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Original Article

microRNA profiling in the hippocampus of isoflurane-anesthetized mice

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Abstract: Postoperative cognitive dysfunction (POCD), which commonly troubles elderly (more than 65-year-old) patients, has been indicated to associate with anesthesia, surgical stress and other conditions. However, little is known about biomarkers responsible for such disorder. The present study was to characterize the microRNA profiling in the hippocampus of isoflurane-anesthetized mice with microRNA microarray profiling and real-time quantitative PCR methods, and the molecular signaling pathway enrichment analysis and Gene Ontology (GO) enrichment analysis for above-mentioned miRNAs were performed to elucidate possible mechanism underlining the toxicity of Isoflurane on neural cells. The microarray analysis indicated that 11 miRNAs (21-23 nucleotides in length), such as mmu-miR-103-3p, mmu-miR-27a-3p, mmu-miR-337-3p and long pre-miRNAs (73-120 nucleotides long), such as mmu-miR-467a-1, mmu-miR-23a, mmu-miR-3067 were significantly downregulated in the hippocampus tissues from Isoflurane-anesthetized mice. On the other side, three miRNAs (mmu-miR-3073a-3p, mmu-miR-6400 and mmu-miR-7684-5p) and two pre-miRNAs (mmu-miR-3070a and mmu-miR-93) were markedly upregulated in the Isoflurane-anesthetized mice hippocampus. The GO enrichment analysis suggested that these miRNAs were closely associated with cognition, detection of external stimulus cell surface receptor signaling pathway and other functional processes. And such molecular signaling pathways as lysosome, extracellular matrix (ECM)-receptor interaction, PI3K-Akt signaling pathway and other molecular signaling pathways were included in the hippocampus of Isoflurane-anesthetized mice. In conclusion, significantly aberrant miRNAs were promoted by Isoflurane in the hippocampus tissues from Isoflurane-anesthetized mice. And such deregulated miRNAs might regulate cognition, extracellular stimulus sensitization and other functional processes, via regulating lysosome, ECM-receptor interaction, PI3K-Akt signaling and other pathways.

Keywords: microRNA profiling, hippocampus, isoflurane, postoperative cognitive dysfunction (POCD)

Introduction

Postoperative cognitive dysfunction (POCD) commonly troubles elderly (more than 65-year-old) patients, including the reduction or deterioration of perception, memory, information analysis, concentration and response [1]. POCD has been indicated to associate with anesthesia [2], surgical stress [3] and other conditions. However, little is known about biomarkers responsible for such disorder. Given the contribution of anesthesia agents to POCD, it is considered that anesthetics poses direct toxicity, via altering calcium homeostasis, promoting systemic inflammatory response, suppressing neuronal stem cell function, accelerating endogenous neurodegenerative processes, as well as inducing cell apoptosis [4, 5]. The production of proinflammatory cytokines, such as TNF-α IL-β and IL-6 was upregulated in mice neurons by isoflurane anesthesia [6], or was downregulated by sevoflurane and desflurane thoracic surgical patients [7]. The learning/memory deficiency-associated amyloid-β peptide concentration was observed in older rats [8] or in cultured neural cells by volatile anesthetics, such as isoflurane, sevoflurane and desflurane [4].

microRNAs (miRNAs) are groups of small single-stranded RNA molecules, ranging 22-24 nucleotides in length, and regulate the expression of approximately 30% genes [9]. microRNA expression profiling in the brain of rat subject to sevoflurane and propofol anesthesia demonstrated that the two widely-used general anesthetics

altered miRNA expression patterns [10]. Therefore, the deregulated miRNAs might be associated with POCD, in terms of anesthesia. Recently, miRNAs have been shown to involve in the pathogenesis of the nervous system diseases [11, 12], such as Huntington's disease [13] and Alzheimer's disease [14, 15]. The abnormal expression of multiple miRNAs was also reported to be associated with the occurrence of neural tube defects [16], with neurobehavioral disorders [17]. We speculated that the deregulated miRNA expression might contribute to POCD.

In the present study, we profiled the expression of miRNAs with microarray technology in the hippocampus of Isoflurane-anesthetized mice. And then performed the analysis of signaling pathway enrichment and of function pathway enrichment of the deregulated miRNAs in the hippocampus of Isoflurane-anesthetized mice. Our study demonstrated significant different miRNA profiling in the hippocampus of Isoflurane-anesthetized mice. And such deregulated miRNAs might be associated with the cognition dysfunction in the Isoflurane-anesthetized mice.

Materials and methods

Ethics statement

The animal care and use were approved by the Institutional Animal Care and Use Committee of Capital Medical University.

Establishment of the isoflurane-anesthetized mice model

The aged male mice (10-12-month old) were purchased from Beijing Experimental Animal Research Center (Vital River Company) and were maintained in pathogen-free and fresh air-supplied facilities under the guidelines of Animal Research: Reporting of In Vivo Experiments (ARRIVE). The aged mice were anesthetized by inhaling 2.5% isoflurane for 3-5 minutes until no active movements and were inhaled with 1.5% isoflurane for another 25 minutes. The control mice were also performed the same two rounds inhalation with fresh air for 3-5 minutes and then for 25 minutes. Once the anesthetized mice were awaking, the two groups of mice were sacrificed by instant destruction of the brain stem for the isolation of mice hippocampus tissues. The whole brains were immediately placed on ice post the head-neck isolation for the hippocampus isolation. The whole hippocampus tissues were stored at -80°C before use.

miRNA microarray

miRNA microarray analyses were performed by Beijing cnkingbio Corp. using hippocampus tissues from the normal or Isoflurane-anesthetized mice. miRNA microarray analyses using Agilent mouse miRNA array. The data of microarray analyses were performed as following. Target genes were respectively predicted by Targets can and Miranda databases and were selected from the intersected target genes from the two databases. Gene Ontology (GO) enrichment analysis was performed to predict the function of deregulated miRNAs and the molecular signaling pathway enrichment was performed to predict the deregulated miRNAs-regulated molecular signaling pathways.

miRNA isolation and quantitative real-time PCR (qRT-PCR)

miRNA samples were extracted using the miRNA Isolation Kit (Sigma-Aldrich, St. Louis, MO, USA). miRNA reverse transcription was performed using miRcute miRNA First-strand cDNA Synthesis kits (Tiangen, Beijing, China) under the guidance of the kit's manual. The qRT-PCR of mmu-miR-103-3p, mmu-miR-337-3p, mmu-miR-344c-3p or mmu-miR-409-5p was performed with the mirVana RT-qPCR miRNA Detection Kit (Ambion, Austin, TX, USA), with each miRNA-specific primer. The miRNA level was presented as a relative level to U6 small RNA (taken as internal control) using the 2-ΔΔCt method.

Gene ontology (GO) enrichment analyses and target prediction

To investigate the biological functions of potential target genes of the deregulated miRNAs, we conducted GO enrichment analysis, according to the GO annotation data for Mus musculus from the UniProt database and matched to target genes via the python language script. The result of GO analysis was ranked with statistical significance by calculating their *P*-values based on hypergeometric distribution with $P < 0.01$ considered to indicate significance,

Table 1. Downregulated miRNAs in the hippocampus of Isoflurane-anesthetized mice

All_Transcript ID (Array Design)	Average array signaling (log_2)		*P* value	FDR *P*-value (All Conditions)
	Control	Isoflurane		
mmu-miR-103-3p	13.223 ± 0.028	13.088 ± 0.058	0.0058	0.1844
mmu-miR-1197	0.688 ± 0.107	0.352 ± 0.057	0.0015	0.3034
mmu-miR-23a	1.013 ± 0.112	0.470 ± 0.109	0.0004	0.2358
mmu-miR-27a-3p	9.884 ± 0.187	9.527 ± 0.215	0.0460	0.3872
mmu-miR-3067	0.556 ± 0.014	0.324 ± 0.175	0.0386	0.4466
mmu-miR-337-3p	3.442 ± 0.648	1.650 ± 0.443	0.0038	0.3912
mmu-miR-344c-3p	4.799 ± 0.248	4.195 ± 0.378	0.0368	0.3872
mmu-miR-409-5p	8.345 ± 0.067	8.031 ± 0.122	0.0041	0.3890
mmu-miR-449a-5p	1.130 ± 0.253	0.881 ± 0.021	0.0977	0.3912
mmu-miR-467a-1	2.750 ± 0.062	2.359 ± 0.127	0.0015	0.3872
mmu-miR-467a-5p	5.560 ± 0.087	5.306 ± 0.101	0.0089	0.2320
mmu-miR-669a-10	1.000 ± 0.132	0.473 ± 0.210	0.0054	0.3890
mmu-miR-669a-11	1.000 ± 0.132	0.473 ± 0.210	0.0054	0.3912
mmu-miR-669a-4	1.000 ± 0.132	0.473 ± 0.210	0.0054	0.3872
mmu-miR-669a-5	1.000 ± 0.132	0.473 ± 0.210	0.0054	0.3912
mmu-miR-669a-8	1.000 ± 0.132	0.473 ± 0.210	0.0054	0.3872
mmu-miR-669a-9	1.000 ± 0.132	0.473 ± 0.210	0.0054	0.3850
mmu-miR-6985-5p	1.275 ± 0.260	0.796 ± 0.146	0.0183	0.4274
mmu-miR-7010-3p	0.893 ± 0.172	0.371 ± 0.147	0.0036	0.4776
mmu-miR-7025-3p	1.441 ± 0.094	0.740 ± 0.220	0.0011	0.1855
mmu-miR-7064	0.776 ± 0.189	0.376 ± 0.005	0.0055	0.3178
mmu-miR-93-5p	9.596 ± 0.014	9.430 ± 0.123	0.0361	0.2769

respectively [18]. The miRWalk database [72] was used to identify target genes of these miRNAs. Genes with $P < 0.05$ were regarded as predicted and validated targets.

Statistical analysis

qRT-PCR results were presented as the mean ± standard error of mean (SEM), and were analyzed by the unpaired two-sample Student's t-test, and $P < 0.05$ (two-tailed) was considered as significantly different.

Results

Differential miRNA profiling in isoflurane-anesthetized mice

Important roles of miRNAs have been suggested by recent studies in the nervous system diseases [11, 12]. To investigate the regulation by Isoflurane on the expression of miRNAs in nervous system, we isolated the hippocampus tissues from Isoflurane-anesthetized mice (10-12-month old), and then profiled the miRNAs expression, with normal mice (10-12-month old) as control. Detailed information about miRNA probes which were utilized in the present study were listed in Supplementary Table 1. It was indicated in **Table 1** that there were 11 miRNAs (21-23 nucleotides in length) which were significantly downregulated in the hippocampus tissues from Isoflurane-anesthetized mice (mmu-miR-103-3p, mmu-miR-27a-3p, mmu-miR-337-3p, mmu-miR-344c-3p, mmu-miR-409-5p, mmu-miR-449a-5p, mmu-miR-467a-5p, mmu-miR-6985-5p, mmu-miR-7010-3p, mmu-miR-7025-3p and mmu-miR-93-5p). And three miRNAs (mmu-miR-3073a-3p, mmu-miR-6400 and mmu-miR-7684-5p) were markedly upregulated in the Isoflurane-anesthetized mice hippocampus (**Table 2**). In addition, some pre-miRNAs have also been profiled in this study. **Table 1** demonstrated that 17 long pre-miRNAs (73-120 nucleotides long) (mmu-miR-467a-1, mmu-miR-23a, mmu-miR-3067, mmu-miR-669a-10, mmu-miR-669a-11, mmu-miR-669a-4, mmu-miR-669a-5, mmu-miR-669a-8, mmu-miR-669a-9, mmu-miR-669a-8, mmu-miR-669a-9, mmu-miR-669a-10, mmu-miR-669a-11, mmu-miR-93, mmu-miR-3070a, mmu-

Table 2. Upregulated miRNAs in the hippocampus of Isoflurane-anesthesized mice

All_Transcript ID (Array Design)	Average array signaling (log_2)		*P* value	FDR *P*-value (All Conditions)
	Control group	Isoflurane group		
mmu-miR-3070a	0.755 ± 0.050	0.969 ± 0.066	0.0020	0.3905
mmu-miR-3073a-3p	0.417 ± 0.107	0.754 ± 0.083	0.0025	0.2395
mmu-miR-6400	0.366 ± 0.016	0.901 ± 0.266	0.0070	0.3119
mmu-miR-7684-5p	1.568 ± 0.527	2.516 ± 0.385	0.0272	0.2673
mmu-miR-93	1.736 ± 0.049	1.860 ± 0.096	0.0616	0.2988

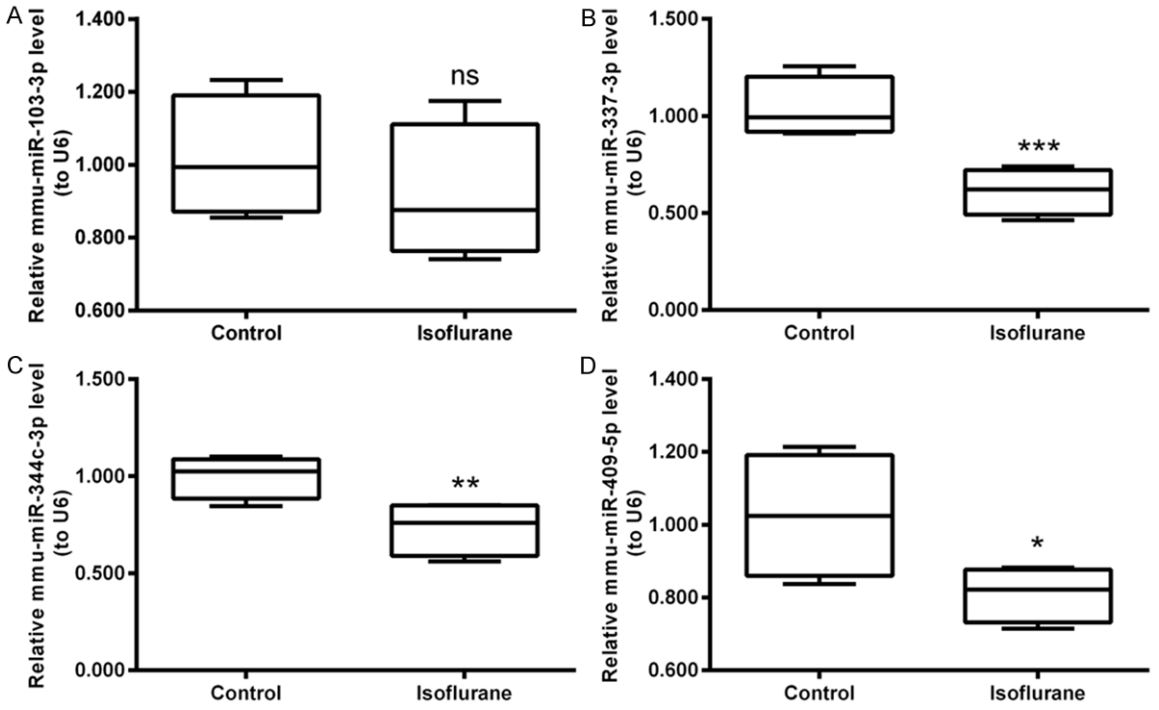

Figure 1. Relative serum levels of mmu-miR-103-3p, mmu-miR-337-3p, mmu-miR-344c-3p and mmu-miR-409-5p in the hippocampus of Isoflurane-anesthesized mice. The relative amounts of mmu-miR-103-3p (A), mmu-miR-337-3p (B), mmu-miR-344c-3p (C), and mmu-miR-409-5p (D) were examined by qRT-PCR in the hippocampus tissues of Isoflurane-anesthesized/control mice. All other samples were expressed as a relative value to the control sample. The sample numbers and the statistical significance were shown respectively. Statistical significance was considered with $P < 0.05$ (*), $P < 0.001$ (***) or no significance (ns).

miR-7064, mmu-miR-1197) were also markedly downregulated (**Table 1**), whereas mmu-miR-3070a and mmu-miR-93 were significantly upregulated (**Table 2**).

Mouse miRNAs validation by qRT-PCR

To evaluate the accuracy of the profiled miRNAs alteration in the Isoflurane-anesthetized mice, we randomly selected four miRNAs (21-23) (mmu-miR-103-3p, mmu-miR-337-3p, mmu-miR-344c-3p, mmu-miR-409-5p) for validation using qRT-PCR. As shown in **Figure 1**, the levels of mmu-miR-337-3p, mmu-miR-344c-3p, mmu-miR-409-5p) were significantly lower in the hippocampus tissues from Isoflurane-anesthetized mice than in the normal control group ($P < 0.001$, $P < 0.01$ or $P < 0.05$). However, the reduction of mmu-miR-103-3p level was not significant. Therefore, the changes of miRNA assayed by qRT-PCR were consistent with the changes profiled by miRNA array analysis.

miRNA target network characterization

To understand how possibly deregulated miRNAs are related to the toxicity of Isoflurane on neural cells, we then performed GO enrichment analysis and molecular signaling pathway enrichment analysis for above-mentioned miR-

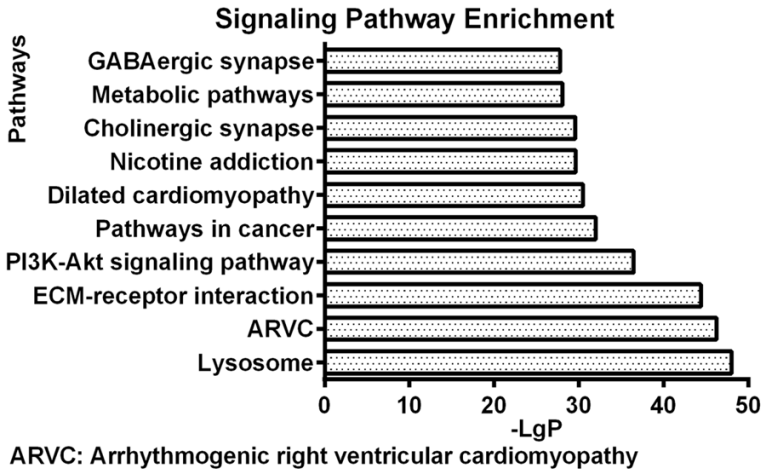

Figure 2. Function pathway enrichment of deregulated miRNAs in the hippocampus of Isoflurane-anesthesized mice. GO enrichment analysis was performed according to the GO annotation data for Mus musculus from the UniProt database and matched to target genes via the python language script. The result of GO analysis was ranked with statistical significance by calculating their *p*-values based on hypergeometric distribution. Cognition: Cognition; DES: Detection of external stimulus; CSRSP: Cell surface receptor signaling pathway; SPP: Sensory perception of pain; Signaling: Signaling; PRMMP: Positive regulation of macromolecule Metabolic process; PMP: Phosphorus metabolic process; SPMS: Sensory perception of mechanical stimulus; RMOD: Regulation of multicellular organismal Development; PRMF: Positive regulation of molecular function; GPCRSP: G-protein coupled receptor signaling Pathway; BA: Biological adhesion; PRDP: Positive regulation of developmental Process; MCSO: Macromolecular complex subunit Organization; PRBP: Positive regulation of biosynthetic process; NP: Neuromuscular process; PTM: Peptidyl-tyrosine modification; HP: Homeostatic process; PRCC: Positive regulation of cell communication; PRNCMP: Positive regulation of nitrogen compound Metabolic process; PRRS: Positive regulation of response to stimulus; LC: Localization of cell; PIT: Potassium ion transport.

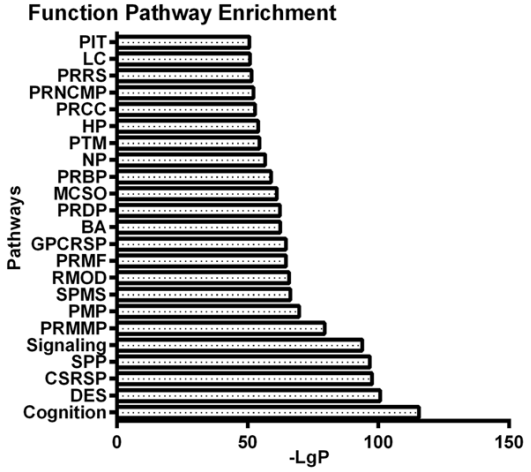

Figure 3. Signaling pathway enrichment of deregulated miRNAs in the hippocampus of Isoflurane-anesthesized mice. The miRWalk database was used to identify target genes of these miRNAs. Genes with $P < 0.05$ were regarded as predicted and validated targets.

NAs as already described in the Materials and methods section. The GO enrichment analysis suggested that these miRNAs were closely associated with cognition, detection of external stimulus cell surface receptor signaling pathway, sensory perception of pain signaling, positive regulation of macromolecule metabolic process and other functional processes (**Figure 2**). The molecular signaling pathway enrichment analysis of the deregulated miRNAs indicated that such molecular signaling pathways as lysosome, extracellular matrix (ECM)-receptor interaction, PI3K-Akt signaling pathway and other molecular signaling pathways were included in the hippocampus of Isoflurane-anesthetized mice (**Figure 3**).

Discussion

The mechanism underlying the inhalational anesthetic-induced neurocognitive impairment is thought to involve neurodegeneration. However, the involved pathways are not entirely clear. The inhalation of isoflurane regulated the central cholinergic system, such as cholinergic receptor insensitivity, affinity, particularly in hippocampus [19], which was in relation to spatial learning and memory impairment [19, 20].

Majority of miRNAs have been increasing shown to be spatially and temporally expressed in the central nerve system [21], and play important roles in nervous system diseases [21, 22]. Therefore, miRNAs might involve in the isoflurane-induced learning and memory impairment. Hippocampi have a close relationship with learning and memory. In the present study we identified a distinct miRNA expression profile in the hippocampal of mice which inhaled isoflurane. There were 11 downregulated 21-23 nt-long miRNAs, such as mmu-miR-103-3p, mmu-miR-27a-3p and mmu-miR-337-3p, and three markedly upregulated miRNAs

(mmu-miR-3073a-3p, mmu-miR-6400 and mmu-miR-7684-5p) in the Isoflurane-anesthetized mice hippocampus. And another 17 pre-miRNAs (73-120 nt-long), such as mmu-miR-467a-1, mmu-miR-23a and mmu-miR-3067 were also markedly downregulated, whereas mmu-miR-3070a and mmu-miR-93 were significantly upregulated. The followed qRT-PCR validated the accuracy of the profiled miRNAs alteration. The levels of mmu-miR-337-3p, mmu-miR-344c-3p, mmu-miR-409-5p were significantly lower.

Moreover, our performance of GO enrichment analysis for above-mentioned miRNAs suggested that these miRNAs were closely associated with cognition, detection of external stimulus cell surface receptor signaling pathway, sensory perception of pain signaling, positive regulation of macromolecule metabolic process and other functional processes. However, it was not clear, which miRNA (s) are specifically regulate the functional process (es). Further molecular signaling pathway enrichment analysis of these deregulated miRNAs indicated the possible involvement of such molecular signaling pathways as lysosome, extracellular matrix (ECM)-receptor interaction, PI3K-Akt signaling pathway. The lysosome disfunction has been indicated to associate with cognition impairment [23]. The asiaticoside of PI3K/Akt/NF-κB signaling in hippocampal has also been recognized to regulate the cognition deficits in various types of diseases [24, 25]. We speculated that the isoflurane-induced lysosome disfunction and the deregulation of PI3K-Akt signaling pathway might contribute to the cognition impairment in mice.

Conclusion

In summary, our results provide the first evidence that isoflurane deregulated miRNA profile in mice hippocampal. And such deregulated miRNAs might be associated with such molecular signaling pathways as lysosome, extracellular matrix (ECM)-receptor interaction, PI3K-Akt signaling pathway, which were associated with the cognition impairment and other functional processes.

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Disclosure of conflict of interest

None.

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References

[1] Rasmussen LS. Defining postoperative cognitive dysfunction. Eur J Anaesthesiol 1998; 15: 761-764.

[2] Qiao Y, Feng H, Zhao T, Yan H, Zhang H, Zhao X. Postoperative cognitive dysfunction after inhalational anesthesia in elderly patients undergoing major surgery: the influence of anesthetic technique, cerebral injury and systemic inflammation. BMC Anesthesiol 2015; 15: 154.

[3] Tian XS, Tong YW, Li ZQ, Li LX, Zhang T, Ren TY, Zhou T, Wang HC, Zhan R, Sun Y, Yan Z, Wang QD, Fan DS, Kong FJ, Guo XY, Xiao WZ, Chui DH. Surgical stress induces brain-derived neurotrophic factor reduction and postoperative cognitive dysfunction via glucocorticoid receptor phosphorylation in aged mice. CNS Neurosci Ther 2015; 21: 398-409.

[4] Hudson AE, Hemmings HC Jr. Are anaesthetics toxic to the brain? Br J Anaesth 2011; 107: 30-37.

[5] Vlisides P, Xie Z. Neurotoxicity of general anesthetics: an update. Curr Pharm Des 2012; 18: 6232-6240.

[6] Wu X, Lu Y, Dong Y, Zhang G, Zhang Y, Xu Z, Culley DJ, Crosby G, Marcantonio ER, Tanzi RE, Xie Z. The inhalation anesthetic isoflurane increases levels of proinflammatory TNF-alpha, IL-6, and IL-1beta. Neurobiol Aging 2012; 33: 1364-1378.

[7] Schilling T, Kozian A, Senturk M, Huth C, Reinhold A, Hedenstierna G, Hachenberg T. Effects of volatile and intravenous anesthesia on the alveolar and systemic inflammatory response in thoracic surgical patients. Anesthesiology 2011; 115: 65-74.

[8] Fodale V, Santamaria LB, Schifilliti D, Mandal PK. Anaesthetics and postoperative cognitive dysfunction: a pathological mechanism mimicking Alzheimer's disease. Anaesthesia 2010; 65: 388-395.

[9] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 2005; 120: 15-20.

[10] Lu Y, Jian MY, Ouyang YB, Han RQ. Changes in rat brain MicroRNA expression profiles follow-

ing sevoflurane and propofol anesthesia. Chin Med J (Engl) 2015; 128: 1510-1515.
[11] Gao J, Wang WY, Mao YW, Graff J, Guan JS, Pan L, Mak G, Kim D, Su SC, Tsai LH. A novel pathway regulates memory and plasticity via SIRT1 and miR-134. Nature 2010; 466: 1105-1109.
[12] Kocerha J, Faghihi MA, Lopez-Toledano MA, Huang J, Ramsey AJ, Caron MG, Sales N, Willoughby D, Elmen J, Hansen HF, Orum H, Kauppinen S, Kenny PJ, Wahlestedt C. MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. Proc Natl Acad Sci U S A 2009; 106: 3507-3512.
[13] Lee ST, Chu K, Im WS, Yoon HJ, Im JY, Park JE, Park KH, Jung KH, Lee SK, Kim M, Roh JK. Altered microRNA regulation in Huntington's disease models. Exp Neurol 2011; 227: 172-179.
[14] Wang X, Wang Z, Wu YY, Tang H, Tan L, Wang X, Gao XY, Xiong YS, Liu D, Wang JZ, Zhu LQ. Melatonin attenuates scopolamine-induced memory/synaptic disorder by rescuing EPACs/miR-124/Egr1 pathway. Mol Neurobiol 2013; 47: 373-381.
[15] Wang X, Wang ZH, Wu YY, Tang H, Tan L, Wang X, Gao XY, Xiong YS, Liu D, Wang JZ, Zhu LQ. Melatonin attenuates scopolamine-induced memory/synaptic disorder by rescuing EPACs/miR-124/Egr1 pathway. Mol Neurobiol 2013; 47: 373-381.
[16] Gu H, Li H, Zhang L, Luan H, Huang T, Wang L, Fan Y, Zhang Y, Liu X, Wang W, Yuan Z. Diagnostic role of microRNA expression profile in the serum of pregnant women with fetuses with neural tube defects. J Neurochem 2012; 122: 641-649.
[17] Kocerha J, Faghihi MA, Lopez-Toledano MA, Huang J, Ramsey AJ, Caron MG, Sales N, Willoughby D, Elmen J, Hansen HF, Orum H, Kauppinen S, Kenny PJ, Wahlestedt C. MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. Proc Natl Acad Sci U S A 2009; 106: 3507-3512.
[18] Qureshi R, Sacan A. Weighted set enrichment of gene expression data. BMC Systems Biology 2013; 7 Suppl 4: S10.
[19] Wang H, Xu Z, Feng C, Wang Y, Jia X, Wu A, Yue Y. Changes of learning and memory in aged rats after isoflurane inhalational anaesthesia correlated with hippocampal acetylcholine level. Ann Fr Anesth Reanim 2012; 31: e61-e66.
[20] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984; 11: 47-60.
[21] Sempere LF, Freemantle S, Pitha-Rowe I, Moss E, Dmitrovsky E, Ambros V. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome Biology 2004; 5: R13.
[22] Welberg L. Neurodegenerative disease: a social role for microRNA. Nature Reviews Neuroscience 2015; 16: 2-3.
[23] Kuratomi G, Saito A, Ozeki Y, Watanabe T, Fujii K, Shimoda K, Inukai T, Mori H, Ohmori K, Akiyama K. Association of the Hermansky-Pudlak syndrome type 4 (HPS4) gene variants with cognitive function in patients with schizophrenia and healthy subjects. BMC Psychiatry 2013; 13: 276.
[24] Yin Z, Yu H, Chen S, Ma C, Ma X, Xu L, Ma Z, Qu R, Ma S. Asiaticoside attenuates diabetes-induced cognition deficits by regulating PI3K/Akt/NF-kappaB pathway. Behav Brain Res 2015; 292: 288-299.
[25] Wang C, Chen T, Li G, Zhou L, Sha S, Chen L. Simvastatin prevents beta-amyloid(25-35)-impaired neurogenesis in hippocampal dentate gyrus through alpha7nAChR-dependent cascading PI3K-Akt and increasing BDNF via reduction of farnesyl pyrophosphate. Neuropharmacology 2015; 97: 122-132.

Supplementary Table 1. Information about miRNA probes utilized in the present study

All_Transcript ID (Array Design)	All_Accession	Alignments	Sequence Length	Sequence
mmu-miR-103-3p	MIMAT0000546	chr11: 35782447-35782469 (+)/// chr2: 131288103-131288125 (+)	23	AGCAGCAUUGUACAGGGCUAUGA
mmu-miR-1197	MI0006305	chr12: 109712317-109712436 (+)	120	GUGAGCUGGAAUCAGCCAGCGUUACCUCAAGGUAUUUGAAGAUGCGGUUGACCAUGGUGUGUACGCUUUAUUUAUGACGUAGGACACAUGGUCUACUUCUUCUCAAUAUCACAUCUCGCC
mmu-miR-23a	MI0000571	chr8: 84208518-84208592 (+)	75	CGGACGGCUGGGGUUCCUGGGGAUGGGAUUUGAUGCCAGUCACAAAUCACAUUGCCAGGGAUUUCCAACUGACCC
mmu-miR-27a-3p	MIMAT0000537	chr8: 84208727-84208747 (+)	21	UUCACAGUGGCUAAGUUCCGC
mmu-miR-3067	MI0014029	chr12: 81166151-81166234 (+)	84	GCCCCAGCUUCCCCAGUUCUCAGGCCCGCUGUGGUGUGAAGUUAGGUUCACCAAGCGGCUGCCCUGGGAGAGGGGGAGGUGUGC
mmu-miR-3070a	MI0014032	chr12: 109587943-109588031 (+)	89	GUGCUGAGUGAGGCUGAGCCCCUGACCUUGAACCUGGGAUCCUAUCCAUGAUCUCCUGGUGCUACCGUCAGGGGUAGAUUCCUUGUCAU
mmu-miR-3073a-3p	MIMAT0014855	chr12: 112109290-112109311 (+)	22	UUGAUGUCCACUGUGACCAUAG
mmu-miR-337-3p	MIMAT0000578	chr12: 109585849-109585869 (+)	21	UUCAGCUCCUAUAUGAUGCCU
mmu-miR-344c-3p	MIMAT0014928	chr7: 61837323-61837345 (-)	23	UGAUCUAGUCAAAGCCUGACAGU
mmu-miR-409-5p	MIMAT0004746	chr12: 109743172-109743194 (+)	23	AGGUUACCCGAGCAACUUUGCAU
mmu-miR-449a-5p	MIMAT0001542	chr13: 113037549-113037570 (+)	22	UGGCAGUGUAUUGUUAGCUGGU
mmu-miR-467a-1	MI0002402	chr2: 10476346-10476418 (+)	73	CUGUGUGCGUAAGUGCCUGCAUGUAUAUGCGUGUAUAUUUUAUGCAUAUACAUACACACACCUACACACACAU
mmu-miR-467a-5p	MIMAT0003409	chr2: 10476355-10476376 (+)/// chr2: 10478812-10478833 (+)	22	UAAGUGCCUGCAUGUAUAUGCG
mmu-miR-6400	MIMAT0025152	chr4: 15942907-15942927 (-)	21	UUCUUGCUGCUUGGUGCUCGC
mmu-miR-669a-10	MI0014073	chr2: 10498911-10498997 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-11	MI0014075	chr2: 10501339-10501425 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-4	MI0014054	chr2: 10479315-10479401 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-5	MI0014055	chr2: 10481761-10481847 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-8	MI0014066	chr2: 10491566-10491652 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-9	MI0014068	chr2: 10494028-10494114 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-6985-5p	MIMAT0027872	chr19: 4263852-4263873 (-)	22	UACUGAGGGGUGGCUGCUAUGU
mmu-miR-7010-3p	MIMAT0027925	chr3: 82106248-82106268 (-)	21	AGGUUCUCCUUUCCUUUGCAG
mmu-miR-7025-3p	MIMAT0027955	chr5: 75176878-75176899 (+)	22	CAGCACAACUCCAGCUCACCAG
mmu-miR-7684-5p	MIMAT0029890	chr15: 82393958-82393978 (-)	21	UCUGGGAAGCCUGGGCAGCAG
mmu-miR-93	MI0000581	chr5: 138165523-138165610 (-)	88	AGUCAUGGGGGCUCCAAAGUGCUGUUCGUGCAGGUAGUGUAAUUACCUGACCUACUGCUGAGCUAGCACUUCCCGAGCCCCCAGGACA
mmu-miR-93-5p	MIMAT0000540	chr5: 138165574-138165596 (-)	23	CAAAGUGCUGUUCGUGCAGGUAG
mmu-miR-669a-8	MI0014066	chr2: 10491566-10491652 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-9	MI0014068	chr2: 10494028-10494114 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-10	MI0014073	chr2: 10498911-10498997 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-669a-11	MI0014075	chr2: 10501339-10501425 (+)	87	UGUGCAUGUGUGUAUAGUUGUGUGUGCAUGUUCAUGUCUAUAUUUGAAUAUACAUAACAUACACACACACGUAUAAACGCAAGCACA
mmu-miR-7064	MI0022913	chr7: 143570671-143570759 (-)	89	GAGGUUCAGAGGCAUAAAGGUCAUUUGCUUCAGUAGUAAAGGCCUGGGCAGGUUUGGAAUGGUCAGCAGGGCCCUUUAUGUCUCUCUAG