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Original Article

Propofol treatment alters microRNA profiling in neurons

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Abstract: Propofol is a short-acting intravenous anesthetics. Whether propofol application and treatment induce destructive or protective effects is still in dispute. MicroRNA alterations have been observed in various physiological and pathological processes. We now report the alterations of microRNA profile after propofol treatment in primarily cultured mouse hippocampal neurons. Among 1908 mouse microRNAs examined, there are 40 microRNAs upregulated and 54 microRNAs downregulated with the cutoff as 2 fold change. Our data shed a light on how propofol may play roles via regulating microRNAs.

Keywords: Propofol, microRNA, anesthetics, Alzheimer's disease, profiling

Introduction

Propofol (2, 6-disopropylphenol) is an intravenous short-acting anesthetics widely used in practices, especially in operating rooms [1]. Propofol has many roles other than anesthetic effects, such as antiemetic, immunomodulatory and anxiolytic roles [1]. Propofol is reported to be protective in cerebral ischemia or ischemia-reperfusion, Parkinson's disease, intracerebral hemorrhage, cerebral resuscitation, ischemia of spinal cords and Alzheimer's disease (AD) [1, 2]. Despite of the observed destructive or beneficial roles of propofol, the exact mechanisms of propofol action still need further investigations.

MicroRNAs (miRNAs) are a group of short noncoding RNAs [3]. There are over one thousand miRNAs identified in mammals [4]. Many miRNAs are conserved and play important roles in rodents, monkeys and humans [5-8]. MiRNAs precursors are first processed into a single-stranded miRNA that interacts with the 3'UTR or 5'UTR area of the complementary mRNA sequences [9], resulting in translation repression or target degradation [10, 11], through which miRNAs regulate the protein level of target genes. A single miRNA can have up to several hundred target genes, and thus, may regulate multiple pathways at the same time [12, 13]. Many miRNAs have demonstrated implications in anesthesia [14-18].

In the present study, the alterations of microRNA profile after propofol treatment in primarily cultured mouse hippocampal neurons were studied. We found that among 1908 mouse microRNAs examined, there were 40 microRNA upregulated and 54 microRNA downregulated with the cutoff as 2 fold change.

Experimental procedures

Cell culture and treatments

Primary neurons were cultured from wild type C57 mouse hippocampus, following the regulations of Peking University Animal Care and Use Committee as previously described [19]. In brief, fresh fetal mouse hippocampal tissues were dissociated with 0.25% trypsin (Invitrogen, Carlsbad, CA), which was then inactivated by 10% decomplemented fetal bovine serum (FBS, HyClone, Logan, UT). The mixture was triturated through pipette to make a homogenous mix-

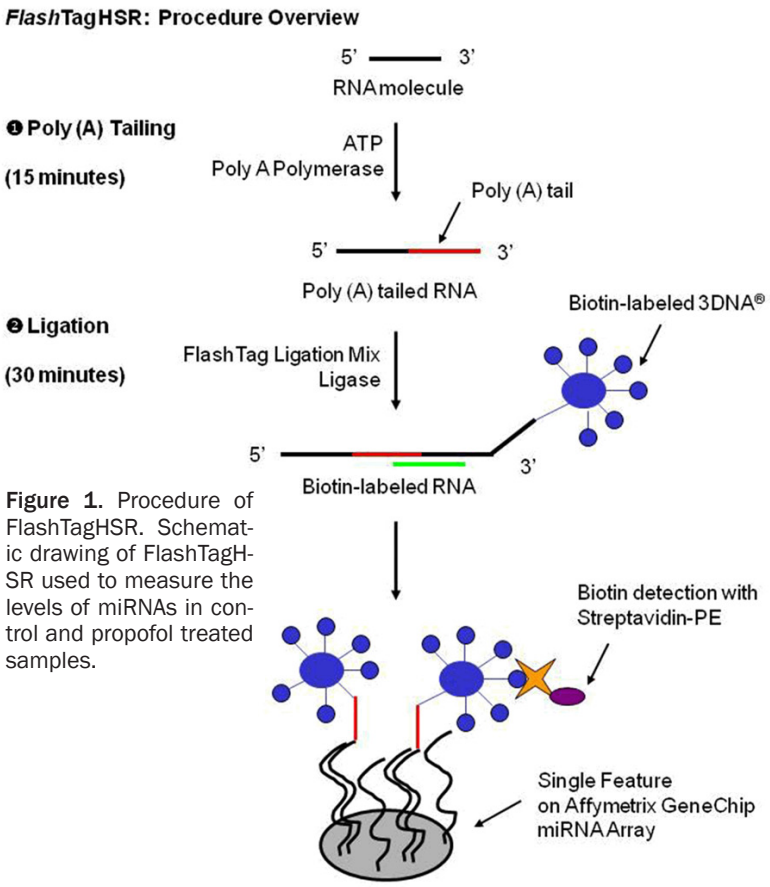

Figure 1. Procedure of FlashTagHSR. Schematic drawing of FlashTagHSR used to measure the levels of miRNAs in control and propofol treated samples.

Table 1. MiRNAs upregulated in propofol treatment

ProbeSetID	Propofol	Control	P/C ratio	Transcript ID (Array Design)
20500273	691.9466	312.6471	2.2132	mmu-miR-9-5p
20500286	78.45807	30.80974	2.5465	mmu-miR-137-3p
20500365	27.39962	13.34035	2.0539	mmu-miR-182-5p
20501211	33.90044	11.80873	2.8708	mmu-miR-365-1-5p
20502356	6.480761	3.181077	2.0373	mmu-miR-448-3p
20502365	20.96932	6.6142	3.1703	mmu-miR-365-2-5p
20503020	14.72497	6.503321	2.2642	mmu-miR-466a-3p
20504162	87.72989	28.18896	3.1122	mmu-miR-486-3p
20504202	27.28932	11.5357	2.3656	mmu-miR-542-5p
20504610	20.7272	8.620374	2.4044	mmu-miR-592-5p
20504618	162.7319	72.05348	2.2585	mmu-miR-1249-5p
20504624	25.65725	11.45431	2.24	mmu-miR-1843a-5p
20504641	14.79067	7.178684	2.0604	mmu-miR-670-5p
20504681	16.23222	7.580155	2.1414	mmu-miR-679-5p
20505713	14.72497	6.503321	2.2642	mmu-miR-466e-3p
20510743	18.6453	7.312149	2.5499	mmu-miR-1943-5p
20510745	17.93054	8.068655	2.2222	mmu-miR-1945
20515460	11.17038	5.491166	2.0342	mmu-miR-3100-5p
20520366	111.317	54.03626	2.06	mmu-miR-5113
20520370	12.77724	6.363966	2.0077	mmu-miR-5119

ture. After filtering the mixture through 70 μm sterilized filters, the flow-through was centrifuged. The pellet was then washed once by phosphate buffer saline (PBS) and once by Dulbecco minimum essential medium (DMEM) in Earle's balanced salt solution containing 0.225% sodium bicarbonate, 1 mM sodium pyruvate, 2 mM $_L$-glutamine, 0.1% dextrose, 1x antibiotic Pen-Strep (all from Invitrogen, Carlsbad, CA) with 5% FBS. Cells were then plated on poly-$_L$-lysine (Sigma, St. Louis, MO) coated plates or glass coverslips at the density of 3×10^6 cells/ml. Neurons were incubated at 37°C in DMEM without phenol red with 5% FBS and with 5% circulating CO_2. Cytarabine was added to culture media 24 hours after plating at 10 μM to inhibit cell growth. Medium was changed every 48 hours. Cells were treated for experiments at 7 days in culture. Propofol was added to the medium at 100 nM for 6 hours. The cells were then lysed and the total RNAs were collected for miRNA profiling.

MicroRNA profiling

MicroRNA levels were measured by CapitalBio (Beijing, China) microarray service by using GeneChip microRNA 2.0 (Affymetrix). The data analysis was done by CapitalBio.

Real-time PCR

Cells were harvested and total RNA was isolated with TRIGene reagent (GenStar BioSolutions Co., Ltd., Beijing, China). Total RNA (2 μg) were reversely transcribed using TransScript II First-Strand cDNA Synthesis SuperMix (Beijing TransGen Biotech Co., Ltd., Beijing, China). Real-time PCRs were done by using TransStart Green q PCR SuperMix UDG (Beijing

20520384	29.2641	13.49704	2.1682	mmu-miR-5129-5p
20524331	50.57885	17.74101	2.851	mmu-miR-6244
20525775	33.51533	14.91819	2.2466	mmu-miR-6907-5p
20525805	38.1338	17.37992	2.1941	mmu-miR-6922-5p
20525821	18.1105	6.496433	2.7878	mmu-miR-6930-5p
20525839	31.88291	14.99428	2.1263	mmu-miR-6939-5p
20525866	10.07331	4.826137	2.0872	mmu-miR-6952-3p
20525919	16.491	6.577157	2.5073	mmu-miR-6978-5p
20525929	37.61599	14.67663	2.563	mmu-miR-6983-5p
20525939	44.50089	19.77448	2.2504	mmu-miR-6988-5p
20525957	19.81134	6.974064	2.8407	mmu-miR-6997-5p
20525993	34.26519	10.72291	3.1955	mmu-miR-7014-5p
20526103	11.5744	5.299487	2.1841	mmu-miR-7068-5p
20526133	103.4043	40.58915	2.5476	mmu-miR-7083-5p
20526141	14.75859	5.717828	2.5812	mmu-miR-7087-5p
20527040	14.42688	5.644851	2.5558	mmu-miR-7212-5p
20527048	26.01753	9.919459	2.6229	mmu-miR-7216-5p
20527078	43.27383	8.203071	5.2753	mmu-miR-7231-5p
20528521	52.40702	10.26006	5.1079	mmu-miR-7036b-3p
20529986	43.43488	19.31721	2.2485	mmu-miR-3473g

Table 2. MiRNAs downregulated in propofol treatment

ProbeSetID	Propofol	Control	P/C ratio	Transcript ID (Array Design)
20500382	5.464223	19.4833	0.2805	mmu-miR-190a-3p
20500392	336.5894	1451.964	0.2318	mmu-miR-195a-3p
20500642	114.8739	427.3714	0.2688	mmu-miR-297a-5p
20500645	32.51185	141.6857	0.2295	mmu-miR-299a-5p
20500883	18.9495	49.48159	0.383	mmu-miR-15a-3p
20500885	80.04687	391.2306	0.2046	mmu-miR-16-1-3p
20501148	11.16825	25.04871	0.4459	mmu-miR-7a-5p
20501149	7.796934	19.34112	0.4031	mmu-miR-7a-1-3p
20501258	5.955464	14.65659	0.4063	mmu-miR-377-5p
20501260	8.962996	26.54212	0.3377	mmu-miR-378a-5p
20501263	4.315138	9.812568	0.4398	mmu-miR-379-3p
20501789	13.23105	36.88714	0.3587	mmu-miR-410-3p
20503024	20.20691	40.87736	0.4943	mmu-miR-468-3p
20504689	10.00902	26.85655	0.3727	mmu-miR-449c-3p
20504704	101.5446	287.0445	0.3538	mmu-miR-669b-5p
20504709	48.71526	171.619	0.2839	mmu-miR-669c-5p
20504716	14.08754	332.708	0.0423	mmu-miR-696
20505710	48.97733	191.426	0.2559	mmu-miR-466c-5p
20505714	166.0447	419.0447	0.3962	mmu-miR-466f-5p
20505717	78.96516	254.7055	0.31	mmu-miR-466h-5p
20505730	121.0542	473.4724	0.2557	mmu-miR-574-5p
20505770	15.14432	34.76015	0.4357	mmu-miR-872-3p
20506719	41.5683	119.5446	0.3477	mmu-miR-669k-5p
20506722	44.80227	193.0294	0.2321	mmu-miR-669d-5p
20506728	39.58652	323.0446	0.1225	mmu-miR-1187

TransGen Biotech Co., Ltd., Beijing, China). Real-time PCR quantifications were run in triplicate for each sample and the average were determined. In order to use the comparative Ct method for relative quantification, the amplification efficiency of target and housekeeping gene must be approximately equal. Quantification was done using the comparative Ct method, expression levels for the target gene was normalized to the GAPDH of each sample [$2^{-\Delta Ct}=2^{-(Ct(target\ gene)-Ct(GAPDH))}$]. Amplification was done for 45 cycles at 95°C for 30 s, 59°C for 30 s, 72°C for 30 s, 95°C for 1 min, 59°C for 30 s and 95°C for 30 s.

Results

Microarray analysis of miRNAs was performed on pooled hippocampal neuron samples (n=3 for control and n=3 for propofol treatment group) using FlashTagHSR procedure (**Figure 1**). Our data shown that, among 1908 mouse microRNAs examined, there were 40 microRNA upregulated (**Table 1**) and 54 microRNA downregulated (**Table 2**) with the cutoff as 2 fold change (**Figure 2A** and **2B**). The most dramatically upregulated miRNAs after propofol treatment were mmu-miR-7231-5p, mmu-miR-7036b-3p, mmu-miR-7014-5p, mmu-miR-365-2-5p and mmu-miR-486-3p. The most dramatically downregulated miRNAs with propofol treatment were mmu-miR-696, mmu-miR-669n, mmu-miR-8095, mmu-miR-1187 and mmu-miR-669e-5p.

The changes of most dramatically altered miRNAs were then confirmed by RT-PCR. As controls, we examined levels of mmu-miR-296-1-5p, mmu-miR-136-3p and mmu-miR-152-5p, which were unchanged with propofol treatment demonstrated by microarray experiment. The data of RT-PCR confirmed that the most dramatically upregulated miRNAs shown by microarray experiment mmu-

20506730	278.3349	689.8877	0.4034	mmu-miR-669f-5p
20506737	194.7155	533.4167	0.365	mmu-miR-466f
20506741	157.3559	591.3853	0.2661	mmu-miR-466j
20506747	37.61597	232.0446	0.1621	mmu-miR-669e-5p
20506750	31.23139	119.7714	0.2608	mmu-miR-467h
20510730	11.9418	37.20062	0.321	mmu-miR-1930-3p
20510755	55.38212	133.9573	0.4134	mmu-miR-669l-5p
20510757	237.3346	762.6684	0.3112	mmu-miR-669m-5p
20510759	55.14551	172.5638	0.3196	mmu-miR-669o-5p
20510765	6.351995	13.36198	0.4754	mmu-miR-1955-5p
20510767	20.17844	202.0446	0.0999	mmu-miR-669n
20515371	3.258923	9.55698	0.341	mmu-miR-3060-5p
20515379	5.312242	16.60332	0.32	mmu-miR-3064-5p
20515417	58.13926	236.3834	0.246	mmu-miR-3082-5p
20515427	237.3346	762.6684	0.3112	mmu-miR-466m-5p
20515482	6.168741	31.327	0.1969	mmu-miR-3105-5p
20521912	4.012059	8.361279	0.4798	mmu-miR-5622-5p
20524675	3.937866	8.491597	0.4637	mmu-miR-6386
20524699	11.82547	28.23739	0.4188	mmu-miR-6405
20525071	4.025704	9.029051	0.4459	mmu-miR-6516-5p
20525759	5.56713	16.12516	0.3452	mmu-miR-6899-5p
20525850	7.643112	15.91766	0.4802	mmu-miR-6944-3p
20525877	6.439501	14.87347	0.433	mmu-miR-6958-5p
20525937	17.8206	40.06075	0.4448	mmu-miR-6987-5p
20526025	210.5446	593.0447	0.355	mmu-miR-7030-5p
20526091	5.281477	11.66391	0.4528	mmu-miR-7063-5p
20526132	4.092182	21.73742	0.1883	mmu-miR-7082-3p
20529955	12.25756	119.2723	0.1028	mmu-miR-8095
20529956	5.389108	15.44339	0.349	mmu-miR-1291

miR-7231-5p, mmu-miR-7036b-3p, mmu-miR-7014-5p, mmu-miR-365-2-5p and 486-3p were increased with propofol treatment (**Figure 3A**). In the other hand, the most remarkably downregulated miRNAs with microarray assay mmu-miR-696, mmu-miR-669n, mmu-miR-8095, mmu-miR-1187 and mmu-miR-669e-p were decreased with propofol treatment detected by RT-PCR (**Figure 3B**). Our data indicated good reliability of the results from microarray analysis.

Discussion

As a commonly used anethesitics, the roles of propofol were studied in various physiolgical and pathological processes. For examples, how propofol may affect AD pathogensis was examined. The effects of propofol on APP processing in rats [20] were investigated. Propofol did not significantly affect the protein and mRNA levels of APP in the brain tissues of rats [20]. These data suggest that propofol may not promote AD neuropathogenesis [20]. In a more recent study, how propofol affected isoflurane-induced caspase-3 activation and isoflurane-induced Aβ aggregation was studied *in vitro* and *in vivo* [2]. In this study, propofol specifically attenuated the isoflurane-induced caspase-3 activation in H4 human neuroglioma cells stably transfected with human full-length APP, and in the brain tissues of AD transgenic mice with higher Aβ levels. However, the attenuation effects of propofol on the isoflurane-induced caspase-3 activation occurred in neither naïve H4 human neuroglioma cells nor the brain tissues of wild type mice with lower Aβ levels [2]. Furthermore, propofol attenuated the isoflurane-induced oligomerization of $A\beta_{42}$, but not $A\beta_{40}$, in H4 human neuroglioma cells overexpressing $A\beta_{40}$ or $A\beta_{42}$. These data suggest that isoflurane may induce caspase activation and apoptosis by increasing $A\beta_{42}$ oligomerization. Propofol blocked isoflurane-induced $A\beta_{42}$ oligomerization, and thus attenuating the isoflurane-induced caspase activation [2].

Although some studies explored the mechanisms through which propofol may play its roles, the regulation of miRNAs by propofol was not investigated. In the present study, we find that there are 40 miRNAs upregulated and 54 miRNAs downregulated with the cutoff as 2 fold change among 1908 mouse miRNAs examined. Our data shed a light on how propofol may play roles via regulating miRNAs.

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Disclosure of conflict of interest

All authors declare no actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning

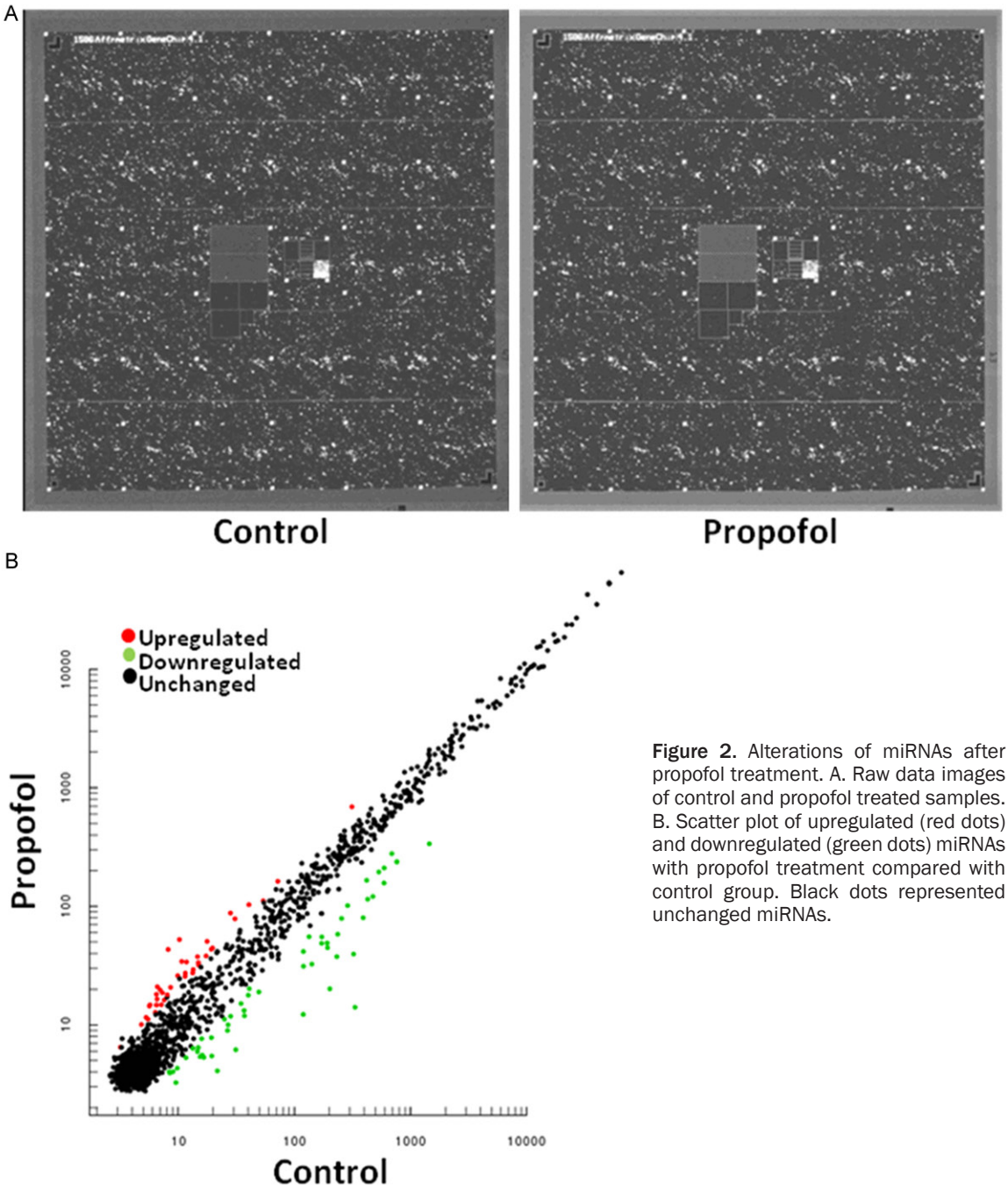

Figure 2. Alterations of miRNAs after propofol treatment. A. Raw data images of control and propofol treated samples. B. Scatter plot of upregulated (red dots) and downregulated (green dots) miRNAs with propofol treatment compared with control group. Black dots represented unchanged miRNAs.

the work submitted that could inappropriately influence (bias) their work.

Authors' contribution

L.S., Y.X., X.C. and D.Z. performed all the experiments and analyzed the data. C.P., T.L. and J.L. conceptualized the study, performed analyses and drafted the manuscript.

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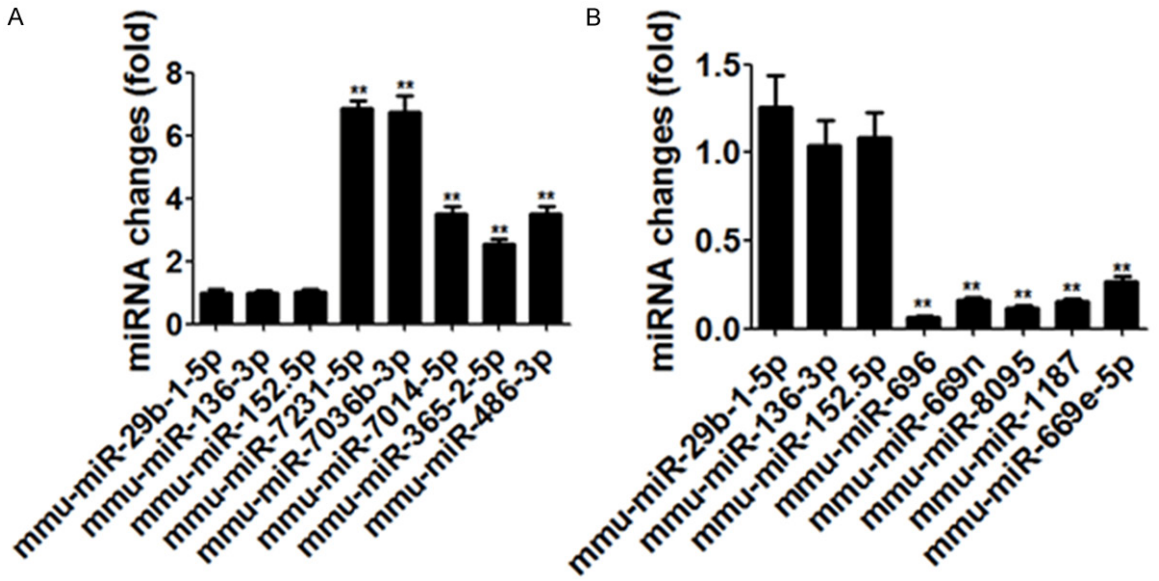

Figure 3. Confirmation of altered miRNAs detected by microarray analysis. A. Most dramatically upregulated miRNAs with propofol treatment in microarray assay were confirmed by RT-PCR. B. Most dramatically downregulated miRNAs with propofol treatment in microarray assay were confirmed by RT-PCR. In both A and B, mmu-miR-29b-1-5p, mmu-miR-136-3p and mmu-miR-152-5p were used as unchanged controls. Data represented mean ± SE (n=3). **: $p<0.01$ compared with the control.

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