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microRNA-124 targets glucocorticoid receptor and is involved in depression-like behaviors

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ABSTRACT

Dysregulation of microRNA (miRNA) has been shown to be involved in early observations of depression. MicroRNA-124-3p (miR-124) is the most abundant microRNA in the brain. Previous studies have shown that miR-124 plays a major role in depression. Here we showed that miR-124 directly targeted glucocorticoid receptor (GR) in HEK 293 cells. In addition, inhibition of miR-124 by its antagomir (2 nmol/every two days) could reverse the decrease of sucrose preference and the increase of immobility time in mice exposed to chronic corticosterone (CORT, 40 mg/kg) injection. Moreover, these effects on behavioral improvement were coupled to the activation of brain-derived neurotrophic factor (BDNF), TrkB, ERK, and CREB, as well as the induction of synaptogenesis and neuronal proliferation. Altogether, our study suggests that miR-124 can be served as a biomarker for depression and a novel target for drug development, and demonstrates that inhibition of miR-124 may be a strategy for treating depression by activating BDNF-TrkB signaling pathway in the hippocampus.

1. Introduction

As one of the most prevalent psychiatric disorders and major public health concerns, depression is featured as anhedonia and low self-esteem (Ma et al., 2016). Approximately 17% of the population suffering from depression and stress-related mood disorders shows a high rate of suicide and a major economic burden (Duman and Voleti, 2012; Kessler et al., 2003; Pincus and Pettit, 2001). However, there is still a poor understanding of the molecular pathophysiology underlying depression.

Both genetic and environmental factors lead to depression. Some evidences have demonstrated that chronic stress which was the major environmental factor could cause deficiency of monoaminergic systems, hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis and the dysfunction of neurotrophin (Brunoni et al., 2008; Elhwuegi, 2004; Rohleder et al., 2010). In recent years, researchers increasingly draw the attention to the neurotrophic hypothesis of depression. Their studies showed that neurotrophin, especially brain-derived neurotrophic factor (BDNF), played a critical role in the pathogenesis and the treatment of depression (Castren and Rantamaki, 2010). However, genetic analyses indicated that the dysregulation of numerous genes were related to genetic susceptibility for depression (Gong and He, 2015; Pezawas et al., 2005).

MicroRNAs (miRNAs or miRs), a class of small non-coding RNAs and approximately 22 nucleotides in length, can directly interact with the 'seed' complementary sequences in 3′untranslated regions (3′UTRs) of the target mRNAs to regulate their expression (Bartel, 2009; Huntzinger and Izaurralde, 2011). The binding of miRNA can decrease the target mRNA stability or inhibit its translation to downregulate the mRNA expression (Bartel, 2009; Eichhorn et al., 2014; Fabian et al., 2010). Recent researches suggest that miRNA widely participates in various system regulations including nervous system and is likely to have key roles in brain function, particularly neurogenesis, neuronal development and plasticity (Cuellar et al., 2008; Kosik and Krichevsky, 2005; Smalheiser and Lugli, 2009). Moreover, obvious evidences have shown there is a dysregulation of miRNA expression in brain of

Abbreviations: miRNA, microRNA; BDNF, brain-derived neurotrophic factor; GR, glucocorticoid receptor; CORT, corticosterone; HPA, hypothalamic-pituitary-adrenal; 3′UTRs, 3′untranslated regions

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depressive-like animals and depressed patients. Consistently, the aberrant miRNA expression and their downstream targets would be modified by antidepressants and physical treatments, such as selective serotonin reuptake inhibitors and electroconvulsive therapy (Belzeaux et al., 2012; Bocchio-Chiavetto et al., 2013; Lopez et al., 2014; Ryan et al., 2013).

MicroRNA-124-3p (miR-124) is one of most abundant microRNAs in brain. Previous studies have shown that miR-124 plays a key effect on adult neurogenesis, neuronal differentiation and synaptic plasticity (Cheng et al., 2009; Makeyev et al., 2007). Recent researches have indicated that there is a higher expression of miR-124 in prefrontal cortex in corticosterone (CORT)-induced depressive-like rats and in serum of major depressive patients (Roy et al., 2017). Moreover, chronic stress could lead to the upregulation of miR-124, and its selective lentiviral-mediated suppression evoked antidepressants-like effects in rats (Bahi et al., 2014).

As an effective method to study the molecular pathogenesis of depression and the effects of antidepressants, depressive-like animal models are always used (Krishnan and Nestler, 2011). CORT-induced depression in rodents is a well-established animal model. The elevated CORT levels would occur as a consequence to stress exposure and could lead to depressive-like phenotype (Sterner and Kalynchuk, 2010). In the present study, we investigated whether miR-124 played a key role in depression, and showed miR-124 antagomir displayed antidepressant-like effects in CORT induced depressive-like mice by sucrose preference test and tail suspension test. Further, to explore the potential neurotrophic role of miR-124 antagomir, CREB, BDNF and its downstream signaling pathways were also assessed.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (24 ± 2 g; 5 weeks old) were purchased from Shanghai Slac Animal Center, PR China. Animals were housed eight per cage (320 × 180 × 160 mm) under a normal 12-h/12-h light/dark schedule with the lights on at 07:00 a.m. The animals were allowed 1 week to acclimatize themselves to the housing conditions before the beginning of the experiments. Ambient temperature and relative humidity were maintained at 22 ± 2 °C and at 55 ± 5%, and the animals were given a standard chow and water ad libitum for the duration of the study. All procedures were approved by the College and performed in accordance with the published guidelines of the China Council on Animal Care (Regulations for the Administration of Affairs Concerning Experimental Animals, approved by the State Council on 31 October 1988 and promulgated by Decree No. 2 of the State Science and Technology Commission on 14 November 1988).

2.2. Drugs and reagents

miR-124 related reagents were purchased from Ribobio Co., Ltd. (Guangzhou, PR China). miR-124 antagomir is a chemically modified, cholesterol-conjugated single-stranded RNA analogue (2′-OMe-modified nucleotides) complementary to miR-124. CORT was purchased from Aladdin Biotech Co., Ltd. (Shanghai, PR China). All reagents used in quantitative real-time PCR (qRT-PCR) were purchased from Sangon Biotech Co., Ltd. (Shanghai, PR China). The mature anti-BDNF antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, USA). The anti-GR, anti-CREB, anti-p-CREB, anti-TrkB, anti-ERK1/2 and anti-p-ERK1/2 antibodies were purchased from Cell Signaling Technology (Beverly, USA). The anti-GAPDH antibody and anti-p-TrkB (Y515) was purchased from Bioworld Technology Inc. (St. Louis Park, USA). The FD Rapid GolgiStain™ Kit was purchased from FD NeuroTechnologies Inc. (Columbia, USA). The dual-luciferase reporter assay kit was purchased from Promega (Madison, USA). All other reagents used in western bolt were purchased from Beyotime Biotech Co. (Shanghai, PR China).

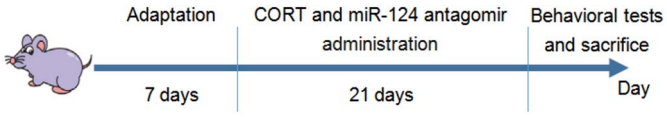

Fig. 1. The timeline of the experiment CORT and miR-124 antagomir treatments were made for 3 weeks and continued during the period of behavioral experiments.

2.3. Drug administration

Mice were randomly divided into four groups (n = 12): the Control-vehicle group (miR-124 antagomir control, 2 nmol/every two days), the Control-miR-124 antagomir group (2 nmol/every two days), the CORT-vehicle group (miR-124 antagomir control, 2 nmol/every two days) and the CORT-miR-124 antagomir group (2 nmol/every two days). The CORT was dissolved in physiological saline containing 0.1% dimethyl sulfoxide (DMSO) and 0.1% Tween-80, and injected subcutaneously (s.c) once daily at a dose of 40 mg/kg. The animals in Control groups were injected with vehicle (physiological saline containing 0.1% DMSO and 0.1% Tween-80). Injections were given between 09:00 a.m. and 11:00 a.m. All drugs were administered at a volume of 10 ml/kg body weight once daily for 21 consecutive days. miR-124 antagomir and its antagomir control were dissolved in physiological saline (4 μl at an injecting rate of 0.5 μl/min) and injected into the lateral ventricle using the following coordinates: 0.6 mm caudal to bregma, 1.5 mm from the midline and 1.5 mm below the dural surface for 3 weeks and then subjected to behavioral tests. All the behavioral tests were performed for all the animals in the same order. Briefly, sucrose preference test was followed by tail suspension test (Fig. 1).

2.4. Sucrose preference test

The sucrose preference test was carried out at the end of 3-week drug treatments. The whole procedure was adopted according to our previous reports. Briefly, before the test, the mice were trained to adapt to sucrose solution (1%, w/v): two bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, the mice were deprived of water and food for 12 h. The test was conducted in which the mice were housed in individual cages and had free access to two bottles containing sucrose solution and water, respectively. After 24 h, the volumes of the consumed sucrose solution and water were recorded. Sucrose preference was calculated using the formula as described below: Sucrose preference = Sucrose consumption / (Water and Sucrose consumption) × 100%.

2.5. Tail suspension test

The tail suspension test was used to evaluate the despair behavior of the animals in our present study. Briefly, mice were individually suspended by tail with a clamp (1 cm from the tip of the end) in a box (25 × 25 × 30 cm) with the head 5 cm from the bottom. Testing was carried out in a darkened room with minimal background noise. Mouse was suspended for a total of 6 min, and the duration of immobility was observed and measured during the final 4 min interval of the test. The test sessions were recorded by a video camera positioned directly above the box. Mice were considered immobile only when they hung passively and completely motionless. The test sessions were scored by an observer blind to treatment.

2.6. Tissue sample collection

After completion of the tail suspension test, mice were sacrificed by rapid decapitation. Whole brains were rapidly removed from mice and chilled in an ice-cold saline solution. Brain region of the whole hippocampus was dissected on a cold plate and frozen in liquid nitrogen