size, and improved neurological outcomes by directly targeting and enhancing DNA repair protein Ku70 (Zhu et al. 2014). In addition, Liu et al. have reported that the inhibition of miR-124 is capable of enhancing the expression of apoptosis-stimulating proteins of the p53 family and reduces neuronal apoptosis and infarction in mouse focal cerebral ischemia (Liu et al. 2013). In the present study, we identified and characterized that miR-134 directly targeted the 3'-UTR of CREB mRNA and that the inhibition of miR-134 enhanced the expression of CREB. Interestingly, Chi et al. reported that miR-134 targets heat shock protein A5 to regulate ischemic injury in vitro and in vivo (Chi et al. 2014). These findings suggested that miR-134 regulates ischemic injury, not only through a single target gene but also through the involvement of different target genes.

miR-134 is the first identified dendritic miRNA that localizes in dendrites and regulates the morphology of dendritic spine associated with synaptic development, maturation, and plasticity (Schratt et al. 2006). They found that overexpression of miR-134 decreased dendritic spine size by directly targeting the 3'-UTR of the LIM domain kinase 1 mRNA (Schratt et al. 2006), which was associated with the actin cytoskeleton reorganization (Arber et al. 1998; Yang et al. 1998). miR-134 has been implicated in many neurological disorders. miR-134 expression levels have been reported to be

enhanced in status epilepticus accompanied with decreased protein levels of LIM domain kinase 1 (Jimenez-Mateos et al. 2012). Upregulation of miR-134 in postmortem human brain tissue from schizophrenia was found (Santarelli et al. 2011). Therefore, the upregulation of miR-134 may be responsible for the pathologies of diseases. The downregulation of miR-134 during ischemia may be a protection mechanism initiated by biology itself to avoid cell injury. Therefore, inhibition of miR-134 provided protective effects against ischemia.

It has been reported that miR-134 expression is regulated by histone deacetylase sirtuin1 (Sirt1) and that Sirt1 deficiency increased the miR-134 expression accompanied with translational repression of CREB (Gao et al. 2010). CREB has been reported to exert critical roles in various diseases including cerebral ischemic models (Hu et al. 1999; Tanaka 2001). The activation of CREB has been demonstrated to be associated with neuronal survival (Walton et al. 1999). Sublethal ischemic preconditioning has been demonstrated to induce CREB expression, exhibiting ischemic tolerance against subsequent severe ischemic injury (Kitagawa 2007; Mabuchi et al. 2001). The activation of CREB upregulated the downstream genes, including BDNF (Tao et al. 1998) and Bcl-2 (Ricchio et al. 1999). Co-expression of p-CREB and Bcl-2 was observed in neurons of brain ischemic area (Tanaka 2001). CREB also promoted neuronal survival through BDNF, which was capable of stimulating CREB phosphorylation and activation (Almeida et al. 2005). Here, we demonstrated that overexpression of miR-134 inhibited CREB expression, whereas inhibition of miR-134 significantly upregulated the protein levels of CREB, as well as CREB phosphorylation, and downstream genes including BDNF and Bcl-2. Furthermore, knockdown of CREB significantly abrogated the protective effect of anti-miR-134 on OGD-induced cell death. In summary, these data imply that CREB plays an important role in ischemic injury regulated by miR-134.

Taken together, our study suggested that miR-134 was a promising target for the prevention of cerebral ischemic injury. However, miRNA-based therapy for the treatment of the cerebral ischemia/reperfusion injury still has limitations. Recombinant AAV for gene delivery has been reported to be capable of passing through the blood brain barrier, which has been implied as a promising vehicle for gene therapy (Foust et al. 2009). In the present study, we have obtained the recombinant AAV carrying anti-miR-134. However, we only investigated their effect in hippocampal neurons subjected to OGD in vitro. Thereby, the further effects and safety of AAV-anti-miR-134 in treatment of cerebral ischemic injury need to be assessed and investigated using animal models in vivo.

Conflict of Interests The authors declare that they have no conflicts of interest.

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