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ORIGINAL RESEARCH

miR-98-5p Prevents Hippocampal Neurons from Oxidative Stress and Apoptosis by Targeting STAT3 in Epilepsy in vitro

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Purpose: Epilepsy is a serious mental disease, for which oxidative stress and hippocampal neuron death after seizure is crucial. Numerous miRNAs are involved in epilepsy. However, the function of miR-98-5p in oxidative stress and hippocampal neuron death after seizure is unclear, which is the purpose of current study.

Methods: Magnesium ion (Mg^{2+})-free solution was used to establish the in vitro epilepsy model in hippocampal neurons. Oxidative stress was exhibited by measuring malondialdehyde (MDA) level and superoxide Dismutase (SOD) activity using enzyme-linked immune sorbent assay (ELISA) kits. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and flow cytometry were applied for the examination of neuron viability and apoptosis, respectively. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) and Western blot were used to evaluate the mRNA and protein levels of miR-98-5p and signal transducer and activator of transcription (STAT3), respectively. The relationship between miR-98-5p and STAT3 was predicted by TargetScan 7.2, and identified by dual-luciferase reporter assay and RNA immunoprecipitation (RIP) assay.

Results: miR-98-5p was decreased in the in vitro epileptic model of hippocampal neurons induced by Mg^{2+}-free solution, whose overexpression rescued oxidative stress and neuron apoptosis in epileptic model. Moreover, overexpression of STAT3, one downstream target of miR-98-5p, partially eliminated the effects of miR-98-5p mimic.

Conclusion: We shed lights on a pivotal mechanism of miR-98-5p in regulating neuron oxidative stress and apoptosis after seizures, providing potential biomarkers for the diagnosis of epilepsy and therapeutic targets for the treatment of epilepsy.

Keywords: epilepsy, oxidative stress, apoptosis, miR-98-5p, STAT3

Introduction

Epilepsy is a chronic non-communicable neurological disorder in the central nervous system (CNS).[1] It has the characteristics of recurrent seizures and high synchronous discharge of brain neurons,[2,3] and is accompanied with comorbidity, depression, mortality, and anxiety.[4] At present, there are approximately 70 million epileptic patients worldwide, and the incidence is increasing year by year. Long-term recurrent seizures induce neuron death and cause brain damage, in which hippocampal neurons are the most severely damaged.[5–7] Hippocampus is a brain structure with special structure and function, which is closely related to learning and memory,[8] and is very sensitive to epileptic discharge in the brain. The damage of hippocampal neurons will further aggravate the course of epilepsy and cause cognitive impairment.[9] Therefore, it is of great importance to investigate the mechanism of epilepsy-induced hippocampal neuron death.

Glutamatergic synapses are the main excitatory synapses in the brain, mediating the transmission of most excitatory synaptic in the CNS.[10] Epilepsy is closely related to the hyperactivity of glutamatergic neurons, which are able to over-activate

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downstream neurons and produce excitatory neurotoxicity.[11,12] N-methyl-D-aspartic acid receptor (NMDAR) is a kind of important ionic glutamate receptors which located in the glutamatergic postsynaptic membrane. When neurons are over-activated, NMDA receptor will mediate a large amount of extracellular calcium ion (Ca^{2+}) influx, and activate cell death pathway.[13,14] Numerous anti-convulsant properties are exerted by NMDA antagonists in animals with acute/chronic epilepsy.[15]

As acknowledged, the increase of intracellular Ca^{2+} leads to excessive accumulation of reactive oxygen species (ROS), which causes oxidative stress in neuron.[16,17] Subsequently, excessive oxidative stress results in protein oxidation, lipid oxidation, DNA damage, other oxidative reactions, and eventually cell death.[18,19]

MicroRNA (miRNA) is a kind of endogenous small non-coding RNAs with 18–24 nucleotides, which can bind target genes, and form RNA-induced silencing complex (RISC) to inhibit the translation of target genes.[20,21] Consequently, miRNAs participate in numerous pivotal physiological processes.[22,23] Massive literatures have reported the relationship between miRNAs and epilepsy,[24,25] eg, miR-101a-3p attenuates pilocarpine-induced epilepsy by repressing c-Fos expression.[26] Moreover, massive miRNAs are involved in Mg^{2+}-free condition induced in vitro epilepsy models in hippocampal neurons, for instance, miR-29a alleviates seizure-induced cell death and inflammation in hippocampal neurons by targeting HMGB1,[27] miR-30b-5p protects against cell proliferation and attenuates apoptosis in hippocampal neurons by targeting GRIN2A.[28] Specifically, in 2017, miR-98-5p was discovered to be down-regulated in the brain samples of post-traumatic epilepsy rats,[29] however, the roles of miR-98-5p in epilepsy remains unclear.

Herein, miR-98-5p expression is downregulated in the Mg^{2+}-free-induced hippocampal neurons; moreover, miR-98-5p prevents neurons from Mg^{2+}-free-induced oxidative stress and apoptosis by targeting STAT3. Current study reveals a novel mechanism of miR-98-5p in regulating neuron survival in epilepsy, which provides an important scientific basis for exploring the mechanism of epilepsy caused neuron death.

Materials and Methods

Cell Culture and Epilepsy Model Establishment

All 2-day postnatal Sprague-Dawley rats were maintained and used. Ethical and legal approval was obtained from the Ethical Commission of Huangshi Central Hospital prior to the commencement of the study. All experiments were performed following guidelines and regulations of the Ethical Commission of Huangshi Central Hospital. Firstly, rats were sacrificed according to standard protocols (100 mg/kg intraperitoneal sodium pentobarbital). Thereafter, hippocampus was dissociated from rats, dissected out in ice-cold PBS, trypsinized (0.25%) and maintained with the whole culture medium (NeuroBasal-A medium, 2% B27 supplement, 0.5 mmol/L glutamine and 10% FBS), before being plated onto poly-D-lysine (50 μg/mL) coated glass coverslips. Cells (2×10^5 cells/mL) were incubated in an incubator containing 5% CO_2 at 37°C and maintained by replacing half medium with fresh culture medium every 4 days.

The well established in vitro epilepsy model in hippocampal neurons was conducted by referring to previous studies.[30,31] In brief, at day 14, the culture medium was removed and harvested as conditioned medium, and primary neurons were appended with Mg^{2+}-free solution (pH 7.3, without $MgCl_2$): 2.5 mM KCl, 145 mM NaCl, 2 mM $CaCl_2$, 10 mM HEPES, 10 mM D-glucose and 0.002 mM glycine, and cultured in an incubator at 37°C for 3 h; thereafter, neurons were restored to the physiological concentration of 1mM $MgCl_2$ by rinse with 3× 1.5 mL of culture medium at 37°C, returned to maintenance feed and incubated at 37°C with 5% CO_2. Hippocampal neurons in control group were treated with culture medium containing 1 mM $MgCl_2$.

Antibodies and Reagents

The antibodies are as follows: rabbit anti-STAT3 primary antibody (Cell signaling technology, #12640), rabbit anti-GAPDH primary antibody (Cell signaling technology, #5174), goat anti-rabbit secondary antibody (Cell signaling technology, #7074).

The regents are as follows: poly-D-lysine (Sigma Aldrich), Neurobasal (Thermo Fisher Scientific), B27 (Thermo Fisher Scientific), GlutaMax (Thermo Fisher Scientific), Penicillin-Streptomycin (Thermo Fisher Scientific), Lipofectamine 2000 (Thermo Fisher Scientific), Trizol (Thermo Fisher Scientific), PrimeScript qRT-PCR Reagent kit

(Takara), SYBR Green Mix (Roche), Dual Luciferase Assay System (Promega Corporation), MDA and SOD ELISA kits (Nanjing Jiancheng Bioengineering Institute), Flow cytometry apoptosis detection kit (BD Biosciences), protease inhibitor cocktail (Sigma-Aldrich), RIPA buffer (Roche), Annexin V-FITC (Thermo Fisher Scientific), Propidium iodide (Thermo Fisher Scientific), ECL Western blotting substrate (Pierce).

Cell Transfection

For transfection experiments, after replacing half medium at 14DIV, 50 nM miR-NC mimic (5'-UCGCUUGGU GCAGGUCGGG-3', GenaPhama, Shanghai, China) or miR-98-5p mimic (5'-UGAGGUAGUAAGUUGUAUUGUU-3', GenaPhama), and 2 μg pcDNA3.1 (GenaPhama) or pcDNA3.1-STAT3 (GenaPhama) was transfected into neurons by Lipofectamine 2000 (Thermo Fisher Scientific). At 4 h later, the culture medium was replaced by mixed conditioned medium (half conditioned culture medium and half fresh culture medium). At 48 h later, cells were harvested for the subsequent experiments.

qRT-PCR

Total RNA was extracted from cultured neurons by Trizol reagent, and reverse transcribed into cDNA by PrimeScript qRT-PCR Reagent kit. SYBR Green Mix kit was used for qRT-PCR experiments. The expression levels of miR-98-5p and STAT3 were analyzed by $2^{-\Delta\Delta Ct}$ method, and normalized to the internal controls U6 and GAPDH, respectively.

Western Blot Analysis

Cultured neurons were rinsed by PBS, and lysed by protease inhibitor cocktail contained RIPA buffer. The protein samples were mixed with SDS loading buffer and loaded to SDS-PAGE gel for electrophoresis onto PVDF membranes. After blocking with 5% BSA in TBST for 1 h at room temperature, the PVDF membranes were incubated with primary antibodies overnight at 4°C, and washed thoroughly with TBST; then incubated with secondary antibodies for 1 h at room temperature. After wash thoroughly, the HRP signals were detected with ECL substrate. The intensity of positive protein band was measured by ImageJ software, and the average value of control group was normalized to 1.

MDA Level and SOD Activity Measurement

Neurons were washed with PBS, and harvested before being lysed by protease inhibitor cocktail contained RIPA buffer on ice for 30 min. Thereafter, neurons were centrifuged at 10,000g for 10 min at 4°C. At last, the supernatant was used for the determination of MDA level and SOD activity with MDA and SOD ELISA kits, respectively.

Flow Cytometric Assay

Cultured neurons were washed by PBS and trypsinized (0.25%) before being harvested from each well. Subsequently, neurons were suspended in 100 μL PBS and mixed with Annexin V-FITC and Propidium iodide, followed by incubation at dark room temperature for 15 min. After washing with PBS, each sample was mixed with SA-FLOUS and incubated at dark 4°C for 20 min. Finally, neurons were analyzed by flow cytometry, and Annexin V-FITC positive cells were considered as apoptotic neurons.

MTT Assay

At 14DIV, each group of neurons was incubated with 250 μL MTT solution and 250 μL fresh Neurobasal medium at 37°C for 3 h. Afterwards, MTT solution was replaced with MTT solvent. Neurons were shaken for 15 min before being subjected to the measurement of the absorbance at 570 nm.

Dual Luciferase Reporter Assays

The binding site between miR-98-5p and STAT3 was predicted by TargetScan 7.2 (http://www.targetscan.org/vert_72/). The 3' untranslated region (3' UTR) sequences of wild type (WT) and mutant (MUT) STAT3 were inserted into pGL3-luciferase reporter plasmids using KpnI and XhoI restriction sites, and the corresponding reconstructed plasmids were named as STAT3-WT and -MUT, respectively. Subsequently, STAT3-WT and -MUT were co-transfected with miR-98-5p

mimic or NC mimic into cultured neurons using Lipofectamine 2000. At 48 h after transfection, cells were trypsinized and transferred to a 24-well plate. The luciferase reporter activities were measured by Dual Luciferase Assay System with Renilla luciferase activity as the reference control.

RNA Immunoprecipitation (RIP) Assay

Nuclei from cells were isolated, lysed and incubated with primary antibodies against anti-Ago2 (Abcam) and anti-IgG (Abcam) at 4°C overnight. Then, RNA immuno-precipitated with RBP was isolated immediately following the addition of protein A agarose (Abcam) and protein G agarose (Abcam). After wash, RNA was purified and reverse transcribed into cDNA. Gene expression was measured by RT-qPCR as aforementioned.

Statistical Analyses

All the data were stated as mean ± S.D and analyzed using SPSS version 17.0. Difference between two groups was measured by unpaired Student's *t* test, and the differences among four groups were measured by one-way analysis of variance analysis followed by Tukey's test. $p<0.05$ was considered as significant difference.

Results

Oxidative Stress and Cell Viability in an in vitro Epilepsy Model in Hippocampal Neurons

Herein, Mg^{2+}-free solution was used to treat 14DIV cultured rat primary hippocampal neurons for 3 h to induce epilepsy in vitro.[30,31] At 20 h later, ELISA and MTT were used to detect the oxidative stress and cell viability of the neurons, respectively.

Lipid peroxidation, due to oxidative stress is involved in some epilepsy types and seizure recurrence.[32] The brain contains high concentration of PUFA which is more prone to lipid peroxidation, therefore, the assessment of MDA level is an important indicator of lipid peroxidation for various diseases. The superoxide dismutases play a crucial role in eliminating superoxide anion radicals (O2•−) generated from extracellular stimulants, including ionizing radiation and oxidative insults together with produced in mitochondrial matrix.[33] Herein, ELISA exhibited significantly higher MDA level and lower SOD activity in Mg^{2+}-free solution treated neurons compared to controls ($p<0.01$, Figure 1A and B).

In addition, MTT exerted significantly lower cell viability in Mg^{2+}-free solution treated neurons compared to controls ($p<0.01$, Figure 1C).

The findings indicated that Mg^{2+}-free solution treated neurons had stronger oxidative pressure and lower cell viability.

Expression of miR-98-5p and STAT3 in an in vitro Epilepsy Model in Hippocampal Neurons

Subsequently, we intended to explore how these aforementioned changes were induced. By searching literatures, it was found that the expression of miR-98-5p in the brain samples of post-traumatic epilepsy rats was decreased;[29] in epileptic

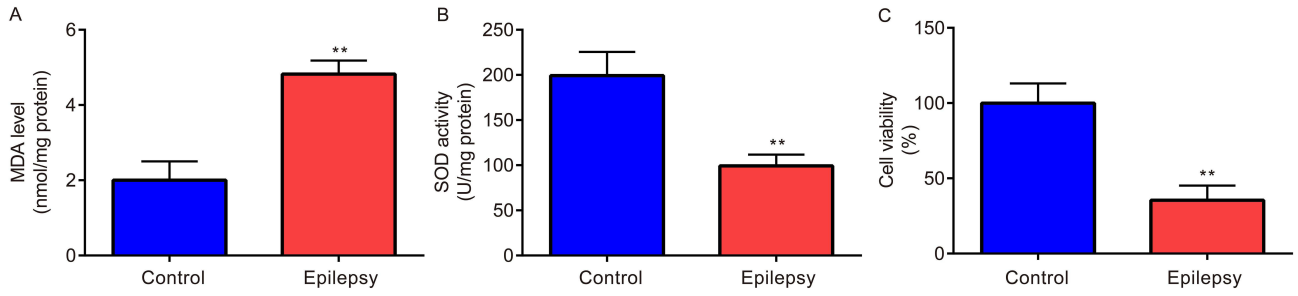

Figure 1 Oxidative stress and cell viability in an in vitro model of epilepsy. (**A** and **B**) ELISA shows that compared to control, MDA level is increased, while SOD activity is decreased by Mg^{2+}-free solution. (**C**) MTT assay shows that compared to control, cell viability is decreased by Mg^{2+}-free solution. n=3. ** $p<0.01$, epilepsy vs control.

cell models, the expression of STAT3 was increased,[34,35] which was closely related to oxidative stress and cell survival.[36,37]

Therefore, qRT-PCR and Western blotting were performed to detect the expressions of miR-98-5p and STAT3 in Mg^{2+}-free solution induced in vitro epilepsy model in hippocampal neurons. Compared with control group, the expression of miR-98-5p decreased significantly ($p<0.01$, Figure 2A), while the mRNA and protein levels of STAT3 increased significantly ($p<0.01$, Figure 2B–D), in Mg^{2+}-free solution treated neurons.

Manipulating miR-98-5p and STAT3 Expression in an in vitro Epilepsy Model in Hippocampal Neurons

To make clear the roles of miR-98-5p and STAT3 in epilepsy, it was needed to achieve their ectopic expressions in hippocampal neurons by miR-98-5p mimic and pcDNA3.1-STAT3. As shown in Figure 3A–D ($p<0.01$), the expressions of miR-98-5p and STAT3 were increased by transfection of miR-98-5p mimic and pcDNA3.1-STAT3, respectively.

Thereafter, we were eager to know whether the expression of STAT3 could be regulated by miR-98-5p. In Figure 4A–C, miR-98-5p mimic ($p<0.05$) significantly attenuated Mg^{2+}-free solution ($p<0.01$) induced upregulation of mRNA and protein levels of STAT3, which were partially eliminated by pcDNA3.1-STAT3 ($p<0.05$).

Collectively, these findings indicated that miR-98-5p was involved in epilepsy by regulating STAT3.

Effects of miR-98-5p and STAT3 on Oxidative Stress, Cell Viability and Apoptosis in an in vitro Epilepsy Model in Hippocampal Neurons

Afterwards, to identify the involvement of miR-98-5p as well as the relation between miR-98-5p and STAT3 in epilepsy induced increase of oxidative stress and decrease of cell viability, miR-98-5p mimic and/or pcDNA3.1-STAT3 were transfected into Mg^{2+}-free solution treated neurons. In Figure 5A–C, ELISA and MTT exhibited that miR-98-5p mimic ($p<0.05$) significantly attenuated Mg^{2+}-free solution ($p<0.01$) induced upregulation of MDA level, downregulation of SOD activity and cell viability, which were partially reversed by pcDNA3.1-STAT3 ($p<0.05$).

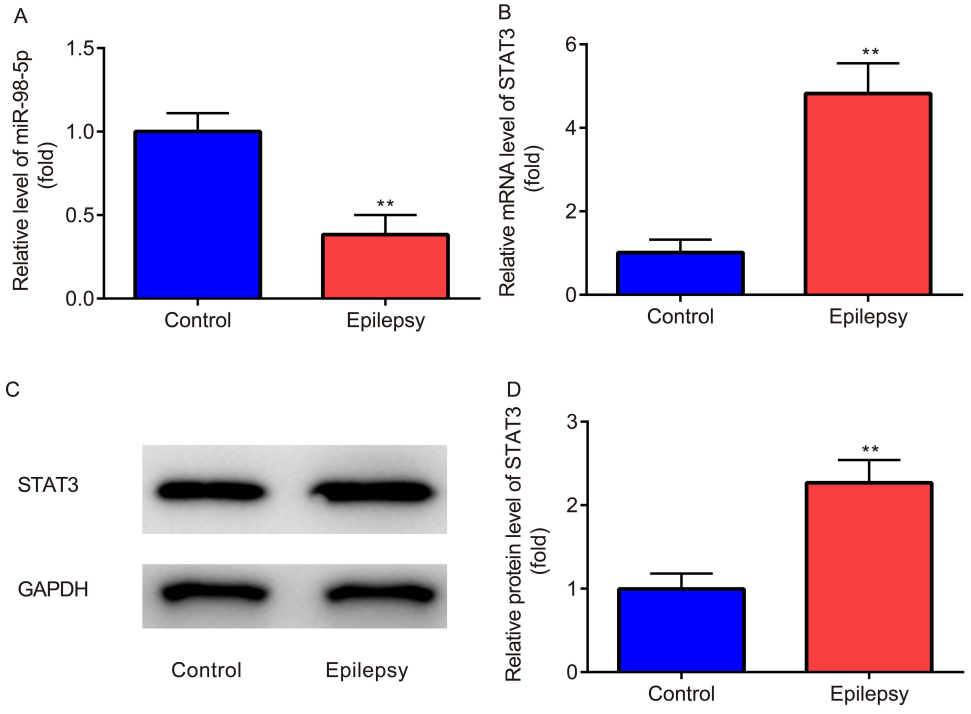

Figure 2 Expression profile of miR-98-5p and STAT3 in an in vitro model of epilepsy. (**A**) qRT-PCR shows that compared with control, miR-98-5p is significantly lowered by Mg^{2+}-free solution. (**B-D**) qRT-PCR and Western blot exhibit that compared with control, the mRNA and protein levels of STAT3 are elevated by Mg^{2+}-free solution. n=3. ** $p<0.01$, epilepsy vs control.

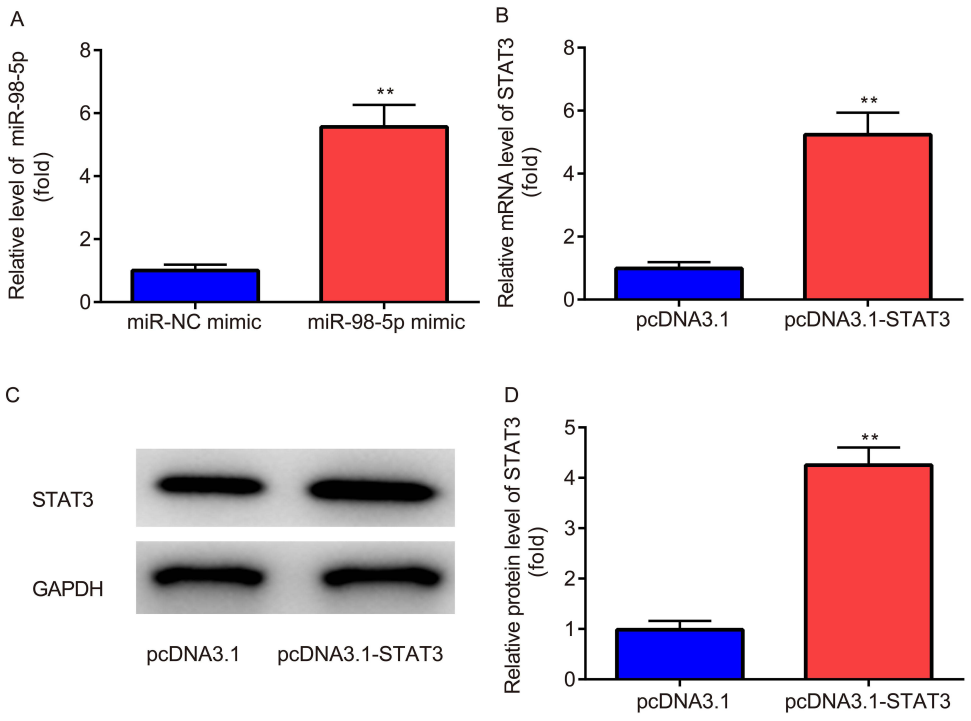

Figure 3 Transfection efficacy of miR-98-5p mimic and pcDNA3.1-STAT3. (**A**) qRT-PCR shows that miR-98-5p expression is significantly elevated by miR-98-5p mimic in comparison with miR-NC mimic. (**B** and **C**) qRT-PCR and Western blot exert that the mRNA and protein levels of STAT3 are elevated by pcDNA3.1-STAT3 in comparison with pcDNA3.1. n=3. ** p<0.01, miR-98-5p mimic vs miR-NC mimic; pcDNA3.1-STAT3 vs pcDNA3.1.

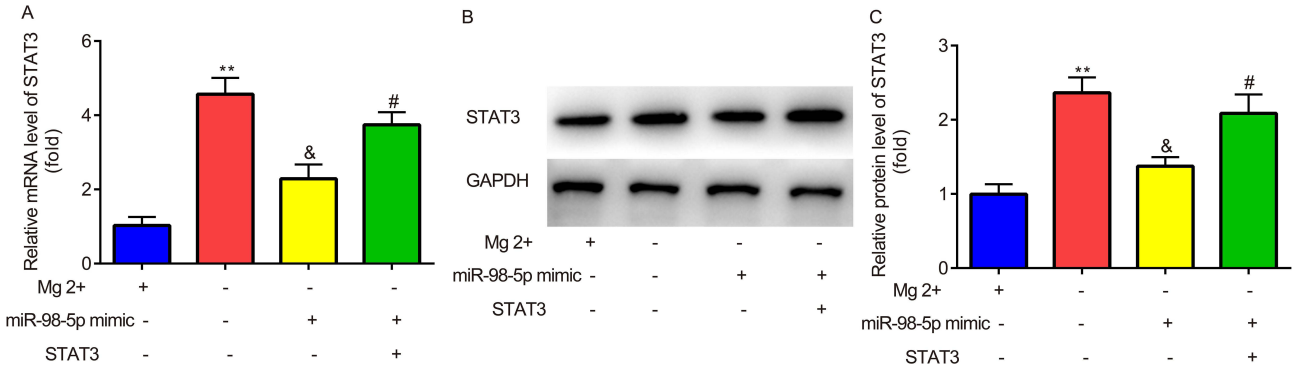

Figure 4 Effects of miR-98-5p on STAT3 in an in vitro model of epilepsy. (**A-C**) qRT-PCR and Western blot exert that miR-98-5p mimic reverses Mg^{2+}-free solution induced upregulation of mRNA and protein levels of STAT3, which are partially eliminated by pcDNA3.1-STAT3. n=3. ** p<0.01, Mg^{2+} free vs control. and p<0.05, Mg^{2+} free+miR-98-5p mimic vs Mg^{2+} free. # p<0.05, Mg^{2+} free+miR-98-5p mimic+pcDNA3.1-STAT3 vs Mg^{2+} free+miR-98-5p mimic.

Cell apoptosis is an important cause of seizure induced neuron loss, and is closely related to oxidative stress and cell viability.[38] Thereafter, we planned to investigate whether miR-98-5p and STAT3 affected Mg^{2+}-free treatment induced cell apoptosis, miR-98-5p mimic and/or pcDNA3.1-STAT3 were transfected into Mg^{2+}-free solution treated neurons. As shown in Figure 6A and B, flow cytometry exerted that, miR-98-5p mimic (p<0.01) significantly attenuated Mg^{2+}-free solution (p<0.001) induced cell apoptosis, which was partially eliminated by pcDNA3.1-STAT3 (p<0.01).

In summary, overexpression of miR-98-5p prevented hippocampal neurons from oxidative stress, cell viability reduction and cell apoptosis, while STAT3 overexpression had the reverse effects.

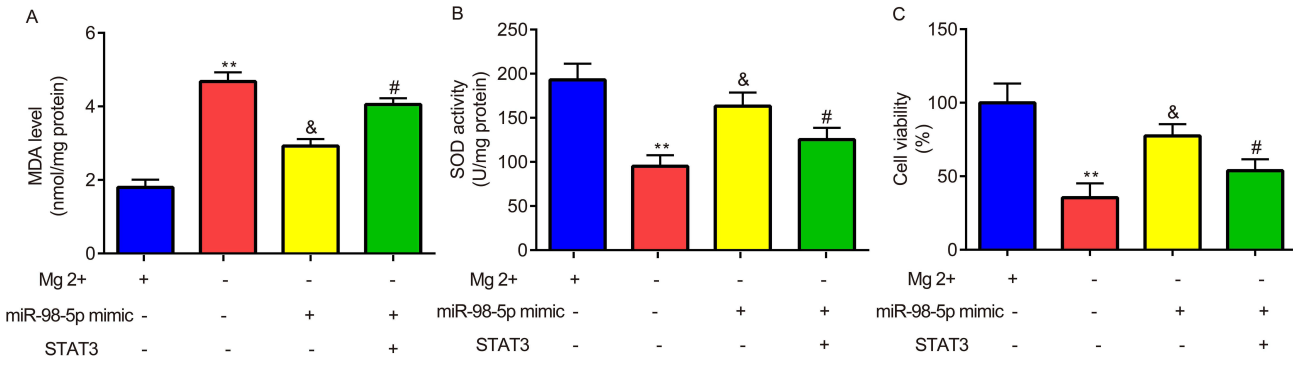

Figure 5 Effects of miR-98-5p and STAT3 on oxidative stress and cell viability in an in vitro model of epilepsy. (**A-C**) ELISA and MTT exhibited that miR-98-5p mimic reverses Mg^{2+}-free solution induced upregulation of MDA level, downregulation of SOD activity and cell viability, which are partially rescued by pcDNA3.1-STAT3. n=3. ** p<0.01, Mg^{2+} free vs control. and p<0.05, Mg^{2+} free+miR-98-5p mimic vs Mg^{2+} free. # p<0.05, Mg^{2+} free+miR-98-5p mimic+pcDNA3.1-STAT3 vs Mg^{2+} free+miR-98-5p mimic.

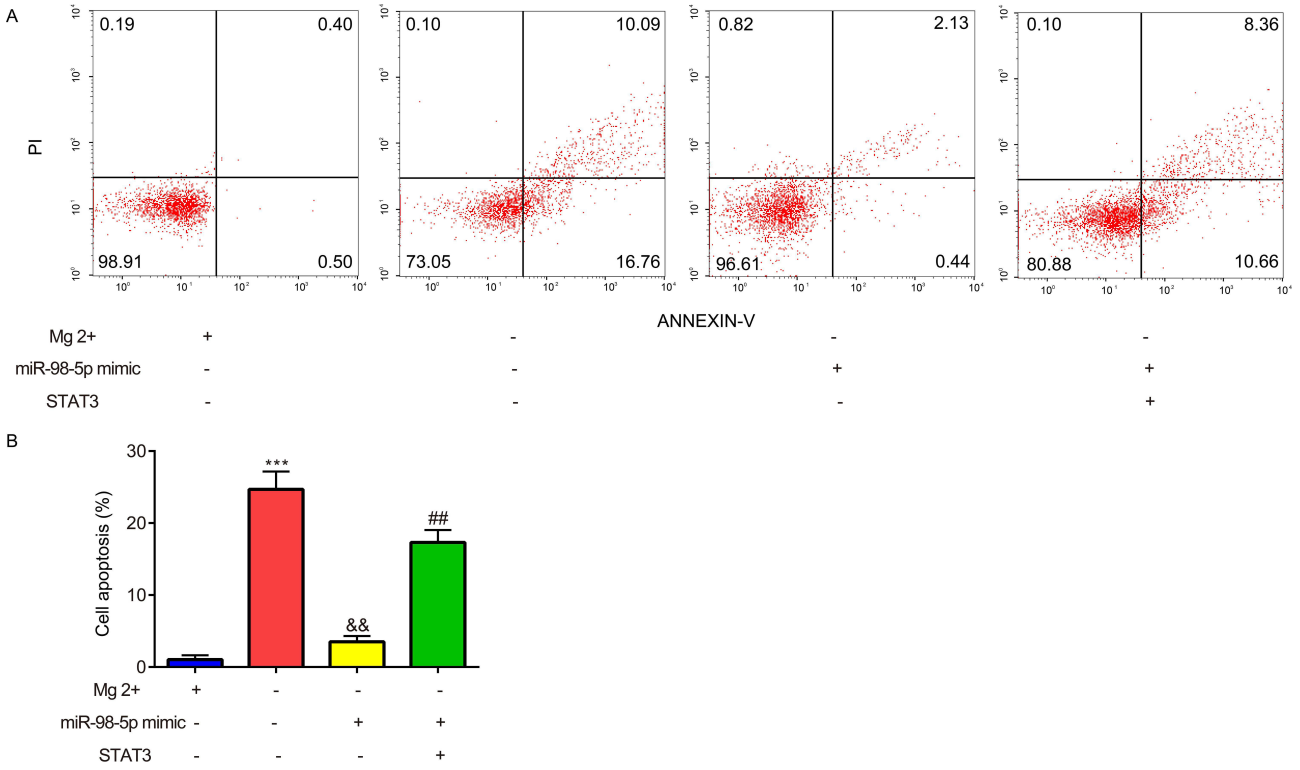

Figure 6 Effects of miR-98-5p on cell apoptosis in an in vitro model of epilepsy. (**A** and **B**) Flow cytometry results show that miR-98-5p mimic reverses Mg^{2+}-free solution induced cell apoptosis, which is partially rescued by pcDNA3.1-STAT3. n=3. *** p<0.001, Mg^{2+} free vs control. and p<0.01, Mg^{2+} free+miR-98-5p mimic vs Mg^{2+} free. ## p<0.01, Mg^{2+} free+miR-98-5p mimic+pcDNA3.1-STAT3 vs Mg^{2+} free+miR-98-5p mimic.

STAT3 is Targeted by miR-98-5p

According to the aforementioned findings, it was found that the expression of STAT3 was regulated by miR-98-5p, and the roles of miR-98-5p and STAT3 in seizure induced oxidative stress and cell apoptosis were completely opposite, demonstrating that STAT3 might be targeted by miR-98-5p.

Consequently, we searched the TargetScan 7.2 website, and found that the 3' UTR of STAT3 was potentially targeted by miR-98-5p (Figure 7A). Next, we designed WT and MUT sequences of STAT3 3'UTR to perform dual luciferase reporter assay, which exhibited that in STAT3-WT transfected neurons, the luciferase activity was significantly lowered by miR-98-5p mimic compared to miR-NC mimic (p<0.05); however, in STAT3-MUT transfected neurons, there was no

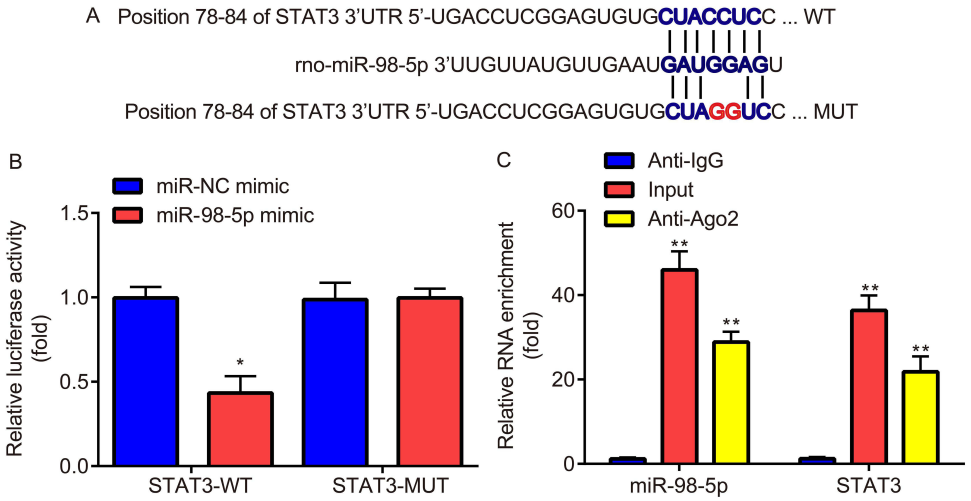

Figure 7 Relation between miR-98-5p and STAT3. (**A**) The sequences of miR-98-5p and STAT3-WT and -MUT. (**B**) Dual luciferase assay shows that miR-98-5p mimic decreases the luciferase reporter activity in STAT3-WT but not STAT3-MUT transfected rat primary hippocampal neurons when compared to miR-NC mimic. (**C**) RIP assay shows increased miR-98-5p and STAT3 enrichment in Anti-Ago2 group in contrast to anti-IgG. n=3. * p<0.05, miR-98-5p mimic vs miR-NC mimic. ** p<0.01, miR-98-5p mimic vs miR-NC mimic; input, anti-Ago-2 vs anti-IgG.

significant difference in the luciferase activity between miR-NC mimic group and miR-98-5p mimic group (p>0.05, Figure 7B). Furthermore, RIP assay displayed that miR-98-5p and STAT3 were highly enriched in the Ago2 group compared to anti-IgG group (p<0.01, Figure 7C). Altogether, these results stated clearly that STAT3 was targeted by miR-98-5p.

Discussion

Herein, miR-98-5p expression was decreased, while STAT3 expression was increased in Mg^{2+}-free induced in vitro epilepsy model in hippocampal neurons. In addition, overexpression of miR-98-5p attenuated Mg^{2+}-free induced phenotypes of increased oxidative stress and cell apoptosis in epilepsy neurons, which was partially abolished by overexpression of STAT3. Altogether, miR-98-5p prevents hippocampal neurons from oxidative stress and apoptosis by targeting STAT3 in epilepsy.

In current study, one of the key findings was that miR-98-5p expression was decreased after Mg^{2+}-free treatment, which was consistent with a previous study showing that miR-98-5p was down-regulated in brain samples of post-traumatic epilepsy rats.[29] The result indicated that, on one hand, decreased expression of miR-98-5p was a co-occurrence phenomenon in epilepsy models, suggesting a biomarker potential of miR-98-5p for epilepsy diagnosis; on the other hand, decreased expression of miR-98-5p was regulated by some early unknown epilepsy-related mechanisms. However, overexpression of miR-98-5p alone did not have any significant effect on control neurons (data not shown), which suggests that miR-98-5p only function under Mg^{2+}-free conditions.

Afterwards, STAT3 expression was discovered to be increased in Mg^{2+}-free solution induced epilepsy model, which was in consistent with previous reports showing increased or overactivated STAT3 after seizure, eg, Choi et al found that nuclear STAT3 was rapidly and constantly activated in the rat hippocampus following kainic acid-induced seizures,[34] Jiang et al reported that STAT3 was elevated in hippocampus of epileptic mice following lipopolysaccharide (LPS)-induced seizures.[35]

In addition, inhibiting the expression of STAT3 by overexpressing miR-98-5p could repress neuronal oxidative stress and apoptosis effectively, while overexpression of STAT3 could promote neuronal oxidative stress and apoptosis. These results indicated that miR-98-5p and STAT3 had opposite functions, STAT3 was a downstream target of miR-98-5p, and STAT3 expression was closely related to neuron viability after seizure. Thus, STAT3 might be a potential therapeutic target for epilepsy, which was further proved by a very recent report in 2023 Jul, where Tipton et al discovered that, in

a mouse line with selective STAT3 knock-out in excitatory neurons, there was inhibited seizure progression, and demonstrated that targeting neuronal STAT3 may be an effective disease-modifying strategy for epilepsy.[39]

miR-98-5p was related to the antioxidant mechanism in various diseases, eg, oxygen-glucose deprivation/reoxygenation (OGD/R)-induced neuronal injury,[40] and cerebral ischemia/reperfusion injury.[41] Additionally, numerous studies have exhibited the facilitation effects of STAT3 in oxidative stress and cell apoptosis in other models, eg, middle cerebral artery occlusion (MCAO) model,[42] and cerebral ischemia-reperfusion injury.[43] However, there is no literature about the relation between miR-98-5p/STAT3 axis and epilepsy-induced oxidative stress, neuron apoptosis. Herein, we reported for the first time that miR-98-5p prevented neurons from oxidative stress and apoptosis by targeting STAT3 in Mg^{2+}-free induced epilepsy model in hippocampal neuron. Moreover, there were several reports about the relationship between miR-98-5p/STAT3 axis and other diseases, for example, overexpression of miR-98 attenuates neuropathic pain by targeting STAT3 in chronic constriction injury rats,[44] miR-98 inhibits cell proliferation and invasion by targeting STAT3 in nasopharyngeal carcinoma,[45] miR-98-5p promotes apoptosis and inhibits migration by targeting STAT3 in A549 cells,[46] which further strengthen the interaction between miR-98-5p and STAT3 in current study.

Altogether, current study identified that miR-98-5p overexpression prevents hippocampal neurons from Mg^{2+}-free-induced cell viability reduction, oxidative stress and apoptosis by targeting STAT3.

Nevertheless, there remain two limitations in the present study: 1. In the in vitro model of epilepsy in cultured hippocampus neurons, the intact intra-hippocampal connectivity is lost, which will be improved in the future study by using the in vitro epilepsy models of whole hippocampus, whole brain, acute slice and organotypic slice, etc, 2. Epilepsy is characterized by recurrent seizures and seizure is quantified by behavior or electrophysiological recordings. However, no electrophysiological recording is displayed in the result part of this manuscript. As a result, more in vitro models of epilepsy will be conducted in the future study to demonstrate the electrophysiological recording of seizure-like events.

Conclusion

Current study reported that the expression of miR-98-5p was decreased, while STAT3 was increased in cultured hippocampal epilepsy neuron model in vitro. What's more, the present study revealed a novel mechanism of miR-98-5p overexpression in preventing neurons from Mg^{2+}-free-induced cell viability reduction, oxidative stress and apoptosis, which were partially abolished by STAT3. In sum, current study provided potential biomarkers for the diagnosis of epilepsy and therapeutic targets for the treatment of epilepsy.

Data Sharing Statement

The data and materials were available from the corresponding author.

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Disclosure

The authors report no conflicts of interest in this work.

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