Regular Article

MicroRNA-146a-5p Downregulates the Expression of P-Glycoprotein in Rats with Lithium–Pilocarpine-Induced Status Epilepticus

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INTRODUCTION

Epilepsy is a common chronic neurological disease, affecting approximately 65 million people worldwide. Although current antiepileptic drugs used in clinical practice have been developed into the third generation, approximately 30% of patients with epilepsy fail to respond to appropriate anti-epileptic drugs (AEDs), imposing a serious psychological and economic burden on the patient. Studies have demonstrated that recurrent seizures induce the expression of P-gp and status epilepticus (SE) could upregulate the expression of P-gp, resulting in drug resistance. MicroRNAs (miRNAs), as endogenous regulators, represent small regulatory RNA molecules that have been shown to act as negative regulators of gene expression in different biological processes. We investigated the impact of miR-146a-5p on the expression of P-gp in status epilepticus rat model. The expression of miR-146a-5p in rat cortex and hippocampus was measured by quantitative RT-PCR at 2 weeks after induction of SE. Meanwhile, we detected the expression of P-gp in the brain of SE rats using Western blotting and immunohistochemistry. Upregulation of miR-146a-5p and overexpression of P-gp were evident at 2 weeks after SE. Moreover, the expression of P-gp was downregulated by injection of miR-146a mimic into the hippocampus. We also detected the expression of interleukin-1 receptor-associated protein kinases-1 (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6) and nuclear factor-kappaB (NF-κB) p65 using Western blotting and immunohistochemistry, which indicated the expression of IRAK1, TRAF6 and NF-κB p-p65/p65 increased in the brain of SE rats, and overexpression of miR-146a-5p could downregulate the expression of IRAK1, TRAF6, NF-κB p-p65/p65 and P-gp. Our study indicated that miR-146a-5p may decrease the expression of P-gp in status epilepticus rats via NF-κB signaling pathway.

Key words status epilepticus; P-glycoprotein; microRNA-146a-5p; drug resistance; nuclear factor-kappaB (NF-κB) signaling pathway

Increasing evidence supports that the efflux transporters, especially P-glycoprotein (P-gp), have vital roles on drug resistance in epilepsy. Overexpression of P-gp in the brain could reduce the anti-epileptic drugs (AEDs) concentration in the epileptogenic zone, resulting in drug resistance. Studies have demonstrated that recurrent seizures induce the expression of P-gp and status epilepticus (SE) could upregulate the expression of P-gp, resulting in drug resistance. MicroRNAs (miRNAs), as endogenous regulators, represent small regulatory RNA molecules that have been shown to act as negative regulators of gene expression in different biological processes. We investigated the impact of miR-146a-5p on the expression of P-gp in status epilepticus rat model. The expression of miR-146a-5p in rat cortex and hippocampus was measured by quantitative RT-PCR at 2 weeks after induction of SE. Meanwhile, we detected the expression of P-gp in the brain of SE rats using Western blotting and immunohistochemistry. Upregulation of miR-146a-5p and overexpression of P-gp were evident at 2 weeks after SE. Moreover, the expression of P-gp was downregulated by injection of miR-146a mimic into the hippocampus. We also detected the expression of interleukin-1 receptor-associated protein kinases-1 (IRAK1) and tumor necrosis factor receptor-associated factor 6 (TRAF6) and nuclear factor-kappaB (NF-κB) p65 using Western blotting and immunohistochemistry, which indicated the expression of IRAK1, TRAF6 and NF-κB p-p65/p65 increased in the brain of SE rats, and overexpression of miR-146a-5p could downregulate the expression of IRAK1, TRAF6, NF-κB p-p65/p65 and P-gp. Our study indicated that miR-146a-5p may decrease the expression of P-gp in status epilepticus rats via NF-κB signaling pathway.

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INTRODUCTION

Epilepsy is a common chronic neurological disease, affecting approximately 65 million people worldwide. Although current antiepileptic drugs used in clinical practice have been developed into the third generation, approximately 30% of patients with epilepsy fail to respond to appropriate anti-epileptic drugs (AEDs), imposing a serious psychological and economic burden on the patient.3) Despite the increasing number of studies that have been performed over the last few decades, the exact mechanism underlying drug resistance in epilepsy remains unclear. Many hypotheses to explain drug resistance have been proposed, among which the transporter hypothesis has been widely recognized. In particular, P-glycoprotein (P-gp), which is encoded by the multi-drug resistance (MDR1) gene in human and mdr1a/mdr1b in rodents, was reported to be closely related to drug resistance in various diseases, including refractory epilepsy. Studies reported the expression of P-gp obviously increased in the blood–brain barrier (BBB) of patients and animal models with refractory epilepsy.2) However, the mechanism of P-gp overexpression has not been established. Recurrent seizures have been shown to induce P-gp expression, and patients with status epilepticus (SE) tend to express high levels of P-gp and develop resistance to treatment.3)

Strategies designed to inhibit the expression and function of P-gp may increase the brain penetration of AEDs. Numerous studies have reported a close correlation between P-gp overexpression and resistance to AEDs in patients with epilepsy and animal models.10) In animal experiments, inhibition of P-gp has been shown to increase the AED concentration in the cerebrospinal fluid (CSF) and decrease the seizure frequency. Several pilot studies also reported that adjunct use of verapamil (a P-gp inhibitor) was efficacious in decreasing seizure frequency in patients with refractory temporal lobe epilepsy (rTLE).5,6) To date, various P-gp inhibitors have been developed, however, the use of exogenous drugs in the clinical is limited due to side effects and a lack of tissue-specificity. Therefore, studies aiming to identify new endogenous molecules targeting P-gp expression and function to reverse drug resistance are urgently needed.

MicroRNAs (miRNAs) are endogenous small non-coding RNAs that are nucleotides in length of 22–25 nt and act as negative regulators of gene expression that silence target mRNAs by binding to the 3’-untranslated region (3’-UTR) of the mRNA. According to recent studies, miRNAs modulate MDR in various diseases, such as hepatocellular carcinoma.7,8) Previous studies demonstrated that miR-146a-5p is one of the most important anti-inflammatory miRNAs. Interleukin-1 receptor-associated kinase 1 (IRAK-1) and tumor necrosis
factor (TNF) receptor-associated factor 6 (TRAF-6), which are key downstream regulatory molecules of Toll like and interleukin-1 receptor signaling pathway, are the main target molecules of mir-146a-5p. Uptregulation of mir-146a was shown to negatively regulate the expression of IRAK-1 and TRAF6, suppressing nuclear factor-kappaB (NF-κB) pathway and impairing inflammatory reaction.\textsuperscript{79} The NF-κB signaling pathway has been implicated in the mechanism regulating the expression of P-gp.\textsuperscript{10,11} Recent studies reported there exist NF-κB p65-binding sites in MDR1 promoter sequence.\textsuperscript{12} Our recent review also indicated several inflammation-associated molecules could regulate the expression of P-gp in the brain via activating NF-κB pathway.\textsuperscript{13} Thus, we speculate that miR-146a-5p might regulate P-gp expression in rats with status epilepticus.

Therefore, the purpose of this study is to investigate whether miR-146a-5p regulates P-gp expression through the NF-κB pathway in rats with status epilepticus. In the present study, we examined the effect of miR-146a-5p on the expression of P-gp in the brain of SE rats and explored the possible mechanism.

MATERIALS AND METHODS

Animals Male Sprague-Dawley (SD) rats weighing 160–180 g were purchased from the Animal Center of Fudan University (Shanghai, China). All animal experiments were conducted in accordance with the Guidelines for Animal Experiments of the Chinese Academy of Medical Sciences and were approved by the Ethics Committee for Animal Care of Jinshan Hospital of Fudan University (2014-16-01). Rats were housed in a standard animal-grade room with five animals in each cage. Rats were maintained at an ambient temperature of 20 ± 2°C, a relative humidity of 60% and a 12:12 h light-dark cycle. Animals were fed a normal laboratory diet and had free access to tap water for one week before modeling.

Grouping and Experimental Procedures Hippocampal cannulation of SD rats was performed with the assistance of stereotactic instrument after anesthesia with 10% chloral hydrate (Shenggong, Shanghai, China). The hippocampus was located from the bregma: anterior–posterior [AP] = −3.7 mm, lateral (L)=±2.0 mm, ventral (V)=+3.5 mm. Three days later, all the rats were randomly divided into 4 groups: Normal Saline (NS), SE + Saline (SE), SE + negative control (NC), SE + miR-146a-5p mimic (miR-146a-5p). Lithium chloride and pilocarpine were used to establish the model of SE.\textsuperscript{14} In brief, lithium chloride (127 mg/kg, Sigma, U.S.A.) was intraperitoneally (i.p.) administered 20–22 h prior to the pilocarpine injection. Pilocarpine was then i.p. injected (20 mg/kg, Sigma). Convulsions were evaluated using Racine’s scale, and only those animals that showed continuous behavioral seizure activity lasting at least 1 h with a score of 4–5 were identified as SE models. After 1 h, all SE rats were then intraperitoneally injected with diazepam (10 mg/kg) to terminate the seizure. The miR-146a-5p mimics and negative control were purchased from Ribobio (Guangzhou, China). Ten nanomoles of miR-146a-5p mimics or NC in 5 μL of phosphate buffered saline (PBS) were injected into the left hippocampus of SE rats, and 5 μL of PBS were injected into the left hippocampus of normal rats and SE rats at 1 h prior to the pilocarpine injections, third and fifth days, respectively.

Immunohistochemistry The levels of P-gp, IRAK1, and TRAF6 were examined using the avidin–biotin-peroxidase method. Sections of the parahippocampal cortex and hippocampus were deparaffinized, rehydrated, and incubated with 1% H₂O₂ for 30 min. After blocking with 5% normal goat serum in PBS at 37°C for 1 h, sections were incubated with anti-P-gp (1:200), anti-IRAK1 (1:100) and anti-TRAF6 (1:200) antibodies overnight at 4°C and then incubated with biotinylated secondary antibodies. Biotinylated antibodies were detected via avidin–biotin conjugation (Elite ABC Kit, Vector Labs, U.S.A.) using 3,3′-diaminobenzidine tetrahydrochloride (DAB Kit, Vector Labs) as the colorimetric substrate. Photomicrographs were captured using a digital camera (Olympus DP20, Tokyo, Japan) linked to an inverted microscope (Olympus BX51).

Quantitative Real-Time PCR Analysis (qPCR) Total RNA was extracted from the brain tissues using TRIZOL Reagent (TaKaRa, Japan). Then, the miR-146a-5p cDNA was synthesized using the Mir-X miRNA First-Strand Synthesis Kit (TaKaRa) while the cDNAs for MDR1 gene was synthesized using the PrimeScript™ RT Master Mix (TaKaRa). Subsequently, quantitative real-time PCR of miR-146a-5p was performed with the Mir-X miRNA qRT-PCR SYBR Kit (TaKaRa) on an Applied Biosystems 7300 instrument (Applied Biosystems, Foster City, CA, U.S.A.). The expression of miRNAs was determined using the 2^{-ΔΔCt} method and normalized to the level of the U6 RNA as an internal quantitative control. Quantitative real-time PCR of MDR1 was performed with SYBR Premix Ex Taq (TaKaRa) to detect the levels of β-actin and MDR1 gene. The expression of MDR1 was determined using the 2^{-ΔΔCt} method and normalized to the level of the β-actin as an internal quantitative control. The primer sequences of MDR1 are as follow. Forward: CTTCGCTGCT ATTCATCACGGA AC, Reverse: CGCTGACGGTCTGTTG TAC TGT TG.

Western Blotting Total protein was extracted from brain tissues with a sodium dodecyl sulfate (SDS) lysis buffer (Beyotime, Shanghai, China) supplemented with 1% phenylmethylsulfonyl fluoride (Beyotime) and phosphatase inhibitors (Beyotime). Equal amounts of proteins were separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes. Then, the membranes were blocked with 5% non-fat milk at room temperature for 1 h and subsequently incubated with primary antibodies against p-NK-κB p-p65 (1:1000, Cell Signaling Technology, U.S.A.), NK-κB p65 (1:3000, Cell Signaling Technology), IRAK1 (1:300, Santa Cruz Biotechnology, U.S.A.), TRAF6 (1:2000, Proteintech, U.S.A.) and P-gp (1:1000, Cell Signaling Technology) overnight at 4°C with gentle shaking. The expression of β-actin (1:5000, Cell Signaling Technology) was used as an internal reference. The appropriate peroxidase-conjugated anti-mouse or anti-rabbit antibodies (1:5000, Proteintech) were incubated with the membranes at room temperature for 1 h. Signals were detected using ECL-Plus (Merck Millipore, Darmstadt, Germany) and quantified using a Bio-Rad 2000 gel imaging system with QUANTITY ONE software (Bio-Rad Laboratories, Hercules, CA, U.S.A.).

Statistical Analysis Statistical analyses were performed using SPSS 22.0 software, including one-way ANOVAs and Pearson’s correlation analyses. Values are presented as...
means ± standard deviation (S.D.), and statistical significance was defined as $p < 0.05$ for all tests.

RESULTS

The Expression of miR-146a-5p in Rats with Lithium–Pilocarpine-Induced SE In the study, we intervened the expression of miR-146a-5p in the brains. Quantitative RT-PCR was used to detect the expression of miR-146a-5p in the parahippocampal cortex and hippocampus under different intervention conditions in vivo. As shown in Fig. 1, the expression of miR-146a-5p was upregulated in the SE and NC groups compared with the NS group (*$p < 0.05$ and **$p < 0.01$ compared with the NS group), while its expression was significantly increased in cerebral cortex and hippocampus tissues of rats in the miR-146a-5p group compared with the NS and SE groups ($^\#p < 0.05$ and ##$p < 0.01$ compared with the NS group).

The Effect of miR-146a-5p on the Expression P-gp in Rats with Status Epilepticus Consistent with the results from previous studies, P-gp expression significantly increased in the parahippocampal cortex and hippocampus of rats in the SE and NC groups compared with the NS group. However, the injection of miR-146a-5p into the hippocampus decreased the expression of P-gp in the cerebral cortex and hippocampus of SE rats compared with the NC groups. In the present study, we assayed P-gp expression using immunohistochemistry, Western blotting and RT-PCR. As shown in the images of immunohistochemical staining presented in Fig. 2A, the levels of P-gp in the cerebral vasculature were increased in the brains of rats in the SE and NC groups (*$p < 0.05$ and **$p < 0.01$ compared with the NS group) but—decreased in the miR-146a-5p group ($^\#p < 0.05$ and ##$p < 0.01$ compared with the NC group). As shown in Figs. 2B–D, levels of the P-gp protein and mRNA were significantly increased in the cerebral cortex and hippocampus of the SE and NC groups (*$p < 0.05$ and **$p < 0.01$ compared with the NS group) but decreased in the miR-146a-5p group ($^\#p < 0.05$ and ##$p < 0.01$ compared with the NC group).

MiR-146a-5p Regulates the Expression of P-gp via the NF-κB Signaling Pathway Our results revealed markedly increased levels of IRAK1, TRAF6 and phosphorylated NF-κB p65/p65 proteins in the SE group and decreased in the miR-146a-5p group, as evidenced by Western blots of the parahippocampal cortex and hippocampus (Figs. 3B–E). We also examined the levels of IRAK1 and TRAF6 using immunohistochemistry and observed similar changes in the levels of these proteins (Fig. 3A). Meanwhile, we assayed the correlation between the levels of P-gp and IRAK1, TRAF6 and NF-κB p-p65/p65 by calculating Pearson’s correlation coefficients. The change in P-gp levels was positively correlated with the levels of IRAK1, TRAF6 and NF-κB p-p65/p65 in the parahippocampal cortex (Fig. 4A a1–c1 $R^2 = 0.6739$, $p < 0.01$; $R^2 = 0.7159$, $p < 0.01$; $R^2 = 0.667$, $p < 0.01$) and the hippocampus (Fig. 4B a2–c2 $R^2 = 0.7203$, $p < 0.01$; $R^2 = 0.8175$, $p < 0.01$; $R^2 = 0.7389$, $p < 0.01$). Based on our results, miR-146a-5p might regulate P-gp expression in the brain of SE models via the NF-κB signaling pathway.

Fig. 1. The Expression of miR-146a-5p in the Cerebral Cortex and Hippocampus of Each Group

Fig. 2. The Expression of P-gp in the Cerebral Cortex and Hippocampus

Immunohistochemical staining for P-gp in the cerebral cortex (A, a–d) and in the hippocampus (A, e–h) in each group. The expression levels of P-gp protein in the hippocampus and in the cerebral cortex were also examined by Western blotting (B and C). The expression of ABCB1 in the cerebral cortex and hippocampus were examined by RT-PCR (D). (*$p < 0.05$ and **$p < 0.01$ compared with the NS group; $^\#p < 0.05$ and ##$p < 0.01$ compared with the NC group).
DISCUSSION

Numerous studies have reported a close correlation between P-gp overexpression and resistance to AEDs in patients with epilepsy and animal models. However, to date, the mechanism underlying the high expression of P-gp has not...
and miR-146a-5p expression is induced by different pro-inflammatory stimuli in various human pathologies. Furthermore, miR-146a-5p has been shown to negatively modulate innate immunity in feedback system. As stated above, the NF-κB signaling pathway modulates P-gp expression, possibly owing to the existence of NF-κB p65 binding sites in the MDR1 promoter sequence. Our recent review indicated some pro-inflammatory-associated molecules induce the expression of P-gp via NF-κB pathway. We thus hypothesized that miR-146a-5p may downregulate P-gp expression by negatively regulating the activity of the NF-κB signaling pathway in rats with status epilepticus.

Recent studies reported intracerebroventricular injection of miR-146a agomir can inhibit NF-κB activity, alleviate neuroinflammation and relieve seizures in SE rats. And miR-146a antagonism has the opposite effect. Intranasal delivery of miR-146a mimics could also delay seizure in model of lithium-pilocarpine induced status epilepticus via inhibiting NF-κB pathway and alleviating the inflammatory reaction in the brain. In our previous study, we performed the experiment on the effect of miR-146a-5p on seizures in rat model of refractory epilepsy, which also indicated hippocampal injection of miR-146a-5p significantly inhibited the abnormal electrical activity of brain and alleviated the hippocampal injury via inhibiting inflammation in the brain. (unpublish) The present study focused on the effect of miR-146a-5p add on the expression of P-gp in the brain of SE rats.

This study revealed increased expression of miR-146a-5p in the brains (both the parahippocampal cortex and hippocampus) of SE rats, consistent with the results from previous studies. The phenomenon may be a compensatory mechanism to combat recurrent seizures. Notably, miR-146a-5p has been identified as a key regulator in the feedback system, and its expression is upregulated by the activation of NF-κB sub-units through a myeloid differentiation factor 88-dependent pathway, which in turn decreases the levels of IRAK1 and TRAF6, alleviating the inflammatory response. Previous studies demonstrated that, the expression of miR-146a-5p is augmented through stimulating by lipopolysaccharide and cytokines including IL-1β and TNF-α, because the NF-κB binding sites in the promoter of miR-146a-5p can be activated by the inflammatory cytokines above. Therefore, activated inflammation can upregulate the expression of miR-146a-5p. Meanwhile, some inflammatory molecules, including pro-inflammatory cytokines (IRAK1, TRAF6) and anti-inflammatory cytokines (complement factor H), are targets of miR-146a-5p. The high expression of miR-146a-5p in turn inhibits IRAK1 and TRAF6, suppresses NF-κB activity, thus alleviating the inflammatory reaction. Aronica et al. reported that the expression of miR-146a was increased at 1 week after SE and persisted in the chronic phase and miR-146a was confirmed to express in reactive astrocytes. The expression of miR-146a in patients with temporal lobe epilepsy was up-regulated mainly in regions where reactive gliosis occurred. Omran et al. reported that the expression of miR-146a-5p and IL-1β were both upregulated in immature rats and children with temporal lobe epilepsy, but in opposite ways. Namely, in acute stage, IL-1β is at its highest level, miR-146a is lowest. And miR-146a is highest in the latent stage, but IL-1β is lowest. In the chronic stage, both miR-146a-5p and IL-1β are increased. These results were consistent with the inflammation-miR-146a feedback regulation.

Fig. 5. The Potential Mechanism by Which miRNA-146a-5p Regulates P-gp Expression in the Brains of SE Rats (Activation Steps Are Represented by “→” and Inhibitory Effects Are Represented by “⊥”).

MiR-146a-5p regulates IRAK1 and TRAF6, which were the classical molecular of the NF-κB pathway. Exogenous miRNA-146a-5p downregulates the NF-κB pathway by IRAK1 and TRAF6, then reduces the expression of P-gp.

Yet been clearly determined. The use of AEDs, the inflammatory response, oxidative stress, prolonged drug exposure and recurrent epilepsy are postulated to contribute to the high expression of P-gp in patients with epilepsy. Moreover, in the present study, it was found that P-gp expression increased in the brain of SE rats, consistent with the results from our previous study. Recurrent seizures exacerbate pathophysiological changes in patients with epilepsy, including oxidative stress and the inflammatory response. Meanwhile, oxidative stress and inflammation have consistently been shown to induce P-gp expression. In previous studies, the abnormal expression of ATP-binding cassette (ABC) transporters in the brains of patients with epilepsy was reported to be associated with glutamate and cyclooxygenase-2 signaling, and continuous exposure to TNF-α could increase P-gp efflux activity. Meanwhile, activation of the mammalian target of rapamycin (mTOR) and mitogen-activated protein kinase (MAPK) signaling pathways were also reported to induce P-gp expression, consistent with the results from our previous study. Activation of the toll-like receptor (TLR)/NF-κB signaling pathway has recently been shown to increase P-gp level in the brain of rats with kainic acid-kindled epilepsy, since the mdr1a promoter sequence contains NF-κB p65-binding sites. The nuclear transcriptional regulatory factor NF-κB has long been considered a prototypical inflammatory marker. As shown in the present study, NF-κB signaling might be closely involved in regulating P-gp expression. However, although exogenous drugs targeting associated pathways to suppress P-gp have been developed, their applications are limited due to side effects and the lack of tissue-specificity.

miRNAs are considered to play vital roles in regulating various physiological and pathological processes. Based on accumulating evidence, miRNAs are related to P-gp-mediated multiple drug resistance in various diseases. As shown in the study by Bao et al., miR-298 downregulates the expression of P-gp by directly binding to the MDR1 3′-UTR and alleviates the doxorubicin chemoresistance of metastatic breast cancer. Meanwhile, previous studies demonstrated miR-146a-5p is one of most important inflammation-associated miRNAs, and miR-146a-5p expression is induced by different pro-inflammatory stimuli in various human pathologies. Furthermore, miR-146a-5p has been shown to negatively modulate innate immunity in feedback system. As stated above, the NF-κB signaling pathway modulates P-gp expression, possibly owing to the existence of NF-κB p65 binding sites in the MDR1 promoter sequence. Our recent review indicated some pro-inflammatory-associated molecules induce the expression of P-gp via NF-κB pathway. We thus hypothesized that miR-146a-5p may downregulate P-gp expression by negatively regulating the activity of the NF-κB signaling pathway in rats with status epilepticus.

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The injection of an miR-146a-5p mimic into the hippocampus down-regulated the expression of P-gp on the blood vessels in the parahippocampal cortex and hippocampus in the present study. Inflammation induces P-gp expression via multiple signaling pathways, including the NF-κB signaling pathway, as above. IRAK1 and TRAF6 are two key cytoplasmic protein kinases that are induced by SE via NF-κB signaling.20) Consistent with a previous report,29) exogenous pathway, as above. IRAK1 and TRAF6 are two key cytoplasmic protein kinases that are induced by SE via NF-κB signaling.20) Consistent with a previous report,29) exogenous miR-146a-5p significantly suppressed IRAK1 and TRAF6 expression in the brain of SE rats in the present study, and the suppression of IRAK1 and TRAF6 by miR-146a-5p reduced the levels of NF-κB p-p65/p65. A strong negative correlation was observed between the levels of P-gp and NF-κB signaling pathway-associated molecules. As stated above, NF-κB p65-binding sites are present in the mdrla promoter sequence, and the activation of the NF-κB signaling pathway decreases P-gp levels.11,12) Thus, miR-146a-5p may decrease P-gp expression via the NF-κB signaling pathway. However, some studies have reported contradictory results. Silencing of miR-146a has been shown to downregulate P-gp expression and alleviate inflammation in epilepsy models.20) According to a recent study, the enhanced miR-146a and reduced prototype miR-146a exert completely opposite effects on regulating inflammatory signaling, which may explain the discrepancy.26) However, the specific explanation for the differences between studies remains unclear and further investigations are required.

Based on our results, miR-146a-5p might downregulate P-gp expression in the brain of SE rats via the NF-κB signaling pathway. This study is the first to show that the overexpression of exogenous miR-146a-5p decreased the P-gp level in the brain of rats with SE, possibly suggesting that miR-146a-5p might be a promising pharmacological treatment for drug resistance. However, our study also has some limitations. We only detected the expression of some NF-κB signaling pathway-associated molecules following the expression of exogenous miR-146a-5p in SE rats, instead of exploring the specific mechanism by which miR-146a-5p regulates the expression of P-gp via the associated signaling pathways. We only evaluated the role of miR-146a-5p mimic on the P-gp expression, but do not knock down miR-146a-5p to reverse evaluation. Therefore, further research is needed to determine the role of miR-146a-5p in regulating P-gp expression.

CONCLUSION

This study provides relevant basic insights into the effect of miR-146a-5p on drug resistance by injecting exogenous miR-146a-5p into rats with status epilepticus. Based on the evidence obtained from this study and previous studies, we concluded that miR-146a-5p may decrease the level of P-gp expressed on the cerebral vessels in SE rats by down-regulating the NF-κB signaling pathway, which suggests that miR-146a-5p might be promising pharmacological treatment for SE induced drug resistance.

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Conflict of Interest The authors declare no conflict of interest.

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