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CARDIAC OVER-EXPRESSION OF MICRORNA-1 INDUCES IMPAIRMENT OF COGNITION IN MICE

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Abstract—Large cohort studies have revealed a close relationship between cognitive impairment and cardiovascular diseases, although the mechanism underlying this relationship remains incompletely understood. In this study, using a transgenic (Tg) mouse model of cardiac-specific over-expression of *microRNA-1-2* (*miR-1-2*), we observed that *microRNA-1* (*miR-1*) levels were increased not only in the heart but also in the hippocampus and blood, whereas its levels did not change in the skeletal muscle of Tg mice compared with age-matched wild-type (WT) mice. Six-month-old Tg mice showed cognitive impairment compared with age-matched WT mice, as assessed using the Morris Water Maze test. The brain-derived neurotrophic factor (BDNF) level and cyclic AMP-responsive element-binding protein (CREB) phosphorylation were also significantly reduced in the hippocampi of the Tg mice, as evaluated by Western blot. Further examination showed that BDNF protein expression was down- or up-regulated by *miR-1* over-expression or inhibition, respectively, and was unchanged by binding site mutations or miRNA-masks for the 3′UTR of *Bdnf*, indicating that this gene is a potential target of *miR-1*. Knockdown of *miR-1* by hippocampal stereotaxic injection of an anti-*miR-1* oligonucleotide fragment carried by a lentivirus vector (lenti-pre-AMO-*miR-1*) led to up-regulation of BDNF expression and prevented the reduction in cognitive performance in the Tg mice without affecting cardiac function. Our findings demonstrate that cardiac over-expression of *miR-1* also induces behavioral abnormalities that may be associated, at least in part, with the down-regulation of BDNF expression in the hippocampus. This study definitely contributes to the understanding of the relationship between cardiovascular disease and cognitive impairment.
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Key words: heart, hippocampus, cognitive impairment, microRNA-1, BDNF.

INTRODUCTION

Cardiovascular diseases, such as hypertension, atrial fibrillation, coronary heart disease and heart failure, have been reported to be closely associated with neurodegenerative diseases and the impairment of cognition (de Toledo Ferraz Alves et al., 2010; Roberts et al., 2010; Monsuez et al., 2011; de la Torre, 2012). However, the underlying mechanisms of these diseases are largely unknown. The understanding of the manner by which cardiac diseases affect cognitive function would yield new therapeutic strategies.

MicroRNAs (miRNAs) are a group of short (~21–23 nucleotides) noncoding RNAs, and their primary function is believed to be either degradation of mRNA or the inhibition of translation, which occurs by the binding of miRNAs to partially complementary mRNA recognition sequences (Bartel, 2004). MiRNAs are also considered biomarkers for several diseases because of their stable presence in various body fluids (i.e., serum, plasma, saliva, urine, breast milk, and tears) (Gibbings et al., 2009; Ai et al., 2010; Cortez et al., 2011; Allegra et al., 2012; Chen et al., 2012b). Although miRNAs are expressed in a tissue/cell-specific manner, recent studies have shown that they are able to mediate cell-to-cell communication after they are secreted into the interstitial space from donor cells and are accepted by recipient cells with functional targeting capabilities through the actions of various transporters (Zernecke et al., 2009; Vickers et al., 2011; Chen et al., 2012a; Redis et al., 2012). Examination of whether miRNAs mediate heart–brain communication and act as a link between heart disease and abnormal brain function would be very interesting and contribute greatly to this field.

MicroRNA-1 (*miR-1*), a muscle-enriched miRNA, has been found to be involved in the development various cardiac diseases, including myocardial infarction induced arrhythmia (Yang et al., 2007), heart failure (Ai et al., 2012), and chronic Chagas disease cardiomyopathy (Ferreira et al., 2014), and it also has roles during coronary bypass surgery (Slagsvold et al., 2014) and apoptosis (Tang et al., 2009). Plasma *miR-1* has been

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Abbreviations: AMI, acute myocardial infarction; BDNF, brain-derived neurotrophic factor; CREB, cyclic AMP-responsive element-binding protein; Ct, threshold cycle; FBS, fetal bovine serum; LCA, left coronary artery; LTP, long-term potentiation; *miR-1*, *microRNA-1*; miRNA, MicroRNA; MWM, Morris water maze; NC, negative control; NRNs, neonatal rat hippocampal and cortical neurons; ODNs, oligodeoxynucleotides; p-CREB, phosphorylated CREB; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; SD, Sprague–Dawley; Tg, transgenic; WT, wild type.

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reported to be a potential biomarker for the prediction of acute myocardial infarction (AMI) (Ai et al., 2010; Cheng et al., 2010b; Liebetrau et al., 2013) and heart failure (Tijsen et al., 2010). A very recent study has reported significant cognitive impairment accompanied by altered β-amyloid metabolism, apoptosis and inflammation in mice at 3 months after ligation of the left coronary artery (LCA) (Hong et al., 2013), indicating an increased risk of dementia following cardiac ischemia. Because *miR-1* is overexpressed in the diseased heart and also functions in non-muscle tissue, such as tumors (Han et al., 2014), we hypothesized that it is likely to be involved in cardiac disease-mediated cognitive impairment.

The purpose of this study was to evaluate whether *miR-1* over-expression in the heart could also induce impairment in cognitive functioning by exerting direct effects on the brain.

EXPERIMENTAL PROCEDURES

Animals

Adult male C57BL/6 mice (6–7 months, 6 M or 7 M) were housed under controlled temperature of (23 ± 1 °C) and humidity (55 ± 5%). Animals were maintained on a 12-h artificial dark–light cycle (lights on at 07:00 A.M.) with food (regular chow) and water available *ad libitum*. *MiR-1* transgenic (Tg) mice were kindly provided by Prof. Xu Gao (Harbin Medical University). Left ventricle, hippocampus and blood samples for quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) and Western blot analysis were obtained from mice after they were anesthetized with sodium pentobarbital (100 mg/kg, i.p.). Mouse death was then confirmed by exsanguination. Hippocampi for primary cell culture were collected from neonatal Sprague–Dawley (SD) rats after administration of 20% isoflurane and confirmation of death by cervical dislocation. All animal procedures were approved by the Institute of Laboratory Animal Science of China (A5655-01). All procedures were carried out in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Oligonucleotide synthesis

MiR-1 mimics for rats (sense: 5′-UGGAAUGUAAAGAAGUGUGUAUGU-3′; antisense: 5′-AUACACACUUCUUUACAUUCCAAU-3′) and AMO-*miR-1* (5′-ACCUUACAUUUCUUCACACAUACA-3′) were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). AMO-1 contains 2′-*O*-methyl modifications. In addition, scrambled RNA was used as a negative control (NC) (sense: 5′-UUCUCCGAACGUGUCACGUAA-3′; antisense: 5′-ACGUGACACGUUCGGAGAAUU-3′). The Brain-derived neurotrophic factor (BDNF)-masking antisense oligodeoxynucleotides (ODNs) were synthesized by Shanghai Sangon Biotech Co., Ltd., China. The sequence of *Bdnf*-ODN-1 was 5′-+C+A+T+TATCGAGGAATGTA+A+T+G+C-3′, which masks the *miR-1* binding site at the positions 213–219 of the 3′ untranslated region (3′UTR) of the *Bdnf* gene; that of *Bdnf*-ODN-2 was 5′-+G+T+A+AACGGAATGTTTTGG+T+T+C+C-3′, which masks the *miR-1* binding site at positions of 407–413 of the 3′UTR of *Bdnf*; and that of *Bdnf*-ODN-3 was 5′-+C+C+T+TTAGGAATGTCTCAA+G+T+A+C-3′, which masks the *miR-1* binding site at the positions 1306–1312 of the 3′UTR of *Bdnf*. The nucleotides or deoxynucleotides at both ends of the antisense molecules were locked by a methylene bridge connecting the 2′-*O*- and 4′-C atoms. Locked nucleic acid (LNA)-modified oligonucleotides (LNA-antimiR-1) was synthesized by Exiqon (Denmark).

Construction of lentivirus vectors

To produce a *miR-1* antisense inhibitor, two single-stranded DNA oligonucleotides were designed as follows: (1) pre-AMO-*miR1* ("top strand" oligo: tgctg ATACATACTTCTTTACATTCCAGTTTTGGCCACTGACTGACTGGAATGTAGAAGTATGTAT) and its complementary sequence ("bottom strand" oligo: cctgATACATACTTCTACATTCCAGTCAGTCAGTGGCCAAAACTGGAATGTAAAGAAGTATGTATc); and (2) an NC ("top strand" oligo: tgctgAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAGTACATTT) and its complementary sequence ("bottom strand" oligo: cctgAAATGTACTGCGTGGAGACGTCAGTCAGTGGCCAAAACGTCTCCACGCGCAGTACATTTc). Double-stranded oligonucleotides (ds oligos) were generated by annealing the above two strands into a pcDNA™6.2-GW/± EmGFP-miR vector and transforming the ligated construct into competent *Escherichia coli*, using a BLOCK-iT pol II miR RNAi expression vector and an EmGFP kit from Invitrogen (Shanghai, China). A pre-miRNA expression cassette was transferred to a Gateway® adapted destination vectors utilizing Pol II promoters to form a new miRNA expression clone containing attR substrates. The vector was identified by analyzing the plasmid sequence (Invitrogen, Shanghai, China). The lenti-pre-AMO-*miR-1* vectors used for the experiments (2.0 μL) contained 1.0×10^8 transducing units (TUs)/mL. Virus suspensions were stored at −80 °C until use and were briefly centrifuged and kept on ice immediately before injection.

Morris Water Maze (MWM)

The maze consisted of a black circular pool of 1.2 m in diameter, which was filled with opaque water (25 ± 1 °C) via the addition of black food pigment. A submerged escape platform (9 cm in diameter, with the top surface positioned 2.0 cm below the water level) was located in the center of the first quadrant. For cued training (three trials per day for six consecutive days), the mice were released into the water facing the side walls, and each mouse was allowed 60 s to find the platform; otherwise, they were guided to the platform and permitted to rest for at least 20 s. After the last cued trial on day 6, the platform was removed from the pool, and each mouse received one 60-s swim probe trial on day 7. The escape latency (s), length of the swim path (cm) swim speed (cm/s), number of times the platform was crossed and swimming distance in target

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