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Research report

MicroRNA-146a down-regulation correlates with neuroprotection and targets pro-apoptotic genes in cerebral ischemic injury in vitro

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ABSTRACT

MicroRNAs (miRNAs) are short, non-coding RNAs that negatively regulate target gene expression, and play an important role in cerebral ischemic injury. MiR-146a has been reported to be highly related to cell invasion, metastasis, immunity, inflammation and apoptosis. Previous studies have indicated that miR-146a can either inhibit or promote apoptosis through different pathophysiological processes. In our previous study, miR-146a in the blood was down-regulated during acute ischemic stroke. However, the connection between miR-146a and acute cerebral ischemic injury and the mechanism underlying the connection remain unclear. Here, we aimed to investigate the role of miR-146a and its possible target genes in human SK-N-SH cells subjected to 16 h of oxygen-glucose deprivation and 12 h of reperfusion (OGD/R) injury. Cells were transfected with miR-146a mimic or inhibitor to alter the expression of miR-146a. MiR-146a in the SK-N-SH cells was down-regulated after OGD/R injury. Moreover, bioinformatics analysis and dual luciferase assays demonstrated that miR-146a directly recognized the 3′-UTR of the pro-apoptotic genes, Caspase7 and Bcl-2-associated transcription factor 1 (Bclaf1). Furthermore, miR-146a over-expression effectively decreased the mRNA and protein expression of Caspase7 and Bclaf1, and aggravated OGD/R-induced cell apoptosis; in contrast, miR-146a down-regulation was neuroprotective. In conclusion, our study revealed that miR-146a contributes to OGD/R injury in vitro, while negatively regulating the pro-apoptotic genes, Caspase7 and Bclaf1. This special mechanism provides new insight into miRNA regulatory networks. In addition, miR-146a may offer a potential therapeutic approach to cerebral ischemic injury.

1. Introduction

Acute ischemic stroke is a major public health problem (Wang et al., 2016), and is characterized by a high incidence rate, sudden onset, rapid progression and serious consequences, such as disability and fatality. Although the medical science develops rapidly, effective therapeutics for acute cerebral ischemic injury are still lacking. Therefore, the development of a novel treatment for acute ischemic stroke is urgently needed, and has become a central focus of the medical community.

MiRNAs are short, non-coding RNAs that can negatively regulate gene expression, by degrading target mRNA or inhibiting translation by combining with the 3′-untranslated region (3′-UTR) of mRNA (Bartel, 2004). MiRNAs may regulate more than 30% of protein-coding genes (Lewis et al., 2005). Several miRNAs can work together to regulate the expression of the same target gene, or one miRNA can regulate several target genes (Lai et al., 2013; Vera et al., 2013). Thus, miRNAs and their corresponding target genes together comprise a complex gene regulatory network. MiRNAs play an important role in various pathological and physiological processes, including cell invasion, metastasis, immunity, inflammation, differentiation, proliferation and apoptosis (Chen et al., 2013; Kong et al., 2012). Growing evidence indicates that cerebral ischemic injury can change miRNA expression in ischemic brain tissue. In addition, these changes can either aggravate or relieve brain damage (Saugstad, 2015). MiR-124 was down-regulated in rats that were subjected to 2 h of middle cerebral artery occlusion (MCAO) and 24 h of reperfusion (Zhu et al., 2014). MiR-

Abbreviations: miRNA, microRNA; OGD/R, oxygen-glucose deprivation and reperfusion; Bclaf1, Bcl-2-associated factor 1; qRT-PCR, quantitative real-time polymerase chain reaction; 3′-UTR, 3′-untranslated region; MCAO, middle cerebral artery occlusion; I/R, ischemia/reperfusion; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum

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29c was down-regulated in rats that were treated with 1 h of electrical stimulation of the fastigial nucleus following 2 h of MCAO and 24 h of reperfusion (Huang et al., 2015). Knockdown of either miR-124 or miR-29c can significantly reduce cell apoptosis and infarct size, and exert a neuroprotective effect. These studies suggest that miRNAs play an important role in the pathophysiological process of cerebral infarction. MiRNAs have also been shown to be stable in serum, and their levels in serum can be measured (Etheridge et al., 2011). In addition, circulating miRNAs can serve as indicators for the diagnosis, progression and prognosis of various diseases (Shalaby and Grotzer, 2015; Yu and Li, 2016; Yue et al., 2016). Thus, miRNA levels in cerebral ischemic tissue and peripheral blood may be closely related under conditions of ischemic stroke.

MiR-146a has received significant attention in recent years. MiR-146a is involved in many pathophysiological functions, including immune responses, invasion, metastasis, inflammation and apoptosis. More importantly, studies have demonstrated an association between miR-146a polymorphisms and ischemic stroke (Liu et al., 2014). MiR-146a was also linked to atherosclerotic function and found to be up-regulated in atherosclerotic plaques in animal and cellular studies as well as in humans (Raitoharju et al., 2011). In our previous study, we found that miR-146a expression in the blood was down-regulated in patients who had experienced acute ischemic stroke, while it was up-regulated in cases of subacute ischemic stroke (Li et al., 2015). However, the function of miR-146a in acute cerebral ischemic injury has not been elucidated. Here, we constructed a SK-N-SH cell-based oxygen-glucose deprivation and reperfusion (OGD/R) model to mimic cerebral ischemic injury. We then transfected the cells with miR-146a mimic or inhibitor to investigate the role of miR-146a during OGD/R injury in vitro and to identify potential miR-146a target genes.

2. Results

2.1. MiR-146a was down-regulated in SK-N-SH cells after OGD/R

In our previous study, we found that miR-146a in the blood of patients with acute ischemic stroke was down-regulated compared with that in healthy controls. To demonstrate this result in our current cell culture system, we detected the level of miR-146a in SK-N-SH cells that were subjected to 16 h of oxygen-glucose deprivation and 12 h of reperfusion. The expression of miR-146a after OGD/R was significantly decreased compared with the level in normal control cells (Fig. 1(A), $P < 0.05$). This outcome was consistent with our preliminary work. Therefore, miR-146a may play an important role in OGD/R injury in SK-N-SH cells.

2.2. MiR-146a directly targets Caspase7 and Bclaf1

MiRNAs can modulate the expression of their target genes. Through bioinformatics analysis, we identified binding sites predicted to be recognized by miR-146a in the 3′-UTR of Caspase7 and Bclaf1 mRNA (Fig. 2(A) and (C)). Furthermore, to verify this prediction, we performed dual-luciferase receptor assays. We constructed a wild-type luciferase reporter vector that encoded the 3′-UTR sequence of Caspase7 or Bclaf1 mRNA and a mutant luciferase reporter vector which miR-146a binding site was mutated (AGUUCUC, the miR-146a binding site, was mutated to UCAAGAG) (Fig. 2(B) and (D)). The vector and miR-146a mimic were transfected together into 293 T cells for 48 h. After transfection with the miR-146a mimic, the luciferase activity in cells that were transfected with the wild-type vector containing the 3′-UTR segment of Caspase7 or Bclaf1 was significantly decreased, compared with that in cells that were transfected with the corresponding non-target control vector, but the activity in cells that were transfected with the mutant vector harboring a mutated segment of Caspase7 or Bclaf1 was not (Fig. 2(E) and (F), $P < 0.01$, $P > 0.05$). These

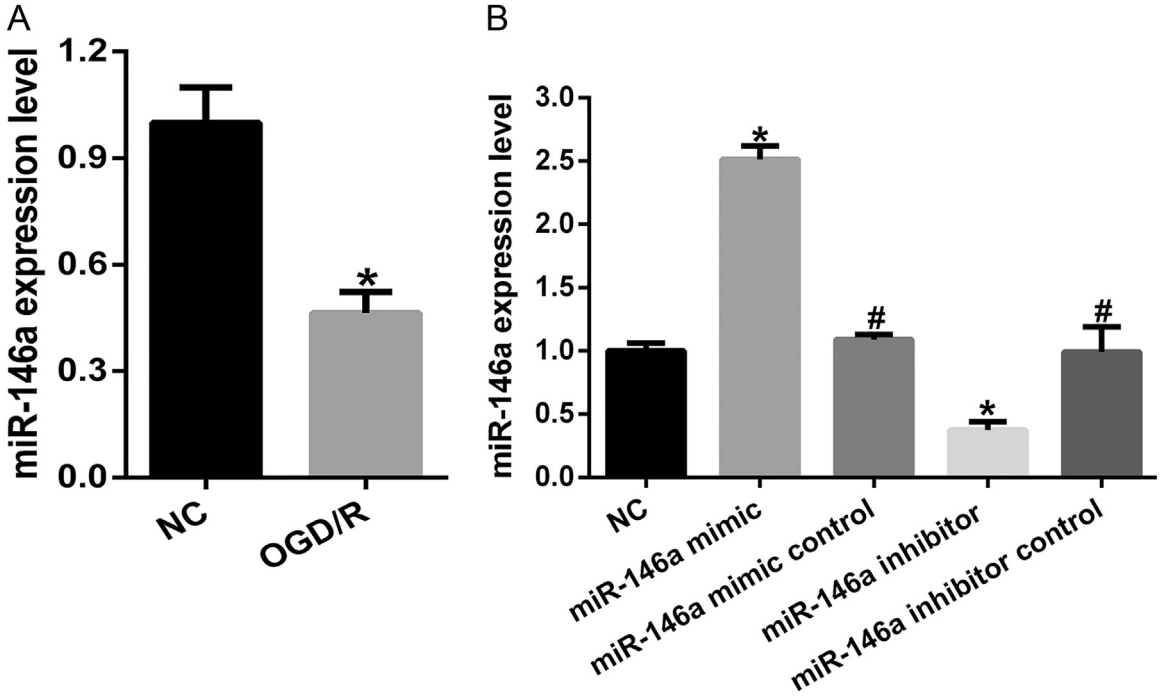

Fig. 1. The expression of miR-146a. (A) The level of miR-146a in SK-N-SH cells was suppressed after the cells were subjected to 16 h of oxygen-glucose deprivation and 12 h of reperfusion (OGD/R). (B) The efficiency of cell transfection with the miR-146a mimic, inhibitor or respective control after 48 h. MiR-146a expression was significantly increased after the cells were transfected with the miR-146a mimic for 48 h, whereas it was decreased following transfection with the miR-146a inhibitor.*$P < 0.05$ vs. NC; #$P > 0.05$ vs. NC. NC: normal control cells.

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