■ Original article

Epileptic Disord 2022; 24 (5): 917-927

Epileptic Disorders

lncRNA UCA1 induces autophagic gene expression via epigenetic regulation mediated by ATG16L1 and miR-132-3p in SH-SY5Y cells treated with retinoic acid

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Received April 25, 2022; Accepted June 18, 2022

ABSTRACT

Objective. Epilepsy is a chronic brain disease with recurrent seizures. Autophagy plays a crucial role in the progression of epilepsy. This study aimed to explore the function and intrinsic mechanism of the long non-coding RNA (lncRNA) UCA1/miR-132-3p/ATG16L1 axis in epilepsy via regulation of autophagy.

Methods. The expression of lncRNA UCA1, miR-132-3p and ATG16L1 was measured in serum from epileptic patients by quantitative RT-PCR. A SH-SY5Y cell model was further constructed using retinoic acid to investigate the UCA1/miR-132-3p/ATG16L1 axis by quantitative RT-PCR, western blotting, fluorescence *in situ* hybridisation, RNA immunoprecipitation, chromatin immunoprecipitation, and a dual-luciferase reporter gene assay.

Results. In the serum of epileptic patients, the level of lncRNA UCA1 and ATG16L1 was reduced and miR-132-3p elevated, compared to controls. Similarly, in the SH-SY5Y cell model, the level of lncRNA UCA1 and ATG16L1 was reduced and miR-132-3p elevated in retinoic acid-treated cells; lncRNA UCA1 was mainly located in the cytoplasm. lncRNA UCA1 overexpression was shown to promote autophagic gene expression, which was reversed by miR-132-3p overexpression. Moreover, autophagic gene expression induced by miR-132-3p knockdown was reversed by ATG16L1 knockdown. Based on precipitation assays, lncRNA UCA1 and miR-132-3p were shown to form a complex with the transcription factor, EZH2, and miR-132-3p was shown to interact with ATG16L1 based on a luciferase assay. Finally, lncRNA UCA1 was shown to negatively regulate miR-132-3p expression, and miR-132-3p was shown to negatively regulate ATG16L1.

Significance. In this cell model, lncRNA UCA1 promotes autophagic gene expression via epigenetic regulation mediated by ATG16L1 and miR-132-3p.

Key words: lncRNA UCA1, ATG16L1, miR-132-3p, epilepsy, autophagy

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doi:10.1684/epd.2022.1477

Epilepsy is a brain disease mainly caused by an excessive discharge of brain neurons, which is associated with seizures caused by paroxysmal and self-limiting supersynchronisation of neurons, characterised as paroxysmal, transient, repetitive and rigid [1-3]. In recent years, autophagy has been confirmed to play a role in the
occurrence and development of epilepsy and plays a crucial role in disease progression [4]. As a potential therapeutic target for epilepsy, autophagy has attracted more and more attention. The treatment of epilepsy has gradually expanded from traditional drug therapy to surgical therapy, gene therapy, cell transplantation, nerve stimulation and other methods, but the therapeutic effects of these methods are still unsatisfactory [5]. The mechanism associated with autophagy in epilepsy requires further elucidation and may thus reveal new targets for diagnosing and treating epilepsy.

Long non-coding RNAs (lncRNAs) are usually defined as RNA molecules >200 ncleotides long [6]. lncRNA is related to the pathogenesis of neuropsychiatric diseases (such as epilepsy) [7, 8]. lncRNA UCA1 regulates neural progenitor cell proliferation and differentiation *in vitro* [9]. Studies have found that lncRNA UCA1 expression is decreased in epilepsy and high expression is associated with suppression of the development of epilepsy [8]. lncRNA UCA1 inhibits hippocampal neuronal apoptosis through the miR-495/Nrf2-ARE pathway, thereby inhibiting pilocarpine-induced epileptic brain injury [10]. However, there are few reports on the regulatory molecular mechanism of lncRNA UCA1 in epilepsy and the effects of lncRNA UCA1 on autophagy, which is worthy of further investigation.

Enhancer of zeste homologue 2 (EZH2) is a key regulator of epigenetic modifications, which exerts its transcriptional inhibitory function mainly by catalysing the trimethylation of Lys27 on histone 3 (H3K27me3) [11, 12]. MicroRNAs (miRNAs) are known to regulate apoptosis, inflammation, autophagy, pyroptosis and other physiological processes [13-16]. Studies have shown that abnormal miRNA expression is an important factor in the development of epilepsy [17, 18]. miR-132 interacts with the p250GAP/Cdc42 pathway and is related to the progression of epilepsy [19]. In addition, miR-132 expression can be repressed by H3K27me3. Experiments have confirmed that the miR-132 promoter region is rich in EZH2 and H3K27me3 binding sites, suggesting that miR-132 downregulation is related to EZH2-mediated histone methylation [20]. lncRNA UCA1 interacts with EZH2 to inhibit downstream target gene expression [21, 22]. These results indicate that lncRNA UCA1 is an important epigenetic regulator, and that it can interact with EZH2, which mediates its effects. It was speculated that miR-132 expression in epilepsy might be linked to lncRNA UCA1-mediated epigenetic regulation, however, its role in epigenetic regulation and whether it affects autophagy in epilepsy models have not been reported.

Autophagy-associated 16-like protein 1 (ATG16L1) is a recombinant protein with multiple functions in autophagy and related processes [23]. ATG16L1 is underexpressed in epilepsy and ATG16L1 can activate downstream signalling pathways, the activation of which can promote autophagy, thus affecting the progression of epilepsy [24]. In addition, miR-132 is predicted to bind ATG16L1, based on Starbase. However, whether miR-132 can affect epileptic autophagy by regulating ATG16L1 expression is unknown.

In this study, based on the above background, serum was collected from epileptic patients to determine the levels of lncRNA UCA1, miR-132-3p and ATG16L1, relative to controls. In addition, the possible role of autophagy mediated by lncRNA UCA1/miR-132-3p/ATG16L1 was explored based on an *in vitro* cell model. lncRNA UCA1 was shown to promote autophagic gene expression via epigenetic regulation mediated by ATG16L1 and miR-132-3p. This study highlights a promising therapeutic strategy for epilepsy.

Materials and methods

Clinical samples

Twenty-four newly diagnosed epileptic patients and volunteers from 2020 to 2021 were selected and serum was collected. Patients with epilepsy were 24 to 50 years old and included nine males and 15 females. All subjects agreed to provide the information required for the study. Approval was obtained from The Second Xiang Ya Hospital of Central South University Ethics Committee before the start of the study.

Cell culture and treatment

The human neuroblastoma cell line, SH-SY5Y, was provided by Procell (CL-0208, Wuhan, China). The culture conditions were minimum essential medium/F-12 containing 10% fetal bovine serum and 1% double antibodies. SH-SY5Y cells were induced with 10 μM retinoic acid (RA) for seven days with a magnesium-free extracellular solution to establish an epilepsy cell model, or were cultured under the same conditions without RA as control.

Cell transfection

oe-UCA1, miR-132-3p mimic, miR-132-3p inhibitor and sh-ATG16L1 and the corresponding negative control

oe-NC, mimic-NC, inhibitor-NC and sh-NC were synthesised by Sangon Biotech (Shanghai, China). The above were transfected into SH-SY5Y cells using Lipofectamine 3000 (Invitrogen) reagent and later induced or not by RA for 48 hours.

Cellular localisation of lncRNA UCA1

The nuclei and cytoplasm were isolated using PARIS Kit (Life Technologies, Carlsbad, CA, USA) [21] according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure the cytoplasmic and nuclear expression levels of lncRNA UCA1. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or U1 was used as a cytoplasmic or nuclear internal reference.

Fluorescence *in situ* hybridisation (FISH)

A FISH kit (BIS-P0001; Guangzhou Bersin Biotechnology Co., Ltd., China) was used to detect the subcellular localisation of lncRNA UCA1 and EZH2 according to a previous report [25]. Slides were treated with digoxin-labelled lncRNA UCA1 and EZH2 probes and observed under a Zeiss LSM880 NLO confocal microscope (Leica, Germany).

RNA immunoprecipitation (RIP) assay and chromatin immunoprecipitation (ChIP)

Binding between lncRNA UCA1 and EZH2 was examined using the EZMagna RIP kit (Millipore, USA) according to the manufacturer's specifications. The precipitated RNA was analysed by qRT-PCR. Binding of EZH2 to the miR-132-3p promoter was determined by qRT-PCR based on a ChIP kit [26]. Different primers were used to detect EZH2 binding sites within the promoter of the *miR-132-3p* gene. Binding sites between miR-132-3p and ATG16L1 were predicted using Starbase.

Dual-luciferase reporter gene assay

To verify the binding between miR-132-3p and ATG16L1, a wild-type (WT) or mutant (MUT) *ATG16L1* fragment was constructed and inserted into the pmirGLO vector (Promega). The recombinant vector was transfected into cells using Lipofectamine 3000 reagent (Thermo Fisher Scientific, USA) according to the manufacturer's instructions, and mimic-NC and miR-132-3p mimic were simultaneously transferred into cells. Finally, the Nano-GlO double-luciferase reporting method (Promega) was used to measure luciferase activity.

▼ **Table 1.** Primer sequences.

Primer name	Primer sequences
lncRNA UCA1 (F)	5′-CTTCTCCATTCAGACCGCCA-3′
lncRNA UCA1 (R)	5′-TTTGAGCTTGGAACTGCCCT-3′
LC3 (F)	5′-GAAGTTCAGCCACCTGCCAC-3′
LC3 (R)	5′-TCTGAGGTGGAGGGTCAGTC-3′
SQSTM1 (F)	5′-CCGCCGCTTCAGCTTCTGCT-3′
SQSTM1 (R)	5′-GTTCCCGCCGGCACTCCTTC-3′
miR-132-3p (RT)	5′-GTCGTATCCAGTGCAGGGTCC GAGGTATTCGCACTGGATACGAC CGACCA-3′
miR-132-3p (F)	5′-CGCGTAACAGTCTACAGCCA-3′
ATG16L1 (F)	5′-CAAGGTGGTGTTCTCTGTGC-3′
ATG16L1 (R)	5′-ACACATTCATGCAACCCACC-3′
U6 (F)	5′-CTCGCTTCGGCAGCACA-3′
U6 (R)	5′-AACGCTTCACGAATTTGCGT-3′
GAPDH (F)	5′-CCAGGTGGTCTCCTCTGA-3′
GAPDH (R)	5′-GCTGTAGCCAAATCGTTGT-3′

qRT-PCR

Total RNA was extracted by Trizol (15596026; Thermo Fisher Scientific). RNA was converted to cDNA using a reverse transcription kit (CW2569; Beijing ComWin Biotech, China). GAPDH or U6 was used as the internal reference; primer sequences are shown in *table 1*. Using 2 µg total cDNA as the template, the $2^{-\Delta\Delta Ct}$ method was utilised to calculate the target gene level.

Western blotting

Proteins were extracted from cells and tissue using RIPA lysis buffer (P0013B; Beyotime). Proteins were adsorbed onto the polyvinylidene fluoride membrane and sealed with 5% skimmed milk solution for two hours. Antibodies against ATG16L1 (67943-1-IG; 1:2000, Proteintech), LC3 (18725-1-AP; 1:2000, Proteintech), P62 (18420-1-AP; 1:2000, Proteintech) and β-actin (66009-1-IG; 1:1000, Proteintech) were incubated overnight at 4°C. Secondary antibodies were incubated and ECL chromogenic exposure was performed. β-Actin was used as an internal reference to detect expression levels.

Statistical analysis

GraphPad 8.0 was used for statistical analysis. Data were expressed as the mean ± standard deviation

(SD). The Student's t-test or one-way analysis of variance was applied to analyse two or multiple groups. The Pearson correlation coefficient was used to analyse the correlation between lncRNA UCA1 and miRNA-132-3p and between miRNA-132-3p and ATG16L1 based on clinical samples. $P < 0.05$ was considered statistically significant.

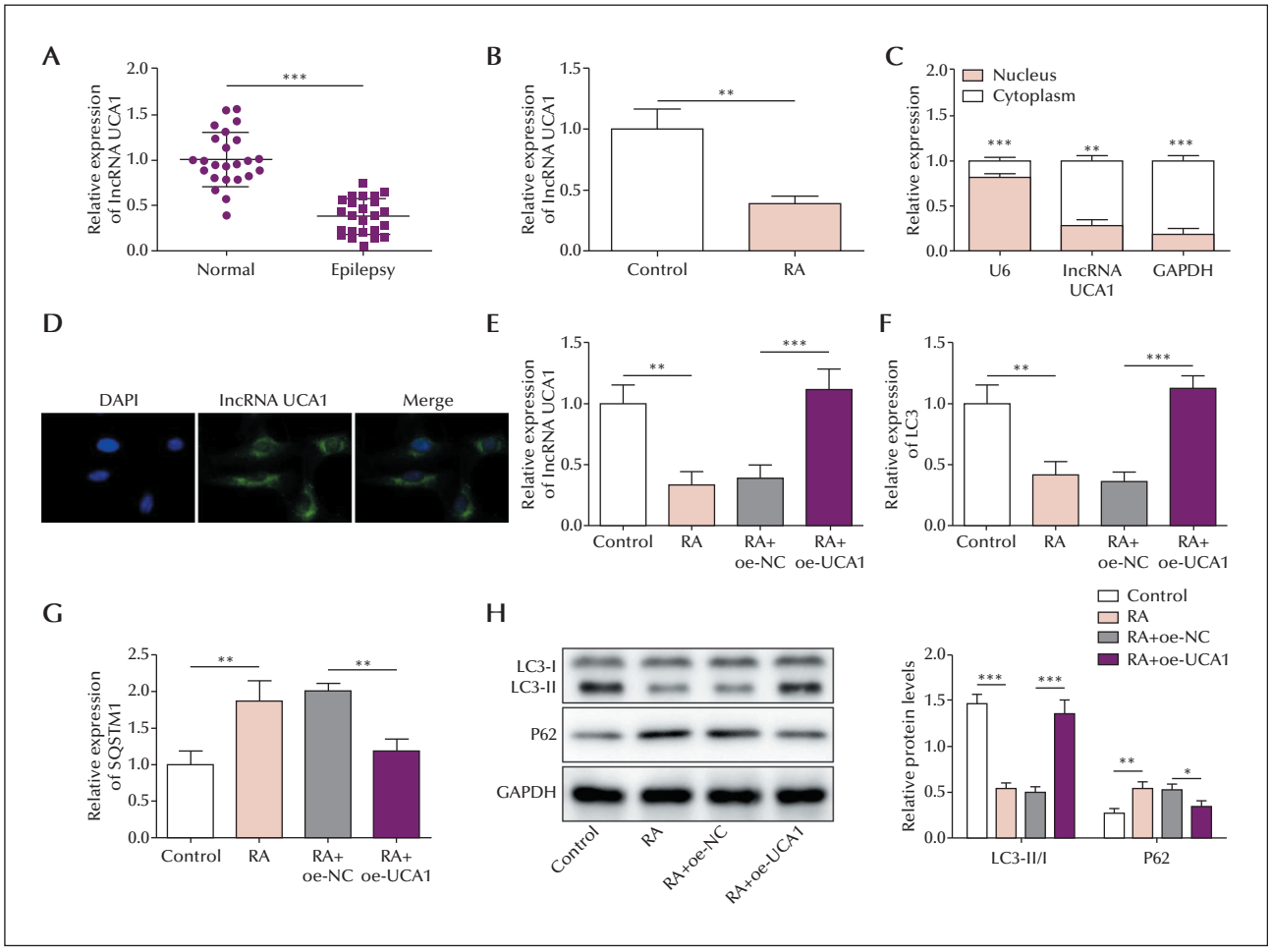
Results

Expression and localisation of lncRNA UCA1

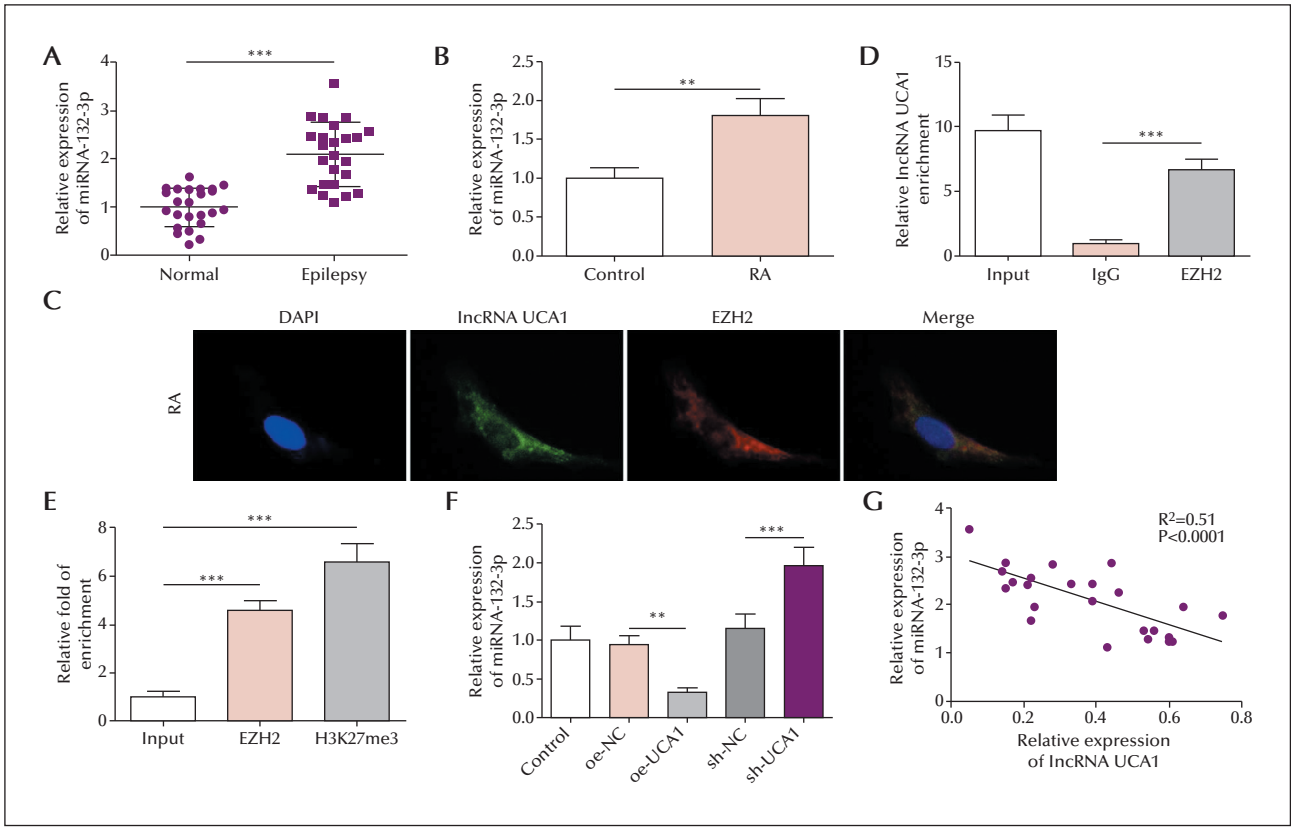
lncRNA UCA1 expression was measured in serum samples of epileptic patients, relative to controls; lncRNA UCA1 expression in the epilepsy group was lower than that in the control group (*figure 1A*). The level of lncRNA UCA1 expression was also investigated in SH-SY5Y cells; the level of lncRNA UCA1 was decreased in RA-treated cells, relative to control (*figure 1B*). Based on cytoplasmic and nuclear RNA extracts, lncRNA UCA1 was shown to mainly localise to the cytoplasm in RA-treated cells based on FISH (*figure 1C, D*).

Effect of lncRNA UCA1 overexpression on LC3 and SQSTM1/P62 in SH-SY5Y cells

lncRNA UCA1 over-expression in RA-treated SH-SY5Y cells was established by transfecting an



■ **Figure 1.** (A) qRT-PCR of lncRNA UCA1 from the serum of epileptic patients ($n = 24$). (B) qRT-PCR of lncRNA UCA1 in SH-SY5Y cells with and without retinoic acid (RA). (C) qRT-PCR of lncRNA UCA1 in cytoplasmic and nuclear extracts of RA-treated SH-SY5Y cells. (D) Localisation of lncRNA UCA1 in RA-treated SH-SY5Y cells by FISH. (E-H) qRT-PCR of lncRNA UCA1 (E), qRT-PCR of LC3 and SQSTM1 (F, G), and western blotting for LC3 and P62 (H) in SH-SY5Y cells over-expressing lncRNA UCA1. Data are expressed as mean ± SD ($n = 3$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.



■ **Figure 2.** (A) qRT-PCR of miR-132-3p from the serum of epileptic patients (n = 24). (B) qRT-PCR of miR-132-3p in SH-SY5Y cells with and without retinoic acid (RA). (C) Colocalisation of lncRNA UCA1 and EZH2 in SH-SY5Y cells by FISH. (D) RNA immunoprecipitation assay for lncRNA UCA1 and EZH2. (E) Chromatin immunoprecipitation assay for EZH2 and miR-132-3p. (F) qRT-PCR of miR-132-3p in SH-SY5Y cells over-expressing lncRNA UCA1 or with lncRNA UCA1 knockdown. (G) Correlation analysis of qRT-PCR for lncRNA UCA1 and miR-132-3p based on serum samples from epileptic patients. Data are expressed as mean ± SD (n = 3). $^{**}p < 0.01$; $^{***}p < 0.001$.

overexpression vector (*figure 1E*). To investigate the effects of lncRNA UCA1 on autophagic gene expression, the levels of LC3 and SQSTM1/P62 were measured. The level of LC3 was notably reduced in RA-treated cells, whereas the levels of SQSTM1/P62 were increased. Over-expression of lncRNA UCA1 caused the level of LC3 to increase, whereas SQSTM1/P62 levels were inhibited (*figure 1F-H*), suggesting that lncRNA UCA1 over-expression promotes autophagy in SH-SY5Y cells.

Expression of miR-132-3p

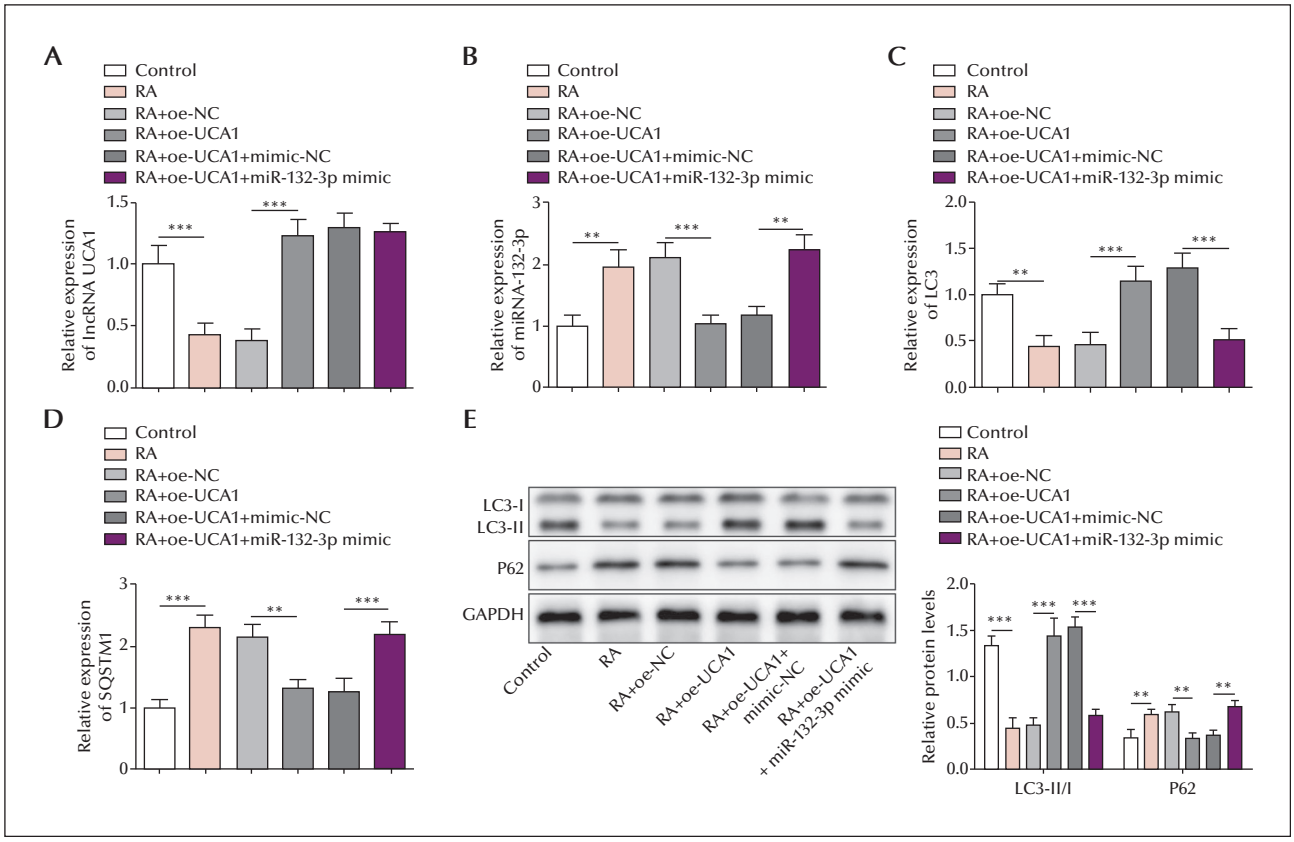
miR-132-3p expression was measured in serum samples from epileptic patients. miR-132-3p levels were increased in the serum of epileptic patients compared to the control group (*figure 2A*). Furthermore, the level of lncRNA UCA1 negatively correlated with that of miR-132-3p (*figure 2G*). In RA-treated SH-SY5Y cells, miR-132-3p levels were also elevated, compared to control (*figure 2B*).

Binding and localisation of EZH2

Based on FISH, lncRNA UCA1 and EZH2 were localised to the nucleus and cytoplasm of SH-SY5Y cells (*figure 2C*). Based on binding assays, EZH2 protein was shown to form a complex with lncRNA UCA1 based on RIP (*figure 2D*), and a complex with miR-132-3p based on ChIP (*figure 2E*).

Effect of lncRNA UCA1 over-expression on miR-132-3p

The level of miR-132-3p was decreased upon over-expression of lncRNA UCA1 and increased upon lncRNA UCA1 knockdown (*figure 2F*). lncRNA UCA1 therefore negatively regulates miR-132-3p expression which may be mediated by EZH2.



■ **Figure 3.** qRT-PCR of lncRNA UCA1 (A), miR-132-3p (B), LC3 (C) and SQSTM1 (D) and western blot analysis of LC3 and P62 expression (E) in retinoic acid (RA)-treated SH-SY5Y cells over-expressing miR-132-3p. Data are expressed as mean ± SD (n = 3). $^{**}p < 0.01$; $^{***}p < 0.001$.

The effect of miR-132-3p over-expression on lncRNA UCA1-induced autophagic gene overexpression

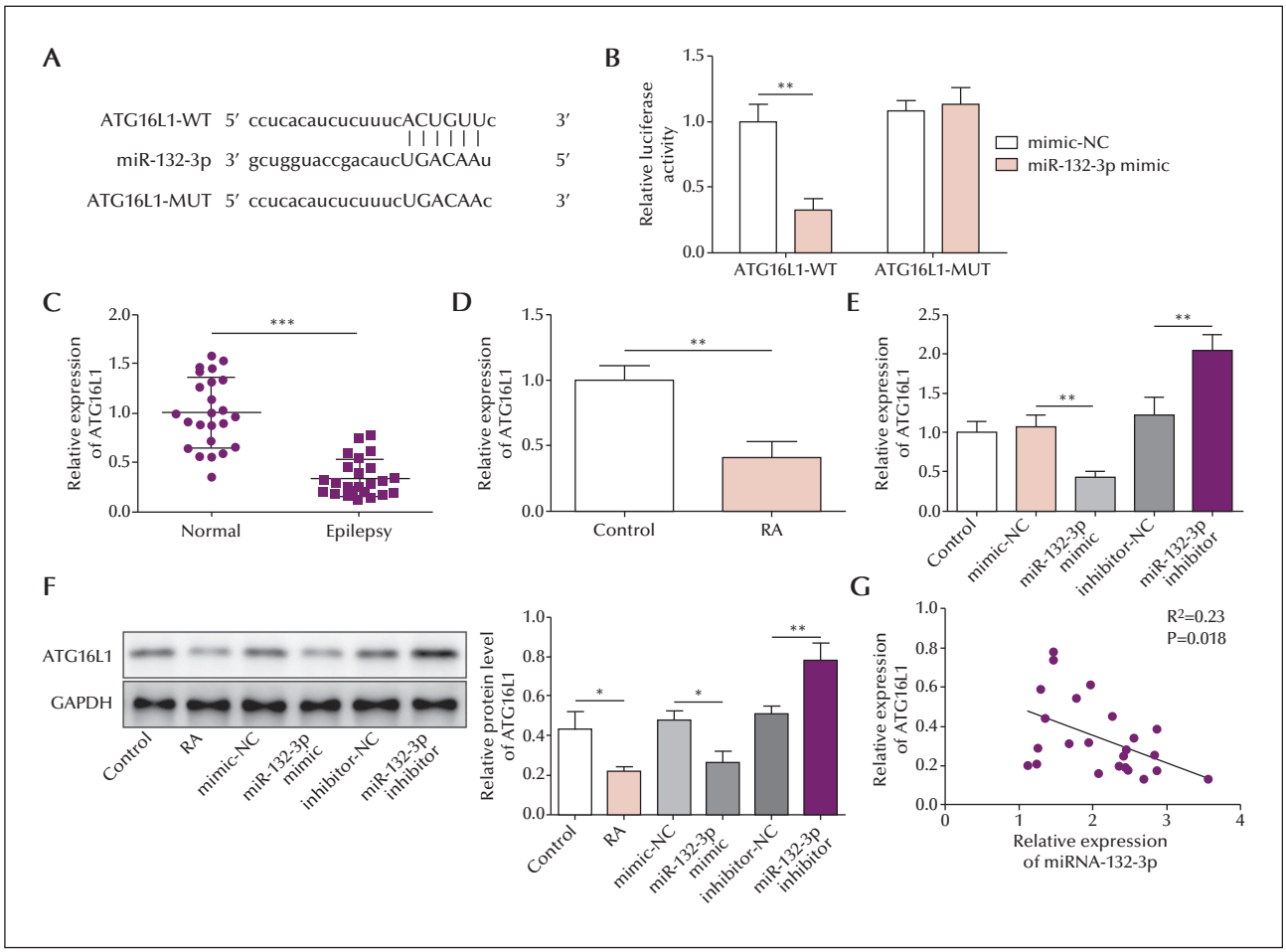
To study the effects of miR-132-3p on SH-SY5Y cells, both miR-132-3p and lncRNA UCA1 were over-expressed; whereas the level of lncRNA UCA1 increased, the level of miR-132-3p decreased after transfection with the lncRNA UCA1 over-expression vector. Upon transfection of miR-132-3p mimic, whereas the level of LncRNA UCA1 was not affected, the level of miR-132-3p increased (*figure 3A, B*). In addition, the level of LC3 increased following lncRNA UCA1 over-expression, whereas the levels of SQSTM1/62 decreased. The effects of lncRNA UCA1 over-expression on LC3 and SQSTM1/P62 levels were reversed by transfecting miR-132-3p mimic (*figure 3C-E*). In brief, miR-132-3p over-expression could reverse the autophagy-promoting effect in SH-SY5Y cells caused by lncRNA UCA1 over-expression.

Expression of ATG16L1

The level of ATG16L1 was shown to be decreased in serum from epileptic patients, compared to controls (*figure 4C*). In SH-SY5Y cells, the level of ATG16L1 was also shown to be decreased in RA-treated cells (*figure 4D*).

Binding between miR-132-3p and ATG16L1

Binding between miR-132-3p and ATG16L1 was predicted based on bioinformatics, revealing a binding site between the two (*figure 4A*). Dual-luciferase reporter gene assays further verified miR-132-3p with ATG16L1 binding. Results showed that the fluorescence activity of cells decreased after cotransfection with ATG16L-WT and miR-132-3p mimic, but was not significantly changed after cotransfection with ATG16L-MUT and miR-132-3p mimic (*figure 4B*).



■ **Figure 4.** (A) Prediction of binding between miR-132-3p and ATG16L1 based on Starbase. (B) Dual-luciferase reporter gene assay to investigate binding between miR-132-3p and ATG16L1. (C) qRT-PCR of ATG16L1 from the serum of epileptic patients (n = 24). (D) qRT-PCR of ATG16L1 in SH-SY5Y cells with and without retinoic acid (RA) (E, F) Expression of ATG16L1 by qRT-PCR (E) and western blotting (F) in SH-SY5Y cells over-expressing miR-132-3p or with miR-132-3p knockdown. (G) Correlation analysis of qRT-PCR for miR-132-3p and ATG16L1 based on serum samples from epileptic patients. Data are expressed as mean ± SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001.

The effect of miR-132-3p overexpression on ATG16L1

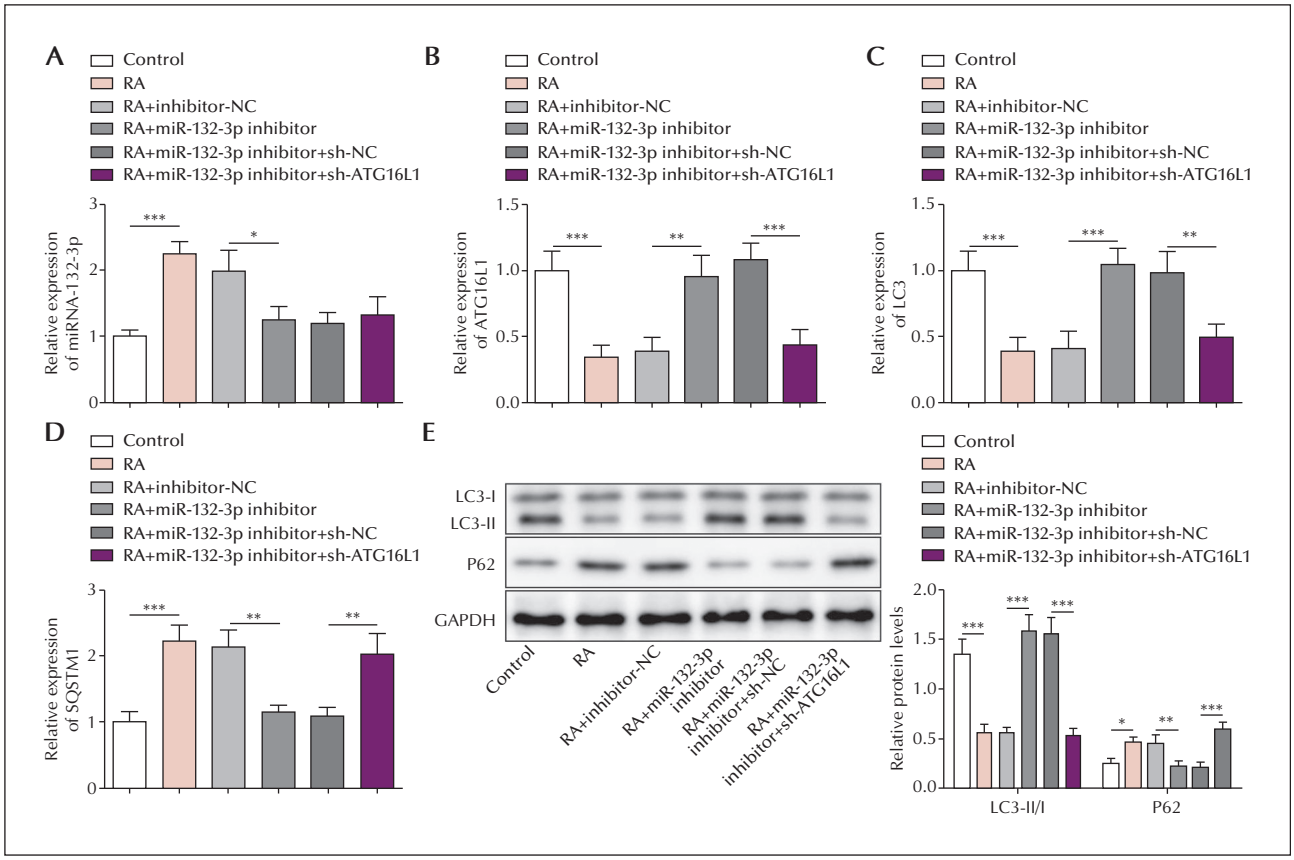
Whereas the level of ATG16L1 was repressed after miR-132-3p overexpression, it was increased in the presence of miR-132-3p inhibitor (*figure 4E, F*). There was therefore a negative correlation between the levels of miR-132-3p and ATG16L1.

Effect of ATG16L1 knockdown on autophagic gene expression induced by miR-132-3p knockdown in SH-SY5Y cells

SH-SY5Y cells were cotransfected with the miR-132-3p inhibitor and sh-ATG16L1 to observe the effects of knockdown of both miR-132-3p and ATG16L1, revealing a decrease of miR-132-3p level and increase of ATG16L1 level (*figure 5A, B*). In addition, the level of LC3 increased and that of SQSTM1/P62 decreased in the presence of miR-132-3p inhibitor. However, upon simultaneous ATG16L1 knockdown, LC3 levels were notably suppressed and SQSTM1/P62 levels were remarkably elevated (*figure 5C-E*), indicating that ATG16L1 knockdown could reverse the autophagy-promoting effect in SH-SY5Y cells induced by miR-132-3p knockdown.

Discussion

Epilepsy is mainly caused by an imbalance between excitation and inhibition of the central nervous



■ **Figure 5.** qRT-PCR of miR-132-3p (A), ATG16L1 (B), LC3 (C) and SQSTM1 (D) and western blot analysis of LC3 and P62 expression (E) in retinoic acid (RA)-treated SH-SY5Y cells with knockdown of miR-132-3p and ATG16L1. Data are expressed as mean ± SD (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001.

system [27]. However, current treatment options have limited effectiveness and can cause problematic adverse reactions. New approaches to treat epilepsy are therefore urgently needed. In this study, the role of the lncRNA UCA1/miR-132-3p/ATG16L1 axis was investigated based on serum samples from patients with epilepsy as well as an *in vitro* cell model. In this epilepsy model, the results suggest that lncRNA UCA1 promotes autophagy which is mediated by ATG16L1 and epigenetic regulation by miR-132-3p. This is a novel finding, as the role of lncRNA UCA1/miR-132-3p/ATG16L1 in autophagy associated with epilepsy has not previously been reported.

In our study, the level of lncRNA UCA1 decreased in serum samples from epileptic patients and in SH-SY5Y cells treated with retinoic acid. More and more evidence indicates that lncRNA is closely related to the progression of epilepsy [3, 28]. Geng *et al.* reported that lncRNA UCA1 was suppressed in epileptic hippocampal tissue and neurons. Moreover, lncRNA UCA1 overexpression suppressed apoptosis of hippocampal neurons by inhibiting miR-495 [10], consistent with this study.

Our results indicate that lncRNA UCA1 overexpression promotes autophagy in SH-SY5Y cells. Autophagy is a highly conserved degradation process that transports dysfunctional proteins, lipids and organelles to lysosomes for degradation [26]. Autophagy is particularly important for post-mitotic and metabolically active cells (such as neurons) [29]. Recent findings suggest that autophagy, as an endogenous self-cleaning pathway in mammals, may be associated with the pathogenesis of epilepsy [30]. Hu *et al.* showed that lncRNA zinc finger antisense 1 knockdown facilitated the activity of status epilepticus in hippocampal neurons and suppressed apoptosis and autophagy [31].

We demonstrate also that lncRNA UCA1 forms a complex with EZH2 and negatively regulates miR-132-3p expression. Moreover, miR-132-3p overexpression may reverse the autophagy-promoting effect in SH-SY5Y cells caused by lncRNA UCA1 overexpression.

This is the first report of the effect of lncRNA UCA1 on factors associated with autophagy in an epilepsy cell model, indicating the role of EZH2 and epigenetic regulation by miR-132-3p. EZH2 modulates various differentially expressed genes in mouse models of acquired epilepsy, and its upregulation in the epileptic brain is considered to be protective [32]. lncRNA can act as a scaffold for various epigenetic proteins (*e.g.* EZH2) [33]. For example, lncRNA HOTAIR regulates the progression of preeclampsia by suppressing miR-106 in an EZH2-dependent manner [34].

Targeting key miRNAs alters brain excitability and inhibits or exacerbates seizures, suggesting that miRNA-based therapies have potential as a treatment for epilepsy [35, 36]. Moreover, miR-132-3p may be the core molecule of tumour-induced epilepsy [37].

In this study, miR-132-3p levels were elevated and ATG16L1 decreased in the serum of epileptic patients and RA-treated SH-SY5Y cells, consistent with previous studies. ATG16L1 is an autophagic protein that is poorly expressed in epilepsy patients and animal/cell models that may promote autophagy and affect the progression of epilepsy [24]. In a kainic acid model of temporal lobe epilepsy, miR-223 affects microglia autophagy through ATG16L1 [24]. miR-223 also inhibits autophagy and facilitates central nervous system inflammation by targeting ATG16L1 [38]. In our study, miR-132-3p was shown to form a complex with ATG16L1 and negatively regulate the level of ATG16L1. Moreover, the low level of ATG16L1 reversed the autophagy-promoting effect in SH-SY5Y cells caused by the low level of miR-132-3p. This is the first report of the possible effects of miR-132-3p on autophagy in epilepsy, in which miR-132-3p binds to ATG16L1 and negatively regulates its expression.

This study, however, has some limitations. This is only a preliminary study and further animal studies are needed to elucidate the association between a low level of lncRNA UCA1 and epilepsy as well as the precise regulatory mechanism of the lncRNA UCA1/miR-132-3p/ATG16L1 axis in epileptic autophagy.

In conclusion, our results suggest that lncRNA UCA1 may promote autophagy in epilepsy mediated by ATG16L1 through epigenetic regulation of miR-132-3p. This study provides clues for further understanding the molecular mechanism of autophagy in epilepsy and offers new targets for treating epilepsy. ■

Key points

- Overexpression of lncRNA UCA1 induces autophagic gene expression in SH-SY5Y cells.
- lncRNA UCA1 forms a complex with EZH2 and reduces the level of miR-132-3p.
- Overexpression of miR-132-3p suppresses autophagic gene expression induced by overexpression of lncRNA UCA1 in SH-SY5Y cells.
- miR-132-3p forms a complex with ATG16L1.
- Knockdown of ATG16L1 suppresses autophagic gene expression induced by miR-132-3p knockdown in SH-SY5Y cells.

Supplementary material.
Summary slides accompanying the manuscript are available at www.epilepticdisorders.com.

Ethics approval and consent to participate.
Ethical approval of this study was granted by the Ethical Review Committee of the Second Xiang Ya Hospital of Central South University.

Availability of data and material.
All data generated or analyzed during this study are included in this article. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Disclosures.
The authors declare no conflicts of interest.

Funding.
This work was supported by the Scientific Research Program of Hunan Provincial Health Commission (No.202103070765).

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TEST YOURSELF

(1) How was the epilepsy cell model constructed in this study?

(2) What were the expression levels for lncRNA UCA1, miR-132-3p and ATG16L1, respectively, in the serum of epileptic patients? What was the correlation between these levels?

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com.