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LncRNA UCA1 alleviates aberrant hippocampal neurogenesis through regulating miR-375/SFRP1-mediated WNT/β-catenin pathway in kainic acid-induced epilepsy

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Temporal lobe epilepsy (TLE) is a chronic disease of the nervous system, associated with increased proliferation in the hippocampus. Urothcarcinoma associated 1 (UCA1) is a long long non-coding RNA that was shown to regulate proliferation and differentiation of neural progenitors *in vitro*. We hypothesised that TLE-associated abnormal proliferation is a consequence of the downregulation of UCA1. This hypothesis was tested in mice with kainic acid (KA)-induced seizures, and then the potential mechanism was explored *in vitro* and *in vivo*. Result showed that the expression of UCA1 and Secreted Frizzled Related Protein 1 (SFRP1) were significantly reduced in hippocampal tissues of epileptic mice, while miR-375 was increased compared with the control group. Pearson correlation analysis showed that UCA1 was positively correlated with SFRP1, while miR-375 was negatively correlated with UCA1 and SFRP1. Besides, UCA1 was overexpressed in mice and the overexpression of UCA1 significantly reversed the abnormal proliferation of hippocampal neurons in epilepsy mice. *In vitro* Luciferase assay showed that UCA1 and *Sfrp1* are both the targets of miR-375, and UCA1 promotes the expression of *Sfrp1* by competitively adsorbing miR-375, thereby inhibiting the activation of the WNT/β-catenin pathway. The inactivation of the WNT/β-catenin pathway prevented the abnormal proliferation of neural progenitors in the epileptic hippocampus. In conclusion, our findings provide a theoretical basis for the clinical application of UCA1.

Keywords: Temporal lobe epilepsy, Urothcarcinoma associated 1, miR-375, hippocampal neurogenesis, Secreted Frizzled Related Protein 1, WNT/β-catenin pathway

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Abbreviations: ANOVA, analyzed by one-way analysis of variance; KA, kainic acid; lncRNAs, long non-coding RNAs; miR-375 inh, miR-375 inhibitor; miRNAs, microRNAs; MUT, mutant; NC inh, NC inhibitor; PFA, paraformaldehyde; SFRP1, Secreted Frizzled Related Protein 1; TLE, Temporal lobe epilepsy; UCA1, Urothcarcinoma associated 1; 3′-UTR, 3 untranslated-untranslated region; WT, wild type

INTRODUCTION

Temporal lobe epilepsy (TLE) is one of the most common chronic diseases of the nervous system, and its clinical treatment is extremely scarce worldwide (Bartolomei *et al.,* 2008). It is well known that TLE is a very common locally related epilepsy, usually drug-resistant (Asadi-Pooya *et al.,* 2017), and known as hippocampal sclerosis, which is characterized by the loss of a large number of neurons and severe glial hyperplasia (Tai *et al.,* 2018). Abnormal neurogenesis and reorganization of neural circuits in the hippocampus cause spontaneous or recurrent seizures of epileptic foci and participate in the process of temporal lobe epilepsy (Cho *et al.,* 2015). Although recent data shows that abnormal neurogenesis is caused by acute attacks or sudden injuries, the mechanism of abnormal neurogenesis in temporal lobe epilepsy is still elusive.

Studies have shown that long non-coding RNAs (long non-coding RNAs, lncRNAs) are also key regulators of the occurrence and development of diseases including epilepsy (Qiao *et al.,* 2018). Urothcarcinoma associated 1 (UCA1) is a lncRNA originally found in bladder transitional cell carcinoma (Fan *et al.,* 2014). Down-regulation of UCA1 plays a role in promoting apoptosis in primary cardiomyocytes by promoting p27 expression (Liu *et al.,* 2015). Recently, UCA1 has been shown to promote the proliferation and differentiation of neural stem cells, suggesting that UCA1 may play an important role in the nervous system (Zheng *et al.,* 2017). Recent studies have found that UCA1 has a certain role in epilepsy. For example, UCA1 inhibits epilepsy and epilepsy-induced brain injury by regulating miR-495/Nrf2-ARE signaling pathway (Zheng *et al.,* 2017). UCA1 inhibits temporal lobe epilepsy star by regulating JAK/STAT signaling pathway Activation of shaped cells (Geng *et al.,* 2018). However, the role and mechanism of UCA1 in abnormal neurogenesis caused by epilepsy has not been investigated.

Recently, the role of small non coding RNAs, especially microRNAs (miRNAs), in epilepsy and neurodegenerative diseases (Alzheimer's disease and Huntington's disease) has attracted great attention (Hébert *et al.,* 2008; Johnson *et al.,* 2008; Wu *et al.,* 2019). MiR-375 has been reported to be involved in neuromodulation. For example, miR-375 inhibits neuronal differentiation by reducing HUD levels (Abdelmohsen *et al.,* 2010), and miR-375 inhibits neuronal apoptosis and promotes growth in cerebral ischemia-reperfusion (Ou *et al.,* 2017). It is reported that miR-375 is up-regulated in the KA-

induced SD epilepsy rat model (Henshall 2013). However, whether the abnormal expression of miR-375 in the hippocampus of patients with temporal lobe epilepsy is related to neuronal hyperplasia has not been reported.

The current study explored the effect of UCA1 on hippocampal neurogenesis in KA-induced epilepsy mice and its potential molecular mechanism. It was found that UCA1 regulates the expression of Secreted Frizzled Related Protein 1 (*Sfrp1*) by targeting miR-375, thereby regulating the WNT/β-catenin signaling pathway and participating in abnormal neurogenesis caused by epilepsy. Our findings provide a theoretical basis for the clinical application of UCA1.

MATERIAL AND METHODS

Animal modeling and grouping

All animal experiments were approved by the Guangxi University of Chinese Medicine Institutional Review Board. All procedures were conducted according to the Guide for the Use of Laboratory Animals (National Academy Press). 24 adult male C57Bl/6 mice (10 weeks, weighing 20–25 g) were obtained from Hunan Slake Jingda Experimental Animal Co. LTD (SCXK(HU)2016-0002). The mice were housed in a constant environment (temperature 25°C) with a 12–12 h light-dark cycle and free accesses of food and water. The mice were divided into two groups (n=12): Control group and kainic acid (KA) group. The modeling is carried out according to the method of Beamer et al., (Beamer *et al.,* 2018). In short, the mice were deeply anesthetized with 5% isoflurane. Then, a guide sleeve was installed on the dura mater of mice and fixed with gutta percha for KA injection. KA was injected into the amygdala under a unilateral stereotaxic microscope (0.3 μg KA in 0.2 μl solvent, pH 7.4). Non epileptic control mice were injected with 2 μl of solvent in the same way. Three days later, the mice were exposed to Ad-vector, Ad-*Uca1* or Ad-sh*Sfrp1*, respectively. Finally, the mice were euthanized by cervical dislocation, and PBS and 4% paraformaldehyde (PFA) were perfused into the brain for analysis.

Isolation of primary mouse hippocampal neurons

After the hippocampal tissue was cut into pieces, D-hank's solution and 0.25% trypsin were added, gently blown and centrifuged, and all supernatant was collected. Sieve with 200 mesh and centrifuge for 5 min at 1500 r/min. Discard supernatant, add medium to resuspend cells. Cells (50 000 cells/cm²) were cultured in Neurobasal Media including 5% FBS, B27, 2 mM GlutaMAX-I, penicillin/streptomycin and 15 mM glucose at 37°C with 5% CO_2 in a humidified incubator, and transfected using Lipofectamine2000 (Invitrogen).

UCA1 overexpression and SFRP1 knockdown

UCA1 gene was integrated into pHBAd-U6 (Wujia, Beijing, China) to construct recombinant vector pHBAd-*U6-UCA1*. Ad-vector was used as a blank control. The successful construction of the vector was confirmed by sequencing and restriction enzyme digestion. The *Sfrp1* shRNA-targeting sequence (sh#1, 5'-CATGGCCTAACGGACGTAAA-3') or control shRNA sequence was inserted into pHBAd-U6 for adenovirus production. Mice in the normal group and KA group were injected with 1×10^7 pfu/10μL purified recombinant Ad-*Uca1* or Ad-sh*Sfrp1* intraperitoneally to overexpress UCA1 or knock down SFRP1.

Cell culture

HEK-293 cells were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China) and maintained in DMEM (Thermo Scientific, San Diego, CA) supplemented with 10% FBS (Thermo Scientific), penicillin (100 U/ml) (Thermo Scientific) and streptomycin (100 μg/ml) (Thermo Scientific) at 37°C with 5% CO_2. All transfections were conducted using Lipofectamine2000 (Invitrogen).

RT-qPCR

The expression of *Uca1*, S*frp1* and miR-375 in the hippocampus was detected. Total RNA was extracted from hippocampus or cells using Qiazol Lysis Reagent (Qiagen). RNAs were reverse transcribed to cDNA and quantified by qPCR using Quant One Step RT-qPCR Kit (SYBR Green) according to the manufacturer's protocol (Tiangen, Beijing, China). Relative quantities were calculated using $2^{\Delta\Delta CT}$ after normalization to *Gapdh*. Primers are as follows:

Uca1, F, 5'-ACTCCGGACTAGCTGCAAGC-3', R, 5-TGCCAACATACAACGTGGT-3';

Sfrp1, F, 5'-TAACTGCGGTAACTTCCTTCAG-3', R, 5-CCTTAAGCTGTTAACGTG-3';

miR-375, F, 5'-CTGGACTATCGTAAACGT-3', R, 5-TAACGTACGTAAACGTGCCG-3';

Brdu staining

Three days after kainic acid administration and after shRNA administration, the mice were injected intraperitoneally with BrdU at 50 μg/g body weight 4 hours before sacrifice to mark the proliferating cells. BrdU-positive cells were detected using BrdU Labeling and Detection Kit I according to manufacturer's procedures (Roche, Indianapolis, IN)

Dual luciferase reporter assay

MiRDB (http://mirdb.org/) and TargetScan (http://www.targetscan.org/vert_72/) were used to predict the targeted binding sites of miR-375 with *Uca1* and *Sfrp1*, respectively. Subsequently, the hypothetical targeting relationship was further confirmed by dual luciferase reporter assay. In Brief, dual luciferase reporter gene vectors containing wild type (WT) sequence of *Uca1* and *Sfrp1* 3 untranslated-untranslated region (3'-UTR) and mutant (MUT) sequence were constructed, which were named PmirGLO-*Uca1*-WT, PmirGLO-*Uca1*-MUT, PmirGLO-*Sfrp1*-WT and PmirGLO-*Sfrp1*-WT, respectively. Then, the recombinant plasmid and miR-375 mimic plasmid or NC plasmid or miR-375 inhibitor plasmid or NC inhibitor plasmid were co-transfected into HEK-293 cells respectively. After 24 hours, the cells were lysed and luciferase activity was detected by dual luciferase reporter gene detection system (E1910, Promega Corp., Madison, WI).

RNA immunoprecipitation (RIP) assay

RIP assay was conducted using magna RNA binding protein immunoprecipitation kit (Millipore) according to manufacturer's instructions. The cell lysate was incu-

bated with human anti-*Uca1* antibody (proteintech) or anti-mouse IgG coupled magnetic bead RIP buffer. Co-precipitated RNA was detected by qRT-PCR. To demonstrate that the detected RNA signals specifically bind to *Uca1*, we examined both total RNA (input control) and IgG controls.

Western blotting

Total protein in hippocampus or HEK-293 cells was lysed using Cell lysis buffer for Western and IP (Beyotime, Shanghai, China) according to the manufacturer's instructions. Cytoplasmic protein and nuclear protein were extracted by cytoplasmic protein extraction kit (BC3740, Solarbio, Beijing, China) and nuclear protein extraction kit (R0050, Solarbio, Beijing, China), respectively. Protein concentration was then measured using BCA Protein Assay Kit (Tiangen, Beijing, China), following protein separation by SDS-PAGE. Thereafter, the protein was then transferred to PVDF membrane, and then blocked with 5% bovine serum albumin. After washed with TBST, the samples were incubated at 4°C overnight with primary antibodies to SFRP1 (1:230, ab4193, Abcam, Cambridge, UK), β-catenin (1:4000, ab6302, Abcam, Cambridge, UK) and β-actin (1:2000, ab8227, Abcam, Cambridge, UK). The next day, after washed with TBST for 10 min, the samples were incubated with Goat Anti-Rabbit IgG H&L (HRP) (ab205718, 1:30 000, Abcam, Cambridge, UK) at room temperature for 1 h. Finally, the blots were analyzed using ImageJ 1.48u software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Data was exhibited as mean ± standard derivation (mean ± SD) and analyzed using SPSS 21.0 (IBM Corp. Armonk, NY, USA). Differences between two groups were analyzed by t-test, while differences between three groups or more were analyzed by one-way analysis of variance (ANOVA) following post *hoc*. Pearson was used to analyze the correlation between *Uca1* and miR-375, miR-375 and *Sfrp1* and *Uca1* and *Sfrp1* expression. $p<0.05$ means significant difference.

RESULTS

Correlation of *Uca1*, miR-375 and *Sfrp1* expression in hippocampus of epileptic mice

In this study, the expressions of *Uca1*, *Sfrp1* and miR-375 in hippocampal tissues of KA-induced epileptic mice were firstly investigated. As shown in Fig. 1A–C, the expression of *Uca1* and *Sfrp1* in hippocampal tissues of epileptic mice were significantly reduced, while the expression of miR-375 was significantly increased compared with the control group. Pearson correlation analysis showed that *Uca1* was positively correlated with SFRP1, while miR-375 was negatively correlated with *Uca1* and SFRP1 (Fig. 1D). These results indicated that abnormal expressions of *Uca1*, miR-375 and *Sfrp1* were related to epilepsy.

Uca1 overexpression reduced abnormal proliferation of neural progenitors in epileptic mice

To investigate the effect of *Uca1* on the proliferation of neural progenitors in epileptic mice, *Uca1* was ectopically expressed. As shown in Fig. 2A, the expression of *Uca1* in hippocampal nerve tissues of the Ad-*Uca1* group was increased sharply in normal mice and epileptic mice compared with the Ad-vector group (Fig. 2A). Besides, BrdU staining showed that the overexpression of *Uca1* in normal mice had no significant effect on the prolif-

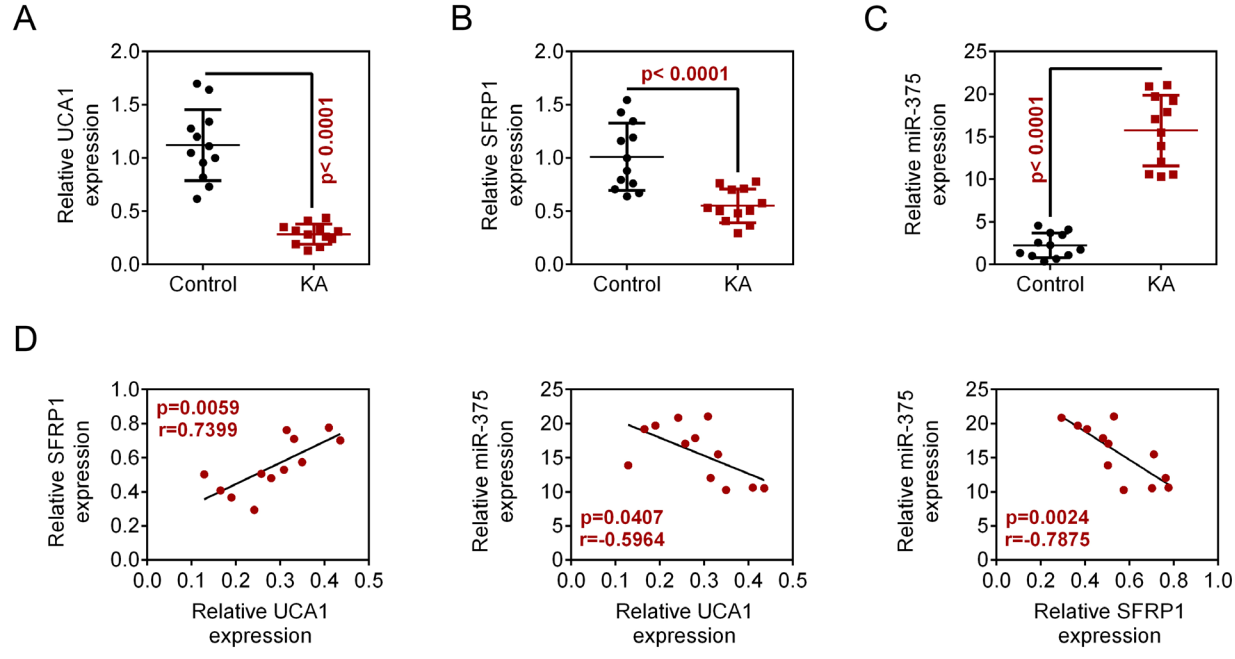

Figure 1. Correlation of *Uca1*, miR-375 and *Sfrp1* expression in hippocampus of epileptic mice.
The mice were divided into two groups (n=12): Control group and KA group. A. The mRNA level of *Uca1* was monitored by RT-qPCR. ($p<0.0001$) B. The mRNA level of *Sfrp1* was monitored by RT-qPCR. ($p<0.0001$) C. The mRNA level of miR-375 was monitored by RT-qPCR. ($p<0.0001$) D. Pearson was used to analyze the correlation between *Uca1* and miR-375, miR-375 and *Sfrp1* and *Uca1* and *Sfrp1* expression. ($p<0.05$)

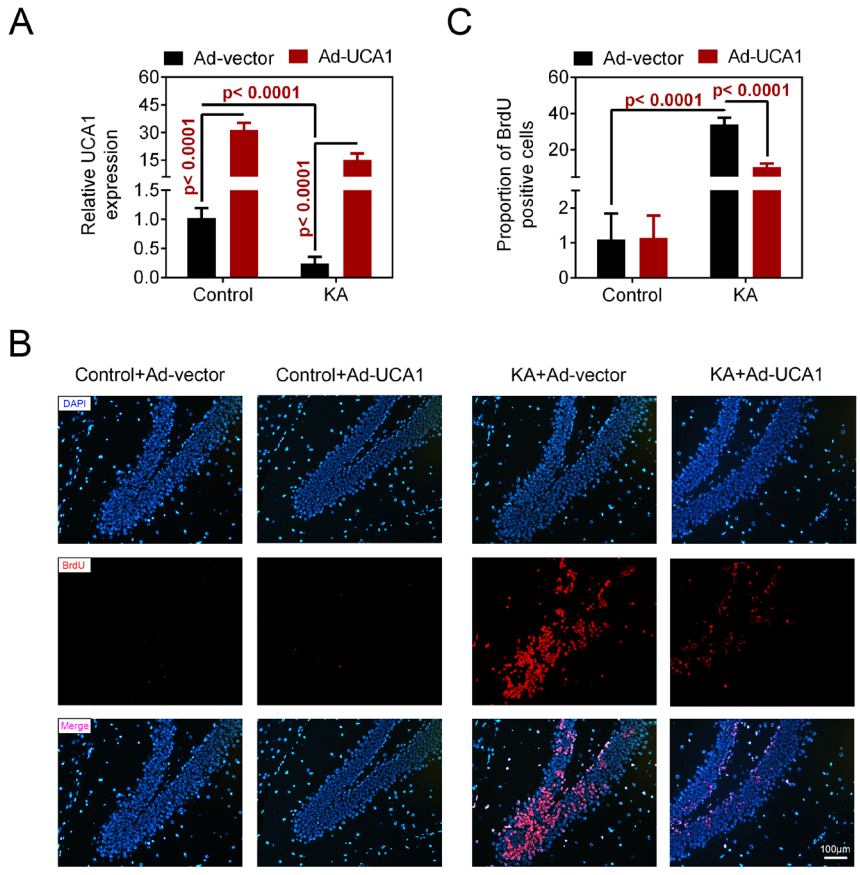

Figure 2. Up-regulation of *Uca1* expression reduced abnormal proliferation of neural progenitors in epileptic mice.
The mice were divided into two groups (n = 12): Control group and KA group. adenovirus Ad-vector or Ad-*Uca1* were infected into normal mice or epileptic mice. A. The mRNA level of *Uca1* was monitored by RT-qPCR. (p<0.0001) B–C. The proliferation of neural progenitors in hippocampus was monitored by Brdu staining. (p<0.0001)

eration of neural progenitors, but in epileptic mice, the number of BrdU positive neurons in Ad-vector group increased sharply. Surprisingly, overexpression of *Uca1* significantly reduced the number of BrdU positive neurons in epileptic mice (Fig. 2B and C). In general, these results showed that up-regulation of *Uca1* expression reduced abnormal proliferation of neural progenitors in epileptic mice.

Uca1 was combined with miR-375

The target binding sites of miR-375 and *Uca1* was predicted by miRDB. (Fig. 3A). Besides, the targeting relationship between miR-375 and *Uca1* was further confirmed by dual luciferase reporter assay and RIP. As shown in Fig. 3B, after co-transfection of *Uca1*-WT and miR-375 mimic into HEK-293 cells with Lipofectamine 3000, the luciferase activity was decreased significantly, while after co-transfection of *Uca1*-WT and miR-375 inhibitor, the luciferase activity of HEK-293 cells was increased (Fig. 3B). RIP assay further showed that compared with IgG, UCA1 was significantly enriched in Ago2, indicating that UCA1 directly binds to Ago2 (Figure 3C). qPCR further showed that *Uca1* overexpression significantly reduced the expression of miR-375 in the hippocampal tissues of epileptic mice, but had no significant effect in normal mice (Fig. 3D). Together, these results suggest that *Uca1* was combined with miR-375.

Uca1 overexpression promoted *Sfrp1* expression through miR-375 to regulate WNT/β-catenin pathway

The targeted binding site of miR-375 to *Sfrp1* was predicted by TargetScan (Fig. 4A). Besides, the targeting relationship between miR-375 and *Sfrp1* was further confirmed by dual luciferase report assay. As shown in Fig. 4B, after co-transfection of *Sfrp1*-WT and miR-375 mimic into HEK-293 cells with Lipofectamine 3000, the luciferase activity of HEK-293 cells was decreased significantly, while after co-transfection of *Sfrp1*-WT and miR-375 inhibitor, the luciferase activity of HEK-293 cells was increased. Besides, after transfection of NC mimics, miR-375 mimic, NC inhibitor (NC inh) or miR-375 inhibitor (miR-375 inh) into neurons with Lipofectamine 3000, respectively, abnormal expression of *Sfrp1* and β-catenin was observed. Western blotting further showed that overexpression of miR-375 significantly inhibited the expression of *Sfrp1* in neurons and promoted the expression of β-catenin, while the low expression of miR-375 showed the opposite effect (Fig. 4C). It is worth noting that *Uca1* overexpression significantly promoted the expression of *Sfrp1*, while suppressing the expression of β-catenin. Interestingly, miR-375 overexpression significantly reversed the effect of high expression of *Uca1* on the expression of *Sfrp1* and β-catenin (Fig. 4D). Together, these results suggest that up-regulation of *Uca1* promoted *Sfrp1* expression through miR-375 to regulate WNT/β-catenin pathway.

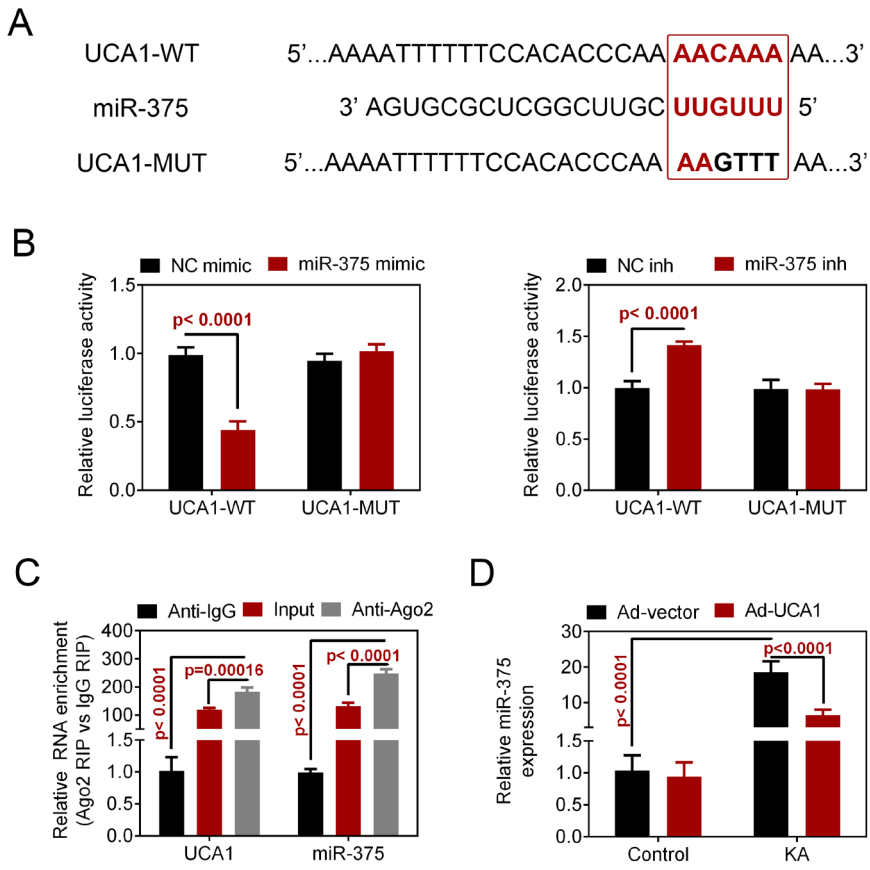

Figure 3. *Uca1* was combined with miR-375.
A. The target binding sites of miR-375 and *Uca1* was predicted by miRDB. B. Luciferase activity was monitored by dual luciferase reporter assay. (p<0.0001) C. The targeting relationship between miR-375 and *Uca1* was further confirmed by RIP assay. (p<0.0001) D. The mRNA level of miR-375 was monitored by RT-qPCR. (p<0.0001)

Uca1 reduced the abnormal proliferation of neural progenitors in epileptic mice by regulating the expression of *Sfrp1*

Finally, this study explored whether *Sfrp1* is involved in the regulation of abnormal proliferation of neural progenitors by *Uca1*. Epileptic mice were injected with Ad-*Uca1* or/and *shSfrp1* three days after kainic acid administration. The protein levels of SFRP1 and β-catenin was monitored by western blotting. As shown in Fig. 5A, *Uca1* overexpression promoted the expression of *Sfrp1* and suppressed the expression of β-catenin. After knockdown of *Sfrp1* by shRNA *in vivo*, the expression of *Sfrp1* was significantly reduced, while the expression of β-catenin was increased. Not only that, *Sfrp1* knockdown reversed the effect of UCA1 on the expression of *Sfrp1* and β-catenin. In addition, functional analysis showed that *Uca1* overexpression inhibited the abnormal proliferation of neural progenitors, while sh-*Sfrp1* showed the opposite effect, and *Sfrp1* knockdown reversed the inhibitory effect of *Uca1* on abnormal neuronal proliferation (Fig. 5B). Taken together, these results indicate that *Uca1* reduces the abnormal proliferation of neural progenitors in epileptic mice by regulating the expression of *Sfrp1*.

DISCUSSION

TLE is one of the most common types of intractable epilepsy, characterized by periodic seizures and unpredictability (Han *et al.,* 2018). Long-term recurrent seizures or epileptic status in the developmental stage can cause many cognitive disorders, such as impairment of learning, memory, language, etc (Jones-Gotman *et al.,* 1997; Boling 2018; Buck & Sidhu 2020). Studies have shown that epileptic seizures are closely related to the abnormalities of hippocampal structure and function (Perkins *et al.,* 2017). Epilepsy can cause the abnormal enhancement of hippocampal neurogenesis which is closely related to cognitive function (Cho *et al.,* 2015). Therefore, it is very important to study the effect of epilepsy on hippocampal neurogenesis and early intervention. In this study, we successfully constructed kainic acid-induced epileptic mice model, and explored the role and potential molecular mechanism of lncRNA *Uca1* in epileptic mice. The results found a new molecular mechanism of *Uca1* in epilepsy, that is, the overexpression of *Uca1* could significantly inhibit the abnormal proliferation of hippocampal neurons by WNT/β-catenin pathway via regulating *Sfrp1* expression. Our findings provided a basis for early intervention of epilepsy.

Epilepsy is closely related to the abnormal regulation of lncRNAs (Qiao *et al.,* 2018; Villa *et al.,* 2019). It was found that 384 or 279 lncRNAs were significantly deregulated in pilocarpine or KA-induced epilepsy mouse models (Lee *et al.,* 2015). *Uca1* is an oncogene and plays an important role in the development of tumors. Notably, *Uca1* has been proved to promote the proliferation and differentiation of neural stem cells (Liu *et al.,* 2015). In epilepsy, Geng et al., found that *Uca1* inhibited the apoptosis of hippocampal neurons, thus inhibiting the brain injury caused by epilepsy (Geng *et al.,* 2018). The study of Wang et al revealed that *Uca1* inhibited

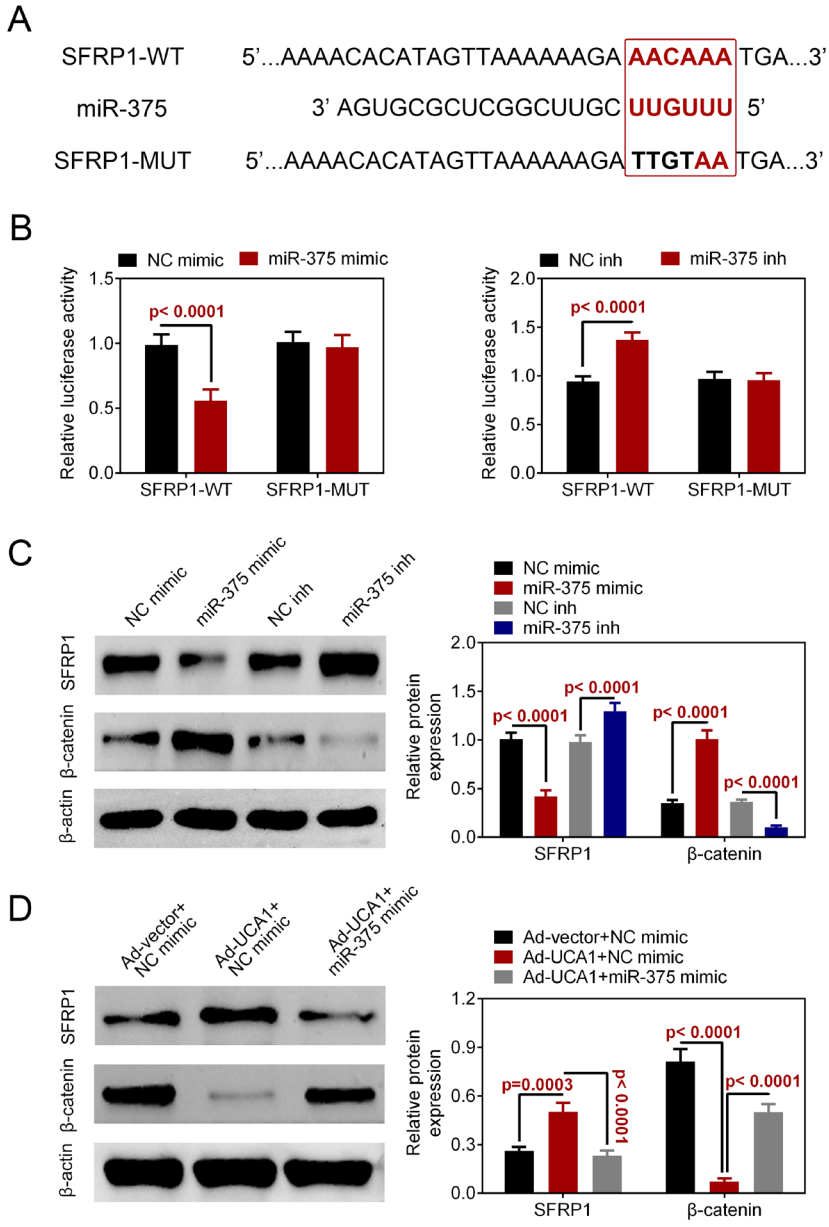

Figure 4. Up-regulation of *Uca1* promoted *Sfrp1* expression through miR-375 to regulate WNT/β-catenin pathway.
The target binding sites of miR-375 and *Sfrp1* was predicted by TargetScan. B. Luciferase activity was monitored by dual luciferase reporter assay. (p<0.0001) C. Neurons were transfected with NC mimic, miR-375 mimic, NC inhibitor or miR-375 inhibitor with Lipofectamine 3000 (Invitrogen), respectively. The protein levels of SFRP1 and β-catenin was monitored by western blotting. D. Neurons were co-transfected with Ad-vector and NC mimic or co-transfected with Ad-*Uca1* and NC mimic or miR-375 mimic. (p<0.001)

the activation of hippocampal astrocytes and improved the learning and memory ability of epilepsy rats, and had a protective effect on neuronal damage caused by KA. Further study clarifies that the role of *Uca1* in epilepsy rats may be achieved by regulating the JAK/STAT signaling pathway (Wang *et al.,* 2020). In addition, Yu et al., found that the expression of *Uca1* in the SD rat epilepsy model established by lithium chloride and pilocarpine was also down-regulated. *Uca1* overexpression can inhibit the epileptic inflammation by regulating the miR203/MEF2C/NF-κB axis (Yu *et al.,* 2020). Consistent with the above results, this study constructed a KA-induced mouse epilepsy model and found that *Uca1* is low-expressed in epileptic mice. And the low expression of *Uca1* caused abnormal proliferation of hippocampal neurons in epileptic mice. After forced overexpression of *Uca1*, the abnormal proliferation of hippocampal neurons was suppressed. Further mechanistic analysis showed that *Uca1* could be targeted to downstream miR-375 to promote the expression of *Sfrp1*, thereby inhibiting the activation of WNT/β-catenin signaling pathway. Overall, this study considers *Uca1* as a potential target for clinical treatment of epilepsy.

There is increasing evidence that miRNAs exhibit abnormal regulation in epilepsy (Jimenez-Mateos *et al.,* 2011; Hu *et al.,* 2012). These abnormally regulated miRNAs mainly participate in the occurrence of epilepsy by regulating cell proliferation and migration, neuroinflammation and neuronal apoptosis (Karnati *et al.,* 2015). MiR-375 is a widely studied miRNA that has been proved to participate in tumorigenesis (Wang *et al.,* 2016; Kang *et al.,* 2018). Studies have shown that

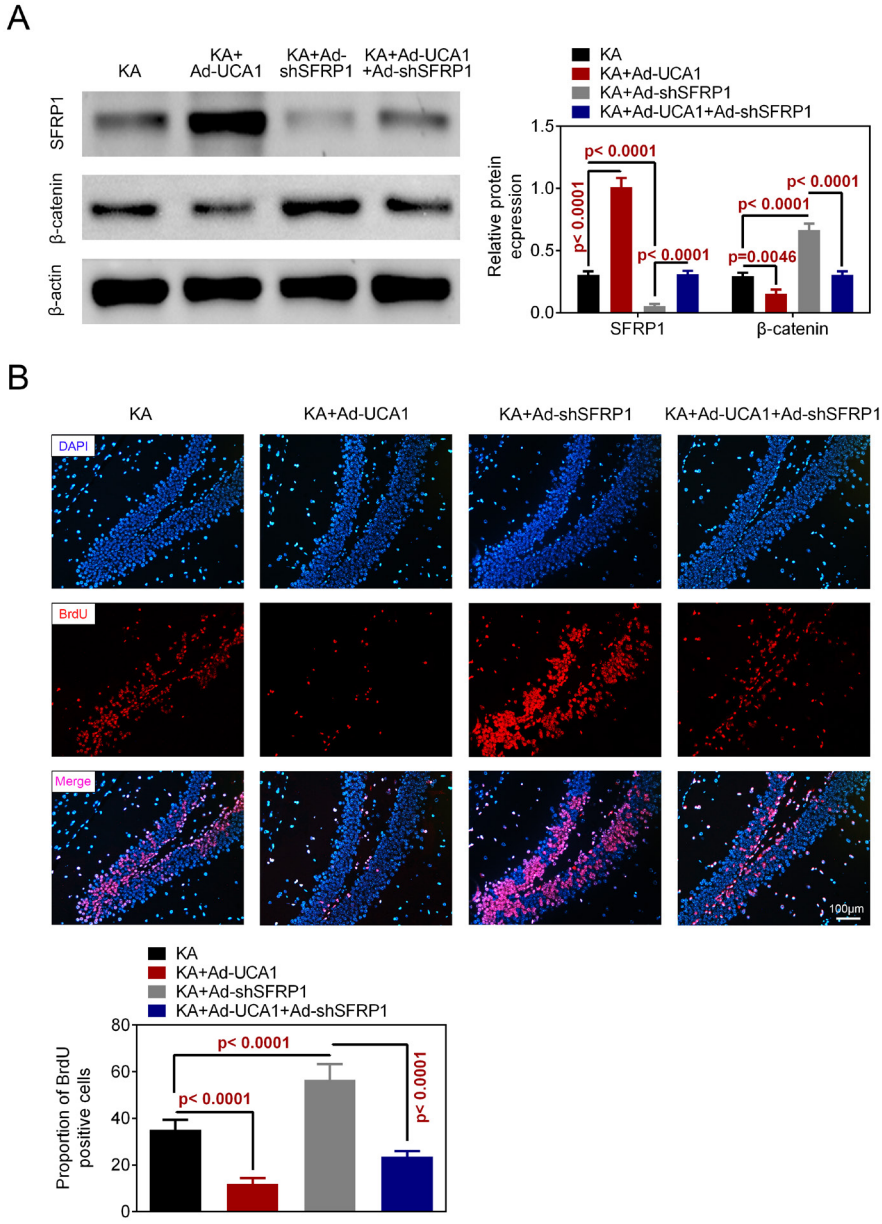

Figure 5. Up-regulation of *Uca1* reduced nerve cell abnormal proliferation through SFRP1/WNT/β-catenin pathway.
Epileptic mice were injected with Ad-*Uca1* or/and sh*Sfrp1* three days after kainic acid administration. A. The protein levels of SFRP1 and β-catenin was monitored by western blotting. (p<0.0001) B. The proliferation of neural progenitors in hippocampus was monitored by Brdu staining. (p<0.0001)

the expression of miR-375 was upregulated in the KA-induced SD epilepsy rat model (Henshall 2013), indicating that the abnormal expression of miR-375 may be related to the occurrence of epilepsy. However, the underlying molecular mechanism of the high expression of miR-375 for epilepsy remains unknown. In this study, a KA-induced epilepsy rat model was constructed, and the expression of miR-375 in the hippocampus tissues of epilepsy mice and normal mice was detected. Consistent with the above results, this study also found that miR-375 was highly expressed in KA-induced epilepsy mice. Further analysis showed that *Uca1* targeted miR-375 and reduced the level of miR-375 in the hippocampus of epilepsy mice, which further inhibited the abnormal proliferation of hippocampal neurone. TargetScan analysis showed that *Sfrp1* was also a target of miR-375. Correlation analysis showed that miR-375 was negatively correlated with the expression of *Uca1* and *Sfrp1*.

SFRP1 is a negative regulator of WNT signaling and dose-dependently regulate the development of midbrain dopamine neurons (Kele *et al.,* 2012). SFRP1 participates in the regulation of WNT/β-catenin pathway by suppressing the accumulation of β-catenin through a GSK-3 dependent mechanism, which interferes with the binding receptor of WNT and FRIZZLED protein (Kawano & Kypta 2003; Galli *et al.,* 2006). The WNT/β-catenin pathway regulates hippocampal neurogenesis, synaptic division, and mitochondrial regulation, and is critical to the development and function of the central nervous system (Rubio *et al.,* 2020). WNT/β-catenin signals modulate epileptic neurogenesis and neuronal death. It also plays a role in the susceptibility of epilepsy and the development of chronic epilepsy, and has been found to be a

promising antiepileptic target for the treatment of epilepsy in the future (Hodges & Lugo 2018). In this study, it was found that miR-375 could promote the activation of WNT/β-catenin pathway by targeting *Sfrp1*, thus promoting the abnormal proliferation of hippocampal neurone in epileptic mice. It is worth noting that UCA1 overexpression can inhibit the activation of WNT/β-catenin pathway and prevent epilepsy by reducing the level of miR-375 and alleviating the inhibitory effect of miR-375 on *Sfrp1*.

In conclusion, we found that *Uca1* was highly expressed in epileptic mice and miR-375 was poorly expressed. Further studies have shown that *Uca1* can promote the expression of *Sfrp1* by reducing the level of miR-375, which further inhibits the abnormal proliferation of neural progenitors in epileptic mice by inhibiting the activation of the WNT/β-catenin pathway.

Acknowledgements

Not applicable.

Competing interests

The authors state that there are no conflicts of interest to disclose.

Ethics approval

Ethical approval was obtained from the Guangxi University of Chinese Medicine Institutional Review Board.

Statement of Informed Consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Limei Diao, Haichun Yu and Huaqiong Li designed the study, supervised the data collection, Yueqiang Hu, Mingfen Li and Qianchao He analyzed the data, interpreted the data, Ling Lu, Huan Li and Xianqiu Liao prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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