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Original Article

Dysregulation of miR-1304-3p in hippocampus and serum of patients with intractable epilepsy

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Abstract: Temporal lobe epilepsy (TLE) is one of the most common intractable epilepsies. Due to its high drug-resistance, pharmacotherapy has had limited success. The multiple drug resistance gene ATP binding cassette subfamily B member 1 (ABCB1) encodes P-glycoprotein, an energy-dependent efflux pump which exports planar hydrophobic substrates from the cell. ABCB1 messenger RNA (mRNA) and P-glycoprotein are already reported elevated in cortical specimens of intractable epilepsy. MicroRNA and long noncoding RNA (lncRNA), have been recognized to be dysregulated, therefore we hypothesize they may modulate drug resistance-related genes. Here, we investigated in hippocampal tissues of TLE patients that ABCB1 mRNA and P-glycoprotein were increased while miR-1304-3p was decreased, whose level had relationships with clinic-pathological manifestations and seizure outcomes after operation. Besides, miR-1304-3p was up-regulated in serum samples and might sever as serum-based biomarker for identification of drug-resistant epilepsy. Moreover, luciferase assays revealed miR-1304-3p could regulate ABCB1 transcriptional as well as translational levels. Furthermore, we found an lncRNA named urothelial cancer associated 1 (UCA1) which was hyper-methylated in TLE, might also bind to miR-1304-3p by luciferase assays. Taken together, our data partly revealed UCA1-miR-1304-3p-ABCB1 axis might contribute to the mechanisms of drug resistance in TLE.

Keywords: microRNA, ABCB1, UCA1, hippocampus, drug-resistant epilepsy

Introduction

Epilepsy is reported to impact 65 million individuals worldwide and render a major burden in seizure-correlated comorbidities, mortality and costs. Although available antiepileptic drugs (AEDs) have substantially increased, about 30-35 percent of patients remain resistant to medical treatment [1]. The mechanisms may be variable and multi-factorial and involve a set of drug-resistant genes.

The ATP-binding cassette (ABC) transporters hypothesis is the mostly investigated theory. Functions of ABC transporters are regulated by transcriptional and post-transcriptional mechanisms, which respond very sensitive to various endogenous and external stimuli, suggesting that they play a pivotal role in central nervous system (CNS) protection. The most widely reported efflux transporter ABCB1 is studied for the first time in pharma-resistance of tumor cells 40 years ago [2]. ABCB1 is involved in numerous CNS related diseases [3-5]. A central control function in a series of events such as oxidative stress, DNA damage, inflammation and seizures comes up to the transcription factor kappa-light-chain-enhancer of activated B cells (NF-κB), an important player in the regulation of ABCB1 [6, 7]. AEDs such as PHT and PB are actually substrates for P-glycoprotein (P-gp) [8]. Even though ABCB1 plays a significant role, the molecular regulation of ABCB1 during drug-resistance remains to be elucidated in epilepsy.

The "competing endogenous RNA" (ceRNA) hypothesis presented how mRNA, transcribed pseudogenes, and lncRNA "talk" to each other using microRNA (miRNA) response elements as letters of a new language, which widely expanded the functional genetic information and played various roles in pathological conditions [9]. Several studies have focused on functions of miRNAs in epilepsy, but how they are involved in drug-resistance of TLE is still unclear [10, 11]. LncRNAs participate in various processes, but the study of their functions in epilepsy has

only just begun. Many lncRNAs are significantly dysregulated in models of epilepsy [12]. In fact, cerebral blood flow in hippocampus during seizure activity was markedly reduced [13]. Then epileptic seizures led to transient brain anoxia, which could exacerbate brain damage [14]. Interestingly, recently study had suggested that many hyper-methylation events were due to hypoxia [15]. LncRNA UCA1 had been for the first time reported to be hyper-methylated in TLE [16], which may be associated with hypoxia [15]. Recent work provided evidence of a UCA1-miR-204-CREB1/BCL2/RAB22A regulatory axis and showed that UCA1 enhanced 5-fluorouracil resistance in colorectal cancer [17]. In addition, by targeting the AKT/mTOR pathway, UCA1 could induce non-T790M acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer [18]. Previously studies had demonstrated that a hypoxia-associated transcription factor hypoxia-inducible factor-1α (HIF1A), which was greatly increased in epileptic brain tissues [19], could directly activate promoters of UCA1 as well as ABCB1 [20, 21]. So we guessed that UCA1-miRNAs-ABCB1 regulatory axis might partly relate to drug-resistance in TLE.

Here, in search for human miRNAs to affect level of ABCB1 from reference [10, 11], we found that a less common miRNA called miR-1304-3p, could regulate ABCB1 transcriptional and translational levels. Moreover, UCA1 might also bind to miR-1304-3p. Furthermore, UCA1-miR-1304-3p-ABCB1 axis might modulate drug resistance.

Materials and methods

Samples and patients

We collected 53 hippocampal tissues of TLE patients and 42 cortical tissues of temporal lobe control cases formalin-fixed paraffin-embedded (FFPE) tissue samples at the Department of Pathology (Ren Ji Hospital) of Shanghai Jiao Tong University from January 2003 to May 2016 retrospectively. All TLE patients had undergone anterior temporal lobectomy plus selective amygdala and hippocampus resection. They all had complex partial seizures (CPS) and were pathologically diagnosed as TLE. All data, including postoperative pathology [22] were obtained from clinical or pathologic records (Supplementary Table 1). All control cases were obtained from non-TLE patients who had undergone surgical therapy for hemorrhage. In these control samples, there were no history of epilepsy and exposure to AEDs (Supplementary Table 2).

We also enrolled serum samples from another 32 drug-resistant and 17 drug-responsive epilepsy patients as well as 17 healthy controls between January 2016 and June 2016. Up to 6 ml whole blood was gathered from each individual, and was conducted for serum isolation within 3 hours after collection by centrifugation for 5 min at 3,000 rpm. At room temperature, followed by centrifugation for 5 min at 12,000 g at 4°C [11]. Serum samples were stored at -80°C and the hemolytic samples were excluded. This study was approved by the Ethical Committee of Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, and written consents were obtained from all patients.

Cell culture and transfection

The human embryonic kidney 293 cells (HEK-293FT) as well as the human glioma cell line U87/DDP which could express P-gp [23] were cultured in Dulbecco's modified Eagle medium (Invitrogen), supplemented with 10% fetal bovine serum (Thermo Fisher Scientific) at 37°C, in 5% CO_2 with saturated humidity. miRNA mimics and negative mimic controls (Mimic-Con, NC) were purchased from Ribobio (Guangzhou, China). Cells were transfected with mimic or NC using Lipofectamine 2000 (Invitrogen Life Technologies).

Western blotting assay

Total proteins from cells or tissues were western-blotted using the monoclonal antibody against ABCB1 (1:800, Santa Cruz). β-actin (1:5000, Sigma) served as a loading control. Horseradish peroxidase (HRP)-labeled secondary antibody (1:1000, Sigma) was used and incubated at room temperature for 1 h. The band densities were quantified by the LICOR Odyssey infrared imaging system (LICOR Bioscience, Nebraska, USA).

Real-time polymerase chain reaction (PCR)

Total RNAs were extracted from FFPE samples using miRNeasy FFPE Kit (Qiagen; 217504), and from fresh serum samples using miRCURY RNA Isolation Kit-Biofluids (Exiqon; 300112).

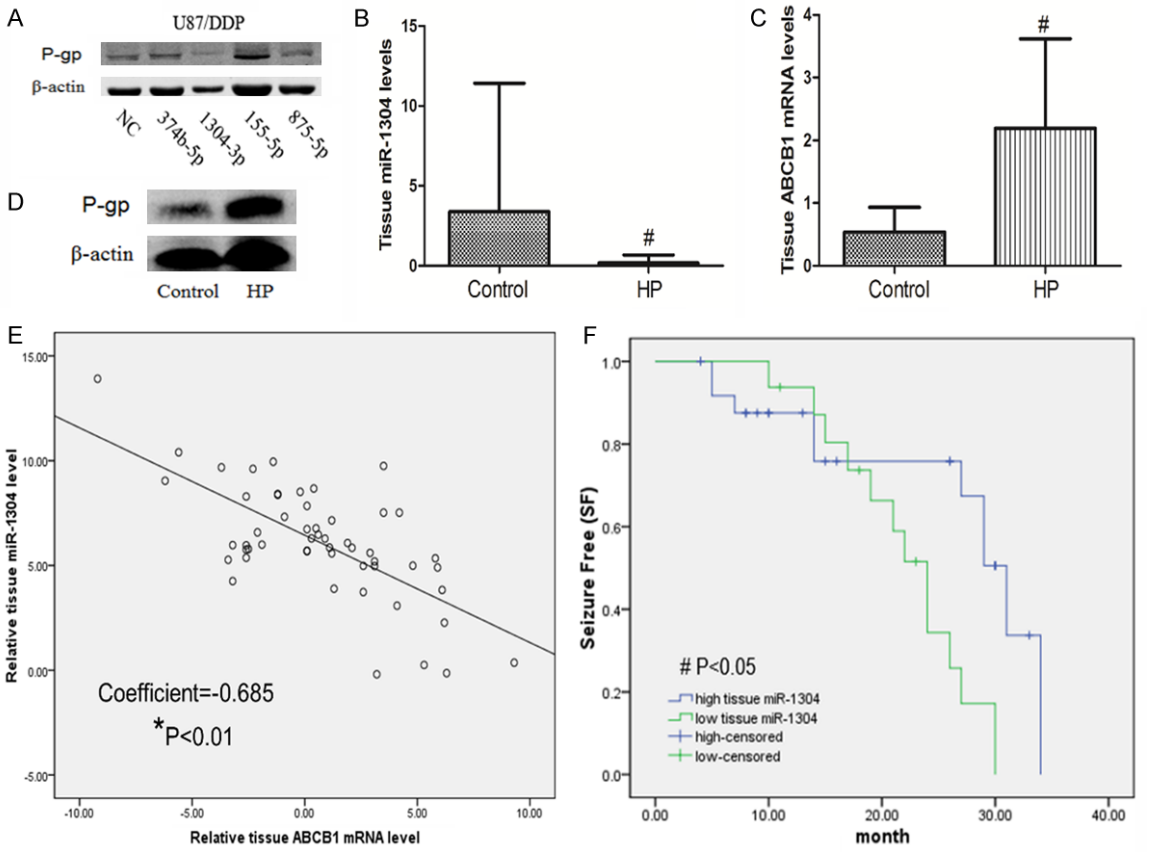

Figure 1. miR-1304-3p and ABCB1 expression levels in hippocampal tissues. A. Western blots of the extracts from U87/DDP cells transfected with the indicated miRNA mimics. B. The expression level of miR-1304-3p. C. The expression level of ABCB1 mRNA. D. Western blots of the extracts from tissues. E. ABCB1 mRNA expression is decreased following forced expression of miR-1304-3p in hippocampal tissues. Pearson's correlation analysis is used. F. Patients with low expression of miR-1304-3p level (n = 26) are obviously less likely to be seizure free than the high expression group (n = 16). #P<0.05. *P<0.01. HP, hippocampus.

After synthesizing cDNAs with Prime Script™ RT reagent Kit (Perfect Real Time) (Takara, RR037A), the levels of miRNAs and ABCB1 mRNA were analyzed using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (Takara, RR820A) and run with Light Cycler 480 (Roche, Germany). Data were analyzed by $2^{-\Delta\Delta CT}$ method. The levels of miRNAs in tissue and serum samples were normalized to U6 and miR-16 respectively. The level of ABCB1 mRNA was normalized to GAPDH. The primer sequences were listed in Supplementary Table 3.

Plasmid construction

ABCB1 3' untranslated region (UTR) sequence was amplified from 293FT genomic DNA and then cloned into the XhoI/NotI site of pmiR-RB-REPORT Vector (RiboBio, Guangzhou, China) (Supplementary 1). The following primers were used to generate specific fragment (384 bp): ABCB1 3' UTR-F, 5' GGCGGCTCGAGACTCTGACTGTATGAGATGTTA 3' and ABCB1 3' UTR-R, 5' AATGCGGCCGCCCAGTCACATGAAAGTTTAG 3'. The mutant plasmid was performed by creating a point mutation in the miR-1304-3p binding site using the Quick Change XL site-directed mutagenesis kit (Stratagene, California, USA). The binding site was mutated into TCACTC. Sequences of the wild-type and mutant vectors were confirmed with restricted digestion and automated DNA sequencing.

The promoter sequence of ABCB1 gene (171 bp) was artificially synthesized according to the reference's primers [24]. Then this sequence was cloned into the NheI/XhoI site of pGL3-basic vector (Promega) (Supplementary 2). The two binding sites of miR-1304-3p were respectively mutated into CTCCGA and AAGCTC.

Table 1. Correlation of clinic-pathologic variables of TLE with miR-1304-3p in hippocampal samples

Clinic-pathological features	Low level (n = 30)	High level (n = 23)	*P* value
Gender			0.933
Male	14	11	
Female	16	12	
Age at onset (yr)			0.487
<15.8	21	14	
>15.8	9	9	
Pre-operative auras			0.305
Absent	14	14	
Present	16	9	
SE			0.805
Absent	27	22	
Present	3	1	
IPI			0.817
Absent	16	13	
Present	14	10	
Duration (yr)			0.817
<14.2	16	13	
>14.2	14	10	
Pre-operative GTCS			0.232
Absent	12	13	
Present	18	10	
MRI (a)			0.829
Normal	11	9	
Abnormal	18	13	
Hippocampus			0.132
Left	18	9	
Right	12	14	
FCD			0.928
I	26	21	
II	4	2	
Frequency (month)			0.477
<5	16	10	
≥5	14	13	
Engel (b)			0.032
I	10	13	
II	10	1	
III	4	1	
IV	2	1	

(a) 2 patients whose data had missed. (b) 11 patients whose data had missed. SE, Status epilepticus; IPI, Initial precipitating incident; GTCS, Generalized tonic clonic seizure; MRI, Magnetic resonance imaging; FCD, Focal cortical dysplasia.

The sequence of UCA1 (2314 bp) was artificially synthesized. Then this sequence was cloned into the XhoI/BglII site of pGL3-CMV-LUC-MCS (Genomeditech, Shanghai, China) (Supplementary 3). But we did not perform mutant plasmids about the binding sites of miR-1304-3p.

Luciferase assays

HEK293FT cells (4×10^3 cells/well) were respectively transfected with pmiR-ABCB1 3' UTR and pGL3 promoter vectors (with either wild-type or mutant-type) and pGL3-CMV-LUC-MCS vector (with either empty or UCA1 sequence) together with 50 nM mimics or controls (RiboBio). And 48 h after transfection, cells were lysed and subjected to luciferase reporter assay system (Promega, Wisconsin, USA). The Firefly luciferase activity was normalized to that of Renilla.

Statistics

Data are expressed as mean ± standard deviation. Two-group comparisons were conducted with unpaired two tailed Student's t test. Pearson correlation coefficient analysis was used to value the relationship of miRNA and ABCB1 mRNA level. Seizure free (SF) probabilities were studied using Kaplan-Meier analyses. Receiver-operating characteristic (ROC) curve and the area under the ROC curve (AUC) were employed to estimate the diagnosis value of serum miRNAs. Bilateral χ^2 test was applied to find the correlations of miRNA with the clinic-pathological manifestations of patients. Each experiment consisted of at least three replicates per condition. SPSS17.0 software was employed for all statistical analysis. $P<0.05$ was considered statistically significant.

Results

MiR-1304-3p expression is decreased in hippocampal tissues

We initially focused on the already known dysregulated miRNAs from reference [10, 11] that might affect the level of ABCB1. Then, prediction using miR and a and TargetScan identified several miRNAs that could potentially bind to 3' UTR of ABCB1 mRNA. We employed western blot to detect P-gp level in U87/DDP cells. Among these miRNAs, we found three miRNAs [10, 11], could decrease the level of P-gp (**Figure 1A**). Then, the levels of these three miRNAs in hippocampal and control samples were analyzed. Only miR-1304-3p was significantly down-regulated (**Figure 1B**), while miR-

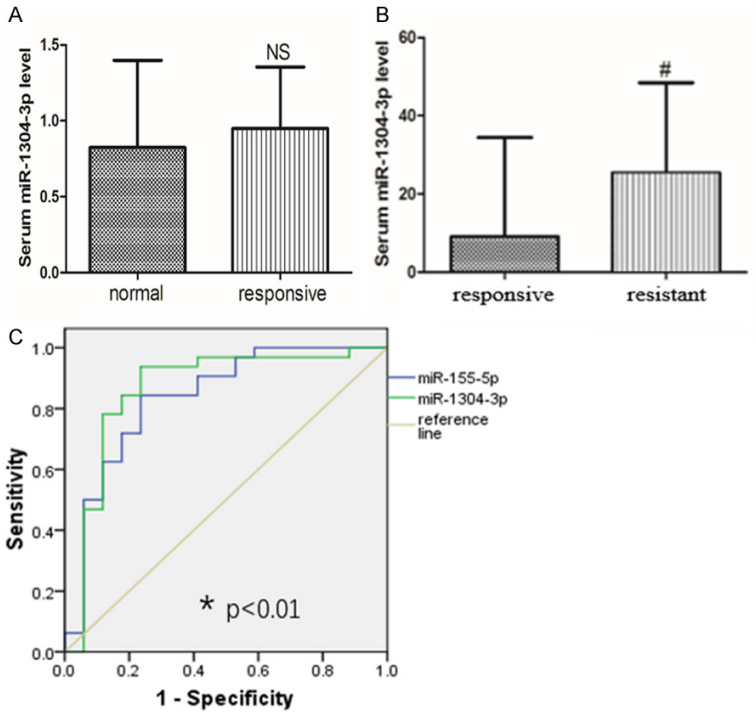

Figure 2. Expression level and diagnostic value of serum miR-1304-3p. A, B. The expression level of miR-1304-3p in serum samples. C. ROC curve analysis using serum miR-1304-3p for discriminating drug-resistant epilepsy from drug-responsive epilepsy. #P<0.05. *P<0.01. NS, no significance. Responsive, drug-responsive epilepsy. Resistant, drug-resistant epilepsy.

155-5p and 875-5p did not distinctly change (Supplementary Figure 1).

Considering the hypothesis that ABCB1 might be a target of miR-1304-3p, we then analyzed the levels of ABCB1 mRNA and P-gp. As shown in **Figure 1C**, ABCB1 mRNA was markedly up-regulated. Similarly, P-gp was also up-regulated in hippocampal tissues (**Figure 1D**). We further tested Pearson correlation coefficient to estimate the association of miR-1304-3p with ABCB1 mRNA. It found that the Pearson correlation coefficient was -0.685, and the inverse association was significant (**Figure 1E**). Taken together, these data significantly implicate that the decrease of miR-1304-3p might play a role in the mechanisms of drug resistance by increasing ABCB1 expression.

MiR-1304-3p expression is associated with seizure outcomes after operation and clinic-pathological manifestations

In the 53 TLE cases, there were 25 males and 28 females with age ranging from 9 to 69 years. All hippocampal tissues were classified into high miR-1304-3p expression group (n = 23) and low expression group (n = 30), according to the median level of all hippocampal tissues (median ΔCT value 6.10). The mean follow-up was 19 months (range, 4 to 34 months), but there were 11 patients whose data had missed. At the time of last follow-up (September 30, 2016), the seizure outcomes using Engel's classification [25] were class I, (54.8%); class II, (26.2%), class III, (11.9%); and class IV, (7.1%). Using Kaplan-Meier survival analysis [25], we tested the relationship between miR-1304-3p level and the percentage of patients rendered seizure free (SF%) after operation. As shown in **Figure 1F**, patients who with low expression of miR-1304-3p were obviously less likely to be seizure free than the high expression group.

Based on the significant result of miR-1304-3p expressionin hippocampal tissues, we further tested the association between its expression and clinic-pathological manifestations in order to better understand the potential role in the diagnosis and progression of TLE. We revealed that the level of miR-1304-3p had relationships with Engel's classification (**Table 1**). Taken together, these results implicate that the decrease of miR-1304-3p might play a role in the development and progression of drug resistance.

The expression level and diagnostic value of serum miR-1304-3p

We inferred the dysregulation of miRNAs in hippocampal tissues would influence that of expression in serum of drug-resistant epilepsy patients. So, we analyzed the serum miR-1304-3p expression in another drug-resistant and drug-responsive samples as well as healthy controls (**Figure 2A**, **2B**). Statistically significant up-regulation between drug-resistant and responsive patients was recognized (**Figure 2B**). Besides, we tested the ROC curve to

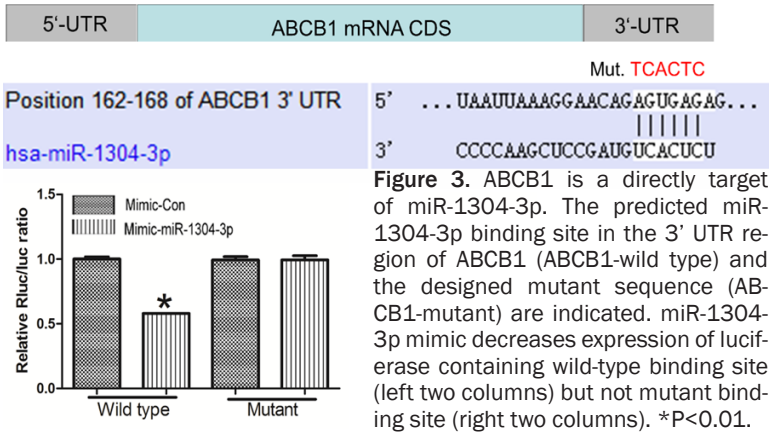

Figure 3. ABCB1 is a directly target of miR-1304-3p. The predicted miR-1304-3p binding site in the 3' UTR region of ABCB1 (ABCB1-wild type) and the designed mutant sequence (ABCB1-mutant) are indicated. miR-1304-3p mimic decreases expression of luciferase containing wild-type binding site (left two columns) but not mutant binding site (right two columns). *P<0.01.

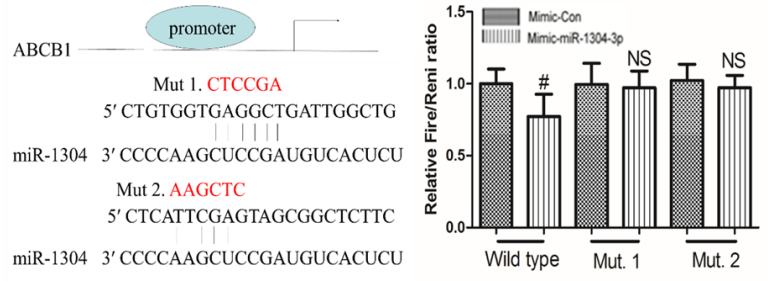

Figure 4. miR-1304-3p can regulate transcription of ABCB1 gene. The predicted miR-1304-3p binding sites in the promoter region of ABCB1 (wild type) and the designed mutant sequences (mutant) are indicated. miR-1304-3p mimic decreases expression of luciferase containing pGL3 wild-type ABCB1 promoter, but not two mutant binding sites. #P<0.05. NS, no significance.

assess its diagnostic value. It revealed that the AUC based on serum miR-1304-3p was 0.862±0.067. At the cut-off point of 0.702 (Yuden index), the sensitivity was 93.8% for serum miR-1304-3p (**Figure 2C**). In all, the expression of serum miR-1304-3p was also dysregulated and the present diagnostic value was positive in drug-resistant epilepsy.

ABCB1 is a directly target of miR-1304-3p

We next explored the mechanism by which miR-1304-3p participated in drug resistance. Alignment of miR-1304-3p with ABCB1 mRNA 3' UTR was shown in **Figure 3**. We then confirmed whether miR-1304-3p could influence the translation of ABCB1 mRNA. As shown in **Figure 3**, the luciferase reporter assay shown that the wild-type 3' UTR of ABCB1 exhibited a decreased translational level in the presence of miR-1304-3p, whereas the mutated 3' UTR did not find a significant response to them. In all, these data revealed that ABCB1 was a directly target of miR-1304-3p.

MiR-1304-3p can also participate in the regulation of ABCB1 transcriptional level

Apart from the inhibitory ability at translational level, we next explored whether miR-1304-3p also had inhibitory ability at transcriptional level of ABCB1 gene. Analysis of the sequence revealed putative two binding sites in the ABCB1 promoter region (171 bp), which were shown in **Figure 4**. The intensity of relative dual-luciferase luminescence in cells co-transfected with pGL3/miR-1304-3p was markedly lower than control group, whereas the two mutated sites did not find a significant response to them (**Figure 4**), suggesting that miR-1304-3p could inhibit the promoter activity of ABCB1 by targeting these two binding sites. Taken together, these data revealed that miR-1304-3p could suppress both transcription and translation of ABCB1.

UCA1 may also bind to miR-1304-3p

To further understand whether UCA1 could interact with miR-1304-3p, we compared the sequence of UCA1 with that of miR-1304-3p using RNA hybrid and found that UCA1 contained seven binding sites of miR-1304-3p (Supplementary 3). Next, we conducted a luciferase construct of UCA1 but did not produce mutated forms. Luciferase assay revealed that UCA1 might bind to miR-1304-3p and suppress its activity (**Figure 5A**). These results revealed that UCA1 might associate with epileptic seizures by interacting with miR-1304-3p via these putative binding sites.

Discussion

Here, we found that miR-1304-3p was decreased in hippocampus of TLE patients and increased in serum samples of drug-resistant epilepsy patients, and revealed a mechanism of direct regulation of ABCB1 transcription and translation by miR-1304-3p. Besides, UCA1

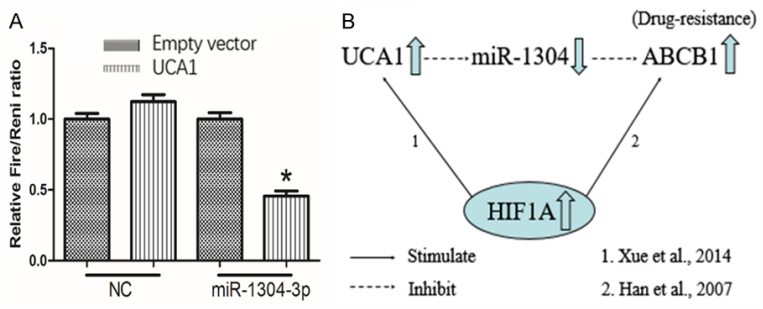

Figure 5. UCA1-miR-1304-3p-ABCB1 axis may modulate drug resistance. A. HEK293FT cells are transfected with NC or miR-1304-3p mimic, then transfected with the luciferase constructs of empty vector or UCA1 sequence. The luciferase activity is analyzed. B. Cartoon shows a summary of the findings. We propose that UCA1 interacts with miR-1304-3p, then miR-1304-3p targets ABCB1 via its promoter and mRNA 3' UTR. Furthermore, previously studies suggest that HIF1A, which is greatly increased in epileptic brain tissues, can directly activate promoters of UCA1 and ABCB1, indicating that HIF1A may participate in the axis of UCA1-miR-1304-3p-ABCB1 in drug-resistant epilepsy. *P<0.01.

might also bind to miR-1304-3p. Furthermore, UCA1-miR-1304-3p-ABCB1 axis might modulate drug resistance.

MiRNAs play important roles in biological processes [26-28]. For the first time, Kan et al. have studied the genome-wide miRNA profiling in human TLE, and the most prominently targeted mRNAs are associated with immune response [10]. MiR-203 is up-regulated in the hippocampal tissues of mice and human and is bound to inhibitory synaptic receptors [10, 29]. In addition, miR-204 is down-regulated in the hippocampal tissues of human and repressed epileptic form discharges [30, 31]. Furthermore, decreased miR-134 level can suppress prolonged seizure and exert neuroprotective actions. However, recognizing whether this is anticonvulsant effect or is truly anti-epileptogenic effect remains require additional clinical experimentation [32]. Recent study reports miR-1304 can suppress non-small cell lung cancer cell growth [24]. Then, identification of miRNAs functions has opened up probably applications in molecular diagnostics and prognostics of TLE [33].

We found that levels of miR-1304-3p were dysregulated in tissue and serum samples, which were consistent with the previously studies [10, 11]. Then, we guessed that it was partly because miR-1304-3p had been released to the blood through impaired blood brain barrier (BBB). Besides, a significant inverse relationship between ABCB1 mRNA and miR-1304-3p was found. In fact, Tishler et al. had already found the ABCB1 mRNA level was more than 10 times in intractable epilepsy [34]. In our study, the decrease of miR-1304-3p leading to high expression of ABCB1 was associated with Engel's classification and SF%. But further bigger studies had to explore whether low expression of miR-1304-3p was an independent signal to predict poor prognosis of TLE patients. Because the specific sequence of miR-1304-3p did not exist on rat as well as mouse, we did not perform the function of miR-1304-3p in models [35]. Therefore, further advanced studies had to investigate whether elevated miR-1304-3p in brain may function as an anti-drug-resistance in the development of TLE.

Mounting studies have detected the possibility of miRNAs as diagnostic biomarkers. For example, serum miR-106b-5p has proved to be novel biomarkers to improve current diagnosis of epilepsy [36]. Besides, miR-301a-3p in the serum of drug-resistant epilepsy patients is found to implicate seizure severity [11]. miR-1304 is dramatically upregulated in hypopharynx cancer patients with paclitaxel-based treatment [37]. Here, we found that serum miR-1304-3p might serve as potential diagnostic biomarkers for drug-resistant epilepsy, since the sensitivities of it was 93.8%. But further bigger studies also needed to confirm this result [11].

The ABC transporters are primarily expressed in capillary vessel endothelial cells, which consist of the primary sections of BBB [38]. The "transporter hypothesis" suggests that drug-resistance may be due to over-expression of efflux transporters at the epileptic zone. ABC transmembrane proteins expel substrates like PHT and PB from the cells against the concentration gradient [8]. Besides ABCB1, multidrug resistance-related protein (also ABCCs) and the breast cancer resistance protein (also ABCG2) are also directly associated with epilepsy [39, 40]. So far, most already reported miRNAs could repress translation. Here, we found miR-

1304-3p not only could suppress translation of ABCB1 mRNA, but also inhibit transcription of ABCB1 gene, even though we had no idea which one was more important (Supplementary Figure 2).

Despite the various roles of lncRNAs, it is not yet much clear whether they are involved in drug-resistance. Suzanne et al. had identified for the first time that four lncRNA were hypermethylated in TLE [16]. Attempt to confirm dysregulation of lncRNA was very hard [16]. However, differential methylation might therefore play different roles in transcriptional regulation of lncRNA in TLE. Previously studies had indicated that HIF1A could directly activate promoters of UCA1 and ABCB1 [20, 21], indicating that an UCA1-miR-1304-3p-ABCB1 axis might modulated drug resistance (**Figure 5B**). In fact, the mechanisms of miRNAs were complex. Apart from miR-1304-3p, other miRNAs might also regulate ABCB1 and be controlled by UCA1. Interesting, several pathways especially axonal guidance seem extremely significant in neurological diseases [30]. Because there were seven binding sites among UCA1-miR-1304-3p, we did not conduct mutant vectors. In all, a better understanding of the importance of specific miRNAs and lncRNAs like UCA1 may yield novel therapeutic targets in TLE.

In conclusion, we identified ABCB1 to be a directly target gene for miR-1304-3p. Besides, UCA1-miR-1304-3p-ABCB1 axis might modulate drug resistance. Considering the fact that there were inadequate evidences apart from luciferase assays, further studies were necessary to reveal the detailed mechanisms, especially the functions of UCA1 and miR-1304-3p in epilepsy.

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Disclosure of conflict of interest

None.

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References

[1] Moshe SL, Perucca E, Ryvlin P and Tomson T. Epilepsy: new advances. Lancet 2015; 385: 884-898.

[2] Juliano RL and Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976; 455: 152-162.

[3] Spudich A, Kilic E, Xing H, Kilic U, Rentsch KM, Wunderli-Allenspach H, Bassetti CL and Hermann DM. Inhibition of multidrug resistance transporter-1 facilitates neuroprotective therapies after focal cerebral ischemia. Nat Neurosci 2006; 9: 487-488.

[4] Kuhnke D, Jedlitschky G, Grube M, Krohn M, Jucker M, Mosyagin I, Cascorbi I, Walker LC, Kroemer HK, Warzok RW, Vogelgesang S. MDR1-P-Glycoprotein (ABCB1) mediates transport of Alzheimer's amyloid-beta peptides-implications for the mechanisms of Abeta clearance at the blood-brain barrier. Brain Pathol 2007; 17: 347-353.

[5] Kooij G, Kroon J, Paul D, Reijerkerk A, Geerts D, van der Pol SM, van Het Hof B, Drexhage JA, van Vliet SJ, Hekking LH, van Buul JD, Pachter JS, de Vries HE. P-glycoprotein regulates trafficking of CD8(+) T cells to the brain parenchyma. Acta Neuropathol 2014; 127: 699-711.

[6] Miller DS. Regulation of ABC transporters blood-brain barrier: the good, the bad, and the ugly. Adv Cancer Res 2015; 125: 43-70.

[7] Miller DS. Regulation of ABC transporters at the blood-brain barrier. Clin Pharmacol Ther 2015; 97: 395-403.

[8] Zhang C, Kwan P, Zuo Z and Baum L. The transport of antiepileptic drugs by P-glycoprotein. Adv Drug Deliv Rev 2012; 64: 930-942.

[9] Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011; 146: 353-358.

[10] Kan AA, van Erp S, Derijck AA, de Wit M, Hessel EV, O'Duibhir E, de Jager W, Van Rijen PC,

Gosselaar PH, de Graan PN and Pasterkamp RJ. Genome-wide microRNA profiling of human temporal lobe epilepsy identifies modulators of the immune response. Cell Mol Life Sci 2012; 69: 3127-3145.

[11] Wang J, Tan L, Tan L, TianY, Ma J, Tan CC, Wang HF, Liu Y, Tan MS, Jiang T and Yu JT. Circulating microRNAs are promising novel biomarkers for drug-resistant epilepsy. Sci Rep 2015; 5: 10201.

[12] Lee DY, Moon J, Lee ST, Jung KH, Park DK, Yoo JS, Sunwoo JS, Byun JI, Lim JA, Kim TJ, Jung KY, Kim M, Jeon D, Chu K and Lee SK. Dysregulation of long non-coding RNAs in mouse models of localization-related epilepsy. Biochem Biophys Res Commun 2015; 462: 433-440.

[13] Choy M, Wells JA, Thomas DL, Gadian DG, Scott RC and Lythgoe MF. Cerebral blood flow changes during pilocarpine-induced status epilepticusactivity in the rat hippocampus. Exp Neurol 2010; 225: 196-201.

[14] Ahn GO, Seita J, Hong BJ, Kim YE, Bok S, Lee CJ, Kim KS, Lee JC, Leeper NJ, Cooke JP, Kim HJ, Kim IH, Weissman IL and Brown JM. Transcriptional activation of hypoxia-inducible factor-1 (HIF-1) in myeloid cells promotes angiogenesis through VEGF and S100A8. Proc Natl Acad Sci U S A 2014; 111: 2698-2703.

[15] Thienpont B, Steinbacher J, Zhao H, D'Anna F, Kuchnio A, Ploumakis A, Ghesquière B, Van Dyck L, Boeckx B, Schoonjans L, Hermans E, Amant F, Kristensen VN, Koh KP, Mazzone M, Coleman ML, Carell T, Carmeliet P and Lambrechts D. Tumour hypoxia causes DNA hypermethylation by reducing TET activity. Nature 2016; 537: 63-68.

[16] Miller-Delaney SF, Bryan K, Das S, McKiernan RC, Bray IM, Reynolds JP, Gwinn R, Stallings RL and Henshall DC. Differential DNA methylation profiles of coding and non-coding genes define hippocampal sclerosis in human temporal lobe epilepsy. Brain 2015; 138: 616-631.

[17] Bian Z, Jin L, Zhang J, Yin Y, Quan C, Hu Y, Feng Y, Liu H, Fei B, Mao Y, Zhou L, Qi X, Huang S, Hua D, Xing C and Huang Z. LncRNA-UCA1 enhances cell proliferation and 5-fluorouracil resistance in colorectal cancer by inhibiting miR-204-5p. Sci Rep 2016; 6: 23892.

[18] Cheng N, Cai W, Ren S, Li X, Wang Q, Pan H, Zhao M, Li J, Zhang Y, Zhao C, Chen X, Fei K, Zhou C and Hirsch FR. Long non-coding RNA UCA1 induces non-T790M acquired resistance to EGFR-TKIs by activating the AKT/mTOR pathway in EGFR-mutant non-small cell lung cancer. Oncotarget 2015; 6: 23582-23593.

[19] Jiang GH, Zhou RJ, He XZ, Shi ZQ, Huang M, Yu JM, Wang X. Expression levels of microRNA-199 and hypoxia-inducible factor-1 alpha in brain tissue of patients with intractable epilepsy. Int J Neurosci 2016; 126: 326-334.

[20] Han HK, Han CY, Cheon EP, Lee J and Kang KW. Role of hypoxia-inducible factor-alpha in hepatitis-B-virus X protein-mediated MDR1 activation. Biochem Biophys Res Commun 2007; 357: 567-573.

[21] Xue M, Li X, Li Z and Chen W. Urothelial carcinoma associated 1 is a hypoxia-inducible factor-1alpha-targeted long noncoding RNA that enhances hypoxic bladder cancer cell proliferation, migration, and invasion. Tumour Biol 2014; 35: 6901-6912.

[22] Palmini A, Najm I, Avanzini G, Babb T, Guerrini R, Foldvary-Schaefer N, Jackson G, Lüders HO, Prayson R, Spreafico R and Vinters HV. Terminology and classification of the cortical dysplasias. Neurology 2004; 62 Suppl 3: S2-S8.

[23] Wang Q, Wang Z, Chu L, Li X, Kan P, Xin X, Zhu Y and Yang P. The effects and molecularmechanisms of MiR-106a in multidrug resistance reversal in human glioma U87/DDP and U251/G cell lines. PLoS One 2015; 10: e0125473.

[24] Guo C, Ding J, Yao L, Sun L, Lin T, Song Y, Fan D. Tumor suppressor gene Runx3 sensitizes gastric cancer cells to chemotherapeutic drugs by downregulating Bcl-2, MDR-1 and MRP-1. Int J Cancer 2005; 116: 155-160.

[25] Foldvary N, Nashold B, Mascha E, Thompson EA, Lee N, McNamara JO, Lewis DV, Luther JS, Friedman AH and Radtke RA. Seizure outcome after temporal lobectomy for temporal lobe epilepsy: a Kaplan-Meier survival analysis. Neurology 2000; 54: 630-634.

[26] Li CG, Pu MF, Li CZ, Gao M, Liu MX, Yu CZ, Yan H, Peng C, Zhao Y, Li Y, Ma ZL, Qi XM, Wang YZ, Miao LL and Ren J. MicroRNA-1304 suppresses human non-small cell lung cancer cell growth in vitro by targeting heme oxygenase-1. Acta Pharmacol Sin 2017; 38: 110-119.

[27] Huang Y, Liu X, Liao Y, Luo C, Zou D, Wei X, Huang Q and Wu Y. MiR-181a influences the cognitive function of epileptic rats induced by pentylenetetrazol. Int J Clin Exp Pathol 2015; 8: 12861-12868.

[28] Liu H, Gao Y, Song D, Liu T and Feng Y. Correlation between microRNA-421 expression level and prognosis of gastric cancer. Int J Clin Exp Pathol 2015; 8: 15128-15132.

[29] Lee ST, Jeon D, Chu K, Jung KH, Moon J, Sunwoo J, Park DK, Yang H, Park JH, Kim M, Roh JK and Lee SK. Inhibition of miR-203 reduces spontaneous recurrent seizures in mice. Mol Neurobiol 2016; [Epub ahead of print].

[30] Kaalund SS, Veno MT, Bak M, Moller RS, Laursen H, Madsen F, Broholm H, Quistorff B, Uldall P, Tommerup N, Kauppinen S, Sabers A, Fluiter K, Møller LB, Nossent AY, Silahtaroglu A, Kjems J, Aronica E and Tümer Z. Aberrant expression of miR-218 and miR-204 in human

mesial temporal lobe epilepsy and hippocampal sclerosis-convergence on axonal guidance. Epilepsia 2014; 55: 2017-2027.
[31] Xiang L, Ren Y, Li X, Zhao W and Song Y. MicroRNA-204 suppresses epileptiform discharges through regulating TrkB-ERK1/2-CREB signaling in cultured hippocampal neurons. Brain Res 2016; 1639: 99-107.
[32] Jimenez-Mateos EM, Engel T, Merino-Serrais P, McKiernan RC, Tanaka K, Mouri G, Sano T, O'Tuathaigh C, Waddington JL, Prenter S, Delanty N, Farrell MA, O'Brien DF, Conroy RM, Stallings RL, De Felipe J and Henshall DC. Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. Nat Med 2012; 18: 1087-1094.
[33] Jimenez-Mateos EM, Engel T, Merino-Serrais P, Fernaud-Espinosa I, Rodriguez-Alvarez N, Reynolds J, Reschke CR, Conroy RM, McKiernan RC, de Felipe J and Henshall DC. Antagomirs targeting microRNA-134 increase hippocampal pyramidal neuron spine volume in vivo and protect against pilocarpine-induced status epilepticus. Brain Struct Funct 2015; 220: 2387-2399.
[34] Tishler DM, Weinberg KI, Hinton DR, Barbaro N, Annett GM and Raffel C. MDR1 gene expression in brain of patients with medically intractable epilepsy. Epilepsia 1995; 36: 1-6.
[35] Enrique A, Goicoechea S, Castaño R, Taborda F, Rocha L, Orozco S, Girardi E and Bruno Blanch L. New model of pharmacoresistant seizures induced by 3-mercaptopropionic acid in mice. Epilepsy Res 2016; 27: 8-16.
[36] Wang J, Yu JT, Tan L, Tian Y, Ma J, Tan CC, Wang HF, Liu Y, Tan MS, Jiang T and Tan L. Genome-wide circulating microRNA expression profiling indicates biomarkers for epilepsy. Sci Rep 2015; 5: 9522.
[37] Xu CZ, Shi RJ, Chen D, Sun YY, Wu QW, Wang T, Wang PH. Potential biomarkers for paclitaxel sensitivity in hypopharynx cancer cell. Int J Clin Exp Pathol 2013; 6: 2745-2756.
[38] Ashraf T, Kao A and Bendayan R. Functional expression of drug transporters in glial cells: potential role on drug delivery to the CNS. Adv Pharmacol 2014; 71: 45-111.
[39] Loscher W and Potschka H. Blood-brain barrier active efflux transporters: ATP-binding cassette gene family. Neuro Rx 2005; 2: 86-98.
[40] Loscher W and Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci 2005; 6: 591-602.

Supplementary Table 1. The clinic-pathological features of 53 patients with TLE

Number	Sex	Age (yr)	Aura	IPI	GTCS before surgery	SE before surgery	Duration (yr)	MRI (a)	AEDs before surgery	Resection tissue (b)	Pathology	Follow up (month)	Engel (c)
1	F	18	Y	N	Y	N	11	Normal	VPA, CBZ, LTG, OXC	L	FCD IB	30	2
2	F	13	N	N	Y	Y	11	Abnormal	VPA, CBZ, GBP	R	FCD IB	4	2
3	F	26	N	N	Y	N	5	Normal	CBZ, OXC, VPA	R	FCD IB	29	1
4	M	32	Y	Y	N	N	2	Abnormal	VPA, OXC, TPM	R	FCD IA, atrophy	7	1
5	M	62	Y	N	Y	Y	15	Abnormal	VPA, LTG, TPM	R	FCD IIB	8	3
6	F	26	Y	Y	Y	N	20	Normal	CBZ, TPM, PHT	L	FCD IA	31	1
7	M	34	N	Y	N	N	1	Abnormal	CBZ, VPA, GBP	L	FCD IA, atrophy	5	1
8	F	24	Y	N	Y	N	13	Normal	LEV, OXC, PB	L	FCD IB	26	3
9	F	18	N	N	N	N	8	Abnormal	VPA, LEV, PHT	R	FCD IB	8	2
10	M	23	N	N	N	N	3	Abnormal	CBZ, VPA, LEV	L	FCD IA		
11	M	19	N	N	Y	Y	11	Normal	VPA, CBZ, LTG, LEV	L	FCD IB	29	1
12	M	26	N	N	Y	N	10	Normal	TPM, OXC, LEV	L	FCD IA		
13	F	36	N	N	Y	N	29	Normal	CBZ, VPA, LTG	L	FCD IB		
14	M	23	Y	N	N	N	8	Abnormal	CBZ, PHT, LTG	L	FCD IA	15	2
15	F	34	Y	Y	N	N	9	Abnormal	TPM, OXC, LEV	L	FCD IB	26	4
16	F	42	N	Y	Y	N	35	Abnormal	CBZ, VPA, TPM	L	FCD IIA, atrophy	10	2
17	F	20	Y	Y	N	N	15	Normal	VPA, PB, TPM, CBZ	R	FCD IB	14	1
18	M	53	N	Y	N	N	2	Abnormal	TPM, LTG, LEV	L	FCD IA	13	4
19	F	36	N	N	Y	N	2	Abnormal	VPA, LTG, CBZ	L	FCD IB	4	2
20	M	20	N	Y	N	N	19	Normal	VPA, PB, CBZ	R	FCD IB	14	1
21	F	25	Y	N	N	N	19	Normal	CBZ, TPM, VPA	L	FCD IIA	30	2
22	F	21	Y	Y	Y	N	12	Abnormal	LEV, VPA, OXC	R	FCD IIB	10	2
23	F	32	N	N	Y	N	30	Abnormal	OXC, TPM, GBP	R	FCD IA	34	1
24	F	38	Y	N	N	N	18	Abnormal	CBZ, VPA, GBP	L	FCD IA, atrophy	5	1
25	M	22	Y	Y	Y	N	18	Abnormal	TPM, PHT, PB	R	FCD IA	27	1
26	M	32	Y	Y	Y	N	23	Normal	PB, PHT, VPA, CBZ	L	FCD IB	9	2
27	M	27	Y	Y	Y	N	24	Abnormal	PHT, CBZ, VPA, TPM	R	FCD IA, atrophy	33	2
28	M	43	N	N	N	N	31	Abnormal	CBZ, VPA, LEV	R	FCD IA		
29	F	31	Y	Y	Y	N	27	Abnormal	CBZ, VPA, PHT	L	FCD IA	16	3
30	M	9	Y	Y	Y	N	9		OXC, PHT, TPM	L	FCD IB	30	3
31	M	18	N	N	Y	N	16	Normal	VPA, PHT, PB	L	FCD IA		
32	F	39	Y	Y	N	N	21	Abnormal	CBZ, LTG, LEV	L	FCD IA, sclerosis	18	2
33	M	24	N	N	N	N	7	Normal	VPA, CBZ, OXC	R	FCD IA	30	1
34	M	39	Y	N	N	N	15	Normal	VPA, CBZ, TPM, PHT	L	FCD IIA	11	4
35	M	40	Y	N	Y	N	28	Normal	VPA, OXC, LTG	R	FCD IA	27	1
36	F	50	N	N	N	N	10	Abnormal	PHT, PB, VPA	L	FCD IA	10	1
37	M	23	Y	N	N	N	16	Abnormal	VPA, OXC, LEV	R	FCD IA, atrophy	19	1
38	F	29	N	N	Y	N	16	Normal	PHT, TPM, CBZ	L	FCD IA		
39	M	21	N	N	Y	N	8	Abnormal	CBZ, VPA, LEV	R	FCD IB	30	1
40	M	20	Y	N	Y	N	5	Normal	OXC, LTG, LEV	R	FCD IB	23	3
41	F	20	N	Y	N	N	9	Normal	VPA, CBZ, LEV, TPM	R	FCD IA	15	1
42	M	41	Y	N	Y	N	30	Abnormal	CBZ, VPA, OXC	L	FCD IA, atrophy		
43	F	25	N	Y	N	N	16	Normal	CBZ, PB, PHT	R	FCD IB		
44	F	48	N	N	Y	N	6	Abnormal	VPA, CBZ, PHT	R	FCD IA, atrophy		
45	F	48	N	N	N	N	4	Abnormal	OXC, LEV, TPM	R	FCD IB	26	1
46	F	19	Y	Y	N	Y	9	Abnormal	OXC, LEV, LTG	L	FCD IA	14	1
47	F	60	Y	Y	Y	N	50		LEV, VPA, OXC	R	FCD IA	24	1
48	M	69	N	Y	N	N	2	Abnormal	CBZ, GBP, PHT	L	FCD IB	17	1
49	F	16	N	Y	Y	N	5	Abnormal	OXC, GBP, LEV	R	FCD IIA	22	1
50	M	20	N	Y	N	N	19	Abnormal	PHT, CBZ, TPM, VPA	L	FCD IA, atrophy	24	1
51	F	23	Y	Y	Y	N	7	Abnormal	CBZ, VPA, LTG	R	FCD IA, sclerosis	21	1
52	M	29	N	N	N	N	5	Abnormal	OXC, CBZ, LEV	R	FCD IA, sclerosis		
53	F	21	N	Y	N	N	6	Normal	VPA, PHT, CBZ	R	FCD IA		

(a) 2 patients whose data had missed. (b) anterior temporal neocortex, amygdala and hippocampus. (c) 11 patients whose data had missed. F, Female; M, Male; Y, Yes; N, No; L, Left; R, Right; IPI, Initial precipitating incident; GTCS, Generalized tonic clonic seizure; SE, Status epilepticus; MRI, Magnetic resonance imaging; AEDs, Antiepileptic drugs; VPA, Valproic acid; CBZ, Carbamazepine; LTG, Lamotrigine; OXC, Oxcarbazepine; GBP, Gabapentin; TPM, Topamax; PHT, Phenytoin; LEV, Levetiracetam; PB, Phenobarbital; FCD, Focal cortical dysplasia.

Supplementary Table 2. The information of 42 control samples

Number	Sex	Age (yr)	Resection tissue (a)
1	M	36	L
2	M	15	L
3	F	57	R
4	M	45	R
5	M	5	R
6	F	20	L
7	M	17	L
8	M	50	R
9	M	50	R
10	M	67	R
11	M	25	L
12	F	67	R
13	M	42	R
14	F	58	L
15	F	65	R
16	M	28	R
17	M	58	L
18	F	41	L
19	M	35	R
20	M	44	R
21	M	71	L
22	M	16	L
23	M	56	R
24	F	20	L
25	M	45	L
26	M	39	R
27	F	36	L
28	F	47	L
29	F	70	R
30	M	56	R
31	F	37	L
32	F	24	R
33	M	34	R
34	M	48	R
35	M	51	R
36	M	46	L
37	M	28	L
38	F	30	L
39	F	63	L
40	M	62	R
41	F	66	R
42	M	60	R

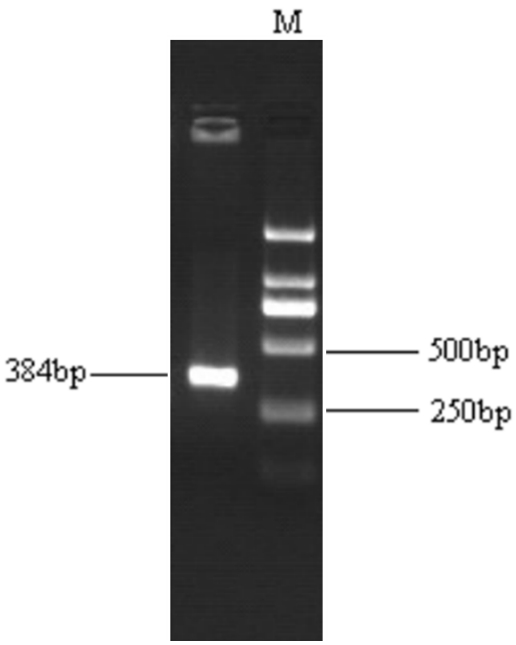
(a) cortical tissue of temporal lobe. F, Female; M, Male; L, Left; R, Right.

Supplementary Table 3. The primer sequences used for qPCR

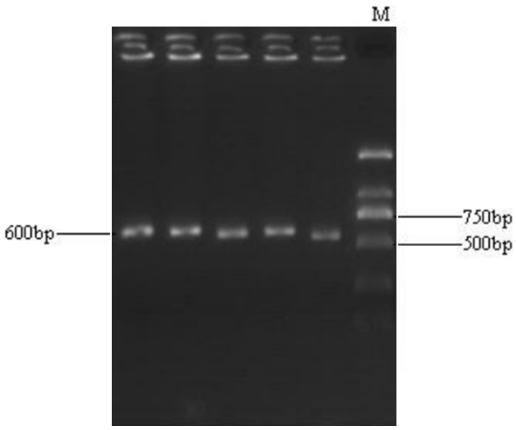
Genes (Homo sapiens)	Primers	Sequences (5'-3')
miR-1304-3p	RT primer	GTCGTATCCAGTGCAGGGTCCGAGG
		TATTCGCACTGGATACGACGGGGTT
	Forward	GCGGTCTCACTGTAGCCTCG
	Reverse	ATCCAGTGCAGGGTCCGAGG
miR-155-5p	RT primer	GTCGTATCCAGTGCAGGGTCCGAGG
		TATTCGCACTGGATACGACACCCCT
	Forward	GCGGCGGTTAATGCTAATCGTGAT
	Reverse	ATCCAGTGCAGGGTCCGAGG
miR-875-5p	RT primer	GTCGTATCCAGTGCAGGGTCCGAGG
		TATTCGCACTGGATACGACCACCTG
	Forward	CCAGGCATGGTATACCTCAGTTTTAT
	Reverse	ATCCAGTGCAGGGTCCGAGG
U6	Forward	CTCGCTTCGGCAGCACA
	Reverse	AACGCTTCACGAATTTGCGT
miR-16-5p	RT primer	GTCGTATCCAGTGCAGGGTCCGAGG
		TATTCGCACTGGATACGACCGCCAA
	Forward	TCGGCGGTAGCAGCACGTAAATA
	Reverse	ATCCAGTGCAGGGTCCGAGG
ABCB1	Forward	TTCACCCAGGCAATGATGTA
	Reverse	CATGGCACCAAAGACAACAG
GAPDH	Forward	GAAGGTGAAGGTCGGAGTC
	Reverse	GAAGATGGTGATGGGATTTC

Supplementary 1. Vector of ABCB1 3' UTR

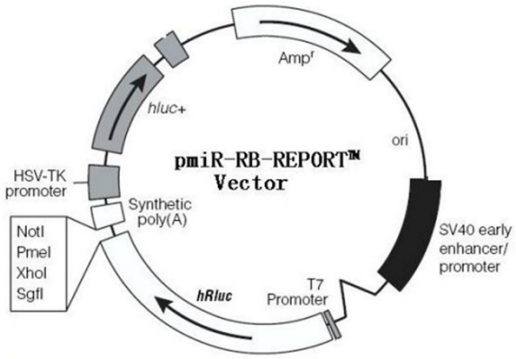
1. Gel electrophoresis of the specific fragment:



2. Gel electrophoresis of the complete vector:



3. Structure:



1) Primers:

ABCB1 3' UTR-F, 5' GGCGGCTCGAGACTCTGACTGTATGAGATGTTA 3'

ABCB1 3' UTR-R, 5' AATGCGGCCGCCCAGTCACATGAAAGTTTAG 3'.

2) GGCGG and AAT were protective sequences.

3) The below underlined nucleotides: primers designing positions.

4) Blue; AGTGAG; hsa-miR-1304-3p binding site.

5) CTCGAG; XhoI.

GCGGCCGC; NotI

6) Primary sequence: (T = U)

GGAGCGCGTGCTGAAGAACGAGCAGTAATTCTAGGCGATCGCTCGAGACTCTGACTGTATGAGATGTTAAATAC-TTTTTAAT

ATTTGTTTAGATATGACATTTATTCAAAGTTAAAAGCAAACACTTACAGAATTATGAAGAGGTATCTGTTTAACATT-TCCTCAG

TCAAGTTCAGAGTCTTCAGAGACTTCGTAATTAAAGGAACAGAGTGAGAGACATCATCAAGTGGAGAGAAATC-ATAGTTTA

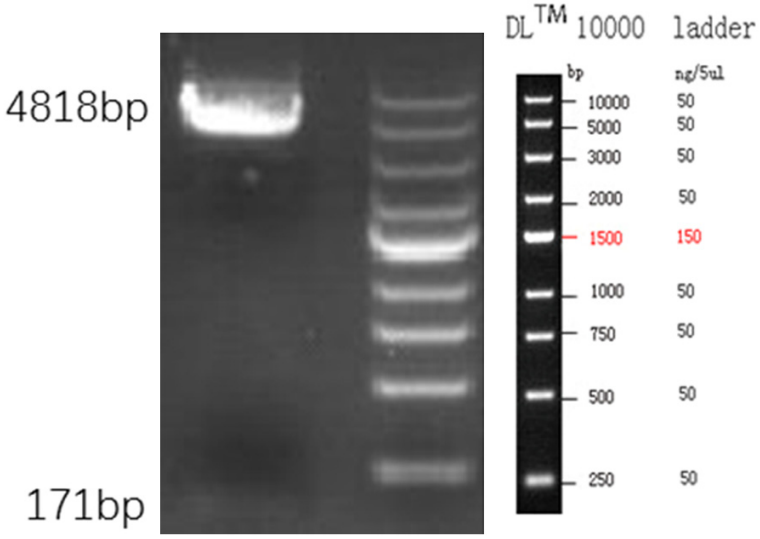
AACTGCATTATAAATTTTATAACAGAATTAAAGTAGATTTTAAAAGATAAAATGTGTAATTTTGTTTATATTTTCCCA-TTTGGAC

TGTAACTGACTGCCTTGCTAAAAGATTATAGAAGTAGCAAAAAGTATTGAAATGTTTGCATAAAGTGTCTATAATA-AAACTAA

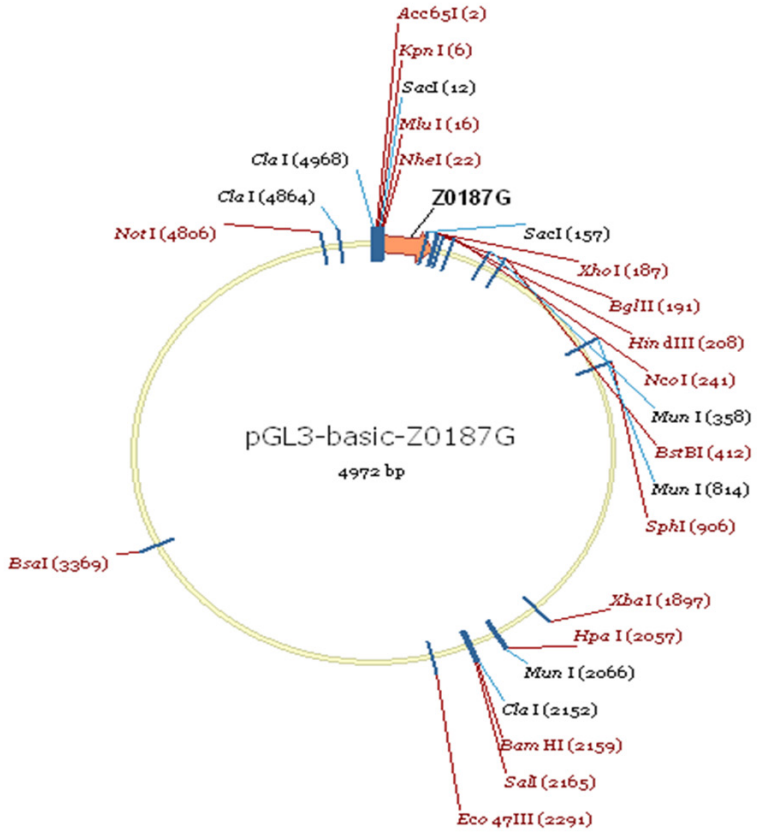
ACTTTCATGTGACTGGGCGGCCGCTGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGT-GTGAGGA

Supplementary 2. Vector of promoter region

1. Gel electrophoresis of the vector:



2. Structure:



1) The below underlined nucleotides: primers designing positions.

2) Red; GAGGCT/TTCGAG; two binding sites of miR-1304-3p.

3) GCTAGC; NheI.

CTCGAG; XhoI.

4) Whole sequence: (T = U)

1	GGTACCGAGC	TCTTACGCGT	GCTAGCGAAT	CAGCATTCAG	TCAATCCGGG
51	CCGGGAGCAG	TCATCTGTGG	TGAGGCTGAT	TGGCTGGGCA	GGAACAGCGC
101	CGGGGCGTGG	GCTGAGCACA	GCCGCTTCGC	TCTCTTTGCC	ACAGGAAGCC
151	TGAGCTCATT	CGAGTAGCGG	CTCTTCCAAG	CTCAACTCGA	GATCTGCGAT
201	CTAAGTAAGC	TTGGCATTCC	GGTACTGTTG	GTAAAGCCAC	CATGGAAGAC
251	GCCAAAAACA	TAAAGAAAGG	CCCGGCGCCA	TTCTATCCGC	TGGAAGATGG
301	AACCGCTGGA	GAGCAACTGC	ATAAGGCTAT	GAAGAGATAC	GCCCTGGTTC
351	CTGGAACAAT	TGCTTTTACA	GATGCACATA	TCGAGGTGGA	CATCACTTAC
401	GCTGAGTACT	TCGAAATGTC	CGTTCGGTTG	GCAGAAGCTA	TGAAACGATA
451	TGGGCTGAAT	ACAAATCACA	GAATCGTCGT	ATGCAGTGAA	AACTCTCTTC
501	AATTCTTTAT	GCCGGTGTTG	GGCGCGTTAT	TTATCGGAGT	TGCAGTTGCG
551	CCCGCGAACG	ACATTTATAA	TGAACGTGAA	TTGCTCAACA	GTATGGGCAT
601	TTCGCAGCCT	ACCGTGGTGT	TCGTTTCCAA	AAAGGGGTTG	CAAAAAATTT
651	TGAACGTGCA	AAAAAAGCTC	CCAATCATCC	AAAAAATTAT	TATCATGGAT
701	TCTAAAACGG	ATTACCAGGG	ATTTCAGTCG	ATGTACACGT	TCGTCACATC
751	TCATCTACCT	CCCGGTTTTA	ATGAATACGA	TTTTGTGCCA	GAGTCCTTCG
801	ATAGGGACAA	GACAATTGCA	CTGATCATGA	ACTCCTCTGG	ATCTACTGGT
851	CTGCCTAAAG	GTGTCGCTCT	GCCTCATAGA	ACTGCCTGCG	TGAGATTCTC
901	GCATGCCAGA	GATCCTATTT	TTGGCAATCA	AATCATTCCG	GATACTGCGA
951	TTTTAAGTGT	TGTTCCATTC	CATCACGGTT	TTGGAATGTT	TACTACACTC
1001	GGATATTTGA	TATGTGGATT	TCGAGTCGTC	TTAATGTATA	GATTTGAAGA
1051	AGAGCTGTTT	CTGAGGAGCC	TTCAGGATTA	CAAGATTCAA	AGTGCGCTGC
1101	TGGTGCCAAC	CCTATTCTCC	TTCTTCGCCA	AAAGCACTCT	GATTGACAAA
1151	TACGATTTAT	CTAATTTACA	CGAAATTGCT	TCTGGTGGCG	CTCCCCTCTC
1201	TAAGGAAGTC	GGGGAAGCGG	TTGCCAAGAG	GTTCCATCTG	CCAGGTATCA
1251	GGCAAGGATA	TGGGCTCACT	GAGACTACAT	CAGCTATTCT	GATTACACCC
1301	GAGGGGGATG	ATAAACCGGG	CGCGGTCGGT	AAAGTTGTTC	CATTTTTTGA
1351	AGCGAAGGTT	GTGGATCTGG	ATACCGGGAA	AACGCTGGGC	GTTAATCAAA
1401	GAGGCGAACT	GTGTGTGAGA	GGTCCTATGA	TTATGTCCGG	TTATGTAAAC
1451	AATCCGGAAG	CGACCAACGC	CTTGATTGAC	AAGGATGGAT	GGCTACATTC
1501	TGGAGACATA	GCTTACTGGG	ACGAAGACGA	ACACTTCTTC	ATCGTTGACC
1551	GCCTGAAGTC	TCTGATTAAG	TACAAAGGCT	ATCAGGTGGC	TCCCGCTGAA
1601	TTGGAATCCA	TCTTGCTCCA	ACACCCCAAC	ATCTTCGACG	CAGGTGTCGC
1651	AGGTCTTCCC	GACGATGACG	CCGGTGAACT	TCCCGCCGCC	GTTGTTGTTT
1701	TGGAGCACGG	AAAGACGATG	ACGGAAAAAG	AGATCGTGGA	TTACGTCGCC
1751	AGTCAAGTAA	CAACCGCGAA	AAAGTTGCGC	GGAGGAGTTG	TGTTTGTGGA
1801	CGAAGTACCG	AAAGGTCTTA	CCGGAAAACT	CGACGCAAGA	AAAATCAGAG
1851	AGATCCTCAT	AAAGGCCAAG	AAGGGCGGAA	AGATCGCCGT	GTAATTCTAG
1901	AGTCGGGGCG	GCCGGCCGCT	TCGAGCAGAC	ATGATAAGAT	ACATTGATGA
1951	GTTTGGACAA	ACCACAACTA	GAATGCAGTG	AAAAAAATGC	TTTATTTGTG
2001	AAATTTGTGA	TGCTATTGCT	TTATTTGTAA	CCATTATAAG	CTGCAATAAA
2051	CAAGTTAACA	ACAACAATTG	CATTCATTTT	ATGTTTCAGG	TTCAGGGGGA
2101	GGTGTGGGAG	GTTTTTTAAA	GCAAGTAAAA	CCTCTACAAA	TGTGGTAAAA

2151	TCGATAAGGA	TCCGTCGACC	GATGCCCTTG	AGAGCCTTCA	ACCCAGTCAG
2201	CTCCTTCCGG	TGGGCGCGGG	GCATGACTAT	CGTCGCCGCA	CTTATGACTG
2251	TCTTCTTTAT	CATGCAACTC	GTAGGACAGG	TGCCGGCAGC	GCTCTTCCGC
2301	TTCCTCGCTC	ACTGACTCGC	TGCGCTCGGT	CGTTCGGCTG	CGGCGAGCGG
2351	TATCAGCTCA	CTCAAAGGCG	GTAATACGGT	TATCCACAGA	ATCAGGGGAT
2401	AACGCAGGAA	AGAACATGTG	AGCAAAAGGC	CAGCAAAAGG	CCAGGAACCG
2451	TAAAAAGGCC	GCGTTGCTGG	CGTTTTTCCA	TAGGCTCCGC	CCCCCTGACG
2501	AGCATCACAA	AAATCGACGC	TCAAGTCAGA	GGTGGCGAAA	CCCGACAGGA
2551	CTATAAAGAT	ACCAGGCGTT	TCCCCCTGGA	AGCTCCCTCG	TGCGCTCTCC
2601	TGTTCCGACC	CTGCCGCTTA	CCGGATACCT	GTCCGCCTTT	CTCCCTTCGG
2651	GAAGCGTGGC	GCTTTCTCAT	AGCTCACGCT	GTAGGTATCT	CAGTTCGGTG
2701	TAGGTCGTTC	GCTCCAAGCT	GGGCTGTGTG	CACGAACCCC	CCGTTCAGCC
2751	CGACCGCTGC	GCCTTATCCG	GTAACTATCG	TCTTGAGTCC	AACCCGGTAA
2801	GACACGACTT	ATCGCCACTG	GCAGCAGCCA	CTGGTAACAG	GATTAGCAGA
2851	GCGAGGTATG	TAGGCGGTGC	TACAGAGTTC	TTGAAGTGGT	GGCCTAACTA
2901	CGGCTACACT	AGAAGAACAG	TATTTGGTAT	CTGCGCTCTG	CTGAAGCCAG
2951	TTACCTTCGG	AAAAAGAGTT	GGTAGCTCTT	GATCCGGCAA	ACAAACCACC
3001	GCTGGTAGCG	GTGGTTTTTT	TGTTTGCAAG	CAGCAGATTA	CGCGCAGAAA
3051	AAAAGGATCT	CAAGAAGATC	CTTTGATCTT	TTCTACGGGG	TCTGACGCTC
3101	AGTGGAACGA	AAACTCACGT	TAAGGGATTT	TGGTCATGAG	ATTATCAAAA
3151	AGGATCTTCA	CCTAGATCCT	TTTAAATTAA	AAATGAAGTT	TTAAATCAAT
3201	CTAAAGTATA	TATGAGTAAA	CTTGGTCTGA	CAGTTACCAA	TGCTTAATCA
3251	GTGAGGCACC	TATCTCAGCG	ATCTGTCTAT	TTCGTTCATC	CATAGTTGCC
3301	TGACTCCCCG	TCGTGTAGAT	AACTACGATA	CGGGAGGGCT	TACCATCTGG
3351	CCCCAGTGCT	GCAATGATAC	CGCGAGACCC	ACGCTCACCG	GCTCCAGATT
3401	TATCAGCAAT	AAACCAGCCA	GCCGGAAGGG	CCGAGCGCAG	AAGTGGTCCT
3451	GCAACTTTAT	CCGCCTCCAT	CCAGTCTATT	AATTGTTGCC	GGGAAGCTAG
3501	AGTAAGTAGT	TCGCCAGTTA	ATAGTTTGCG	CAACGTTGTT	GCCATTGCTA
3551	CAGGCATCGT	GGTGTCACGC	TCGTCGTTTG	GTATGGCTTC	ATTCAGCTCC
3601	GGTTCCCAAC	GATCAAGGCG	AGTTACATGA	TCCCCCATGT	TGTGCAAAAA
3651	AGCGGTTAGC	TCCTTCGGTC	CTCCGATCGT	TGTCAGAAGT	AAGTTGGCCG
3701	CAGTGTTATC	ACTCATGGTT	ATGGCAGCAC	TGCATAATTC	TCTTACTGTC
3751	ATGCCATCCG	TAAGATGCTT	TTCTGTGACT	GGTGAGTACT	CAACCAAGTC
3801	ATTCTGAGAA	TAGTGTATGC	GGCGACCGAG	TTGCTCTTGC	CCGGCGTCAA
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3951	ATCCAGTTCG	ATGTAACCCA	CTCGTGCACC	CAACTGATCT	TCAGCATCTT
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4151	GATACATATT	TGAATGTATT	TAGAAAAATA	AACAAATAGG	GGTTCCGCGC
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4351	GCCGGCTTTC	CCCGTCAAGC	TCTAAATCGG	GGGCTCCCTT	TAGGGTTCCG
4401	ATTTAGTGCT	TTACGGCACC	TCGACCCCAA	AAAACTTGAT	TAGGGTGATG
4451	GTTCACGTAG	TGGGCCATCG	CCCTGATAGA	CGGTTTTTCG	CCCTTTGACG
4501	TTGGAGTCCA	CGTTCTTTAA	TAGTGGACTC	TTGTTCCAAA	CTGGAACAAC
4551	ACTCAACCCT	ATCTCGGTCT	ATTCTTTTGA	TTTATAAGGG	ATTTTGCCGA

4601	TTTCGGCCTA	TTGGTTAAAA	AATGAGCTGA	TTTAACAAAA	ATTTAACGCG
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4701	GCGCAACTGT	TGGGAAGGGC	GATCGGTGCG	GGCCTCTTCG	CTATTACGCC
4751	AGCCCAAGCT	ACCATGATAA	GTAAGTAATA	TTAAGGTACG	GGAGGTACTT
4801	GGAGCGGCCG	CAATAAAATA	TCTTTATTTT	CATTACATCT	GTGTGTTGGT
4851	TTTTTGTGTG	AATCGATAGT	ACTAACATAC	GCTCTCCATC	AAAACAAAAC
4901	GAAACAAAAC	AAACTAGCAA	AATAGGCTGT	CCCCAGTGCA	AGTGCAGGTG
4951	CCAGAACATT	TCTCTATCGA	TA		
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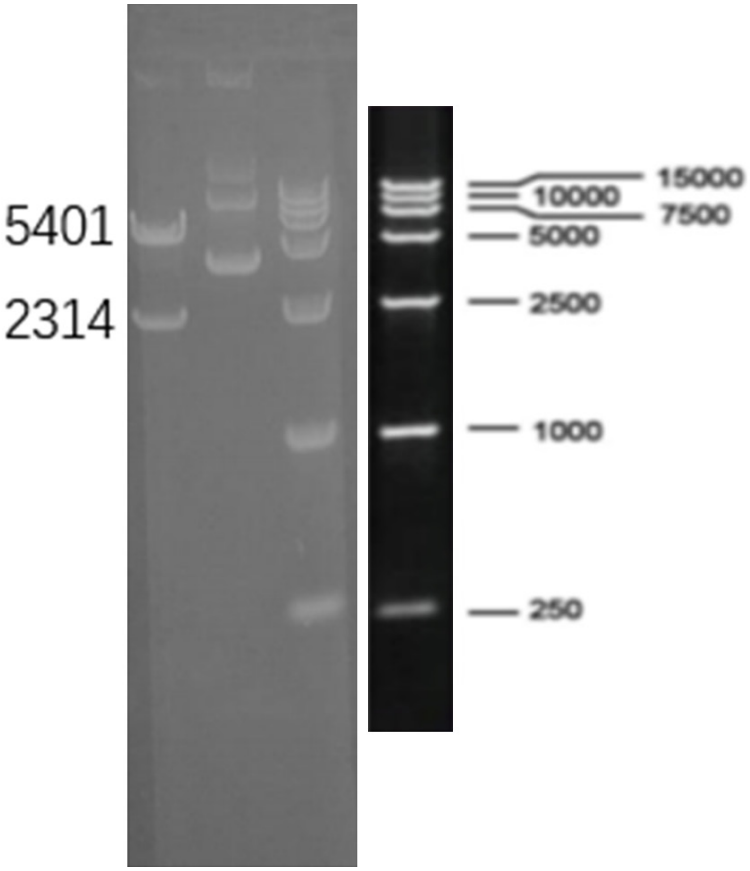
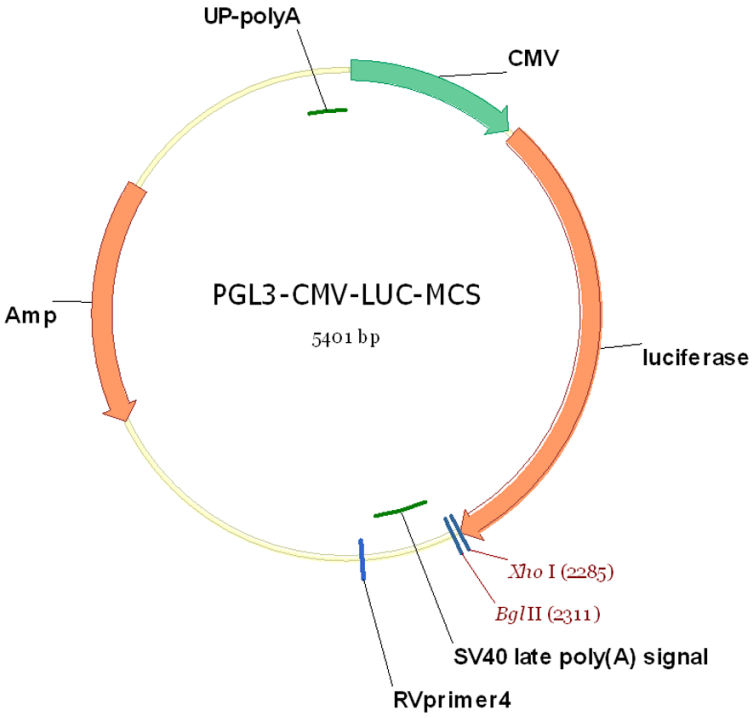
5051					
5101					
5151					
5201					
5251					
5301					

Supplementary 3. Vector of UCA1

1. Gel electrophoresis of the vector:



2. Structure:



1) CTCGAG; XhoI.

AGATCT; BglII.

2) Whole sequence: (T = U)

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gtttcatgtggttttcctttcctttccttatatgtgaaaaggtgacagaaaagaaatcttcctaaaagagtcagAGATCT

3) Positions of putative miR-1304-3p binding sites:

186-190;

290-295;

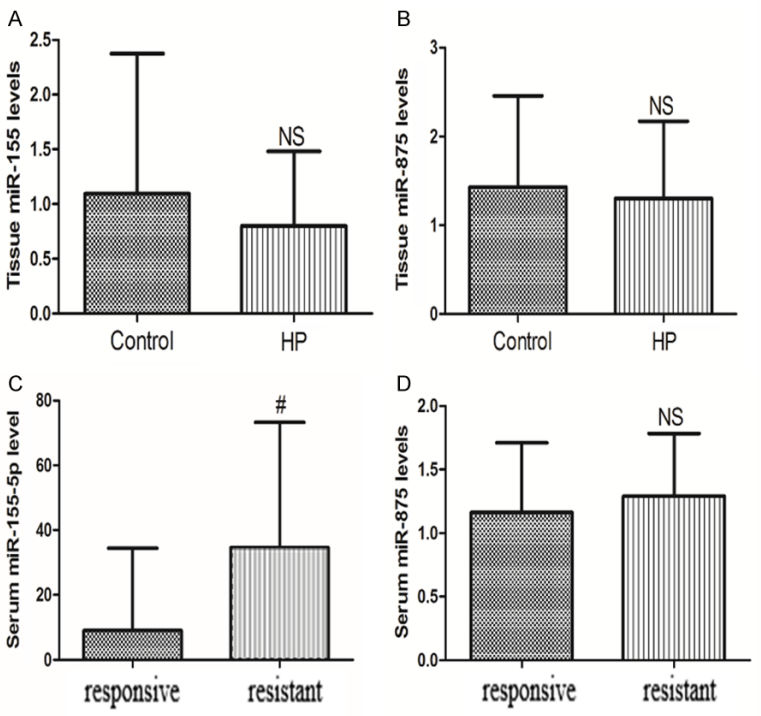
563-568;

642-647;

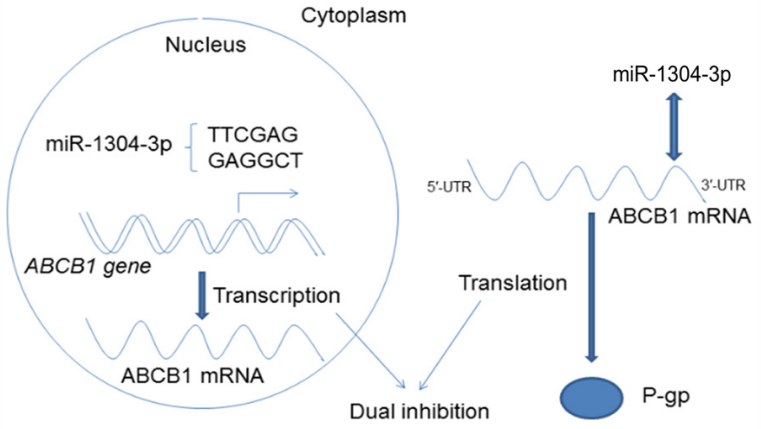
841-845;

1485-1490;

2236-2240.



Supplementary Figure 1. A, B. The expression levels of miR-155-5p and 875-5p in hippocampal tissues. C, D. The expression levels of miR-155-5p and 875-5p in serum samples. #P<0.05. NS, no significance. HP, hippocampus. Responsive, drug-responsive epilepsy. Resistant, drug-resistant epilepsy.



Supplementary Figure 2. miR-1304-3p not only can suppress translation of ABCB1 mRNA, but also inhibit transcription of ABCB1 gene.