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miR-182 (microRNA-182) suppression in the hippocampus evokes antidepressant-like effects in rats

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ABSTRACT

Depression is a serious and potentially life-threatening mental disorder with unknown etiology. Emerging evidence shows that brain-derived neurotrophic factor (BDNF) and microRNAs (miRNAs) play critical roles in the etiology of depression. However, the molecular mechanisms are not fully understood. Expression of miR-182 and BDNF in the hippocampus were analyzed in a chronic unpredictable mild stress (CUMS) model. Male Wistar rats received bilateral intra-hippocampal infusions of BDNF- and miR-182-expressing (miR-182) or miR-182-silencers (si-miR-182) lentiviral vectors (LV). miR-182 upregulation was correlated with decreased BDNF expression in the hippocampus of a CUMS model. Accordingly, an anti-depressant like effect was observed when LV-BDNF or LV-si-miR-182 was injected into the hippocampus. Moreover, BDNF and its target gene cyclic AMP responsive element binding protein 1 (CREB1) decreased following LV-miR-182 injection and increased upon LV-si-miR-182 injection in rat hippocampus and cultured neuronal cells. In contrast, miR-182 overexpression exacerbated depression-like behaviors and decreased BDNF. Further, luciferase reporter evidence confirmed BDNF was a miR-182 target. Taken together, the current results reveal a potential molecular regulation of miR-182 on BDNF and the pronounced behavioral consequences of this regulation.

1. Introduction

Depression prevalence is expected to become the second leading cause of disease burden (after HIV) by 2030 (Mathers and Loncar, 2006). This growth in prevalence will result in heavy social and economic burdens. Despite extensive research, the exact etiology of depression remains elusive and diagnostics uncertain.

Evidence has shown that certain aspects of depression result from maladaptive stress-induced neuroplastic changes in specific neural circuits of the central nervous system (CNS), the hippocampus may be a core factor in the pathophysiology of depression (Nestler et al., 2002). Abnormal neural plasticity may be related to alterations in of neurotrophic factor levels. Among these is brain-derived neurotrophic factor (BDNF), which plays a central role in plasticity (Masi and Brovedani, 2011, Mitchelmore and Gede, 2014). Stress exposure decreased BDNF expression and, if exposure was persistent, and caused eventual atrophy of the hippocampus. This took place in humans suffering from chronic depression. In turn, BDNF regulation may reverse stress-induced deficits in structural and synaptic plasticity in the brain, yielding increased ability to cope with environmental challenges that may precipitate or exacerbate depressive episodes (Castren and Rantamaki, 2010a, b, Leal et al., 2014). Low BDNF and depression were probably causally related because antidepressants indeed increase BDNF signaling and synthesis in the hippocampus (Schmidt and Duman, 2010). BDNF levels decreased in the blood of depressed patients and renormalized with successful antidepressant treatment (Sen et al., 2008). Chronic peripheral administration of BDNF produced antidepressant and anxiolytic behavioral responses in animal models, increased the survival rate of newborn neurons, and increased BDNF mediated signaling in the adult hippocampus (Schmidt and Duman, 2010). In contrast, antidepressant agents failed to exert behavioral responses in transgenic animals with decreased brain BDNF levels or inhibited BDNF-TrkB signaling (Saarelainen et al., 2003). Further studies showed that BDNF bound to the neurotropic tyrosine kinase receptor 2 (NTRK2; also called TrkB) on neurons, leading to the phosphorylation of the nuclear transcription factor cyclic AMP responsive element binding protein 1 (CREB1). This process resulted in the synthesis of a variety of proteins that were important in neuronal function. In animal models, expression of CREB1 resulted in changes reminiscent of antidepressants (Svenningsson et al., 2007) .

MicroRNAs (miRNAs) are a class of short, non-protein coding RNAs, capable of decreasing mRNA translation by binding to 3′untranslated region (3′ UTR) of these structures (Bahi et al., 2014). Evidence has shown miRNAs alterations in the serum of depressed patients,

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modulating their vulnerability to depression and antidepressant responses (Lopez et al., 2014, Smalheiser et al., 2012). Changes in the levels of BDNF-related miRNAs and their target genes have been identified in the pathophysiology of depression, response to stress and antidepressant response. Higher levels of the BDNF-related miR-132 have been shown to correlate to higher depression scores (Li et al., 2013). BDNF-targeting microRNAs (miR-16 and miR-124) have been shown to be upregulated by stress in the rat hippocampus and associated with subsequent induction of depressive-like symptoms (Bahi et al., 2014, Bai et al., 2012). BDNF-targeting miR-30a-5p and miR-206 has been shown to modulate the upregulation of BDNF following treatment with the antidepressants paroxetine and ketamine (Angelucci et al., 2011, Yang et al., 2014). In clinical research, patients with depression showed lower serum BDNF, and higher serum miR-132 and miR-182, levels (Li et al., 2013). Recently, miR-1, miR-10b, miR-155, and miR-191 were demonstrated to be novel regulators of BDNF (Varendi et al., 2014). All of these miRNAs help clarify the pathogenesis of depression and may be useful as biomarkers or therapies.

In the present study, we found that miR-182 was enriched in a mouse model of CUMS and associated with decreased BDNF. Lentiviral-mediated miR-182 reduction induced the expression of BDNF and was accompanied by an anti-depressant like effect. In contrast, elevated miR-182 exacerbated depression-like behavior and decreased BDNF expression. BDNF expression in the hippocampus caused an anti-depressant like phenotype. Collectively, these results suggest that miR-182 is associated with the pathophysiology of depression and may be a potential target for new antidepressant treatments.

2. Materials and methods

2.1. Animals

Male Wistar rats (~200 g) were purchased from the Shanghai Slac Animal Center, China. Animals were housed independently in standard plexiglas cages, under a normal 12 h light/dark schedule. The animals were allowed 1 week to acclimatize to the housing conditions before the experiments. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%, respectively. The animals were given free access to standard chow and water for the duration of the study. All procedures were approved by the Institute for Experimental Animals of Jiangsu University.

2.2. Chronic unpredictable mild stress (CUMS) protocol

The stress scheme was slightly modified from the previous study (Yi et al., 2014). Briefly, rats in CUMS groups were exposed to different stressors, including 24 h food deprivation, 24 h water deprivation, 5 min cold swimming (at 6 °C), 1 min tail pinch (1 cm from the end of the tail), physical restraint for 2 h, exposure to rat odor (removal of the cage containing the experimental rats from the procedure room and placing the experimental rats into cages in which cats had been held) for 1 h and overnight illumination. One of these stressors (in random order) was implemented every day for 4 weeks. Control rats were housed under identical conditions in a separate room and had no contact with the stressed animals. Rats were submitted to the behavioral test before they were sacrificed.

2.3. Lentivirus vectors

The lentivirus-derived plasmid (pTK431), driven by the cytomegalovirus (CMV) promoter, carried the transgenes for BDNF (using the following primers: 5′-CGCGGGATCCATGACCATCCTTTTCCTT-3′, and 5′-GCCGCTCGAGCTACAGGTCCTCCTCTGAGATCAGCTTCTGTCTTCCCCTTTTAA-3′). The empty vector (LV-Mock) was used as a negative control. LV-miR-182 (10^8 TU/ml) and LV-si-miR-182 (10^8 TU/ml) were purchased from the Gene Pharma Company (Shanghai, China).

2.4. Stereotaxic injection of LV-Mock, LV-BDNF, LV-miR-182 and LV-si-miR-182

All viral injections were performed in accordance with the previous study (Bahi, Chandrasekar, 2014). For stereotaxic surgery, rats were anesthetized with a ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively, i.p.) and installed in a stereotaxic frame. Using a precision Hamilton micro-syringe with a 26 G needle, rats were bilaterally infused with 1 ml viral solution using the following coordinates: hippocampus [1st injection: 4.8 mm posterior to Bregma, 2.5 mm lateral the medial suture, 3.5 mm ventral to the skull surface. 2nd injection: 4.8 mm posterior to Bregma, 5 mm lateral the medial suture, 6 mm ventral to the skull surface]. Rats were left to recover for 1 week before being exposed to CUMS.

2.5. Depression-like behavioral tests

2.5.1. Novelty-suppressed feeding test (NSFT)

The NSFT was carried out after 4 weeks of CUMS exposure. The testing apparatus consisted of a plastic box (35 cm x 35 cm). Food was withheld from the rats for 24 h before the test. At the beginning of the test, a single pellet of food was placed on a white paper platform positioned at the center of the box. We placed a rat in a corner of the maze and immediately started a stopwatch. Interest measurements began when the mouse reached for the food with its forepaws and began eating. As a control value, we measured the home-cage food consumption within 90 min of the test's completion.

2.5.2. Sucrose preference test (SPT)

Before the test, the mice were trained to adapt to a sucrose solution (1%, w/v). Two bottles of sucrose solution were placed in each cage for 24 h, and 1 bottle of sucrose solution was then replaced with water for 24 h. After the adaptation, rats were deprived of water and food for 24 h. During the test, rats were housed in individual cages and had free access to 2 bottles containing sucrose solution and water. After 24 h, sucrose solution volumes and water consumption were measured.

2.5.3. Forced swim test (FST)

The FST was conducted 24 h after the SPT, as originally reported (Porsolt et al., 1977). Glass beakers were first filled with tap water (22-26 °C). Rats were then placed into the test beaker and were unable to escape or rest by touching the bottom of the beaker. Sessions lasted 6 min (360 s), and the duration of immobility was recorded. Mobility was defined as any movement beyond that necessary to maintain the head above water. Data were expressed as the mean time of immobility, swimming and climbing within the 6 min observation period.

2.6. Primary hippocampal neuronal cultures

Primary hippocampal neuronal culture was prepared as previously described (Fath et al., 2009). Hippocampi were first carefully dissected from rat embryo brain. The neuronal cells were then dissociated with trypsin (Gibco) digestion. The dissociated cells were re-suspended in a neurobasal medium (Gibco) (B27 19 final, Gibco) containing 2 mM GlutaMAX. Cells were diluted to 100 cells/ml. A total of 3 ml (approximately 300 cells) of cell suspension was added to each 35-mm poly-D-lysine-coated plate and cultured for 2 h in 5% CO_2 at 37 °C. The plating medium was then replaced with 3 ml of neurobasal/B27 medium, and the culture was continued in 5% CO_2 and 21% O_2. The primarily cultured cells were allowed to grow for 2 weeks before treatment. After the cultured neurons were treated with LV-miR-182 or LV-simiR-182 for 48 h, BDNF and CREB1 protein expression levels were analyzed by Western blot.