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ORIGINAL PAPER

Inhibition of miR-219 Alleviates Arsenic-Induced Learning and Memory Impairments and Synaptic Damage Through Up-regulating CaMKII in the Hippocampus

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Abstract

Epidemiological investigations and experimental studies indicate that chronic arsenic exposure can reduce learning and memory function. However, the underlying mechanism of this effect remains largely unknown. Emerging evidence suggests that microRNA (miRNA) play an important role in toxicant exposure and a regulatory role in cognitive function. In this study, we observed that subchronic arsenic exposure induced impairment of learning and memory and significantly up-regulated miRNA-219 (miR-219) expression in the mouse hippocampus. Furthermore, the expression of CaMKII, an experimentally validated target of miR-219, was decreased in the mice exposed to arsenic. Suppression of miR-219 by adeno-associated viral (AAV)-delivered anti-miR-219 prevented the arsenic-induced impairment of learning and memory and relieved the pathological changes in the synaptic structure of the hippocampus. Furthermore, we observed that the NMDA receptor subunit 2 (NR2) and the memory-related proteins c-Fos and c-Jun were up-regulated by inhibition of miR-219 in the mouse hippocampus. Taken together, the results of this study indicate that inhibition of miR-219 regulates arsenic-induced damage in the structure of the hippocampus and impairment of learning and memory, possibly by targeting CaMKII. Suppression of miR-219 may be a potential strategy to ameliorate arsenic-induced neurotoxicity.

Keywords Arsenic · miR-219 · Learning and memory · Hippocampus · CaMKII

Introduction

Arsenic has been recognized worldwide as a serious inorganic contaminant in drinking water. In arsenic-contaminated areas, the arsenic concentration in drinking water or groundwater often ranges from 0.25 to 2.1 ppm and even exceeds 4.0 ppm in certain severely contaminated areas of China [1–3]. Chronic arsenic exposure is associated with diseases of the cardiovascular, gastrointestinal, respiratory, neurological, hematopoietic, endocrine, genitourinary and cutaneous systems. Epidemiological studies show that chronic exposure to inorganic arsenic via drinking water results in a dose-dependent reduction in the intellectual functions of children [4, 5]. In animals exposed to arsenic, deficits in learning tasks and changes in behavior have been observed [6, 7]. Taken together, these findings indicate that arsenic induces neurotoxic effects in the central nervous system, including impairing learning and memory. However, the molecular mechanisms of arsenic-induced reductions in learning and memory ability remain unknown. Recently, a

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series of studies have been conducted to determine the role of microRNAs (miRNAs) in toxicant-induced neurotoxicities and the mechanisms involved [8–10]. miRNAs are a class of evolutionarily conserved, small (~23 nt), noncoding RNAs that post-transcriptionally regulate gene expression [11]. miRNAs are enriched in the central nervous system and have attracted considerable attention as central players in synaptic plasticity, a cellular mechanism that is thought to underlie many complex brain functions, including learning and memory [12–15]. Several miRNAs (for example, miR-134, miR-125, miR-138, miR-132, miR-219 and miR-29) have been shown to regulate dendritic spine morphology and synaptic plasticityby regulating the translation of the p250GAP, NA2B and CREB genes [16].

miR-219 is a conserved miRNA expressed in both rodent and human brains but not in other tissues [17, 18]. It was reported that miR-219 appears to be an integral component of the NMDAR signaling cascade and was down-regulated in induction and maintenance of long-term potentiation (LTP) [19]. It has been reported that many neurotoxicants, such as sevoflurane and perfluorooctane sulfonic acid, can disturb gene expression of miR-219 [20, 21]. These findings indicate that miR-219 may be a target of environmental toxicants. However, studies on the toxic effect of arsenic on expression of miRNAs in the hippocampus are highly limited.

miR-219 negatively regulates the translation of calcium/calmodulin-dependent protein kinase II (CaMKII) [22]. Previous studies have shown that CaMKII plays an important role in arsenic-induced neurotoxcity [23]. In this study, we show that in arsenic-exposed mice, alterations of hippocampal miR-219 expression regulate arsenic-induced impairment of learning and memory, and we provide the first evidence of the effect of inhibition of miR-219 on arsenic-induced learning and memory impairment through regulation of CaMKII.

Materials and Methods

Animal Treatment and Sample Preparation

A total of 128 adult male Kunming mice (9 weeks old) with body weights ranging from 27.5 to 32.4 g were purchased from the Experimental Animal Center of Dalian Medical University. The mice were housed in standard polycarbonate cages on a 12 h dark-light cycle and maintained on a standard laboratory diet with water available ad libitum. The tap water at Dalian (China) contains approximately 0.0005–0.05 ppb arsenic. After a 1-week adaptation, the mice were randomly assigned into four groups (n = 32 for each group). The first group received distilled water alone for 90 days (control group) [24]. The second group received 4.0 ppm As_2O_3 (Beijing Chemical Works) through drinking water for 90 days (As group). The third group received 4.0 ppm As_2O_3 through drinking water for 90 days, and the animals were inoculated with AAV anti-miR-219 on 60th day (As + AAV-anti-miR-219 group). The fourth group received 4.0 ppm As_2O_3 through drinking water for 90 days and the animals were inoculated with AAV control on 60th day (As + AAV-control group). The experimental diagram was shown in Fig. 1. The weights of the mice were measured every 7 days, and the volumes of water consumed were measured every 2 days. The body weights and the water consumption were no significantly differences among these groups (Fig. 2). General consumption results were similar to other reports indicating that a 30 g mouse consumes 4–7 ml of water daily [25]. Based on these estimations, in our experiment, a 30 g mouse consumed between 12.16 and 21.28×10^{-3} mg As/day, which is equivalent to 0.41–0.71 mg As/(kg day). The experiments were performed in accordance with the Animal Guidelines of Dalian Medical University and in agreement with the Ethical Committee of Dalian Medical University.

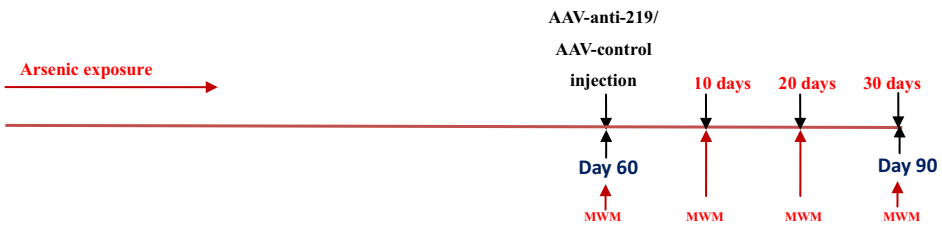

Fig. 1 The experimental diagram shows the timeline of AAV-anti-219 injection, and behavior tests (Morris water maze test—MWM). After 60-day exposure to arsenic, AAV-delivered anti-miR-219 or control sequence was stereotaxically injected into the hippocampal CA1 region of mice followed by arsenic treatment for 30 days continuously. Alterations in learning and memory ability of the mice were evaluated by MWM for four times. Five from each group were killed under anesthesia after each MWM test, the hippocampus tissues of them used for real-time PCR, western blot or observation via transmission electron microscopy (TEM)

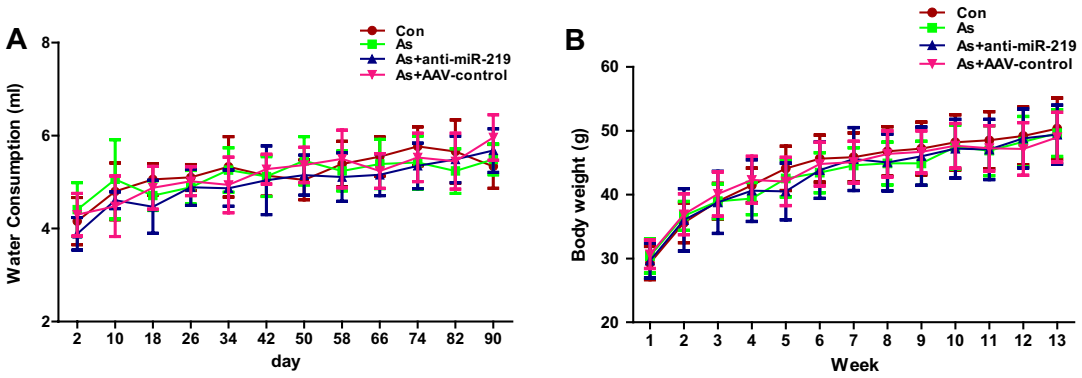

Fig. 2 Water consumption (**a**), and body weight (**b**) of mice exposed to arsenic-containing drinking water (n = 16)

MWM Test

Mice from each group (n = 8) were tested for their spatial learning ability using the MWM test. The device is geographically divided into four equal quadrants (N, S, E, and W). Briefly, the test included the navigation test and the spatial probe test [26]. In a hidden platform test, a circular transparent escape platform (10 cm diameter and 29 cm height) was submerged 1 cm below the water surface and always located in the center of the N quadrant. Before the hidden platform test, 2 days of adaptive training should be taken, and make sure that all the mice to a criterion of performance before running the hidden platform tests. The hidden platform test lasted 4 days. Mice were given four trials daily for four consecutive days. During the training period, each mouse was gently released from one of four different positions in each quadrant and allowed to swim freely for 90 s, and the time spent to reach the platform (escape latency) was recorded as acquisition latency. If the mouse failed to reach the platform within 90 s, it was gently guided onto the platform for 30 s and subsequently removed from the pool. The escape latency to identify the hidden platform was collected as the parameter of learning function.

On the fifth day, the mice were given a spatial probe test, in which the platform was taken away, and each mouse was placed at one point in the S quadrant. The mouse was allowed to navigate freely in the pool for 60 s. The time swimming in the target quadrant, which had previously contained the hidden platform, was recorded as the parameters of memory retention. The swimming time and the swimming distance in the target quadrant were calculated by a smart video tracing system (NoldusEtho Vision system, version 5, Everett, WA, USA).

In Vivo Hippocampal Injections

The AAV for mouse anti-miR-219 (AAV-anti-miR-219, AGAATTGCGTTTGGACAATCA) and the control sequence (AAV-control, TTCTCCGAACGTGTCACGT) were synthesized by Shanghai GenePharma. The titer of the AAV was 1×10^9 TU/ml. AAV-delivered anti-miR is antisense oligonucleotides with a sequence perfectly complementary to their target. When introduced into cells, the oligonucleotides bind to and inhibit endogenous miRNA molecules. Virus suspensions were stored at − 80 °C until use and were briefly centrifuged and kept on ice immediately before injection.

After anesthesia, mice were placed into a stereotaxic frame (RWB Life Science). Two tiny holes (0.05 mm in diameter, one per hemiphere) were drilled in the skull (from bregma: dorso-ventral, − 1.7 mm; medio-lateral, ± 1.1 mm; anterio-posterior, − 2.1 mm). Two microliters (10,000 TU/μl) anti-miR-219 or AAV control were injected into CA1 of hippocampus using a 5 μl Hamilton syringe with a 33 gauge tip needle (Hamilton). The needle was subsequently maintained in place for another 2 min after injection and withdrawn very slowly to prevent backflow of solution. The accuracy of injection sites was identified by Evans blue (Sigma) stereotaxic injection directly into the hippocampus CA1 subfield.

Ultrastructure of the Synapses

After 90 days of arsenic exposure, the mice were anesthetized with sodium pentobarbital via i.p. injection. The brain tissue was removed, and hippocampus samples from each group were separated (− 2 mm at the anterior/posterior axis, ± 1.8 mm at the lateral/medial axis and − 1.5 mm at the dorsal/ventral axis) (Paxinos and Franklin, 2001), fixed with 4% glutaraldehyde, and cut into pieces measuring 1 mm^3 after cooling. Samples were later fixed with 2% osmium tetroxide, dehydrated, soaked and embedded through a graded acetone series, and finally dual stained with uranyl acetate–lead citrate. Finally, the samples were cut into ultrathin slices (500–700 nm). The ultrastructure of the synapses was observed via TEM (JEM-2000EX, Olympus, Tokyo,

Japan), and the thickness of the PSD was measured using Guldner's method [27].

Real-Time PCR Analysis

miRNA was extracted from the mouse hippocampus tissue using RNAiso Plus according to the manufacturer's instructions (Takara, Japan). 1 μg of total RNA was reverse transcribed using a reverse transcription kit (Takara, Japan). Real-time PCR amplification was performed with a SYBR Green PCR kit (Takara, Japan) using the TP800 Real-Time PCR Detection System (Takara, Japan). The primers for the selected genes are shown in Table 1. The efficiencies of primers are from 96.7 to 99.2%. U6 was used as internal control. The reaction conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 12 s, 60 °C for 40 s. The data were analyzed using the $2^{-\Delta\Delta CT}$ method.

Western Blotting

Western blot analysis was performed to detect the protein expression of CaMKII signaling proteins, including CaMKII, NR2, c-Fos and c-Jun. β-actin was used as a control. Briefly, hippocampus samples were homogenized in ice-cold RIPA Tissue Protein Extraction Reagent (Beyotime, China) supplemented with 1% proteinase inhibitor mix and incubated at 4 °C for 1 h. After incubation, debris were removed via centrifugation at 12,000×*g* for 15 min at 4 °C, and the lysates were stored at − 80 °C until use. The total protein concentration in the lysates was determined using a BCA protein assay kit (Beyotime, China). The samples employed for Western blotting contained 30 μg of protein from hippocampal tissue in each lane. The proteins were mixed with an equal volume of SDS-PAGE loading buffer, separated via SDS-PAGE under non-reducing conditions using 10% SDS-PAGE gels and electrotransferred to Hybond-P polyvinylidene fluoride membranes (Millipore, France). The membranes were blocked with blocking buffer containing defatted milk powder for 1 h and incubated overnight at 4 °C with 1μg/ml of anti-rabbit CaMKII (1:1000, Abcam, USA), c-Fos (1:1000, Abcam, USA) and c-Jun (1:1000, Abcam, USA) antibodies. The membrane was washed three times with Tris-buffered saline containing 0.05% Tween-20 (TBST) for 15 min and later incubated at room temperature for 1 h with horseradish peroxidase-conjugated IgG (1:5000, Sigma, USA). The resultant signals were visualized using an enhanced ECL chemiluminescence kit (Beyotime,China) and quantified densitometrically using a UVP BioSpectrum Multispectral Imaging System (Ultra-Violet Products Ltd, Upland, CA).

Immunofluorescence Staining

The mice were perfused with PBS followed by 4% paraformaldehyde. After perfusion, mice were decapitated, and the brains were carefully removed. The brains were fixed in 4% paraformaldehyde at 4 °C for approximately 4 h and stored in 30% PBS-buffered sucrose solution for 72 h. The brains were sectioned into coronal slices (8 μm), washed in PBS, and incubated with 4′,6-diamidino-2-phenylindole (DAPI) (1:500, Beyotime, China) in a dark place for 5 min. Sections were subsequently rinsed with PBS three times. Green fluorescent protein (GFP) gene was used as an expression reporter. The GFP and DAPI fluorescence in these sections of the hippocampus were monitored to confirm successful transfection using a fluorescence microscope (×400 magnification, Leica, Germany).

Statistical Analysis

Data are presented as the mean ± standard error of mean (SEM). All data were analyzed with SPSS 23.0 for Windows. Student's *t* test or one-way ANOVA followed by post hoc tests, were performed where appropriate, and in all instances a p value of less than 0.05 was considered to be statistically significant.

Table 1 Primers used in real-time PCR

Gene	Forward primer	Reverse primer
miR-320-3p	GCCAAAAGCTGGGTTGAG	GCAGGGTCCGAGGTATTC
miR-27a-3p	GTCGCTACGAGTTCACAGTGGCTAAGT	ATCCAGTGCAGGGTCCGAGGTATTC
miR-125a-3p	GCCACGACACAGGTGAGGTTCTTG	ATCCAGTGCAGGGTCCGAGGTATTC
miR-294	CGACCACTGACTCAAAATGGAGGC	CAGTGCAGGGTCCGAGGTATTC
miR-199a-3p	GCAGTACTGGATACAGTAGTCTGCACATTG	ATCCAGTGCAGGGTCCGAGGTATTC
miR-219	CGACTTCAGGTGATTGTCCAAACGC	ATCCAGTGCAGGGTCCGAGGTATTC

Results

miR-219 Expression Is Up-Regulated in the Hippocampus After Arsenic Exposure

After 60-days exposure to arsenic, alterations in learning and memory ability of the mice were evaluated by MWM test. In the navigation test, the arsenic exposed group showed that the escape latency of the mice was significantly prolonged compared with the control group ($p < 0.05$, Fig. 3a). In the probe test, the time spent in the target quadrant in the arsenic exposed group was significantly shorter compared with the control group ($p < 0.05$, Fig. 3b). These findings indicate that arsenic exposure results in an impairment of mouse learning and memory.

miRNAs have been shown to regulate diverse brain functions, including learning and memory. When we started the study, we performed a literature search and observed six miRNAs that have been reported to mediate synaptic plasticity in brain, as well as in neurotoxicity by environmental toxins exposure [19, 20, 28–30]. We next screened the levels of these related miRNAs in the hippocampus of the arsenic-exposed mice to determine whether any of these miRNAs may be involved in the action of arsenic. As shown in Fig. 3c, arsenic exposure significantly increased the expression level of miR-219.

miR-219 Inhibition Attenuates the Impairment of Learning and Memory Induced by Arsenic Exposure

To examine the specific contribution of miR-219 to learning and memory ability related to arsenic exposure, the mice were administered with arsenic for 60 days, and AAV-delivered anti-miR-219 or control sequence was stereotaxically injected into the hippocampal CA1 region of mice followed by arsenic treatment for 30 days continuously. The expression of virus-derived reporter GFP and its co-localization with the nuclear marker DAPI were observed (Fig. 4a), indicating that the AAV was successfully transfected. Real-time PCR analysis showed that the miR-219 level decreased gradually, and 22.2, 28.6 and 39.9% reductions were detected in the hippocampus tissues on 10, 20 and 30 days after AAV anti-miR-219 injection, respectively (Fig. 4b–d). Furthermore, we evaluated the effect of miR-219 inhibition on learning and memory ability. As shown in Fig. 4e–g, in the MWM test, the escape latency of the mice to locate the platform on day 10 after anti-miR-219 injection showed no significant differences compared with the As + AAV-control group, while the escape latency to locate

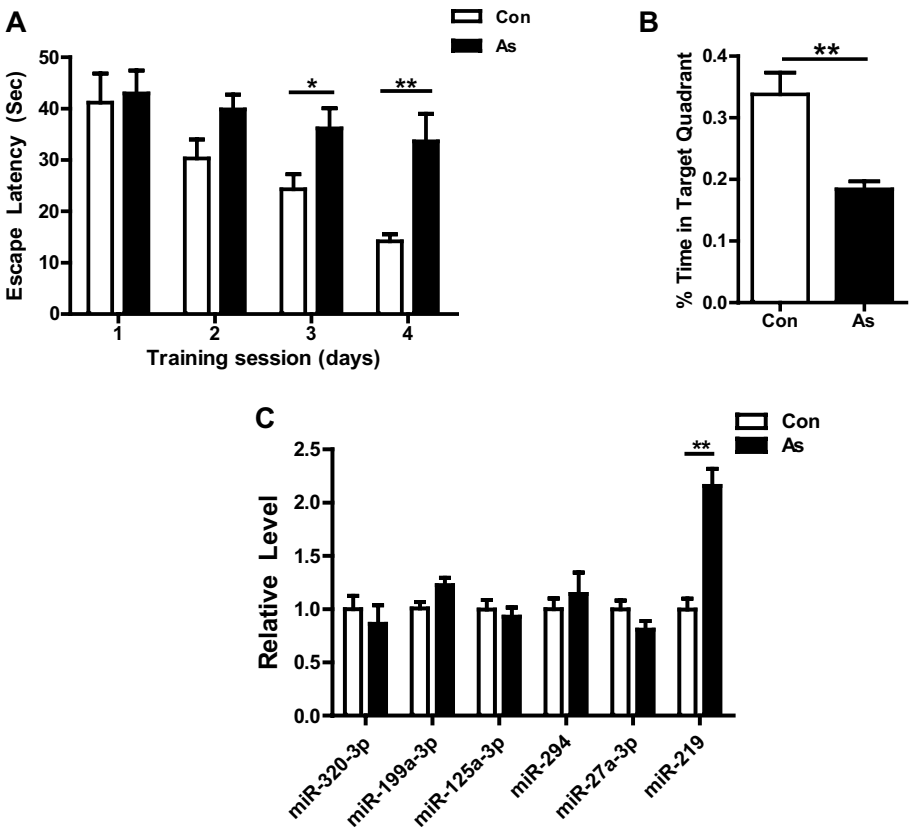

Fig. 3 Expression of miR-219 in hippocampus and learning and memory ability of mice exposed to arsenic. Mice exposed to 4 ppm As2O3 in drinking water for 60 days. After the treatment, the MWM tests were performed to analyze the learning and memory ability of mice. a Escape latency to reach the platform (s) in the navigation test; b Percentage of time spent in the target quadrant in the probe test (n = 8 for each group). Total RNAs were isolated, and the levels of six miRNAs were determined by Real-Time PCR. c The miRNA expression ratios between arsenic-treated groups and control groups are shown. Data are expressed as the mean ± SD of three independent experiments. *$p < 0.05$ compared to the control group; **$p < 0.01$ compared to the control group

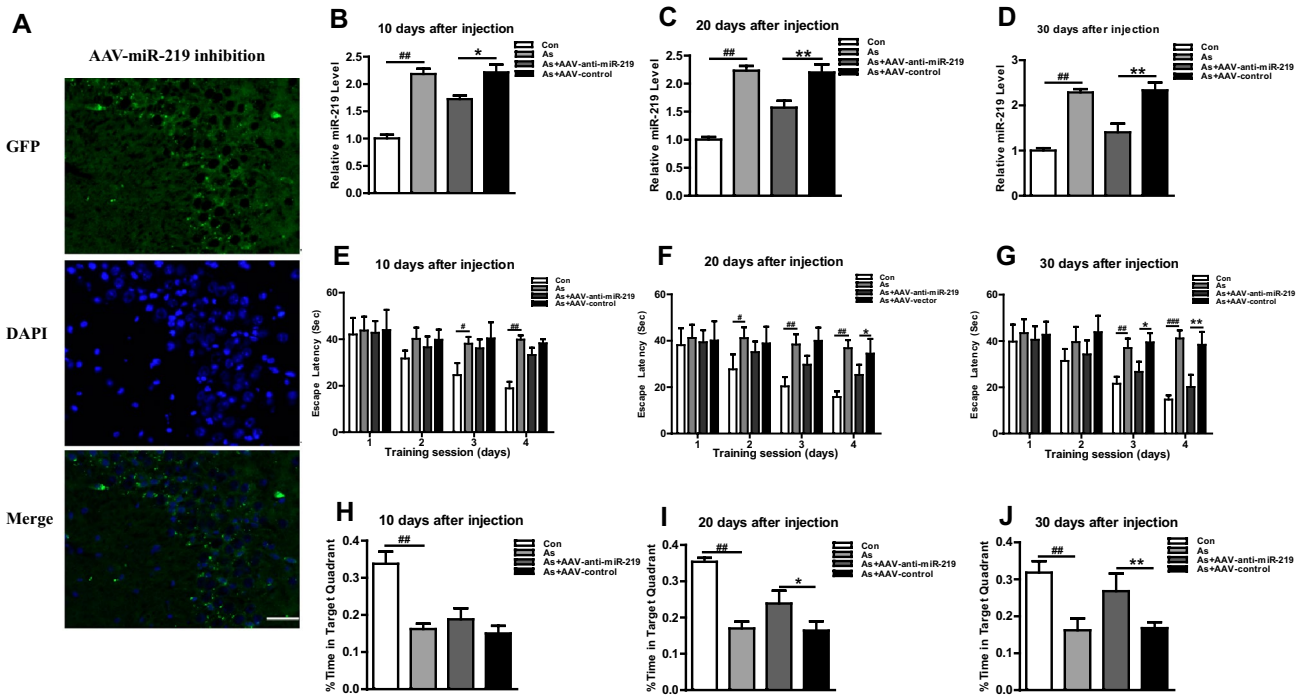

Fig. 4 Inhibition of miR219 affects arsenic induced impairment of learning and memory abilities. **a** Cell localization of anti-miR-219 AAV infected cells (green), nucleus using DAPI antibody (blue), scale bar = 50 μm. The expression of miR-219 was measured at 10 days (**b**), 20 days (**c**) and 30 days (**d**) day after injection of anti-miR-219 in mice hippocampus. Data are expressed as the mean ± SD of three independent experiments. $^{\#\#}p < 0.01$ compared to the control group; $^{*}p < 0.05$, $^{**}p < 0.01$, significantly different compared with As + AAV-control group. The MWM test was performed at the 10, 20, and 30 days after injections of anti-miR-219 (n = 8). Escape latency of mice at the 10 days (**e**), 20 days (**f**), and 30 days (**g**) day after injections of anti-miR-219. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$compared to the control group; $^{*}p < 0.05$ and $^{**}p < 0.01$, compared to As + AAV-control group. Percentage of time spent at the 10 days (**h**), 20 days (**i**), and 30 days (**j**) day after injections of anti-miR-219 in the target quadrant in the probe test. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$compared to the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared to the As + AAV-control group. (Color figure online)

the platform 20 days ($p < 0.05$) and 30 days ($p < 0.01$) after anti-miR-219 injection was significantly decreased. In the probe test, the swimming time in the fourth quadrant 10 days after anti-miR-219 injection showed no significant differences compared with the As + AAV-control group, while the swimming time in the fourth quadrant 20 days ($p < 0.05$) and 30 days ($p < 0.01$) after anti-miR-219 injection was significantly increased (Fig. 4h–j).

Effect of miR-219 Inhibition on Synaptic Ultrastructure in the CA1 Region of the Hippocampus of Mice Exposed to Arsenic

The synapse is the key structure in transmitting neural information and is correlated with learning and memory function. It has been reported that arsenic exposure can lead to ultrastructure abnormalities of the synaptic structure in the brain [7, 31]. Therefore, we examined the synaptic ultrastructure in the hippocampus after miR-219 inhibition under electron microscopy, as shown in Fig. 5A. The synapses in the control group exhibited integrated and clear structure. The presynaptic area was abundant with vesicles and the postsynaptic area was adhered by even density substances (Fig. 5a). Compared to the control group, the PSD thickness was decreased, and the synaptic cleft width widened in the synapses in the hippocampus of the mice in the As group (Fig. 5b). Treatment with anti-miR-219 obviously improved the neuronal synaptic ultra-structure (Fig. 5c) compared to the mice in the As + AAV-control group (Fig. 5d).

Quantitatively, compared to the control group, the PSD thickness was decreased, and the synaptic cleft width was increased significantly in the synapses of the hippocampus of the As group. Treatment with anti-miR-219 increased the PSD thickness and decreased the synaptic cleft width significantly compared to the As + AAV-control group (Fig. 5B).

The Use of an Antisense for miR-219 Increases CaMKII Expression in the Hippocampus of Mice Exposed to Arsenic

Next, we sought to further explore the role of miR-219 in arsenic-induced impairment of learning and memory. It has been reported that miR-219 targets CaMKII to regulate NMDA receptor function [22]. Furthermore, both the

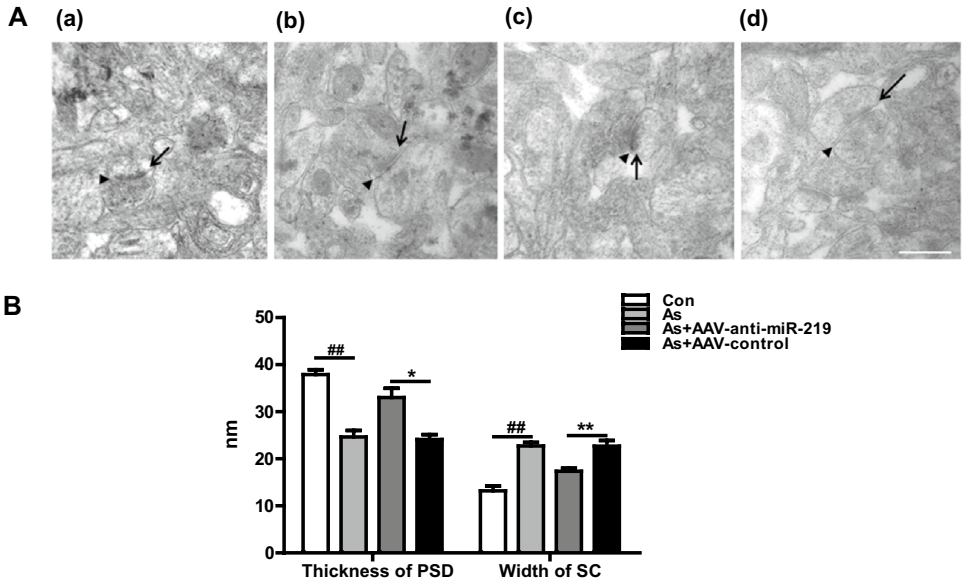

Fig. 5 Effects of miR-219 inhibition on synaptic ultrastructure in hippocampus of mice exposed to arsenic. Comparison of synaptic structure in the mice hippocampus among groups (**A**). PSD, postsynaptic density (arrows); SC, synaptic cleft (arrowheads). (a) Control group: Ultra structure of synapse in the hippocampus of mice without arsenic exposure. (b) As group: Ultra structure of synapse in the hippocampus of mice exposed to 4 ppm As_2O_3 for 90 days. (c) As + AAV-anti-miR-219 group: Ultra structure of synapse in the hippocampus of mice exposed to 4 ppm As_2O_3 for 90 days and received the AAV-anti-miR-219 at 60 days of exposure. (d) As + AAV-control group: Ultra structure of synapse in the hippocampus of mice exposed to 4 ppm As_2O_3 for 90 days and received the AAV-control at 60 days of exposure. Scale bar = 500 nm. Quantitatively changes in the PSD thickness and SC width of synaptic in the mice hippocampus among groups (**B**). $^{\#\#}p<0.01$ compared to the control group; $^{*}p<0.05$, $^{**}p<0.01$ compared to the As + AAV-control group

CaMKII family and NR2 were key regulators of arsenic-induced impairment of learning and memory. Therefore, we chose to evaluate the potential role of CaMKII as a target of miR-219 in arsenic-induced impairment of learning and memory. Western blot analysis showed that the protein expression of CaMKII in hippocampus tissues was significantly lower in groups exposed to 4 ppm As_2O_3 than that in control (Fig. 6a–c). The CaMKII level increased gradually, and 17.8, 63.4 and 88.4% increases were detected in the hippocampus tissues 10, 20 and 30 days after anti-miR-219 injection, respectively (Fig. 6a–c), compared with the protein level in the mice of the As + AAV-control group. Moreover, the NR2 level was increased 30 days after anti-miR-219 injection (Fig. 6d) compared with the protein level in mice of the As + AAV-control group. As a downstream protein of CaMKII and a marker of protein synthesis related to long-term memory and synaptic plasticity, expression of c-Fos and c-Jun protein in mouse hippocampus at 30 days after miR-219 inhibition was investigated to further explore the effect of miR-219 inhibition on arsenic-induced impairment of learning and memory. As shown in Fig. 6d, compared to the control group, c-Fos and c-Jun protein expression levels in the hippocampus were significantly decreased in the group exposed to arsenic. Furthermore, arsenic-induced decrease of c-Fos and c-Jun protein expression was significantly alleviated by anti-miR-219 compared to the As + AAV-control group.

Discussion

miRNAs are powerful regulators of neuronal gene expression that contribute to both physiological and pathological processes [32]. These small noncoding RNAs bind recognition motifs in multiple target mRNAs and silence expression through post-transcriptional mechanisms, such as translational repression or transcript destabilization, enabling them to serve as master regulators of transcriptional networks [33]. miRNAs regulate multiple neurological mechanisms, including brain development, cognition, and synaptic plasticity [34]. Recent reports have shown a strong connection between miRNA modulation and neurotoxicity. An et al. reported that lead (Pb) could induce brain synaptic abnormitiesand learning and memory impairment related to the dysregulation of specific miRNAs and subsequently dysregulate the target gene expression [9]. Aluminum (Al) is considered to be an environmental factor involved in the pathogenesis of Alzheimer's disease [35]. Pogue et al. showed that aluminum–sulfate exposure elevated the expression of miR-146a in a co-culture model of human neuronal

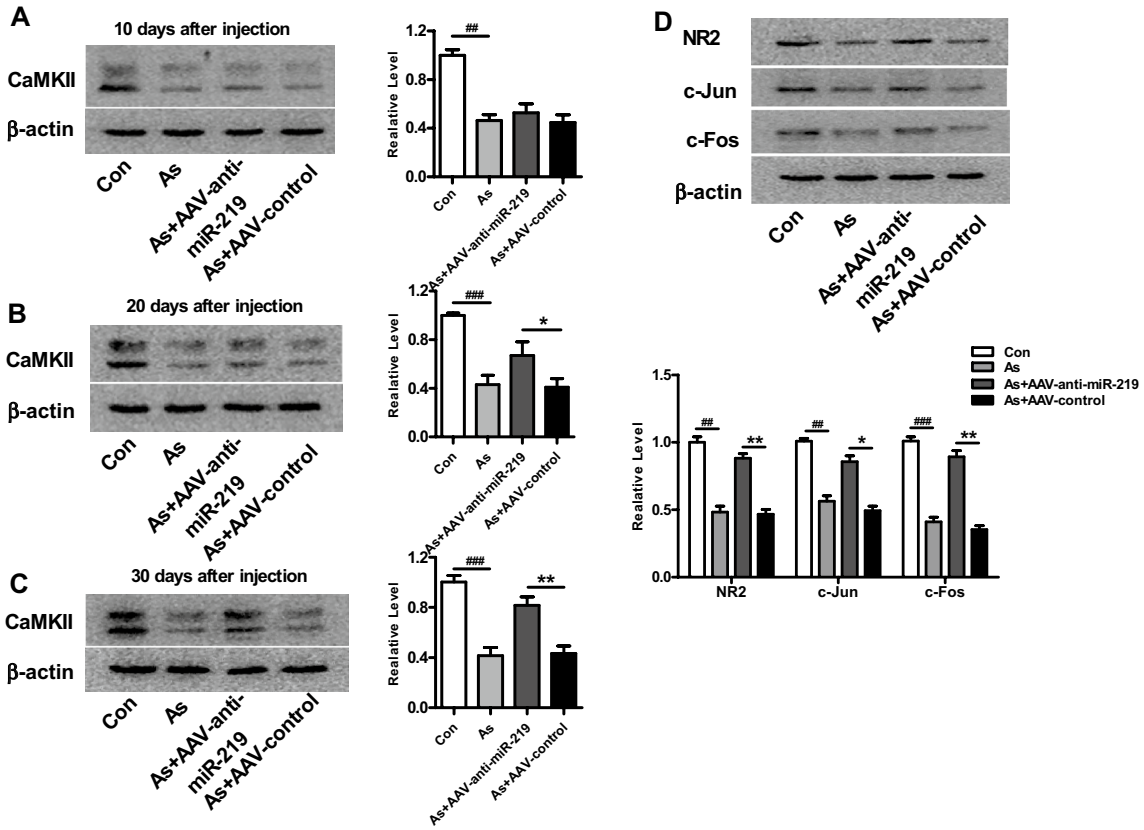

Fig. 6 Effects of miR-219 inhibition on the altered expression of CaMKII protein induced by arsenic exposure. The expression of CaMKII was measured on 10 days (**a**), 20 days (**b**) and 30 days (**c**) day after injection of anti-miR-219 in mice hippocampus by western blot. Data are expressed as the mean ± SD of three independent experiments. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ compared to the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared to As + AAV-contorl group. **d** The expression of NMDAR 2(NR2), c-Fos and c-Jun was measured on 30 days after injection of anti-miR-219 in mice hippocampus by western blot. Data are expressed as the mean ± SD of three independent experiments. $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$compared to the control group; $^{*}p < 0.05$, $^{**}p < 0.01$ compared to As + AAV-control group

and glial cells [36]. Li et al. observed that perfluorooctane sulfonic acid affected miRNA expression, including miR-22, and induced synaptic dysfunctions [37]. These findings demonstrate that miRNAs are key regulators in underlying environmental toxin-induced neurotoxicity and suggest that specific miRNAs may act as new molecular targets for neurotoxicity prevention and relief.

miR-219 has been previously recognized as a brain-specific miRNA [38]. This mRNA has been observed to mediate various neuronal processes and is deregulated in neuronal dysfunction [19]. In this study, we observed that the gene expression of miR-219 was increased by arsenic administration. Suppression of miR-219 by stable inhibition using AAV-delivered anti-miR-219 ameliorated the arsenic-induced impairment of learning and memory. We show that miR-219 may be involved in arsenic-induced neurotoxicity.

It has been reported that the number, morphology, and composition of dendritic spines comprise the structural basis for synaptic plasticity during learning and memory [39]. Several miRNAs enriched in the brain participate in localized modifications of protein translation that regulate the structural changes underlying plasticity [16, 40]. Ultrastructural observations have shown the destroyed synapses and ultrastructure alterations in animals administered arsenic [7]. The results from our previous studies demonstrated that mice exposed to arsenic showed destroyed postsynaptic ultrastructure in the brain [31]. Luo et al. reported ultrastructural changes in hippocampal neurons in arsenite-exposed rats [41]. However, in this study, anti-miR-219 obviously improved the synaptic ultrastructure in the hippocampus after the administration of arsenic. Thus, it is implied that the destroyed synaptic structure of the hippocampus by arsenic exposure might be inhibited by suppression of miR-219.

To identify a potential learning and memory-relevant target gene of miR-219, three independent prediction programs, including miRanda, PicTar, and TargetScan, were performed. A total of 62 genes were predicted to be miR-219 targets (data not shown). Certain of these target genes (e.g., Cpeb3 and Plk2) were reported to have contributed to learning and memory processes [42, 43]. CaMKII has conserved

miR-219 binding sites within its 3_UTR (bp, 1227–1233) [22, 44], and hippocampal CaMKII is important in arsenic-induced impairment of learning and memory [41]. Therefore, we chose to evaluate CaMKII and its potential role as a target of miR-219 in arsenic-induced impairment of learning and memory. Our previous study determined that subchronic exposure to arsenic significantly down-regulated gene and protein expression of CaMKII in mouse brain tissue [31]. Wang et al. reported that arsenic induces neurotoxicity in astrocytes by suppressing the expression of the CaMKII protein [23]. These studies have shown that CaMKII played an important role in arsenic-induced neurotoxicity. However, to the best of our knowledge, few studies have investigated the mechanism of arsenic-mediated down-regulated expression of CaMKII in the hippocampus.

CaMKII activation depends on Ca^{2+}/calmodulin and is a multifunctional protein kinase highly expressed in the CNS. In the CNS, CaMKII has been shown to play a crucial role in gene expression, memory processing, learning and neuroplasticity [27, 32, 33]. CaMKII inhibition was reported to attenuate LTP, the long-lasting form of synaptic plasticity which may represent ways of encoding "memories" in the brain [45]. Activation of CaMKII [46–48] sustains the molecular organization of postsynaptic density and initiates the biochemical cascade that potentiates synaptic plasticity [49]. CaMKII subsequently activates the expression of some immediate early genes (c-Fos and c-Jun) and transcription factors (CREB). The transcription factors change the expression of nuclear genes and ultimately cause the synthesis of different proteins [50–52]. It has been reported that miR-219 modulates NMDA receptor-mediated neurobehavioral dysfunction [22]. The change of NMDA receptor expression following miR-219 disturbance may result from the changes of CaMKII [53]. Furthermore, NR2 is involved in impairment of learning and memory by arsenic exposure [41]. Our current study showed that arsenic exposure was associated with the down-regulation of the NR2 subunit in the mouse hippocampus, which was consistent with a previous report [54]. The expression of NR2 was up-regulated after inhibition of anti-miR-219. Since NMDA receptor was not the target gene of miR-219, the change of NR2 level following miR-219 suppression may result from the changes of CaMKII. The level of CaMKII protein was increased significantly compared to the arsenic exposure group. However, the expression of CaMKII mRNA in the mouse hippocampus was not significantly changed after miR-219 suppression. Therefore, the reduction in CaMKII might be induced by miR-219 binding and preventing translation. Moreover, the decreased protein expression of c-Fos and c-Jun in the hippocampus following arsenic exposure was up-regulated by miR-219 inhibition. We suggest that hippocampal miR-219 inhibition in arsenic-induced deficit of learning and memory acts by regulating the function of CaMKII signaling.

Conclusions

Taken together, the results of this study reveal the role of miR-219 inhibition in the hippocampus and elucidate a novel neuroprotective strategy against arsenic neurotoxicity. However, as miR-219 has hundreds of target genes, we cannot exclude the possibility that other target genes are involved in the context of arsenic-induced impairment of learning and memory, and we only investigated the effect of miR-219 inhibition in hippocampus. Therefore, further studies are still needed to elucidate the precise mechanisms of anti-miR-219 involved in attenuating arsenic neurotoxicity using other brain regions and other target genes of miR-219.

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