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Dynamic regulation effect of long non-coding RNA-UCA1 on NF-kB in hippocampus of epilepsy rats

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Abstract. – OBJECTIVE: We aimed to discuss the mechanism of occurrence and progression of epilepsy through analyzing the expression changes of UCA1 and NF-Kb in temporal hippocampus and UCA1 in peripheral blood in rats with epilepsy induced by lithium chloride-pilocarpine.

MATERIALS AND METHODS: The lithium chloride-pilocarpine-induced epilepsy rat model was established; 1, 7, 14, 30, and 60 d after status epilepticus were selected as the time points of research. The expression levels of UCA1 and NF-kB in the hippocampus of rats and UCA1 in peripheral blood were detected and analyzed using quantitative Real-time PCR (qRT-PCR). The differences and correlations between expression levels of UCA1 and NF-kB at each time point of research in experimental group and control group were analyzed statistically.

RESULTS: Results showed that mRNA expression levels of UCA1 and NF-kB in brain tissues in experimental group were higher than those in control group at each time point. The change trend of expression levels of UCA1 and NF-kB with time was consistent. The expression level of UCA1 in peripheral blood in experimental group at each time point was higher than that in control group, and mRNA expression level of UCA1 in peripheral blood in experimental group was positively correlated with that in brain tissue.

CONCLUSIONS: The expressions of UCA1 and NF-Kb are in the dynamic change in the formation of epilepsy, suggesting that UCA1 may participate in the pathogenesis of epilepsy, so as to provide a potentially feasible new direction for guiding the clinical diagnosis and treatment of epilepsy.

Key Words:
Epilepsy, Hippocampus, Receptor activator of nuclear factor-kappa B.

Introduction

Epilepsy is a kind of nervous system disease of repeated convulsions caused by abnormal neurons excitation[1]. Status epilepticus (SE) can lead to acute and persistent central nervous system damage, and damage the hippocampal neurons[2]. After an acute phase of SE, ischemia and hypoxia, edema and inflammatory response can occur in hippocampus, inducing the release of excitatory amino acid, influx of sodium and calcium ion and other changes in mechanism, causing the neuronal damage (including hippocampus neuron loss, neuronal apoptosis, gliocyte proliferation, fiber sprouting, hippocampal sclerosis, etc.), which is the main reason for the occurrence and development of chronic spontaneous epilepsy. Finally, refractory temporal lobe epilepsy (TLE) can be formed[3]. The incidence rate of refractory TLE accounts for about 25% of the total number of epilepsy population, and the effects of a variety of antiepileptic drugs on patients with this type of epilepsy are not ideal[4]. Therefore, it is of great clinical significance to control and reduce the hippocampal neuron loss after SE and inhibit the occurrence of refractory epilepsy. The activation of nuclear transcription factor (NF-kB) can efficiently induce the extensive gene expression and regulate the transcription of cytokine target genes, playing a regulatory role in inflammation and immune responses[5]. Although a few literatures have reported that the epileptic seizure is accompanied by the expression of NF-kB in the brain, induces the neuronal death and activation of glial cells, and promotes the pathological changes in the brain of epilepsy rats[6], there are few reports on the regulatory mechanism related to NF-kB in epilepsy. Long non-coding RNA (lncRNA) is a kind of ncRNA with a length greater than 200nt and a lack of the ability to encode protein. lncRNA is generally divided into intergenic lncRNA, intragenic RNA, circular RNA, competitive endogenous RNA, ultra-conserved transcriptional region, and antisense RNAs, etc[7]. Compared with RNA of encoded protein, lncRNA has shorter

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length, less exons, weak coding capacity, and tissue or cell specificity; compared with mRNA, lncRNA is less conserved in close species[8]. At present, there are more and more studies on the function of lncRNA, and it is found that its function is complicated and can be involved in each stage of regulating gene expression and plays a role via influencing the molecular level: (1) chromatin structure; (2) transcriptional activity; (3) mRNA stability; (4) mRNA processing after transcription; (5) mRNA translation[9]. In recent years, more and more studies have also found that lncRNA is expressed in the brain with tissue specificity, and it is closely related to the brain development, neuronal differentiation, and nervous system diseases, etc.[10], such as nervous system degenerative disease, cerebral ischemia, gliomas, and epilepsy. Urothelial cancer associated 1 (non-protein coding), also known as UCA1, is a long non-coding RNA, which is well-known to be upregulated in bladder cancer[11]. It is believed to function in regulation of embryonic development and in cancer invasion and progression. It regulates the expression of several genes involved in tumorigenesis and/or embryonic development[12]. Previous studies have found that UCA1 exhibits abnormal methylation in the hippocampus removed in the surgical operation of TLE patients[13], but its possible mechanism under SE was not further studied. Therefore, this research, through the detection and analysis of expression level of each index in temporal hippocampus and peripheral blood in epilepsy rats, analyzed whether UCA1 induced or aggravated epilepsy via the interaction with NF-kB, so as to further reveal the mechanism of long non-coding RNA in the occurrence and progression of epilepsy, and provide a potentially feasible new direction and target for guiding the clinical diagnosis and treatment.

Materials and Methods

Experimental animals and grouping

50 healthy male Sprague-Dawley (SD) rats aged 6-8 weeks weighing 220 ± 30 g were provided by Experimental Animal Center of People's Hospital of Yucheng City, and fed in separate cages at room temperature of 20-25°C, relative humidity of 50-60% and artificial 12 h day/night cycle lighting. This study was approved by the Animal Ethics Committee of Animal Center of People's Hospital of Yucheng City. The rats were randomly assigned to experimental group (n=45) and control group (n=5) using the random number table method. The experimental group was divided into l d group, 7 d group, 14 d group, 30 d group and 60 d group (chronic epilepsy) after status epilepticus (SE). 5 rats were randomly enrolled in each group. Rats in control group received the non-epileptogenic drug during modeling, and all rats in control group were enrolled.

Establishment of Lithium Chloride - Pilocarpine SE Model

Rats in experimental group were intraperitoneally injected with lithium chloride (about 125 mg/kg). After 18-20 h, pilocarpine (Pilo) was intraperitoneally injected (20 mg/kg). Pilocarpine and lithium chloride were prepared with fresh sterile saline solution. After the injection of pilocarpine, the behavior change of rats was observed and the degree of epileptic seizure was graded according to Racine criteria[14]. The behavior test and EEG (Sirius BB EEG diagnostic system, EB Neuro) were performed at 1, 7, 14, 30, and 60 d after modeling to determine whether it was successful. Successful epilepsy induction group: rats reached Racine IV-V grade and entered SE for more than 30 min, paroxysmal or persistent abnormal discharge in EEG based on sharp wave or spike wave; if there was no seizure or it failed to reach Racine IV-V grade, pilocarpine was injected at 10 mg/kg/time and repeated for 6 times. The animals with Grade IV or above were also enrolled into the successful epilepsy induction group. Unsuccessful epilepsy induction group: it failed to reach the criteria after the injection of pilocarpine for 6 times. The unsuccessful epilepsy induction group and dead rats were eliminated, and complemented in the subsequent experiments. Rats in control group were intraperitoneally injected with 0.9% saline (125 mg/kg) and the behavioral changes were observed.

Specimen collection

Each rat in experimental group was intraperitoneally injected with 10% chloral hydrate at 5 ml/kg; then, they were sacrificed, the brains were taken out, and blood was collected from the femoral vein. The brain tissues on both side of hippocampus were bluntly isolated on normal saline ice surface and stored in a liquid nitrogen tank at -180°C. Total RNA was extracted from the blood samples by Trizol method and stored in a refrigerator at -80°C. The brain tissue and blood samples in hippocampus of rats in control group were also collected and stored according to the above method.

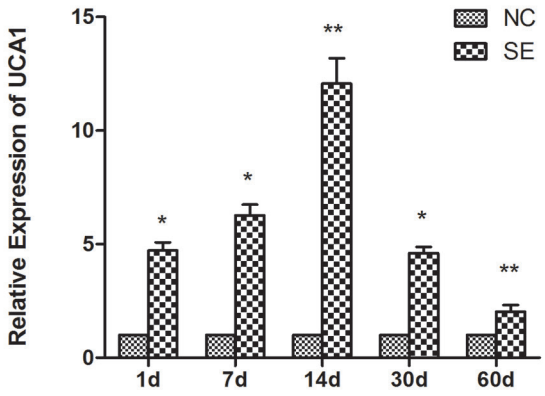

Figure 1. Expression of UCA1 in brain tissue in hippocampus at each time point detected at 1 d, 7 d, 14 d, 30 d and 60 d after SE, respectively. Total RNA was detected by qRT-PCR and GAPDH was used as an internal control. Data are presented as the mean ± SD of three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$.

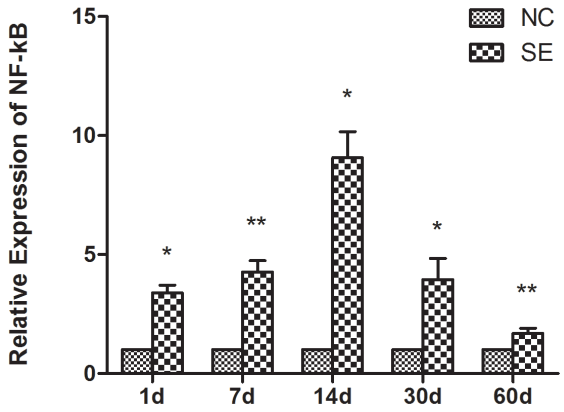

Figure 2. Expression of NF-kB in brain tissue in hippocampus at each time point detected at 1 d, 7 d, 14 d, 30 d and 60 d after SE, respectively. Total RNA was detected by qRT-PCR and GAPDH was used as an internal control. Data are presented as the mean ± SD of three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$.

RNA Extraction and qRT-PCR

Total RNA was extracted from collected frozen tissue samples and animal blood specimens by using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. Reverse Transcription Kit (TaKaRa, Otsu, Shiga, Japan) was used to synthesize cDNAs. Levels of mRNA were quantified by SYBR Green Real-time PCR and normalized to GAPDH using the following primers: for lncRNA-UCA1, forwards, 5'- ACC TCA ACC CAA AGG CAG TC -3' and reverse, 5'- GCC TTT GTG CCG CTA CTT TT -3'; for NF-kB, forward, 5'- CGA TCT GTT TCC CCT CAT CT-3' and reverse, 5'- ATT GGG TGC GTC TTA GTG GT -3; and for

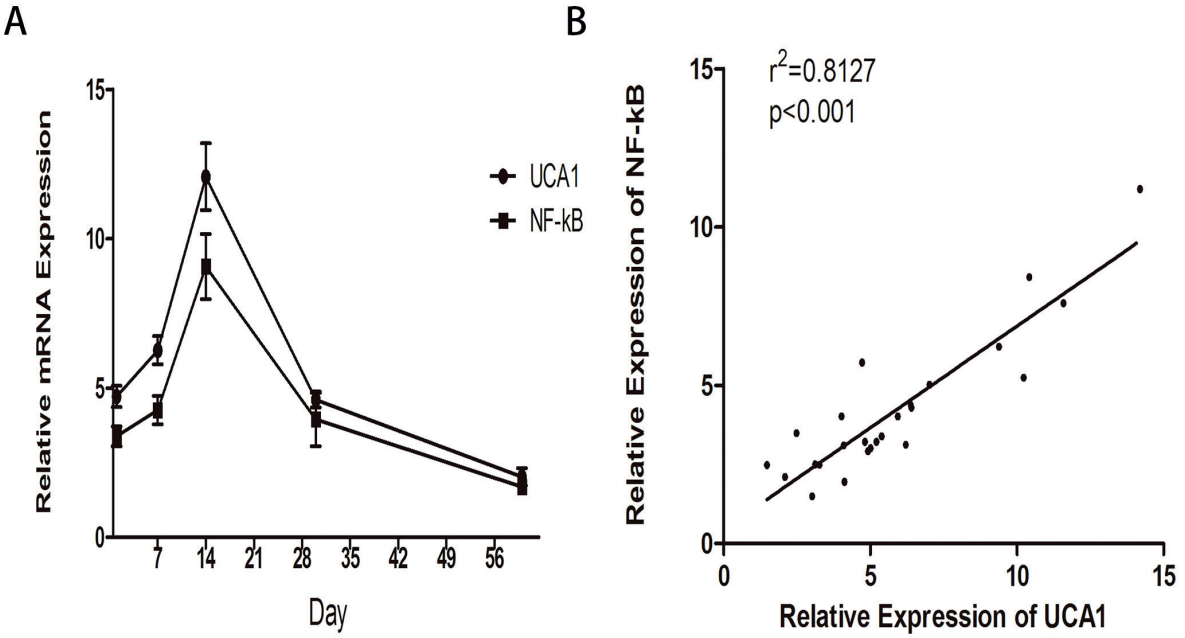

Figure 3. Analysis of correlation between expressions of UCA1 and NF-kB in hippocampus, detected at 1 d, 7 d, 14 d, 30 d and 60 d after SE, respectively. ***A,*** The change trend of expressions of UCA1 and NF-kB with time was consistent: ***B,*** UCA1 expression was positively correlated with NF-kB expression. Data are presented as the mean ± SD of three independent experiments. $^{***}p<0.001$.

GAPDH, forward, 5'-CGG AGT TGT TCG TAT TCG G-3' and reverse, 5'-TAC TAG CCG ATG ATG GCA TT-3'. QRT-PCR was performed by the ABI 7500 system (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, statistical data was presented with Graph PAD prism software, and quantitative data was presented as mean ± SD. The independent samples *t*-test was used to perform statistical analysis. The regression and correlation analysis was analyzed using the Spearman *X2* test. The relative expression of mRNA was measured using the method of 2-ΔΔCT. Results was seemed as statistically significant at $p<0.05$.

Results

Animal behavior observation

After the intraperitoneal injection of lithium chloride, the animal behavior of rats in experimental group did not change significantly. 5-30 min after the intraperitoneal injection of pilocarpine, the rats in experimental group began to have the peripheral cholinergic reactions, such as piloerection, salivation, tremble, tears of blood, etc. At the same time or after that, animal behaviors occurred, such as gazing, chewing, nasal inspiration, exploration, wet dog shake, repeated head and neck upward, followed by blink, hemifacial spasm, nodding, etc. Finally, they suffered from the repeated bilateral forelimb clonus with upright, falling or flipping, and some animals might suffer from limb stiffness clonus, etc. The onset was not frequent at first, but increased as time goes by. There were 45 SD rats used for the lithium chloride-pilocarpine-induced epilepsy, 37 of which reached Racine IV-V grade and entered status epilepticus (SE). After entering SE, 10 SD rats died for convulsions. 5 SD rats were sacrificed at 24 h after inducing epilepsy successfully, and their brains were taken out; 5 SD rats were sacrificed at 7 d, and their brains were taken out; 5 SD rats were sacrificed at 14 d, and their brains were taken out; 5 SD rats were sacrificed at 30 d, and their brains were taken out; 5 SD rats were sacrificed at 60 d (chronic epilepsy), and their brains were taken out. Also, EEG monitoring was performed for epilepsy rats in experimental group at each time point, and the sharp wave, spike wave, or spike and slow wave, could be recorded. No epileptiform discharge wave was detected in rats in normal control group. In this study, the success rate of inducing SE of lithium chloride-pilocarpine-induced epilepsy model was 82.2%.

Detection of UCA1 Expression Change in the Formation of Epilepsy by qRT-PCR

The expressions of UCA1 in brain tissue in temporal hippocampus of rats in experimental group and control group were detected by qRT-PCR at 1, 7, 14, 30 and 60 d after SE, respectively. The results showed that the expression level of UCA1 in experimental group at each time point was higher than that in control group, reached the peak at 14 d after SE, and fell at 30 d and 60 d (chronic epilepsy) after SE; the differences were statistically significant (Figure 1).

Detection of NF-kB Expression Change in the Formation of Epilepsy by qRT-PCR

The expressions of NF-kB in brain tissue in temporal hippocampus of rats in experimental group and control group were detected by qRT-PCR at 1, 7, 14, 30 and 60 d after SE, respectively. The results showed that the expression level of NF-kB in experimental group at each time point was higher than that in control group, reached the peak at 14 d after SE, and fell at 30 d and 60 d (chronic epilepsy) after SE; the differences were statistically significant (Figure 2).

Analysis of Correlation Between Expressions of UCA1 and NF-kB in Hippocampus

In order to investigate whether UCA1 and NF-kB interacted in the hippocampus and caused the occurrence and progression of epilepsy, we analyzed whether there was a correlation between the expression levels of them. The results showed that the change trend of expression levels of UCA1 and NF-kB in temporal hippocampus was consistent (Figure 3A), and the comprehensive analysis of expressions of UCA1 and NF-kB at each time point showed that they were positively correlated, and the differences were statistically significant (Figure 3B). Therefore, it may be roughly deduced that UCA1 induces or aggravates epilepsy through the interaction with NF-kB.

Detection of UCA1 Expression in Peripheral Blood of Animals

In order to further study and investigate the potential possibility of UCA1 as the diagnosis

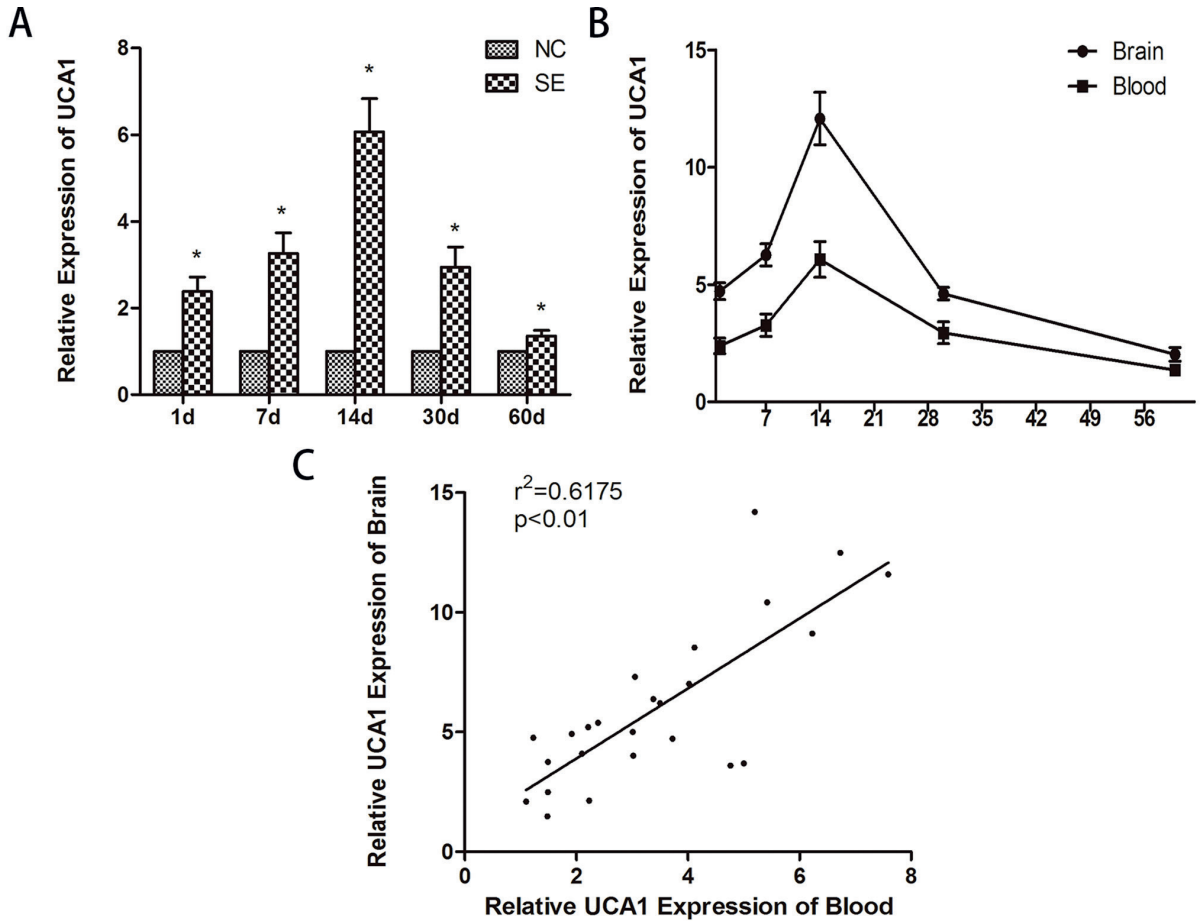

Figure 4. Expression of UCA1 in peripheral blood at each time point, detected at 1 d, 7 d, 14 d, 30 d and 60 d after SE, respectively. ***A,*** The expression of UCA1 in experimental group at each time point was higher than that in control group; ***B,*** The change trend of expressions of UCA1 in peripheral blood and brain tissue was consistent; ***C,*** The expressions of UCA1 in peripheral blood and brain tissue were positively correlated. Data are presented as the mean ± SD of three independent experiments. $^{***}p<0.01$.

and treatment target of epilepsy, the peripheral blood of rats in experimental group and control group at 1, 7, 14, 30 and 60 d after SE was drawn and the expression level of UCA1 was detected by qRT-PCR. In addition, the correlation between UCA1 expression level in peripheral blood of experimental group and that in temporal lobe brain tissue of rats at the same time point was analyzed and compared. The results showed that UCA1 expression level in experimental group at each time point was higher than that in control group, reached the peak at 14 d after SE and fell at 30 d and 60 d (chronic epilepsy) after SE (Figure 4A). At the same time, UCA1 expression trends in peripheral blood and brain tissue of experimental group were almost consistent (Figure 4B), and the comprehensive analysis of expressions of UCA1 in peripheral blood and brain tissue at each time point showed that they were positively correlated and the differences were statistically significant (Figure 4C).

Discussion

NF-κB is a kind of nuclear transcription factor with a wide range of biological effects, involved in the occurrence and development of a variety of diseases. In 1986, Sen et al[15] initially found that NF-κB is a kind of protein that binds to the specific κB site of immunoglobulin κ light chain gene enhancer. NF-κB is widely present in the cytoplasm of quiescent cells. When the cells are stimulated by the environment, the specific inhibitory protein-super inhibitor IκB can be phosphorylated and inactivated, so NF-κB is activated into the nu-

cleus; then NF- KB binds to the GGGRNNYYCC element of the enhancer of some genes in the nucleus, initiating or regulating the transcription of early response genes and participating in inflammatory response, cell proliferation and apoptosis. Lerner et al[16] studied the inflammation and immune changes of hippocampus in SE and found that the sign of NF-κB activation produced by long-term seizures is the increased NF-κB expression and nuclear translocation, and the inflammatory cascade caused by epileptic seizures can lead to overexpression of NF-κB in astrocytes. In view of the complexity of NF-κB activation in the pathogenesis of epilepsy, its signal transduction system has become one of the current research hotspots. At present, the role of lncRNA in nervous system disease has attracted more and more attention from the outside world. It is found in microarray data analysis that lncRNA expression in brain tissue is different in patients with Alzheimer's disease (AD) compared with healthy people, most of which are intergenic lncRNA. For example lncRNAn341006 significantly down-regulated in AD can significantly affect the protein ubiquitination pathway[17]. It is found in the study that NURR1, closely related to the occurrence, development and survival of dopaminergic neurons in the midbrain, can regulate the expression of AS Uchl1, and the expression of AS Uchl1 was significantly down-regulated in the neurochemical model *in vitro* and *in vivo* of patients with Parkinson's disease (PD)[18]. The abnormal expression of lncRNA is also found in epilepsy; for example, the expression of brain-derived neurotropic factor (BDNF) in high-peak region in brain of epilepsy patients is up-regulated, but the expression of lncRNA-BDNFOS is significantly decreased. *In vitro* cell experiments have showed that BDNFOS can negatively regulate BDNF[19]. This study aimed to investigate the mechanism of lncRNA in epilepsy. In this study, brain tissues in temporal hippocampus of rats in experimental group and control group at each time point were detected quantitatively first, and it was found that mRNA expression levels of UCA1 and NF-kB in experimental group were significantly higher than those in control group. Also, it was found that the change of their expression levels with time was consistent; so it can be deduced that there is an interaction between UCA1 and NF-kB, acting on the development or progression of epilepsy, which provides a new idea and target for the molecular biology study on lncRNA in epilepsy or nervous system disease. Furthermore, mRNA level of UCA1 in peripheral blood of rats was also detected, and the expression level of UCA1 in peripheral blood of experimental group was found to be higher than that of control group. Moreover, mRNA expression levels of UCA1 in peripheral blood and brain tissue of experimental group were positively correlated. According to these data, the detection of lncRNA change in peripheral blood can be served as a predictive index of lncRNA change in brain tissue. In order to really apply to clinical diagnosis and treatment, it is needed to not only expand the sample size and repeat the animal experiments, but also verify through a large number of clinical tests. Once the results are proved to be effective and considerable, it is easy to be operated in the clinical diagnosis and treatment of epilepsy, and can quickly guide the next treatment, which has a very significant clinical significance.

Conclusions

We showed that UCA1 in epilepsy rats induces or aggravates epilepsy through the interaction with NF-kB, and the UCA1 expression in peripheral blood of epilepsy rats was positively correlated with that in brain tissue. Our study provides a new basis for revealing the role of long non-coding RNA in the occurrence and progression of epilepsy and provides a potentially feasible new direction and target for guiding the clinical diagnosis and treatment of epilepsy.

Conflict of interest

The authors declare no conflicts of interest.

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