Int J Clin Exp Pathol 2014;7(8):5000-5007
www.ijcep.com /ISSN:1936-2625/IJCEP0001118

Original Article

Upregulation of miR-137 protects anesthesia-induced hippocampal neurodegeneration

Changshun Huang, Xingcai Zhang, Jungang Zheng, Chunru Chen, Yijun Chen, Juan Yi

Department of Anesthesia, The First Hospital of Ningbo City, 59 Liuting St., Ningbo 315010, Zhejiang, China

Received June 15, 2014; Accepted July 28, 2014; Epub July 15, 2014; Published August 1, 2014

Abstract: Purpose: Ketamine is commonly used in pediatric anesthesia but may cause neurodegeneration in young brains. The aim of the study is to use an animal model to characterize the role of microRNA 137 (miR-137) in ketamine-induced neurodegeneration in neonatal hippocampus. Methods: Young Sprague-Dawley Rats (1 month old) was systemically administrated with ketamine (75 mg/kg) for 3 days. TUNEL assay was used to assess the ketamine-induced neurodegeneration of hippocampal CA1 neurons, quantitative real-time PCR (qRT-PCR) to assess the expression of miR-137 and Morris water maze test (MWM) to assess the damaged memory function. Alternatively, lentivirus over-expressing miR-137 was injected into hippocampus before ketamine administration, and the subsequent effects of miR-137 upregulation on ketamine-induced hippocampal neurodegeneration and memory dysfunction were investigated. Furthermore, the direct downstream target of miR-137, CDC42, was down-regulated by siRNA injection into hippocampus. The effects of CDC42 inhibition on hippocampal apoptosis and memory function were also investigated. Results: Excessive ketamine treatment resulted in severe apoptosis in hippocampal CA1 neurons, downregulation of miR-137 in hippocampus and significant long-term memory dysfunction. Conversely, pre-treatment of overexpressing miR-137 protected hippocampal neurodegeneration and memory loss. The molecular target of miR-137, CDC42 was down-regulated by ketamine in hippocampus. Knocking down hippocampal CDC42 exerted an apoptotic effect on hippocampal neurons and memory loss, similar to the effect of ketamine treatment. Conclusions: Our results demonstrated that miR-137 played an important role in regulating ketamine induced hippocampal neurodegeneration, possibly through CDC42.

Keywords: Anesthesia, ketamine, hippocampal neurodegeneration, miR-137, CDC42

Introduction

Ketamine is an antagonist of noncompetitive NMDA receptor [1], primarily used as an anesthetic medicine in both adults and children [2-4]. In recent years, emerging evidences have revealed that repeated ketamine administration during anesthesia would actually result in significant neurotoxicity and brain damage in young animals and humans [5-7]. Specifically in hippocampus, it had been shown in either *in vitro* or *in vivo* animal experiments that, repeated or high concentration of ketamine administration induced excessive neural activities, severe hippocampal neurodegeneration and deteriorated memory functions [8-10]. Unfortunately, the exact mechanisms or associated molecular pathways of anesthesia-induced cortical/hippocampal neurodegeneration and subsequent memory dysfunction are largely unknown. Thus, finding the molecular regulators responsible for the induction (or inhibition) of ketamine-induced neurodegeneration would certainly help to seek appropriate therapeutic strategy to prevent or reverse anesthetic-induced cortical neurodegeneration in both young and adult patients.

MicroRNAs (miRNA) are a group of short-sequenced, noncoding RNAs that suppress nucleus translation to endogenously silence targeted coding messenger RNAs [7, 11]. MicroRNAs are expressed in various cortical regions, including hippocampus [12, 13], and have been shown to play important roles in regulating cortical development, function and pathology [14-17]. Among many of the hippocampal miRNAs, microRNA 137 (miR-137) has been shown to be enriched during hippocampal development while its inhibition resulted in delayed neuronal maturation [18]. Functionally, hippocampal miR-137 de-regulation may be associated with the pathogenesis and development of Alzheimer's disease [19, 20]. However,

the full scope of molecular mechanisms of miR-137 in hippocampal development remains elusive.

In our current study, we intended to investigate whether miR-137 might be involved in the process of anesthesia-induced hippocampal neurodegeneration caused by repeated ketamine administration in an *in vivo* rat model. We examined the effect of ketamine administration on the change of molecular expressing profiles of miR-137 in hippocampus. Functional essay of lentivirus-administrated miR-137 up-regulation was applied to investigate the role of miR-137 on regulating ketamine-induced hippocampal neurodegeneration and memory dysfunction. Finally, the direct target of mi-137, CDC42 was down-regulated through siRNA inhibition to further investigate the molecular mechanisms involved in anesthesia-induced hippocampus neurodegeneration.

Materials and methods

Ethics statement

All procedures in the study were reviewed and approved by the Institutional Animal Care and Use Committee at 1st Hospital of Ningbo City, Ningbo, Zhejiang Province, China.

Animals and anesthesia treatment

Sprague-Dawley rats were housed at the Animal Culture Facility of 1st Hospital of Ningbo City, in accordance with the institutional guidelines by the Institutional Animal Care and Use Committee at 1st Hospital of Ningbo City, Ningbo, Zhejiang Province, China. The *in vivo* induction of ketamine-related hippocampal neurodegeneration was performed at the age of 1-month, based on the method modified from a previous study [9]. Ketamine hydrochloride (Shanghai Bio-Tech, Shanghai, China) was diluted in normal saline. Right before anesthesia, rats were separated from their mothers and were carried out of daily ketamine intraperitoneal injection at a concentration of 75 mg/kg for 3 consecutive days [9]. Normal saline without the mixture of ketamine was also intraperitoneally injected rats as parallel control experiments.

Terminal transferase dUTP nick end labeling (TUNEL) assay

One day after the 3rd injection of ketamine, the rats were deeply anesthetized and quickly conducted with cardiac perfusion with 4% paraformaldehyde. The hippocampi were extracted and cut into 10 μM sections. TUNEL staining was conducted on hippocampal CA1 neurons with an in situ apoptosis detection kit (Roche Applied Science, USA) according to the manufacturer's protocols. Primary antibody against neuronal nuclear antigen (NeuN, 1:100, Shanghia Bio-Tech, Shanghai, China) was also used to identify CA1 neurons in the sectioned slices. Apoptotic CA1 neurons were identified as the percentage of TUNEL positive neurons among all NeuN-positive neurons within 0.01 mm^2 area.

Quantitative real-time reverse transcription-PCR (qRT-PCR)

Total RNA and miRNA fractions were isolated from hippocampal tissues with Trizol reagent according to manufacturer's protocol (Invitrogen, USA). Total RNA concentrations were measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies) at 260 and 280 nm (A260/280), and examined with an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Quantitative real-time reverse transcription-PCR (qRT-PCR) assays were done using the TaqMan miRNA Assay according to manufacturer's protocol (Applied Biosystems, USA). The amplification conditions were 40 cycles of 15 s at 95°C and 1 min at 60°C. The expression level of miR-137 was normalized by housekeeping gene U6, and expression level of CDC42 normalized by GAPDH.

Morris water maze (MWM) test

At age of 2 months, memory functions of rats, including spatial learning and memory abilities, were evaluated by the Morris water maze test. A circular water pool was set up in a soundproof room and the water temperature was maintained at 25°C. A platform (1.5 cm × 1.5 cm) was put in the middle of the pool and 1 cm below water surface. A visual cue of flashing green light was positioned above the platform to guide the animal swim to the target. A video recording system was also positioned above the pool to track the movement and timing of animals. Rats were given two training trials per day for five consecutive days before the experimental Morris water maze test. During test, rats were released into the water maze. The distance and time to reach the platform were recorded for each trial. If the rats did not reach

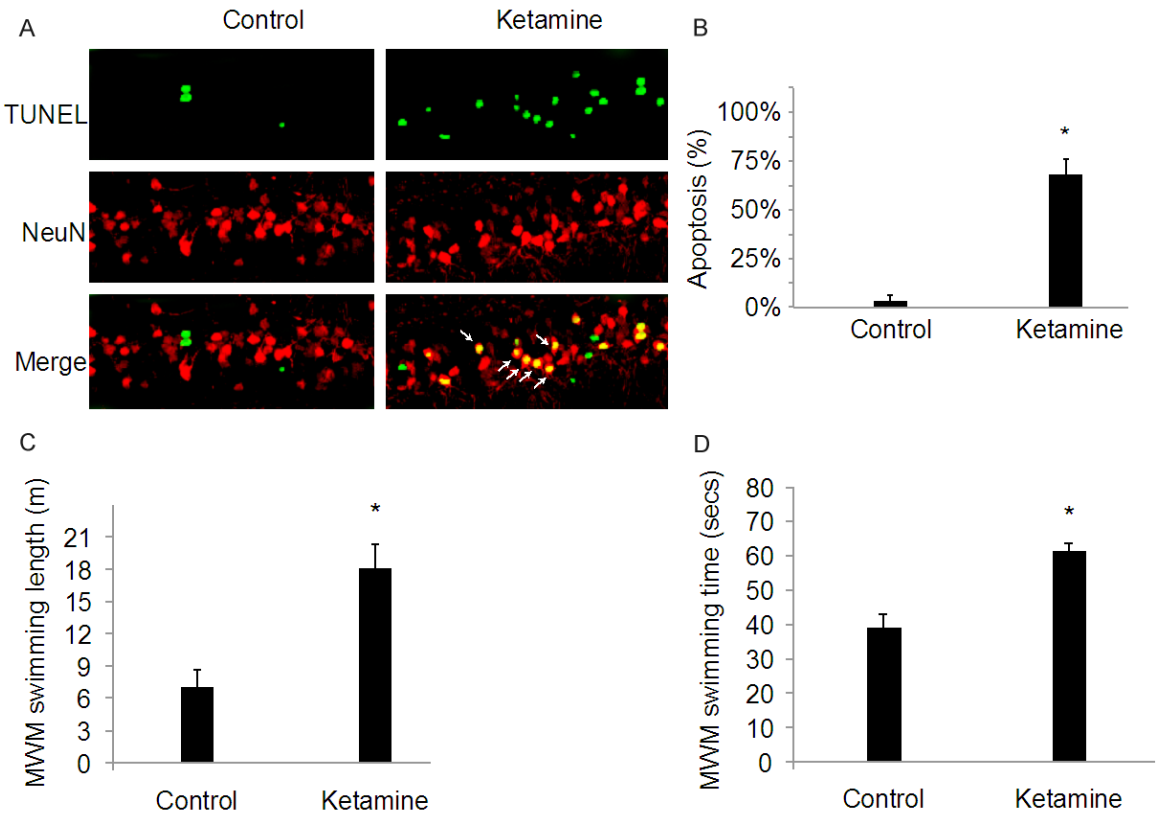

Figure 1. Repeated ketamine administration induced hippocampal apoptosis and memory dysfunction One month old rats were I.P. injected with daily dose of 75 mg/kg ketamine for 3 days. Normal saline was injected in control group of rats. (A) Twenty-four hours after ketamine administration, hippocampi were extracted and the CA1 region was performed with TUNEL staining (green) to identify apoptotic cells. CA1 neurons were identified by NeuN (red) staining. On merged images, the overlapped cells indicated apoptotic CA1 neurons (white arrows). (B) Quantitative measurement demonstrated the percentage of apoptotic CA1 neurons was significantly higher in ketamine-treated hippocampi than in control hippocampi (*: $P < 0.05$). At 2-month of age, Morris water maze test (MWM) was used to assess the memory function of rats. Both the swimming length (C) and swimming time (D) were increased in the rats treated with ketamine than in control rats (*: $P < 0.05$).

platform within 120 seconds, they were guided to do so and the time to reach platform was set to be 120 seconds. The rats were allowed to rest freely on the platform for 20 seconds.

Lentivirus construction to over-express miR-137

The oligonucleotides of rat miR-137 mimics and its non-specific control were synthesized by Ribobio (RiboBio, Shanghai, China). The coding sequences were then amplified and cloned into pCDH-CMV-MCS-EF1-coGFP constructs (System Biosciences, USA) to generate miR-137 mimics oligonucleotide vector and its non-specific control vector. Then, according to manufacturer's instruction, the lentiviral expression constructs and pPACK packaging plasmid mix were co-transfected into 293T cells and viral particles of miR-137 mimics (miR137_mimics) and non-specific control (miR137_NC) were collected and the titer was determined.

SiRNA to knock down CDC42

The specific CDC42 siRNA (CDC42_siRNA) and its non-specific scrambled siRNA (NC_siRNA) were purchased from Stanta Cruz (Santa Cruz Biotechnology, USA). The working concentration to be injected into hippocampus was 20 μM.

Hippocampal injection

One day before ketamine administration, the lentiviruses of miR137_mimics and miR137_NC were injected cortically on the right side of the cortex. After rats were deeply anesthetized, a micro-hole (10 μM × 10 μM) on the skull was opened above the position of hippocampus. A

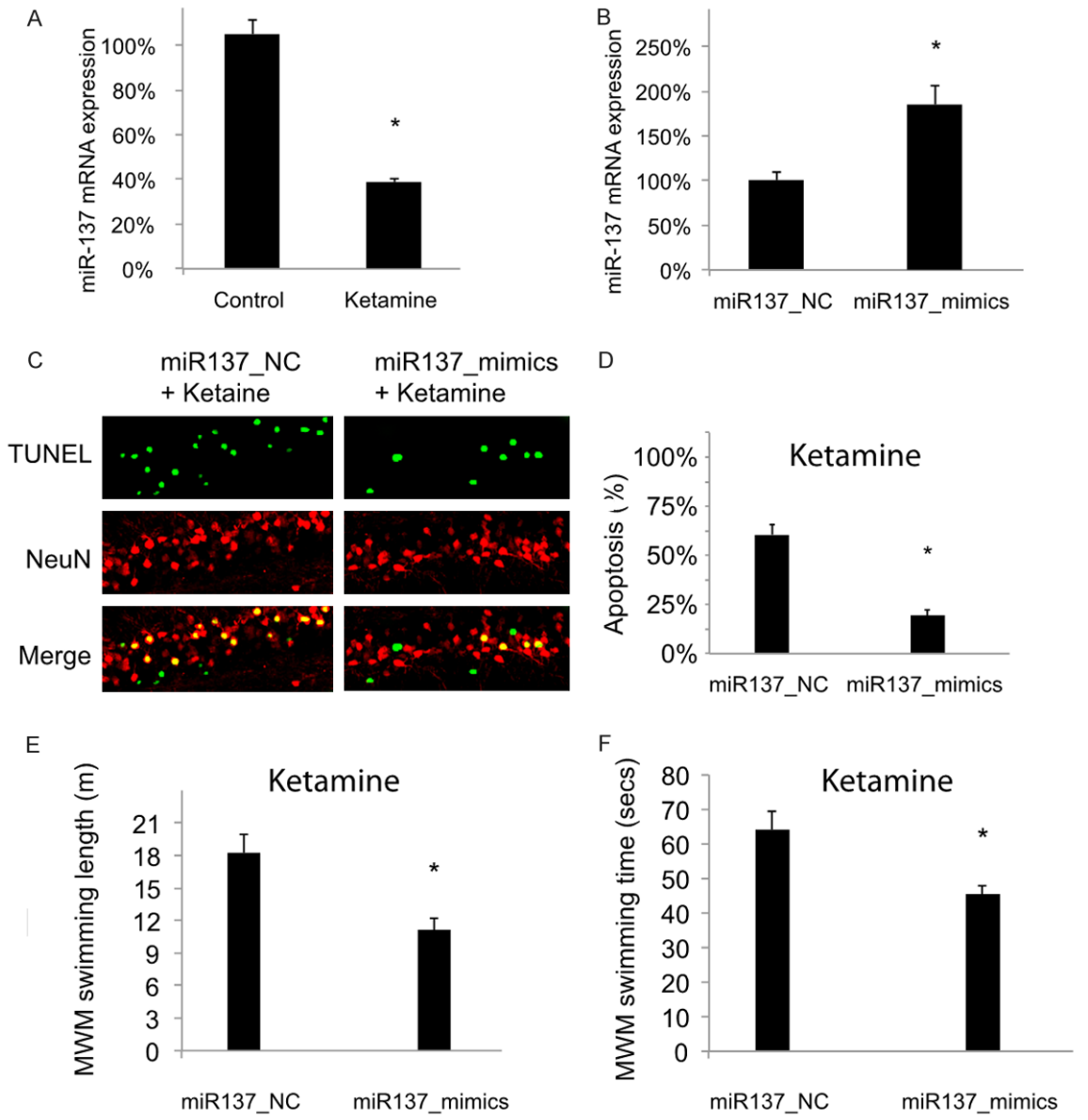

Figure 2. miR-137 was down-regulated by ketamine and miR-137 overexpression reduced anesthesia-induced hippocampal neurodegeneration. (A) Twenty-four hours after ketamine administration, rat hippocampi were extracted and the mRNA expression of miR-137 was examined by qRT-PCR. The value was normalized to the one under control condition (*: $P < 0.05$). (B) The efficiency of hippocampal injection of lentivirual vector to over-express miR-137 (miR137_mimics) was examined by qRT-PCR. In control group, non-specific lentivirus of miR-137 (miR137_NC) was injected into rat hippocampi (*: $P < 0.05$). One day before ketamine administration, rats received hippocampal injection of either miR137_NC or miR137_mimics lentiviruses. Then, twenty-four hours after the 3rd administration of ketamine, TUNEL staining was performed (C) and the apoptotic CA1 neurons were quantified (D). At 2-month of age, Morris water maze test (MWM) was used to assess the memory function of rats. Both the swimming length (E) and swimming time (F) were recorded. (*: $P < 0.05$)

Hamilton micro-surgically gauged syringe was applied to inject 5 μL of lentivirus of miR137_mimics, or miR137_NC at the coordinates assessed from bregma and skull surface: anteroposterior -3.0 mm, lateral +1.9 mm, and vertical -1.9 mm. For siRNAs induction, 10 μL of CDC42_siRNA (20 uM) or its non-specific control siRNA (NC_siRNA, 20 μM) was injected into hippocampi.

Statistical analysis

Statistic analysis was done by a windows-based SPSS software (version 12.0). All data were

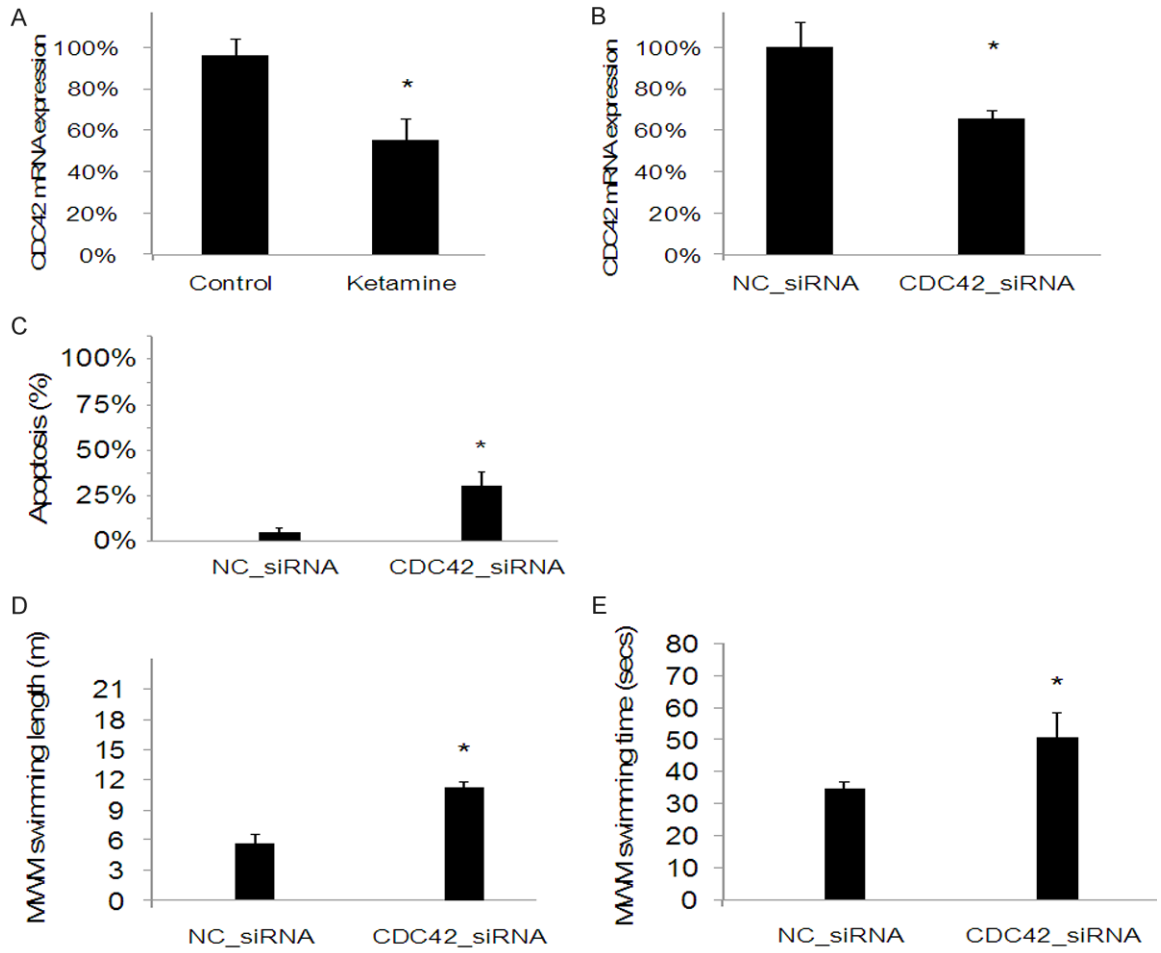

Figure 3. CDC42 inhibition promoted hippocampal neurodegeneration. (A) Twenty-four hours after ketamine administration, rat hippocampi were extracted and the mRNA expression of CDC42 was examined by qRT-PCR. The value was normalized to the one under control condition (*: $P < 0.05$). (B) The efficiency of hippocampal knocking down CDC42 (CDC42_siRNA, 20 μM) was examined by qRT-PCR. In control group, non-specific scrambled siRNA (NC_siRNA, 20 uM) was injected into rat hippocampi (*: $P < 0.05$). (C) Twenty-four hours after the hippocampal injection of siRNAs, TUNEL stainings were performed on two groups of hippocampi to compare the apoptotic CA1 neurons (*: $P < 0.05$). At 2-month of age, Morris water maze test (MWM) was used to assess the memory function after hippocampal injections of siRNAs. Both the swimming length (D) and swimming time (E) were recorded. (*: $P < 0.05$).

presented as mean ± standard deviations. The statistic differences were measured with a student's t-test. Statistical significance was determined if $P < 0.05$.

Results

Repeated anesthesia induced hippocampal neurodegeneration

We used a modified protocol, based on previous studies [9, 10], to induce hippocampal neurodegeneration by repeated anesthesia administration *in vivo*. One-month-old Sprague-Dawley rats were intraperitoneally (I.P.) administrated with one dose of 75 mg/kg ketamine per day for 3 days. Parallel control rats were injected with same amount of normal saline but without ketamine. Twenty-four hours after the last administration of ketamine, rats were sacrificed and the hippocampi were extracted and examined with TUNEL staining (**Figure 1A**). We found that, in control hippocampus sparse TUNEL positive cells (green) were observed in CA1 region but not overlapped with NeuN-positive CA1 neurons (red). However, in ketamine-treated hippocampus, significant number of CA1 neurons was identified to be positive to TUNEL staining (white arrows). Quantitative analysis of comparing the percentages of apoptotic CA1 neurons in ketamine-treated hippo-

campi vs. control (non-ketamine) hippocampi confirmed that repeated anesthesia resulted in significant apoptosis in hippocampal CA1 neurons. (**Figure 1B**, *: $P < 0.05$).

The long-term effect of repeated ketamine administration was evaluated at the age of 2-month through a Morris water maze (MWM) test. The results showed that ketamine-treated rats spent significantly longer length (**Figure 1C**) and time (**Figure 1D**) to swim to the platform, as compared to rats without ketamine administration.

Thus, both short-term TUNEL staining and long-term MWM test demonstrated that repeated administration of ketamine induced hippocampal neurodegeneration.

Repeated anesthesia induced miR-137 down-regulation

We then investigated whether the expression profile of miR-137 was modulated during the process of ketamine-induced hippocampal neurodegeneration. Twenty-four hours after ketamine administration, hippocampi were extracted. The expression level of hippocampal miR-137 messenger RNA was examined by qRT-PCR, and compared with the expression level of hippocampal miR-137 mRNA in control animals that just received normal saline (no ketamine) I.P. injections for 3 days. The result showed that ketamine induced significant down-regulation of miR-137 in hippocampus (**Figure 2A**).

Upregulation of miR-137 protected ketamine-induced hippocampal neurodegeneration

Since the expression level of hippocampal miR-137 was down-regulated by repeated anesthesia, we asked whether up-regulating miR-137 would in turn regulate the process of hippocampal neurodegeneration induced by repeated ketamine treatment. For that purpose, we constructed lentivirual vector to over-express miR-137 (miR137_mimics) and injected it into rat hippocampus one day before ketamine administration. Parallel control experiment of injecting non-specific lentivirus (mi137_NC) was also performed. We examined the efficiency of lentivirual injection by qRT-PCR and found that miR137_mimics did up-regulate the expression level of miR-137 mRNA in hippocampus, as compared to the lentivirual injection of miR137_NC (**Figure 2B**, *: $P < 0.05$).

After rats received hippocampal injection of lentiviruses and subsequent 3-day ketamine I.P. administration, TUNEL staining was performed in twenty-four hours to examine the effect of up-regulating miR-137 on anesthesia induced hippocampal neurodegeneration. The result showed that fewer CA1 neurons were TUNEL positive in hippocampi treated with miR137_mimics, than in hippocampi treated with miR137_NC (**Figure 2C**). Quantitative measurement of the percentage of apoptotic CA1 neurons confirmed that over-expressing miR-137 protected the hippocampal apoptosis induced by ketamine (**Figure 2D**, *: $P < 0.05$). We further examined the long-term effect of up-regulating miR-137 on ketamine-induced memory dysfunction. One month after lentivirual pre-treatment and ketamine administration, rats were examined with MWM test. The result showed that the rats with up-regulated hippocampal miR-137 performed much better than the rats without hippocampal miR-137 upregulation, as they used shorter swimming distance (**Figure 2E**) and less swimming time (**Figure 2F**) to find the platforms.

Thus, both our short-term and long-term *in vivo* results demonstrated that in both short-term and long-term experiments that miR-137 upregulation in hippocampus had protective effect on anesthesia-induced neurodegeneration.

Repeated anesthesia down-regulated CDC42 in hippocampus

It has been shown in the literatures that CDC42 is the direct target of miR-137 regulation [21-23]. In the present study, we asked whether CDC42 was also playing a role in the regulation of miR-137 on anesthesia-induced hippocampal neurodegeneration. By qRT-PCR, we examined the expression of CDC42 after ketamine administration and found that repeated anesthesia induced significant down-regulation of CDC42 in hippocampus (**Figure 3A**, *: $P < 0.05$).

Downregulation of CDC42 induced hippocampal neurodegeneration

We then investigated whether CDC42 played direct role in regulating hippocampal neurodegeneration. CDC42 was genetically knocked down in hippocampi by cortical injection of siRNAs. Both the CDC42 specific siRNA (CDC42_

siRNA, 20 μM) and its non-specific scrambled siRNA (NC_siRNA, 20 μM) were cortically administrated into rat hippocampi, respectively. Twenty-four hours later, qRT-PCR showed that CDC42_siRNA was able to specifically down-regulate hippocampal expression of CDC42, as compared to control siRNA (**Figure 3B**, *: $P < 0.05$). TUNEL staining was also performed. It demonstrated that, more apoptotic CA1 neurons were induced in hippocampi treated with CDC42_siRNA, as compared to the hippocampi treated with NC_siRNA (**Figure 3C**, *: $P < 0.05$). Finally, one month after siRNA treatment, rats were examined with MWM test. It showed that the rats with hippocampal CDC42 down-regulation had deteriorated memory function with longer swimming distance (**Figure 3D**), and extended swimming time (**Figure 3E**) to find platform.

Thus, the results demonstrated that in both short-term and long-term experiments that CDC42 down-regulation in hippocampus had proliferative effect on neurodegeneration, similar to the effect induced by repeated anesthesia administration.

Discussion

Ketamine is primarily used in both adult and pediatric anesthesia. Recent evidence revealed that repeated administration of ketamine elevated cortical neural activities, resulted in neurotoxicity, thus delayed or deteriorated the maturation of young brain in both animal and human studies. It is of great interest, both clinically and academically, to dissect the underlying mechanisms of anesthesia-induced cortical neurodegeneration.

In current study, we first established an *in vivo* animal model showing that repeated I.P. administration of ketamine (75 mg/kg) in one-month old rat induced significant neuronal apoptosis in hippocampal CA1 regions, as well as severe memory dysfunctions at 2-month age (**Figure 1**). This result is in line with previous *in vitro* and *in vivo* studies demonstrating hippocampal neurotoxicity by ketamine administration [8, 9].

Furthermore, we intended to examine whether miR-137 was playing an important role in modulating anesthesia-induced neurodegeneration in hippocampus. We constructed a lentivirus containing miR-137 mimic oligonucleotides and successfully over-expressed miR-137 in hippocampus (**Figure 2A**). Then, we conducted hippocampal injection of miR137_mimics before ketamine administration, and discovered that ketamine-induced hippocampal CA1 neuron apoptosis was significantly reduced, and memory loss markedly rescued by miR-137 upregulation (**Figure 2B-F**), suggesting that miR-137, through its upregulation, played critical role in protecting anesthesia-induced hippocampal neurodegeneration. A recent study had demonstrated that miR-137 was developmental upregulated in hippocampus, whereas down-regulation of miR-137 resulted in delayed dendritic maturation of hippocampal neurons [18]. Along with our results showing protective effect against neurodegeneration by over-expressing miR-137, both studies may point to a similar functional role of miR-137 upregulation in regulating the maturation or pathology of hippocampal development.

As shown in many of the literatures, CDC42 is the direct target of miR-137 [21-23]. Thus, in the present study, we intended to further understand the molecular pathways involved in the regulation of miR-137 by investigating the role of CDC42 during the process of anesthesia-induced hippocampal neurodegeneration. We found that CDC42 was down-regulated by ketamine administration, as well as siRNA-induced CDC42 down-regulation resulted in hippocampal apoptosis and memory loss in animals (**Figure 3**). This result suggested that, miR-137 and CDC42 might be coordinately regulated during anesthesia-induced neurodegeneration in hippocampus, as both of them were down-regulated by ketamine administration. Contrary to our hypothesis, studies from carcinoma showed that miR-137 negatively regulated CDC42 [21]. Thus, if the findings of our study could be confirmed further by biochemical investigation on the hippocampal interaction between miR-137 and CDC42, it may present a distinct yet new mechanism of miRNA regulation in hippocampus than in cancer.

Taken together, our data presented a novel finding that upregulating miR-137 was able to protect ketamine-induced neurodegeneration in rat hippocampus. Although the experimental condition of repeated administration of ketamine was unlikely to be the case in clinical applications, the results of our study may help

on seeking targeted therapeutic methods to prevent or rescue anesthesia-induced cortical degeneration in human patients.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Changshun Huang, Department of Anesthesia, The First Hospital of Ningbo City, 59 Liuting St., Ningbo 315010, Zhejiang, China. Tel: 86-54-9078432; E-mail: 1328634997@qq.com

References

[1] Olney J, Labruyere J, Wang G, Wozniak D, Price M and Sesma M. NMDA antagonist neurotoxicity: mechanism and prevention. Science 1991; 254: 1515-1518.

[2] Deasy C and Babl FE. Intravenous vs intramuscular ketamine for pediatric procedural sedation by emergency medicine specialists: a review. Paediatr Anaesth 2010; 20: 787-796.

[3] Green SM and Johnson NE. Ketamine sedation for pediatric procedures: Part 2, Review and implications. Ann Emerg Med 1990; 19: 1033-1046.

[4] White PF, Way WL and Trevor AJ. Ketamine--its pharmacology and therapeutic uses. Anesthesiology 1982; 56: 119-136.

[5] Brambrink AM, Orfanakis A and Kirsch JR. Anesthetic neurotoxicity. Anesthesiol clin 2012; 30: 207-228.

[6] Dong C and Anand K. Developmental neurotoxicity of ketamine in pediatric clinical use. Toxicol lett Jun 20; 220: 53-60.

[7] Olney JW, Wozniak DF, Jevtovic-Todorovic V, Farber NB, Bittigau P and Ikonomidou C. Drug-induced apoptotic neurodegeneration in the developing brain. Brain Pathol 2002; 12: 488-498.

[8] Huang L, Liu Y, Zhang P, Kang R, Li X, Bo L and Dong Z. In vitro dose-dependent inhibition of the intracellular spontaneous calcium oscillations in developing hippocampal neurons by ketamine. PLoS One 2013; 8: e59804.

[9] Huang L, Liu Y, Jin W, Ji X and Dong Z. Ketamine potentiates hippocampal neurodegeneration and persistent learning and memory impairment through the PKCgamma-ERK signaling pathway in the developing brain. Brain Res 2012; 1476: 164-171.

[10] Hayashi H, Dikkes P and Soriano SG. Repeated administration of ketamine may lead to neuronal degeneration in the developing rat brain. Paediatr anaesth 2002; 12: 770-774.

[11] Li L and Liu Y. Diverse small non-coding RNAs in RNA interference pathways. Methods Mol Biol 2011; 764: 169-182.

[12] Juhila J, Sipila T, Icay K, Nicorici D, Ellonen P, Kallio A, Korpelainen E, Greco D and Hovatta I. MicroRNA expression profiling reveals miRNA families regulating specific biological pathways in mouse frontal cortex and hippocampus. PLoS One 2011; 6: e21495.

[13] Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M and Greenberg ME. A brain-specific microRNA regulates dendritic spine development. Nature 2006; 439: 283-289.

[14] Lau P and Hudson LD. MicroRNAs in neural cell differentiation. Brain Res 2010; 1338: 14-19.

[15] Konopka W, Kiryk A, Novak M, Herwerth M, Parkitna JR, Wawrzyniak M, Kowarsch A, Michaluk P, Dzwonek J, Arnsperger T, Wilczynski G, Merkenschlager M, Theis FJ, Kohr G, Kaczmarek L and Schutz G. MicroRNA loss enhances learning and memory in mice. The Journal of neuroscience : the official journal of the Society for Neuroscience. J Neurosci 2010; 30: 14835-14842.

[16] Iyengar BR, Choudhary A, Sarangdhar MA, Venkatesh KV, Gadgil CJ and Pillai B. Non-coding RNA interact to regulate neuronal development and function. Front Cell Neurosci 2014; 8: 47.

[17] Follert P, Cremer H and Beclin C. MicroRNAs in brain development and function: a matter of flexibility and stability. Front Mol Neurosci 2014; 7: 5.

[18] Smrt RD, Szulwach KE, Pfeiffer RL, Li X, Guo W, Pathania M, Teng ZQ, Luo Y, Peng J, Bordey A, Jin P and Zhao X. MicroRNA miR-137 regulates neuronal maturation by targeting ubiquitin ligase mind bomb-1. Stem Cells 2010; 28: 1060-1070.

[19] Geekiyanage H and Chan C. MicroRNA-137/181c regulates serine palmitoyltransferase and in turn amyloid beta, novel targets in sporadic Alzheimer's disease. J Neurosci 2011; 31: 14820-14830.

[20] Lukiw WJ. Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. Neuroreport 2007; 18: 297-300.

[21] Chen Q, Chen X, Zhang M, Fan Q, Luo S and Cao X. miR-137 is frequently down-regulated in gastric cancer and is a negative regulator of Cdc42. Dig Dis Sci 2011; 56: 2009-2016.

[22] Liu M, Lang N, Qiu M, Xu F, Li Q, Tang Q, Chen J, Chen X, Zhang S, Liu Z, Zhou J, Zhu Y, Deng Y, Zheng Y and Bi F. miR-137 targets Cdc42 expression, induces cell cycle G1 arrest and inhibits invasion in colorectal cancer cells. Int J Cancer 2011; 128: 1269-1279.

[23] Zhu X, Li Y, Shen H, Li H, Long L, Hui L and Xu W. miR-137 inhibits the proliferation of lung cancer cells by targeting Cdc42 and Cdk6. FEBS Lett 2013; 587: 73-81.