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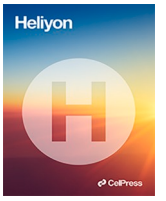

SREBP1 deficiency diminishes glutamate-mediated HT22 cell damage and hippocampal neuronal pyroptosis induced by status epilepticus

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ABSTRACT

Status epilepticus (SE) is a life-threatening disorder that can result in death or severe brain damage, and there is a substantial body of evidence suggesting a strong association between pyroptosis and SE. Sterol regulatory element binding protein 1 (SREBP1) is a significant transcription factor participating in both lipid homeostasis and glucose metabolism. However, the function of SREBP1 in pyroptosis during SE remains unknown. In this study, we established a SE rat model by intraperitoneal injection of lithium chloride and pilocarpine in vivo. Additionally, we treated HT22 hippocampal cells with glutamate to create neuronal injury models in vitro. Our results demonstrated a significant induction of SREBP1, inflammasomes, and pyroptosis in the hippocampus of SE rats and glutamate-treated HT22 cells. Moreover, we found that SREBP1 is regulated by the mTOR signaling pathway, and inhibiting mTOR signaling contributed to the amelioration of SE-induced hippocampal neuron pyroptosis, accompanied by a reduction in SREBP1 expression. Furthermore, we conducted siRNA-mediated knockdown of SREBP1 in HT22 cells and observed a significant reversal of glutamate-induced cell death, activation of inflammasomes, and pyroptosis. Importantly, our confocal immunofluorescence analysis revealed the co-localization of SREBP1 and NLRP1. In conclusion, our findings suggest that deficiency of SREBP1 attenuates glutamate-induced HT22 cell injury and hippocampal neuronal pyroptosis in rats following SE. Targeting SREBP1 may hold promise as a therapeutic strategy for SE.

1. Introduction

Status epilepticus (SE) is a severe central nervous system disorder characterized by prolonged seizures or multiple seizures with the

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incomplete return to baseline [1]. It poses a serious threat to human health with its high morbidity and mortality, and can induce significant neuronal damage to the hippocampus [2]. The hippocampus is a vital structure in the pathophysiology of SE and has been extensively studied in both human and experimental SE. Hippocampal neurons are highly sensitive to epileptic discharges and are more susceptible to cell death [3,4]. Studies have demonstrated that the aberrant discharge activity of hippocampal neurons may contribute to epileptic seizures and play a crucial role in the onset and progression of this disease [5,6].

The neuronal excitotoxicity triggered by epileptic seizures can lead to an excessive release of glutamate [7]. Glutamate, a neurotransmitter released by excitatory neurons, is important in various of physiological and pathological brain processes [8,9]. It has been extensively demonstrated that glutamate-mediated excitotoxicity contributes to the neurobiology of epileptic seizures [10]. Convincing evidence suggests that sustained elevation of extracellular glutamate promotes epileptic seizures and is considered the primary cause of excitotoxicity or neuronal death during seizures [11–13]. However, the underlying mechanisms by which sustained elevation of extracellular glutamate leads to hippocampal neuronal damage in SE remain incompletely unclear.

Mounting evidence indicates that neuroinflammation is closely linked to the pathophysiology of SE, leading to significant neuronal damage [14,15]. Both animal and clinical studies have shown an increase in proinflammatory cytokines in the brain tissues of rodent epilepsy models, as well as in the serum or cerebrospinal fluid of patients with epilepsy [14,16]. As the connection between proinflammatory cytokines and neuronal damage becomes clearer, scientists are paying more attention to pyroptosis, a newly discovered form of programmed cell death that relies on the activation of inflammasome pathways [17]. When the inflammasomes are triggered, procaspase-1 undergoes autocatalysis to produce activated caspase-1. Subsequently, active caspase-1 cleaves IL-18, IL-1β, and other proinflammatory factors. Additionally, it stimulates gasdermin D (GSDMD) to generate GSDMD N-terminal fragments, leading to cell swelling and pyroptosis [18]. An increasing number of studies suggest that pyroptosis contributes to the pathophysiology of SE via inflammasome activation, although the exact mechanism remains unclear.

Our previous study demonstrated the activation of the PI3K-AKT-mTOR pathway following SE. We found that increased phosphorylation of proline-rich Akt substrate of 40 kDa (PRAS40) can activate the mTOR pathway and inhibit autophagy [19]. Furthermore, accumulating evidence indicated that inhibition of mTOR pathway could be proposed as a novel therapeutic approach for SE [20].

Sterol regulatory element-binding proteins (SREBPs) , as the downstream target of mTOR pathway [21–23], may play an important role in SE. It has been demonstrated that SREBP1 is primarily involved in the uptake and synthesis of fatty acids, while SREBP2 predominantly regulates the expression of genes required for cholesterol synthesis [24,25]. Research suggests that SREBP1 plays a significant role in neuronal development through glia-neuron interactions [26,27]. Mary et al. reported that SREBP1 is involved in the structural plasticity of the hippocampus, suggesting its critical role in hippocampal functioning [28]. Nevertheless, few studies have been conducted on the contribution of SREBP1 in SE.

Therefore, the current study was undertaken to evaluate the role of SREBP1 in glutamate-induced HT22 cell injury in vitro and hippocampal neuronal pyroptosis following SE in vivo. Additionally, we sought to explore the potential underlying mechanism.

2. Materials and methods

2.1. Antibodies and reagents

Antibodies against SREBP1 (AF6283), Cleaved Caspase-1 (AF4022), TMS1/ASC (DF6304) were obtained from Affinity Bioscience (Cincinnati, OH, United States). ProteinTech Group provided the anti-NLRP1 (12256-1-AP), anti-IL-18 (10663-1-AP), anti-BAX (60267-1-Ig), anti-RIPK1 (17519-1-AP), and anti-RIPK3 (17563-1-AP). The following proteins' antibodies were bought from Cell Signaling Technology (Beverly, Massachusetts, United States): p-PRAS40 (Thr246; 2997), PRAS40 (2691), p-P70S6K (Thr389; 9234), NLRP3 (15101), P70S6K (14130), p-mTOR (Ser2481; 2974), GPX4 (59735), mTOR (2983), p-4EBP1 (Thr37/46; 2855), Cleaved Caspase3 (9661), 4EBP1 (9452). Antibodies for NLRP1 (sc-390133), GAPDH (sc-365062) were obtained from Santa Cruz (CA, United States). Anti-GSDMDC1 (NBP2-33422, 1:1000) was purchased from Novus Biologicals (Minnesota, United States). Lithium chloride (L9650) was bought from Sigma-Aldrich (St. Louis, MO, USA). Pilocarpine (S4231), Scopolamine (S2508), Glutamate (S4721) and Afuresertib (S7521) were obtained from Selleck Chemicals (Shanghai, China).

2.2. Experimental animals and treatments

All animal studies were approved by the Experimental Animal Ethics Committee of the Basic Medical College of Fudan University (approval no. 20170223-066). Adult male Sprague-Dawley rats weighing between 200 and 250 g were acclimatized to the laboratory environment for 1–2 weeks prior to the induction of status epilepticus (SE) using pilocarpine. The SE model was established using the previously published methods outlined in Ref. [19]. Briefly, the rats were injected with lithium chloride (127 mg/kg; i.p.), and after 18 h, SE was induced by injecting pilocarpine (30 mg/kg, i.p.). Scopolamine (1 mg/kg) was injected 1 h before pilocarpine injection to antagonize peripheral cholinergic stimulation. Seizure severity was graded using the Racine scale [29]. Only rats that progressed to 4 or greater were selected and were terminated after 2 h using pentobarbital sodium (40 mg/kg; i.p.). Rats in the control group obtained the same amount of vehicle solvent in the same manner. In addition, Akt/mTOR inhibitor Afuresertib (0.4 mg/kg; i.p.) was pretreated 6 h before seizures in SE + inhibitor (SE + inh) group. The rats in SE group and SE + inh groups were sacrificed at 4 h with pentobarbital sodium (400 mg/kg; i.p.) following SE. During the experiment, 8 rats were randomly allocated to the control group, 12 rats to the SE group, and 10 rats to the SE + inh group. However, 4 rats from the SE group and 2 rats from the SE + inh group died during the modeling process, resulting in only 8 rats in each group for the remainder of the experiment.

2.3. Hematoxylin & Eosin (H&E) and Nissl staining

The rat hippocampal tissue was fixed in 4 % buffered formalin for 24 h following by embedding in paraffin and sliced into 4 μm-thick. Paraffin sections were dewaxed and dehydrated in a gradient alcohol. Histologic sections of hippocampal tissues were stained with hematoxylin & eosin (H&E) for histological analysis. For Nissl staining, paraffin sections were immersed in Nissl staining solution (Beyotime, Shanghai, China) at 37 °C for 10 min, followed by two rinses with distilled water. Images were acquired using a light microscope.

2.4. Immunohistochemistry (IHC)

IHC staining in hippocampal tissues was performed as our previously described (14). After sacrificing the rats with lethal pentobarbital sodium, the hippocampal tissues were fixed in 4 % paraformaldehyde overnight at 4 °C. Subsequently, the brains were embedded in paraffin and sectioned in the coronal plane into 4 μm thick slides. The sections of the hippocampus were then subjected to antigen retrieval by heating to 98 °C for 15 min using a citrate buffer in a microwave. To eliminate endogenous peroxidase activity, the sections were treated with 3 % H_2O_2 for 15 min at room temperature. After blocking with 3 % bovine serum albumin (BSA) for 30 min, the anti-SREBP1 antibody was applied overnight at 4 °C. Following this, the sections were incubated with the corresponding HRP-conjugated secondary antibodies for 30 min at 37 °C. Finally, the sections were visualized by incubating with a 3,3-diaminobenzidine solution at room temperature, and images were captured using a light microscope.

2.5. Cell culture and treatment

HT22 cell lines were cultured in DMEM with 10 % FBS in a humidified atmosphere at 37 °C supplemented with 5 % CO_2. The use of glutamate (Glu) treatment on HT22 cells is commonly employed as an in vitro model to study excitotoxicity or neuronal injury in hippocampal neurons. To induce cell damage, HT22 cells were treated for 24 h with 1 mM, 5 mM, and 10 mM Glu.

2.6. Cell transfection

To knock down SREBP1, HT22 cells were transfected with 25 nM of either SREBP1 target siRNA (siSREBP1) or control nonspecific siRNA (siNC) (Sangon, Shanghai, China) with Lipo8000™ Transfection Reagent (Beyotime, Shanghai, China). After 24 h, the cells were treated with Glu at indicated doses. SREBP1 transfection effectiveness was assessed using western blotting.

2.7. Cell viability assays

After cell transfection of siSREBP1 and siNC were completed as mentioned above. HT22 cells were incubated in fresh medium containing 5 mM Glu for 24 h. Then, HT22 cells were incubated with Calcein/PI (Beyotime, Shanghai, China) and observed using fluorescence microscopy.

2.8. Immunofluorescence (IF) staining

NLRP1, NLRP3 and SREBP1 were detected by IF staining. In brief, HT22 cells were fixed in 4 % paraformaldehyde for 30 min and permeabilized in 0.2 % Triton X-100 for 15 min. After that, cells were incubated with primary antibodies against NLRP1, NLRP3, and SREBP1 at 4 °C overnight and following secondary antibodies. The cell nuclei were probed by DAPI (Beyotime, Shanghai, China) for 10 min. Finally, the results were visualized under a fluorescence or confocal laser scanning microscope.

2.9. Western blot analysis

Western blot analysis was conducted according to our previous publication [19]. Briefly, rat hippocampal tissues were collected after sacrifice, and HT22 cells were also included as a sample. Total proteins were extracted using RIPA buffer (Beyotime, Shanghai, China) supplemented with phosphorylase inhibitors and protease inhibitors. The protein concentration was determined using the BCA Protein Assay kit (Beyotime, Shanghai, China). Subsequently, the protein samples were separated by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride membrane. After blocking with 5 % BSA for 1 h at room temperature, the membranes were incubated overnight at 4 °C with the corresponding primary antibody. Following that, the membranes were treated with horseradish peroxidase (HRP)-conjugated secondary antibodies against mouse or rabbit for 1 h at room temperature. The protein signals were detected using an ECL chemiluminescence kit (34096; Thermo Fisher Scientific, San Francisco, CA, USA). The signal intensities were quantified using the ImageJ v1.28 program (National Institutes of Health), and the protein expressions were normalized to GAPDH.

2.10. Statistical analysis

Data were presented as mean ± standard error of the mean (SEM) and analyzed using SPSS 17.0 (IBM) and GraphPad Prism 8.0 (Graphpad Software, lnc.). After confirming normal distribution with the skewness and kurtosis statistic test, an independent sample *t*-

test was used to analyze differences between two groups. One-way ANOVA followed by LSD post hoc test was performed for the comparison of multiple groups. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Histological analysis of hippocampus and change in apoptosis-associated protein after SE

After constructing the rat model of pilocarpine-induced SE, we performed H&E staining (Fig. 1A) and Nissl staining (Fig. 1B) to assess the histological changes in the hippocampal tissue of both the control and SE groups. Histological analyses revealed no significant pathological changes in the hippocampal tissue of the SE group compared to the control group, except for a mildly dispersed distribution of neurons in the hippocampus. Additionally, we detected the apoptosis-related protein cleaved-caspase3 (Cle-cas3) level (Fig. 1C and D) in the hippocampal tissue of both groups and found that it was significantly higher ($p = 0.023$) in the SE group compared to the control group.

3.2. SREBP1 was upregulated after SE

To investigate the change in SREBP1 expression following SE, a rat pilocarpine-induced SE model was established. A Western blot analysis of total protein of the hippocampal tissue demonstrated a substantial rise ($p = 0.0103$) in the expression of SREBP1 following SE (Fig. 2A and B). Immunohistochemistry (IHC) was performed to visualize the localization of SREBP1 in the cytoplasm of hippocampal neurons, and it was observed that the SE group exhibited markedly higher levels of SREBP1 compared to the control group (Fig. 2C).

3.3. SREBP1 was regulated by AKT/mTOR signaling pathway and may be associated with hippocampal neuronal pyroptosis induced by SE in vivo

Evidence indicated that neural cell death exacerbates the onset and progression of SE [30]. Next, to explore the molecular

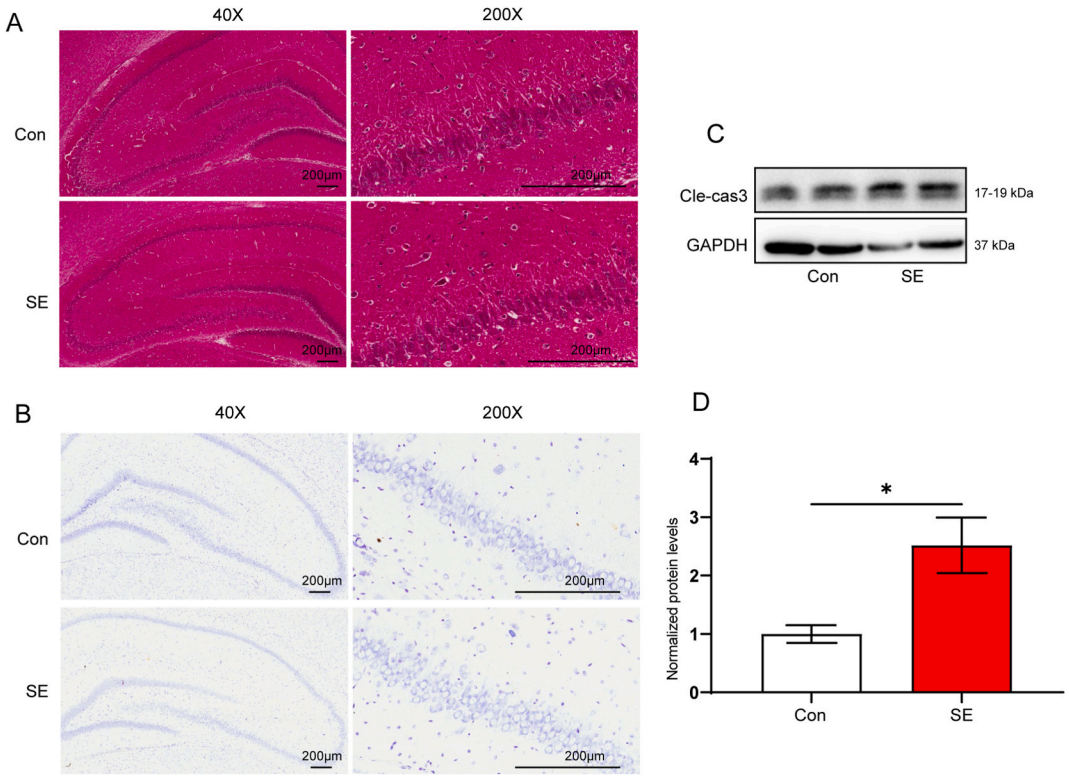

Fig. 1. Histopathological changes in the hippocampus following SE and alteration in apoptosis-related protein. Histological tests comparing the hippocampal H&E staining (A) and Nissl staining (B) between the SE group and the control group, scale bar = 200 μm. The left images were 40× magnification and the right images were 200× magnification. (C) The expression of cleaved-caspase3 (Cle-cas3) in the hippocampus was detected by western blotting in both the SE and control groups. (D) Summary of Western blot results. The values were showed as the mean ± SEM (n = 4). *p < 0.05 compared with control group. SE, status epilepticus; Con, control; Cle-cas3, Cleaved-caspase3.

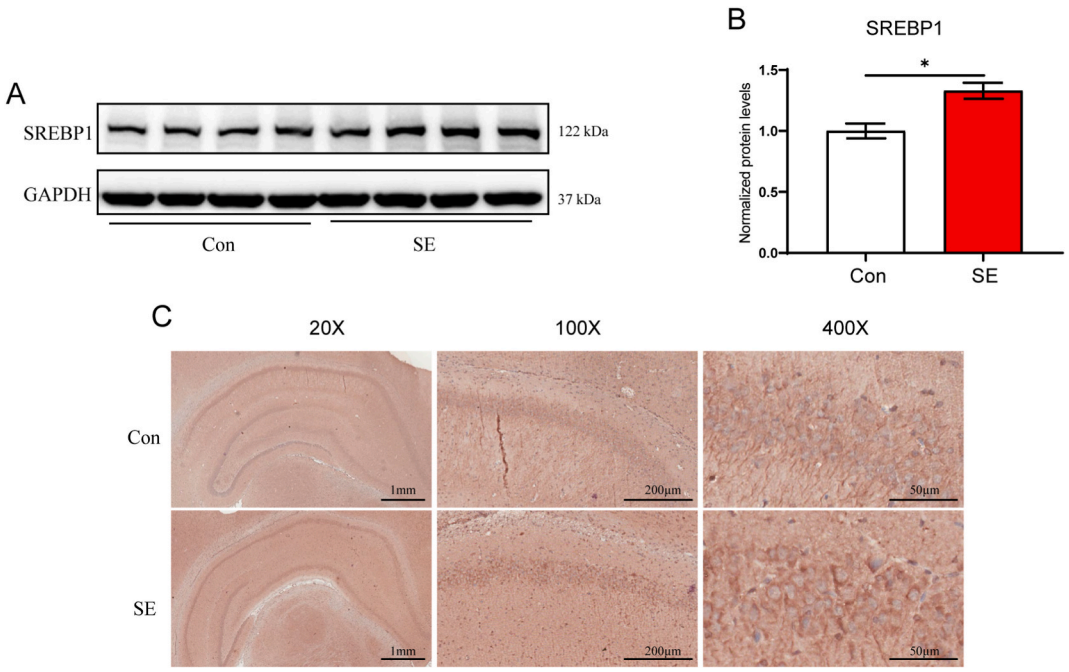

Fig. 2. SREBP1 was increased in the rat model of SE. (A) The effect of SE on SREBP1 expression in vivo. (B) Summary of Western blot results. (C) Representative IHC pictures of SREBP1 in the SE and control groups. The left images were captured at a magnification of 20 × with a scale bar of 1 mm, the middle images were captured at a magnification of 100 × with a scale bar of 200 μm, and the right images were captured at a magnification of 400 × with a scale bar of 50 μm for each staining. The values were showed as the mean ± SEM (n = 4). *p < 0.05 compared with control group. SE, status epilepticus; Con, control; SREBP1, Sterol regulatory element binding protein 1.

mechanism of SE-induced hippocampal neuron death, we examined the changes in several main forms of cell death, including apoptosis, necroptosis, ferroptosis, and pyroptosis. As depicted in Fig. 3A and B, the levels of apoptosis-related proteins (BAX, Cleaved caspase-3) and pyroptosis-related molecules (NLRP3, GSDMD-N, ASC, IL-18, and Cleaved caspase-1) were significantly elevated in the SE group. However, there were no significant changes observed in the ferroptosis-related marker GPX4 or necroptosis markers (RIPK1, RIPK3) compared to the control group. Meanwhile, inactivation of Akt/mTOR signaling by Afuresertib in the SE + inh group could effectively alleviate the pyroptosis rather than apoptosis, which was accompanied by a reduction in SREBP1 expression.

The mTOR pathway exerts a key role in the pathogenesis of SE [31]. Our previous study demonstrated that Akt/mTOR pathway was activated, which was related to the combination of PRAS40 with 14-3-3 scaffold [19]. Furthermore, we found that the phosphorylation levels of PRAS40, mTOR, as well as the well-known downstream substrates of mTORC1, p70S6K, and 4EBP1, were significantly elevated in the SE group compared to the control group (Fig. 3C and D). Moreover, the inhibition of Akt/mTOR signaling using Akt inhibitor Afuresertib could reverse the increased expression of SREBP1 induced by SE, suggesting that Akt/mTOR signaling pathway may regulate the activity of SREBP1. These results support the notion that SE causes pyroptosis in the hippocampus, and that SREBP1 is involved in pyroptosis of hippocampus neurons mediated by the Akt/mTOR signaling pathway.

3.4. Glutamate (Glu) altered SREBP1 expression in HT22 cells

SE can lead to an excessive release of glutamate, and sustained elevation of extracellular glutamate leads to hippocampal neuronal damage in SE [11–13]. To further explore the role of SREBP1 in Glu-mediated neuronal damage in hippocampal and the relationship between SREBP1 and SE-induced pyroptosis, an in vitro cell injury model was established by treating HT22 mouse hippocampal neuronal cells with Glu. The HT22 cells were subjected to various concentrations of Glu for 24 h. The results obtained from Western blot analysis indicated a significant and dose-dependent increase in the protein levels of SREBP1 in Glu-treated HT22 cells (Fig. 4A and B). Notably, the change in SREBP1 expression caused by 5 mM Glu was particularly pronounced and therefore utilized in subsequent experiments conducted in this study. Additionally, SREBP1 expression in Glu-treated HT22 cells was also visualized using immunofluorescence (IF) staining (Fig. 4C and D). These findings shown that SREBP1 was elevated in HT22 cells treated with Glu.

3.5. SREBP1 knockdown alleviated Glu-induced inflammasomes activation and pyroptosis in HT22 cells

To elucidate the role of SREBP1 in Glu-treated HT22 cells, we employed siRNA to silence the expression of SREBP1. As shown in Fig. 5A and B, siSREBP1-2 and siSREBP-3 could significantly reduce the expression of SREBP1, and they were used in subsequent experiments. To investigate the Glu-mediated neuronal damage, cell viability was detected using Calcein/PI staining in HT22 cells. As

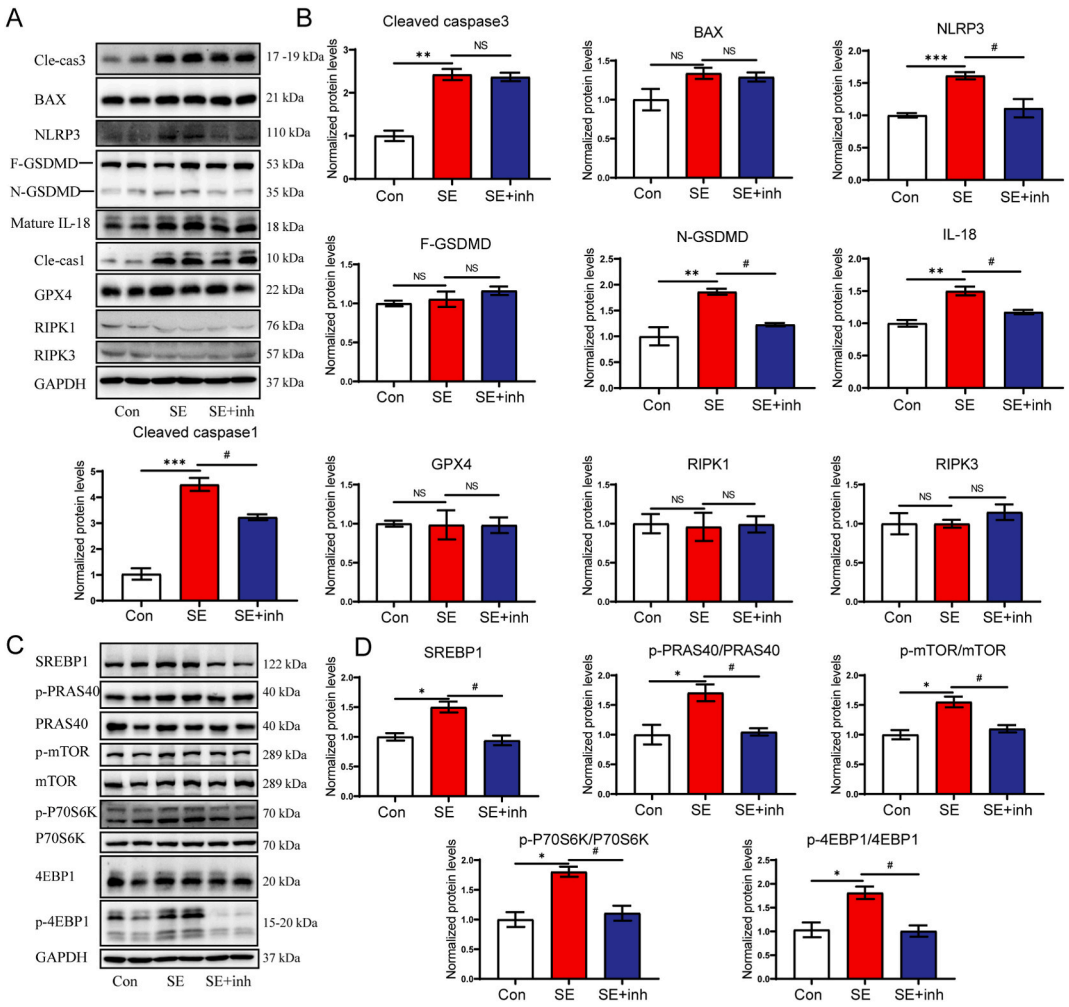

Fig. 3. AKT/mTOR signaling pathway was activated and inhibition of AKT/mTOR signaling could block hippocampal neuronal pyroptosis induced by SE, along with the downregulation of SREBP1. (A) Western blot images of apoptosis-related proteins (BAX, Cleaved caspase3), ferroptosis-related marker GPX4, pyroptosis-related proteins (NLRP3, GSDMD-N, ASC, IL-18 and Cleaved caspase-1), and necroptosis markers (RIPK1, RIPK3) expression in multiple groups. (B) Quantification of the results of A. (C) Western blot analysis of SREBP1 and the AKT/mTOR signaling pathway relative molecules, including PRAS40, p-PRAS40, p-P70S6K, P70S6K, p-mTOR, mTOR, p-4EBP1 and 4EBP1. (D) Quantification of SREBP1 and the AKT/mTOR signaling pathway expression. The values were showed as the mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 compared with control group, and #p < 0.05 compared with SE + inh group. NS, no significance. Con, control; inh, inhibitor; SE, status epilepticus; p-, phosphorylated; P70S6K, p70 S6 kinase; 4EBP1, eIF4E-binding protein 1; Cle-cas3, Cleaved caspase3; Cle-cas1, Cleaved caspase-1; F-GSDMD, full-length gasdermin D; N-GSDMD, N-terminal of gasdermin D; RIPK1, receptor-interacting protein kinase 1; IL-18, interleukin-18; RIPK3, receptor-interacting protein kinase 3; GPX4, glutathione peroxidase 4.

depicted in Fig. 5C, elevated Glu can lead to HT22 cell death. However, the knockdown of SREBP1 using siRNA resulted in a reduced cell death ratio in Glu-treated HT22 cells compared to the control group. These results indicated inhibition of SREBP1 could reduce the Glu-mediated cellular damage. Further research found that Glu could increase significantly the expression of pyroptosis-related protein, such as NLRP1, NLRP3, ASC, GSDMD-N, IL-18, and cleaved caspase-1 (Fig. 5D and E). Notably, administration of SREBP1-targeting siRNA (siSREBP1) effectively attenuated these changes. Moreover, IF assay also shown that the elevations of NLRP1 and NLRP3 caused by Glu were significantly diminished by siSREBP1 (Fig. 5F and G). Collectively, these results demonstrated that blocking SREBP1 impedes Glu-induced activation of NLRP1 or NLRP3 inflammasomes and subsequent pyroptosis in HT22 cells.

3.6. Co-localization of SREBP1 and NLRP1 was induced in HT22 cells treated with Glu

Finally, we endeavored to investigate the underlying mechanisms of SREBP1-mediated pyroptosis and inflammasome activation. Our confocal IF analysis revealed a significant degree of co-localization between SREBP1 and NLRP1 in Glu-treated HT22 cells (Fig. 6). This observation strongly suggests that the inhibition of SREBP1 could potentially counteract Glu-induced cell death in HT22 cells by suppressing inflammasome activation and pyroptosis through modulating the interaction between SREBP1 and NLRP1.

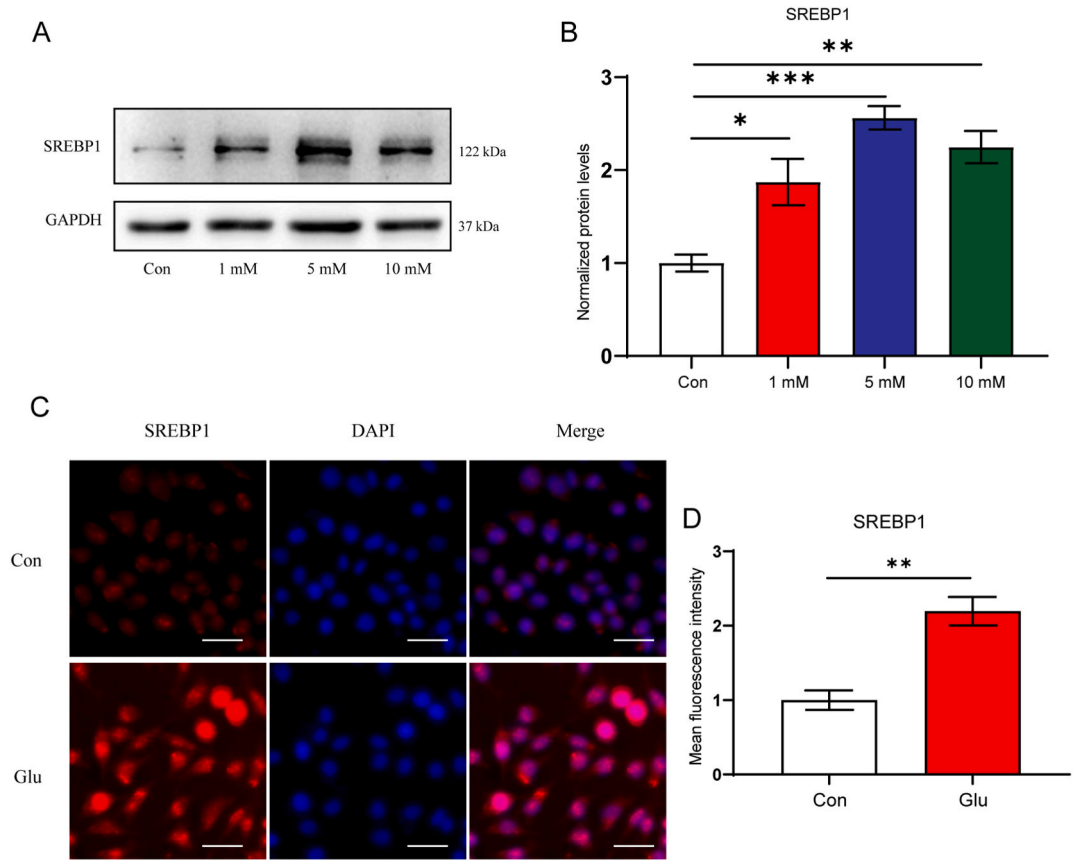

Fig. 4. Glu increases SREBP1 expression in HT22 cells. (A) Western blot images of SREBP1 level in HT22 cells incubated with different dose of Glu (1 mM, 5 mM, and 10 mM) for 24 h. (B) Summary of Western blot results. (C) IF analysis of SREBP1 level in HT22 cells incubated with 5 mM Glu for 24 h. Scale bar, 50 μm. (D) SREBP1 fluorescence intensity quantification in the control and Glu groups. The values were showed as the mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 compared with control group. Glu, glutamate.

4. Discussion

Status epilepticus (SE) is a prevalent neurological disorder that can lead to severe disability and mortality [32]. SE can lead to an excessive release of glutamate, which can lead to significant damage to hippocampal neuronal cells in SE [11–13]. In this study, we aimed to investigate the molecular mechanisms underlying SE-related hippocampal neuronal damage, especially SE-induced Glu damage to hippocampal neuronal cells. To achieve this, we established a rat model of early-stage SE and a glutamate (Glu)-induced cell damage model using HT22 cells. Hippocampus-derived HT22 cells do not possess functional ionotropic glutamate receptors, making them an excellent cellular model for investigating glutamate-mediated cell death excluding excitotoxicity [33,34]. Our results showed that SE and Glu-mediated HT22 cells had abnormally high levels of SREBP1 and pyroptosis. Furthermore, we conducted a more comprehensive investigation and found that the mTOR signaling pathway regulated the expression of SREBP1. Most likely due to the relatively short duration of SE, we did not detect hippocampal neuronal death through H&E and Nissl staining. However, we observed that the administration of afuresertib significantly prevented the SE-induced increase in various markers of neuronal pyroptosis (such as IL-18, cleaved caspase-1 etc.) that could potentially contribute to neuronal death. More importantly, we noted that the co-localization of SREBP1 and NLRP1 occurred in Glu-treated HT22 cells by confocal immunofluorescence analysis. Therefore, these findings suggested that SREBP1 may be involved in SE/Glu-induced hippocampal neuronal death, and positively regulated the pyroptosis and inflammasomes activation, which may be due to the interaction of SREBP1 and NLRP1.

Emerging evidence suggests that the initiation and progression of SE can lead to neuronal cell loss in various brain regions, particularly the hippocampus [35]. According to current research, apoptosis, necroptosis, ferroptosis, and pyroptosis are the main types of programmed cell death. In our study, we investigated the expression of these different types of programmed cell death in our SE rat model. Apoptosis-related markers (BAX, Caspase3), necroptosis markers (RIPK1, RIPK3), ferroptosis-related marker GPX4 and pyroptosis markers (NLRP3, GSDMD, ASC, IL-18 and Caspase-1) were detected in the hippocampal tissues of rats with SE induced by pilocarpine, 4 h after the onset of SE. Interestingly enough, we found that the changes of pyroptosis-related proteins were more pronounced than other programmed death-related markers in the early stages of SE. This may suggest inflammation has a greater effect on nerve cell damage in the early stages of SE. Besides that, the necroptosis and ferroptosis did not change in our model. It may indicate

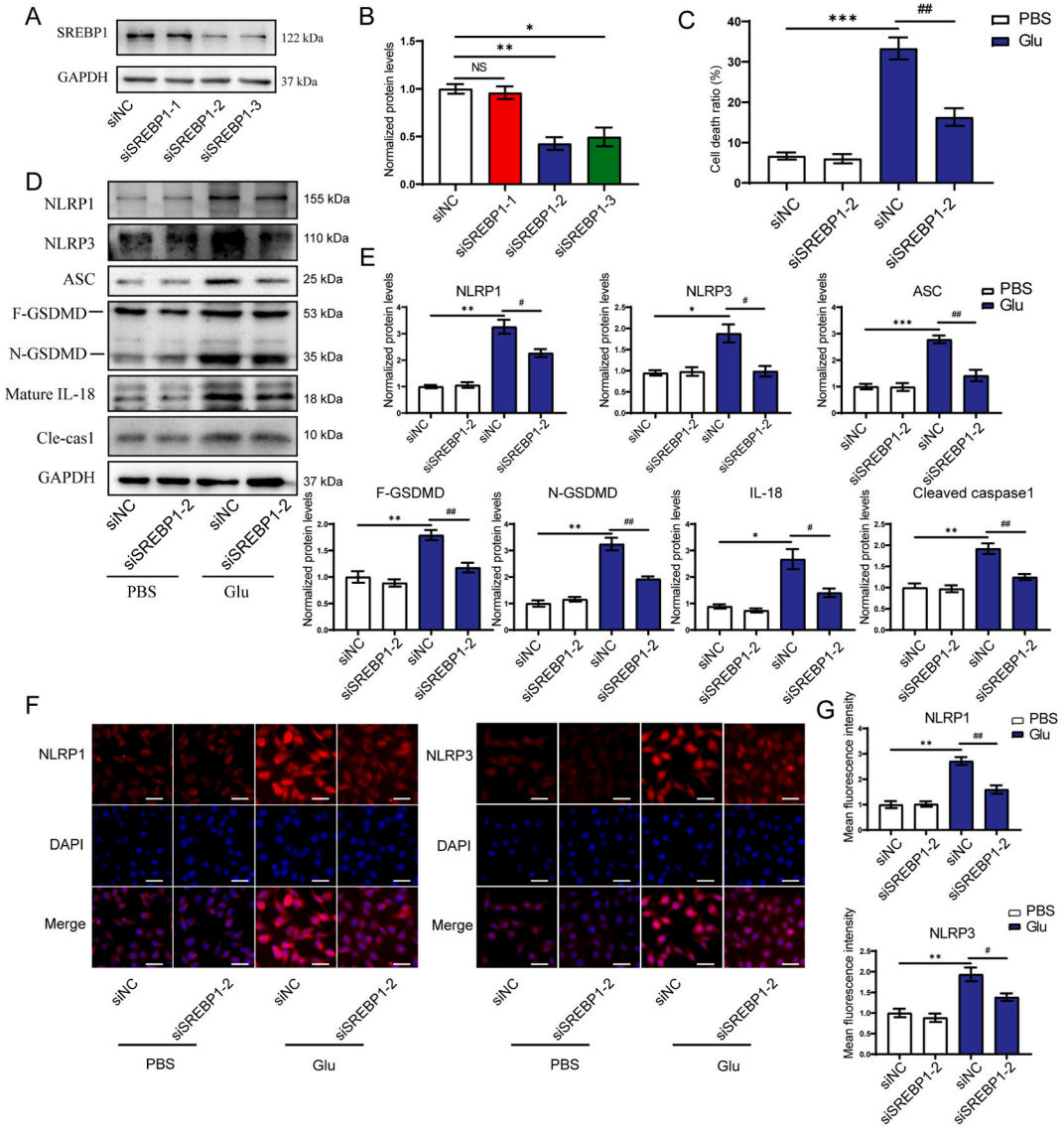

Fig. 5. SiRNA-mediated knockdown of SREBP1 attenuates Glu-induced HT22 cell death, inflammasomes activation and pyroptosis in vitro. The siSREBP1 or siNC was transfected into HT22 cells. Then cells were treated with 5 mM Glu for 24 h. (A) The transfection efficiency of SREBP1 was measured by Western blot analysis. (B) Quantification of knockdown efficiency of SREBP1 by siRNA. (C) Cell viability was detected using Calcein/PI staining in HT22 cells. (D) The expression of pyroptosis-related proteins was captured by Western blot analysis. (E) Quantification of the results of D. (F) IF analysis of NLRP1 and NRP3 expression in HT22 cells. Scale bar, 50 μm. (G) NLRP1 and NRP3 fluorescence intensity quantification in the control and Glu groups treated with siSREBP1 or siNC. The values were showed as the mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 compared with siNC + PBS group and #p < 0.05, ##p < 0.01 compared with siNC + Glu group. NS, no significance. siSREBP1, SREBP1 target siRNA; siNC, control siRNA; Cle-cas1, Cleaved caspase-1; IL-18, interleukin-18.

RIP1-RIP3 necrosis complex and iron-dependent programmed cell death are not responsible for the early SE-induced cellular damage. In contrast to our study, Dong-Qi et al. found that SE can induce programmed necrosis in hippocampal neurons, and inhibiting programmed necrosis has a protective effect against hippocampal neuronal damage [36]. The reason for the disparity in our study results may be attributed to the different modeling approach using kainic acid induction in their study, as well as differences in the stage of SE. Furthermore, our study also revealed a certain degree of increase in the level of hippocampal neuronal apoptosis induced by SE. Consistent with our study findings, Xing et al. reported that SE also leads to an increase in hippocampal neuronal apoptosis, and this phenomenon can be attenuated by propofol [37]. Therefore, considering the more pronounced changes in pyroptosis-related molecules, indicating that pyroptosis contributes to the development of SE and plays a greater role in the early stage of SE.

Pyroptosis is a form of inflammation-related programmed cell death that is characterized by the release of some proinflammatory factors, such as IL-18, IL-1β [17,38]. Based on current research about canonical pyroptotic death, pyroptosis was mediated by inflammasome and caspase-1. The inflammasomes including NLRP3 and adaptor protein ASC were interacted to activate caspase-1

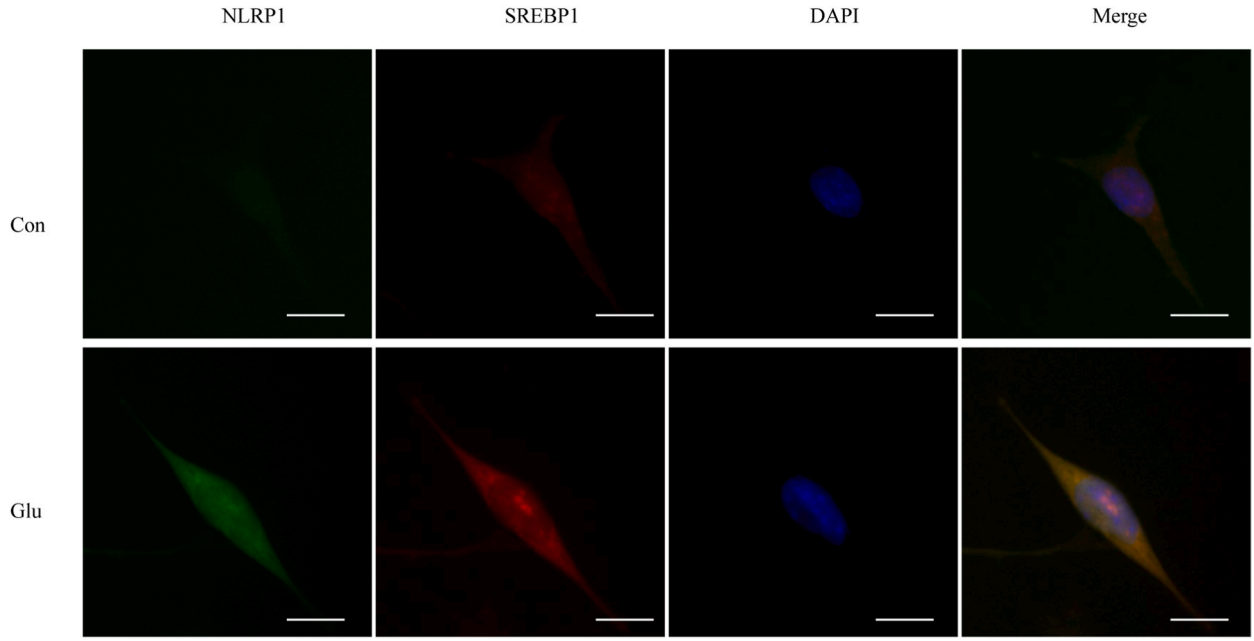

Fig. 6. The co-localization of SREBP1 and NLRP1 was showed by confocal IF analysis. Typical confocal fluorescence images show the influence of Glu on the co-localization of SREBP1 and NLRP1. Scale bar, 20 μm.

[39]. IL-18 and IL-1β was cleaved by active caspase-1 and released from the pores formed by GSDMD to damage cell [18,40]. Accumulating evidence suggests that inflammation is involved in the progression and pathogenesis of epilepsy. NLRP1 and NLRP3 are the two most extensively studied members of the inflammasome, and their activation during epilepsy has been observed in both basic and clinical studies [41]. NLRP3 has been found to be significantly increased in both neurons and microglia of the epileptic brain [42, 43], and inactivation of NLRP3 has been shown to have anti-epileptic and neuroprotective effects [44,45]. These findings provide strong evidence that NLRP3 inflammasome is involved in epilepsy through neuroinflammation. In addition to epilepsy, there have been several studies showing that pyroptosis occurs in SE. Shuai et al. has also shown that NLRP3 inflammasome components were upregulated following SE [46]. Consistent with the above research, our results showed that caspase-1, IL-18, GSDMD, NLRP3, and ASC were all elevated in Glu-induced HT22 cell injury in vitro, as well as in hippocampal neuronal damage following SE in vivo. These results indicated pyroptosis occurred in SE, which might be associated with the SE-induced elevation of Glu. In addition, the expression of NLRP1 was also detected in this study, which was elevated in HT22 cells incubated with Glu. It is worth noting that NLRP1 inflammasome can also stimulate caspase-1 and cause IL-18 and IL-1β production, leading to pyroptosis [47]. Activation of the NLRP1 inflammasome has been observed in patients with mesial temporal lobe epilepsy (TLE), and inhibition of NLRP1 can reduce pyroptosis-related neuronal loss and alleviate the severity of seizures in an animal model of TLE [48]. Therefore, the upregulation of both NLRP1 and NLRP3 can activate caspase-1 signaling, contributing to hippocampal neuronal pyroptosis.

Evidence suggests that SREBP1 was involved in neuronal cell death. Taghibiglou et al. has shown that there was a direct causative relationship between SREBP1 and neuronal cell death in vivo model of stroke [49]. Additionally, increased SREBP1 has been found to significantly contribute to the apoptosis of motor neurons in amyotrophic lateral sclerosis [50]. As far as we know, there are few studies investigated the role of SREBP1 in hippocampal neurons and the association between SREBP1 and pyroptosis induced by SE. To illustrate the function of SREBP1 in pyroptosis following SE. Glu-induced HT22 cell was built, which was often used as in vitro model to study excitotoxicity or cell injury. Our results showed SREBP1 and pyroptosis-related proteins were dramatically elevated in HT22 cells treated with Glu and hippocampal neurons following SE.

Downregulation of SREBP1 alleviated the expression of pyroptosis-related markers, and effectively protected neuronal cells against pyroptosis. Collectively, these findings support the involvement of SREBP1 in glutamate-induced HT22 cell injury and hippocampal neuronal damage following SE through its impact on pyroptosis.

Besides that, we also found SREBP1 may play a regulatory role between mTOR and pyroptosis in SE and glutamate-mediated excitotoxicity models. Abnormal activation of mTOR is considered as potential pathogenesis of SE [51]. Our previous study also has shown that PI3K-AKT/mTOR pathways were activated to regulate the autophagy following SE [19]. There is substantial evidence supporting the involvement of the AKT/mTOR signaling pathway in the regulation of SREBP1, with SREBP1 being identified as a downstream target of mTOR [21–23]. In this study, AKT/mTOR inhibitor Afuresertib was pretreated in rat model of SE. Consistent with our findings, SREBP1 was regulated by AKT/mTOR signaling pathway. Inactivation of mTOR pathway suppressed the increase of SREBP1 due to SE, as well as the activation of NLRP3 inflammasome and pyroptosis following SE in rats. These results suggest that SREBP1 plays a pivotal role in mediating the crosstalk between mTOR and pyroptosis in the SE model. However, further investigations are required to elucidate the specific interactions involved.

The current study offered the first proof that upregulation of SREBP1 contributes at least partially to hippocampal neuronal pyroptosis caused by SE and Glu. To further investigate the underlying mechanism linking SREBP1 and pyroptosis in the hippocampus, we conducted confocal immunofluorescence analysis to observe the co-localization of SREBP1 and NLRP1 in Glu-treated HT22 cells. The results suggest that SREBP1-mediated pyroptosis may involve the interaction between SREBP1 and NLRP1. Supporting our findings, Silvia et al. revealed that SREBP1 could regulate NLRP1 inflammasome expression in peripheral arterial disease [52]. Admittedly, the specific mechanisms underlying SREBP1-induced pyroptosis and the interaction with NLRP1 need to be clarified in future work.

Our current research has several limitations. Firstly, the precise mechanism driving the increase of SREBP1 in response to SE has not been examined in our investigation, and it is therefore important to conduct further studies on this topic. Secondly, although we found that inhibition of SREBP1 can alleviate hippocampal neuronal pyroptosis caused by pilocarpine-induced SE in vivo, and glutamate-induced neurotoxicity in vitro using HT22 cells, it is important to note that glutamate-treated HT22 cells do not truly mimic the complex nature of SE in vivo. This difficulty in accurately modeling epilepsy in vitro is a common challenge in current research. Furthermore, more samples, including human samples, are needed to further prove our conclusions.

In conclusion, the current work revealed that SREBP1-mediated pyroptosis is involved in the mechanism of Glu-induced HT22 cell injury and hippocampal neuronal impairment in rats after SE. Additionally, SREBP1-induced pyroptosis may be aided by the interaction between NLRP1 and SREBP1. These results provide valuable insights into the critical role of SREBP1 in the occurrence of pyroptosis following SE, highlighting the potential of targeting SREBP1 as a promising therapeutic strategy for SE.

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Xing Ye: Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Jun-Yi Lin:** Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Ling-Xia Chen:** Formal analysis, Writing – original draft. **Xue-chun Wu:** Formal analysis, Methodology. **Kai-Jun Ma:** Formal analysis. **Bei-Xu Li:** Funding acquisition, Methodology, Supervision, Writing – review & editing. **You-Xin Fang:** Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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