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Research Report

miR-21 alleviates secondary blood–brain barrier damage after traumatic brain injury in rats

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ABSTRACT

Our recent studies have identified increased expression of miR-21 in brain following traumatic brain injury (TBI), which alleviated brain edema that related to the blood–brain barrier (BBB) leakage. To analyze the potential effect of miR-21 on secondary BBB damage after TBI, we employed the fluid percussion injury rat model and manipulated the expression level of miR-21 in brain. We found that miR-21 level in brain microvascular endothelial cells (BMVECs) in lesioned cerebral cortex can be upregulated or downregulated by intracerebroventricular infusion of miR-21 agomir or antagomir. Upregulated miR-21 level conferred a better neurological outcome of TBI, and alleviated TBI-induced secondary BBB damage and loss of tight junction proteins. To explore the molecular mechanism underlying this protective effect, we detected the impact of miR-21 on the expression of Angiopoietin-1(Ang-1) and Tie-2, which can promote the expression of tight junction proteins and amplify BBB stabilization. We found that miR-21 exerts the protective effect on BBB by activating the Ang-1/Tie-2 axis in BMVECs. Thus, miR-21 could be a potential therapeutic target for interventions of secondary BBB damage after TBI.

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1. Introduction

Traumatic brain injury (TBI) is one of the leading causes of injury induced death and disability, especially in adolescence and young adults. Approximately 10 million TBI cases are reported globally each year, which makes TBI to be the third most common cause of disease burden by 2020 (Feigin et al., 2013).

The primary insult of TBI results in neural damage, intracerebral hemorrhage and primary blood–brain barrier (BBB) disruption, which cause a series of pathological event including neural apoptosis and necrosis, hematoma compression, impaired local blood supply and metabolic disorders in the central nervous system (CNS) (Blennow et al., 2012). These pathological events interact with each other and develop concurrently. They lead to secondary brain damage, characterized by secondary BBB damage which happens from hours to days post-injury (Marmarou, 2007). BBB protects the CNS from pathogenic microorganism and other high molecular weight substances in blood circulation in physiological state. Its damage results in neural damage, intracranial hypertension, brain edema and even brain hernia that leads to poor neurological prognosis (Zweckberger et al., 2006). From this, improving secondary BBB damage is significant to attenuate the pathological change in injured brain and improve the prognosis after TBI.

The clinical therapeutic methods on TBI mainly include oxygen inhalation therapy, decompressive craniectomy, evacuation of intracranial hematoma, and dehydrant treatment. These methods are not aimed at directly reducing secondary BBB damage, thus its therapeutic effect on secondary BBB damage is limited. The high-dose glucocorticoid pulse therapy was supposed to be effective in alleviating secondary BBB damage after TBI. However, a clinical trail (MRC CRSAH) involved 10008 patients conducted in more than 40 countries demonstrated that corticosteroids does not have any therapeutic effect on TBI patients in spite of the time of administration after injury (Edwards et al., 2005). The hypothermia therapy had been applied to treat secondary BBB damage. But a clinical trial supported by the National Institute of Health indicated that hypothermia therapy is ineffective on patients over 45 (Mena et al., 2011). Consequently, exploring new therapeutic strategies for secondary BBB damage is crucial for the clinical treatment of TBI.

In recent years, researchers began to put emphasis on the molecular therapy, like VEGI (Sethi et al., 2009) and Apolipoprotein E (Zheng et al., 2014) therapy, in treating secondary BBB damage. Our group has focused on studying the roles of miRNAs in TBI. We have reported that the expression level of miR-21 in brain was increased in response to TBI, and that it contributes to improving the neurological outcome while inhibiting apoptosis and promoting angiogenesis in brain (Ge et al., 2014; Lei et al., 2009). We also observed that upregulated miR-21 level in brain after TBI alleviated brain edema that related to the BBB leakage (Ge et al., 2014). Based on these findings, we performed this research to clarify the function and mechanism of miR-21 on secondary BBB damage. The results are expected to open a new avenue of therapeutic strategies for secondary BBB damage after TBI by manipulating miRNA levels.

2. Results

2.1. *Altered miR-21 expression in brain microvascular endothelial cells (BMVECs) in lesioned cerebral cortex after TBI and intracerebroventricular infusion of miR-21 oligomers*

We detected the BMVECs-expressed miR-21 level in lesioned cerebral cortex at different time-points from 0 h to 14 d post-injury using qRT-PCR (Fig. 1). The miR-21 level of *injury ctl* group was increased at 6 h post-injury, reached the peak level at 48 h post-injury, then gradually declined to baseline at 14 d post-injury. Compared with the *injury ctl* group, the expression level of miR-21 was increased or decreased in the *agomir* or *antagomir* group at 6 h, 24 h, 48 h and 72 h post-injury. These data indicated that TBI leads to upregulation of miR-21 level in BMVECs in lesioned cerebral cortex, which can be manipulated (upregulated or downregulated) by intracerebroventricular infusion of miR-21 agomir or antagomir.

2.2. *Upregulation of miR-21 level in brain improved the neurological outcome of TBI*

We performed the modified Neurological Severity Score (mNSS) test to evaluate the neurological outcome of TBI rats. A lower score in the mNSS test demonstrates a better neurological function. As shown in Fig. 2, there was no difference in the neurological score among all experimental groups at 24 h post-injury, indicating that rats in different groups had relatively comparable injuries (one-way ANOVA, $F=0.224$, $P=0.924$). The recovery of neurological function

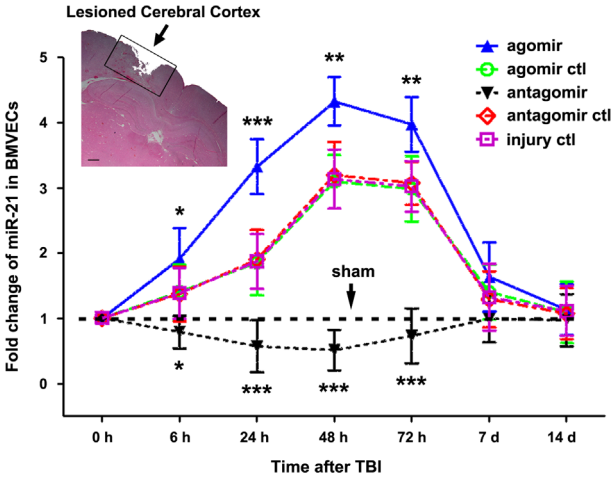

Fig. 1 – Altered BMVECs-expressed miR-21 level in lesioned cerebral cortex after TBI and miR-21 oligomers intervention. The miR-21 level in BMVECs from 0 h to 14 d post-injury were determined by qRT-PCR. The quantitative data were analyzed using the $2^{-\Delta\Delta Ct}$ method, in which miR-21 level of sham rats (presented as the dotted line) were used as controls. Note that TBI led to upregulation of miR-21 level in BMVECs, which can be upregulated (or downregulated) by intracerebroventricular infusion of miR-21 agomir (or antagomir). The data are expressed as mean ± SD. Scale bar: 500 μm. ($n=6$) ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus the *injury ctl* group).