Aberrant expression of miR-153 is associated with overexpression of hypoxia-inducible factor-1α in refractory epilepsy

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Evidence suggest that overexpression of hypoxia-inducible factor-1α (HIF-1α) is linked to multidrug resistance of epilepsy. Here we explored whether aberrant expression of HIF-1α is regulated by miRNAs. Genome-wide microRNA expression profiling was performed on temporal cortex resected from mesial temporal lobe epilepsy (mTLE) patients and age-matched controls. miRNAs that are putative regulator of HIF-1α were predicted via target scan and confirmed by real-time quantitative polymerase chain reaction (RT-qPCR). Mimics or miRNA morpholino inhibitors were transfected in astrocytes and luciferase reporter assay was applied to detect HIF-1α expression. Microarray profiling identified down-regulated miR-153 as a putative regulator of HIF-1α in temporal cortex resected from surgical mTLE patients. RT-qPCR confirmed down-regulation of miR-153 in plasma of mTLE patients in an independent validation cohort. Knockdown of miR-153 significantly enhanced expression of HIF-1α while forced expression of miR-153 dramatically inhibited HIF-1α expression in pharmacoresistant astrocyte model. Luciferase assay established that miR-153 might inhibit HIF-1α expression via directly targeting two binding sites in the 3′ UTR region of HIF-1α transcript. These data suggest that down-regulation of miR-153 may contribute to enhanced expression of HIF-1α in mTLE and serve as a novel biomarker and treatment target for epilepsy.

Epilepsy is one of the most prevalent neurological disorders worldwide that affects about 1.5–2% of the world population. It has been shown that 30–40% patients with epilepsy are pharmacoresistant to multiple anti-epileptic drugs (AEDs).1–3 Mesial temporal lobe epilepsy (mTLE), a special form of epilepsy, is particularly relevant due to its high frequency of therapeutic resistance. However, the molecular mechanisms underlying multidrug resistance of epilepsy remain largely unknown4–6.

Hypoxia-inducible factor 1α (HIF-1α) is the main transcription factor responsible for the cellular adaptation to hypoxia7. Recently, growing evidence indicates HIF-1α signaling is involved in various physiological processes in the brain8–10 as well as pathogenesis of various neurological diseases such as ischemic and hypoxic encephalopathy11. Activated HIF-1 is shown to transcriptionally enhance the expression of multidrug transporters such as P-glycoprotein (P-gp) in astrocytes, resulting in decreased accumulation of AED in the brain12. Consistent with these notions, we previously found that expression levels of HIF-1α and P-gp are coordinately elevated in hippocampus and temporal lobe of patients with mTLE as well as pharmacoresistant TLE rat model kindled by coriaria lactone13–16. Accordingly, targeting HIF-1α represents an attractive strategy to enhance the efficacy of current therapies of refractory epilepsy.

However, how HIF-1α expression is regulated during epileptogenesis and drug resistance formation remains largely unknown. MicroRNAs (miRNAs) are small, noncoding RNA species that play critical roles in regulating protein expression via inhibiting translation and/or mRNA degradation17. Previous studies suggest that HIF-1α expression is tightly regulated by multiple miRNAs in different tissues/cells. For example, it has been shown that miR-20b and miR-199a may regulate HIF-1α expression in MCF-7 breast cancer cells18 and cardiomyocytes19.
respectively. Notably, recent study suggested that expression levels of numerous miRNAs are significantly dysregulated after status epilepticus in rat20. This raises the possibility that abnormally expressed miRNAs which contribute to the pathogenesis of epilepsy and enhanced expression of HIF-1α could be, at least in part, caused by loss of expression of some regulatory miRNAs.

To examine the possible role of miRNA in refractory epilepsy, we performed a genome-wide miRNA expression profiling studies to identify aberrantly expressed miRNAs in temporal cortex tissues isolated from mTLE patients. Via target prediction, we screened for significantly down-regulated miRNAs that are putative regulatory of HIF-1α expression. We then validated aberrant expression of these miRNAs in a larger validation cohort, and used functional assays to investigate their roles in regulating HIF-1α expression. These findings have largely extended the current concepts of mTLE pathogenesis to miRNA-mediated gene expression regulation.

Results

miR-153 is down-regulated in temporal cortex of mTLE patients. As shown in Fig. 1a, of all 428 miRNA expressed in temporal cortex, 65 miRNAs were down-regulated and 29 miRNAs were up-regulated in mTLE patients with a fold change >1.5 (see Supplementary Tables S1 and S2 for detailed information on patients and microRNA results). Targetscan software was applied to identify down-regulated miRNAs that are putative regulator of HIF-1α gene and four miRNAs with robust binding sites in HIF-1α transcript, including miR-153, miR-543, miR-194 and miR-494, were identified (Fig. 1b). Previous studies found miR-153 expression was significantly down-regulated in drug-resistant K562 cells21 while miR-494 played a critical role in multidrug resistance in SW480 cells22. Accordingly, in the present study, we focused on the function of miR-153 and miR-494 in regulating HIF-1α expression and refractory epilepsy. Consistent with the microarray data, real-time PCR analysis in 32 surgical mTLE cases and 18 controls confirmed miR-153 and miR-494 were both down-regulated in temporal cortex of patients with mTLE (see Table 1 and Fig. 2 for patients information and PCR results).

miR-153 is down-regulated in plasma of mTLE patients. Recent studies suggested miRNAs are highly stable in blood23,24. Consistent with data from brain tissue, we observed significantly reduced expression of miR-153 in plasma of 32 surgical patients with mTLE patients as compared with 18 surgical controls (p < 0.001) (Fig. 3a). In contrast, no significant difference in miR-494 expression was observed between these surgical patients and controls (p = 0.14) (Fig. 3b). Furthermore, down-regulation of miR-153 in plasma was validated in overall 56 mTLE patients with/without surgery compared to 101 healthy non-surgical controls (p < 0.001) (see Table 2 and Fig. 3c for patients information and PCR results), while no significant difference of plasma miR-494 level was found (p = 0.43) (Fig. 3d).
Such as fasting glucose and alanine aminotransferase (model 3).

Together, these results established that HIF-1α expression is repressed by miR-153.

As expected, HIF-1α expression was significantly up-regulated as a result of miR-153 inhibition (Fig. 4c), but not by siRNA. These findings suggest that decreased miR-153 levels in plasma may play a critical role in drug-resistant epilepsy and may serve as a potential diagnostic marker and therapeutic target for refractory epilepsy.

miR-153 directly targets HIF-1α 3’UTR. A 217bp fragment of HIF-1α 3’-UTR containing the two possible miR-153 target sites was cloned and inserted after the luciferase reporter gene (Fig. 5a, WT). We also constructed three mutant vectors with impaired miR-153 binding site 1, 2 or both, respectively (Fig. 5a, MUT-1~3). Of note, co-transfection of luciferase reporters and miR-153 mimics or control revealed that, miR-153 significantly decreased the relative luciferase activity of WT, MUT-1 and MUT-2, but had no effect on MUT-3 (Fig. 5b). These results supported the role of miR-153 in directly inhibiting HIF-1α expression, and suggested that both binding sites are essential for miR-153-induced inhibition of HIF-1α expression.

Discussion

The key finding of our study is that miR-153 is significantly down-regulated in both temporal cortex tissue and plasma of patients with mTLE. Bioinformatics analysis predicted and our results determined that HIF-1α is a direct target of miR-153. Overexpression of miR-153 in rat astrocytes inhibit HIF-1α transcription while inhibition of miR-153 significantly up-regulated HIF-1α expression. These findings suggest that decreased miR-153 may play a critical role in drug-resistant epilepsy and may serve as a potential diagnostic marker and therapeutic target for refractory epilepsy.

Overexpression of multidrug resistance gene 1 (MDR1), specifically P-gp in brain astrocytes decreases accumulation of AEDs in the brain, which has been widely accepted as a crucial factor in drug-resistant epilepsy25,28. Numerous studies on tumor pharmacoresistance mechanism suggest MDR1 expression is modulated by HIF-1α27,28. In our previous study, we found HIF-1α and P-gp are coordinately overexpressed in hippocampus and temporal lobe of patients and animal models with refractory epilepsy29, which was again confirmed in this study. Activated HIF-1α is shown to enhance the transcription of P-gp in astrocytes, leading to lower concentration of AED in the brain32. Silencing HIF-1α by siRNA could reversely increase the sensitivity of transfected cells in different drug-resistant models31,29. Collectively, it suggests that HIF-1α may be a core factor involving drug-resistance of epilepsy and may be a potential therapeutic intervention strategy. However, the underlying mechanism of HIF-1α deregulation in epilepsy remains poorly understood.

MicroRNAs, a class of small noncoding RNA molecules that function as post-transcriptional regulators of gene expression by sequence complementarities with their target mRNA molecules, have been identified as key regulators in almost all aspects of cellular processes, such as cell proliferation, differentiation, apoptosis and...
cellular response to stimulus. Recent studies support that aberrant alterations of miRNAs have been associated with a variety of neurological disorders, including epilepsy. However, it still remains largely unknown how miRNAs contribute to multidrug resistance in epilepsy. In this study, we performed a genome-wide miRNA expression profiling and identified 65 down-regulated miRNAs and 29 up-regulated miRNAs in temporal cortex tissue isolated from patients with refractory mTLE. By targeting different mRNAs, these miRNAs might play important roles in fine-tuning of signaling pathways that control normal brain development and drug resistance in refractory epilepsy.

In this study, both miR-153 and miR-494 were found down-regulated in the temporal cortex of patients with mTLE and predicted as a putative regulator of HIF-1α gene with robust binding sites in HIF-1α transcript by bioinformatics analysis. However, only miR-153 was shown also decreased in the peripheral plasma. Similarly, previous literature reported dysregulation of miR-494 in pathogenesis of epilepsy in both animal models and clinical studies. However, to the best of our knowledge, this is the first work that shows decreased miR-153 level is related to drug-resistant epilepsy through mediating overexpression of HIF-1α. The discrepancies with previous studies may due to different inclusion criteria and standard for the surgery selection of patients with mTLE and varied technics and standards applied to screen for significantly dysregulated miRNAs. In addition, application...
of different animal models and/or brain regions, extraneous effects such as racial, regional difference and other individual characteristics may also affect the profiling of miRNA abundance. Thus, the results required to be further validated in the future.

Using miRNA overexpression, miRNA function inhibition and luciferase assay, we demonstrated that overexpression of miR-153 resulted in up-regulation of HIF-1α, which is immediately linked to pharmacoresistance via inducing expression of numerous drug transporters and decreasing accumulation of AEDs in the brain. Furthermore, inhibition of miR-153 significantly decreased the HIF-1α expression. Although gene expression is regulated at multiple transcriptional and post-transcriptional levels, our data provides clear evidence that miR-153 is a critical regulator of HIF-1α expression and may serve as a potential therapeutic strategy for drug-resistant epilepsy.

miRNAs might easily penetrate the blood–brain barrier (BBB) and are highly stable in blood. Several previous studies confirmed selected miRNAs expression in injured brain tissues and blood are highly correlated. In line with results from brain tissue, we found that abundance of plasma miR-153 is significantly reduced in the same surgical patients as well as a larger cohort of mTLE patients as compared with controls. On the contrary, plasma miR-494 showed no difference between patients and controls. Multivariable linear regression analysis showed the reduced miR-153 in plasma is an independent risk factor associated with mTLE, suggesting miR-153 might be used as a robust biomarker for drug-resistant epilepsy.

Table 2. Clinical information of overall 56 patients with mTLE and 101 healthy controls included for analysis of plasma miR-153 level. SPS: simple partial seizure; CPS: complex partial seizure; sGTCS: secondary generalized tonic-clonic seizure; AEDs: anti-epileptic drugs. AEDs used by patients include: phenobarbital, phenothiazine, carbamazepine, lamotrigine, levetiracetam, valproate acid, topiramate, oxcarbazepine and clonazepam.

Table 3. Multiple linear regression analyses of the association between miR-153 and mTLE. Model 1: unadjusted; Model 2: adjusted for gender and age; Model 3: adjusted for all Baseline characteristics examined. Association between different variables and epilepsy are shown as Standard β (p-value).
exposure, supporting its role in protecting against teratogenic effects of chemical exposure in neural cells. In a study by Doxakis et al., researchers also found down-regulation of miR-153 might lead to alpha-synuclein accumulation, which contributes to the pathogenesis of Parkinson disease. As suggested previously, each miRNA can potentially regulate hundreds of target genes, with the prediction that more than one-third of all human genes may be regulated by miRNAs. Further studies are needed to explore other functions of miR-153 in epileptogenesis and drug-resistance using both patient samples and epilepsy animal models.

There are several limitations of our study. Firstly, the number of brain samples used to validate the microRNA profiling is relatively limited and no hippocampus tissue was detected due to difficulty in tissue achieving especially the normal hippocampus as controls. Secondly, although we demonstrate miR-153 can regulate HIF-1α in rat astrocytes in vitro, animal models of mTLE should be used in the future to further confirm our findings. Thirdly, as we have mainly focused on miR-153 in this study, the role of other deregulated miRNAs, such as miR-543 and miR-494, in the epileptogenesis and/or drug-resistance should be further investigated in the future.

Figure 4. MiR-153 inhibits protein expression of HIF-1α. (a) Putative miR-153 binding sites predicted by targetscan in 3′-UTR of human HIF-1α gene. (b,c) Transfection of miR-153 mimics significantly reduces expression of HIF-1α in astrocytes as compared with control transfection with vehicle or miR-328 mimics. (d,e) Transfection of miR-153 inhibitor significantly enhanced expression of HIF-1α in astrocytes as compared with transfection with vehicle or miR-328 inhibitor.
In conclusion, our results suggest that miR-153 is significantly down-regulated in temporal cortex and plasma of patients with refractory epilepsy. This might be linked to up-regulation of HIF-1α in the patients' brain tissue and provide a promising therapeutic target for refractory epilepsy. As increasing number of miRNAs in regulating pathogenesis of epilepsy have been discovered, we suggest that this connection may be widespread. Considering the fact that different brain subfields may function differentially and have various contributions to the establishment of an epileptic state, future studies with detailed miRNA profiling in each structure would certainly facilitate data interpretation, and lead to the identification of genetic subtypes and their association with treatment response.

Methods
mTLE patients and control group. 56 patients who were diagnosed as mTLE and had undergone treatment in West China Hospital from Jan 2010 to Jan 2013 were included in this study. 32 of 56 patients with mTLE had undergone anterior temporal lobectomy in our hospital. All patients were comprehensively evaluated and met the definition of mTLE: (I) seizure semiology consistent with mTLE, usually with epigastric/autonomic/psychic auras, followed by complex partial seizures; (II) electroencephalography confirmed the seizure onset zone in the temporal lobe; (III) Magnetic resonance imaging showed no lesions other than uni- or bilateral hippocampal atrophy (reduced hippocampal dimensions and increased T2 signal); (IV) clinical histopathological examination consistent with hippocampus sclerosis; and (V) no evidence of dual pathology identified by any assessment.

Figure 5. MiR-153 directly targets 3'-UTR of human HIF-1α gene. (a) Schema of luciferase reporter constructs used in the luciferase assays. A 217 fragment of HIF-1α 3'-UTR containing two possible miR-153 target sites predicted by TargetScan was inserted after the luciferase gene. The “X” sign over the gray boxes represents the deleted miRNA-binding sites. ‘WT’ stands for wild type and ‘MUT’ for mutant. (b) Dual luciferase assays of astrocytes cotransfected with miR-153 mimics and luciferase reporters containing WT or MUT HIF-1α 3'-UTR. Normalized firefly luciferase activity (firefly luciferase activity/Renilla luciferase activity) was calculated. Histograms show mean ± SD values of the relative luciferase activity of cells transfected with miR-153 with respect to those transfected with vehicle only or control miRNA. For each transfection, luciferase activity was averaged from six replicates. *P < 0.001, student's t-test.
In addition, 18 patients who had undergone surgical treatment for head trauma or cerebral hemorrhage and 101 age-matched healthy controls were also included. The study was approved by the Ethics Committees of West China Hospital. Informed consent was obtained from each patient and all experiments were performed in accordance with relevant guidelines.

**Brain tissue collection and RNA extraction.** Temporal cortex tissues resected from 32 patients with refractory mTLE who had undergone anterior temporal lobectomy were collected. The 18 control samples of temporal neocortical tissues without abnormal pathological changes were obtained from neurosurgery department of the same hospital. The control group included 5 head trauma and 13 cerebral hemorrhage cases. None of patients in control group was ever diagnosed of epilepsy or seizures. Resected brain tissues were immediately minced to small pieces and frozen in liquid nitrogen for further studies. For miRNA expression analysis, small RNAs from temporal cortex tissue from patients or controls were isolated using miRNA extraction kit (Qiagen) according to manufacturer's instruction. For detection of HIF-1α mRNA in brain tissue, RNA was extracted according to Trizol method (Invitrogen, USA).

**Plasma collection and RNA Extraction.** 5 ml blood samples from all 56 mTLE cases, 18 surgical control cases and 101 healthy controls were collected in EDTA-containing tubes. Whole-blood samples were centrifuged at 1800g for 20 min at 4°C after blood collection, and the upper plasma phase was carefully transferred into microcentrifuge tube. A second centrifugation at 1600g for 10 min at 4°C to remove additional cellular debris and minimize contamination of cell-free nucleic acids derived from damaged blood cells. For long storage, plasma frozen in aliquots was kept at −80°C until further analysis. Total RNA was extracted from 100μl of plasma samples using TRizol LS reagent (Life Technologies) according to manufacturer's instructions. 3μl synthetic Caenorhabditis elegans miRNA cel-miR-39 (5′-UCACCGGGUGMAAAUACGCUUG-3’, 10nM) was used as spike-in control and added directly to each sample. RNA was re-suspended in 30μl of nuclease-free water and tested using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific).

**MicroRNA expression profiling.** The GeneChip miRNA 1.0 array (Affymetrix) was used for the miRNA expression profiling in 5 patients with refractory mTLE who had undergone epileptic surgery and 3 age-matched surgical controls. Equal amount of small RNAs isolated from different patients or controls were mixed to generate a pool of patient and a pool of control. 1μg of small RNAs in each pool were used for microarray analysis using GeneChip miRNA Array. Array hybridization, washing, and scanning of the slides were carried out by Beijing Capitalbio Corporation, inc. Microarray raw data were analyzed using the GeneSpring software (Agilent). Signals from different microarrays were normalized by mean gene expression value. Fold change >1.5 was used as a cut-off to identify up- and down-regulated miRNAs between different groups. We used online tools Targetscan (http://www.targetscan.org) for target prediction.

**Real-time quantitative PRC.** For detection of HIF-1α in brain tissue, PCR primers were designed and synthesized by Shanghai Shenggong (China). The β-actin was taken as an internal control. RT-qPCR was performed with the FTC2000 PCR system (Funglyn, Canada). The following conditions were used for amplification: 94°C for 2 min; 94°C for 20 s; 50°C for 20 s; and 60°C for 30 s for 45 cycles. The relative expression levels were calculated using the ΔΔCt method. For detection of miR-153 in brain/plasma sample, 10μl purified RNA was reverse transcribed using mature miRNA-specific primers and miScript II RT Kit (Qiagen) following the manufacturer’s protocol. The q-PCR assay was carried out in a 10μl reaction mixture using miScript SYBR Green PCR Kit (Qiagen) and the following thermal cycling condition: 95°C for 10 min, 40 cycles of PCR amplification at 95°C for 15 s, 60°C for 1 min. U6 and cel-miR-39 were used as internal controls to normalize miRNA in brain and plasma, respectively. The relative miRNA expression in each individual sample calculated by ΔΔCt method.

**Rat astrocyte culture and pharmacoresistant astrocyte model establishment.** Rat astrocytes were isolated as described in our previous study and were identified by immunocytochemical staining with glial fibrillary acidic protein (GFAP). All procedures were carried out in accordance with the Chinese Animal Welfare Act and approved by the responsible governmental agency at Sichuan University.

Pharmacoresistant astrocyte model was built as described in our previous study. Briefly, coriaria lactone (CL), a compound extracted from the plant loranthus or coriaria sinica maxim which can incur epilepsy in animals, was provided by the Pharmaceutical Company of Sichuan University (coriaria lactone injection ampoules, 1 ml = 55 mg; lot no. 980715) and was added to medium (12 ml/ml) to induce rat astrocytes to express HIF-1α. After 12 days induction, rat astrocytes grown under these conditions had high P-gp over-expression and optimal cell morphology were used in subsequent experiments.

**Transfection.** miRNA mimics or morpholin inhibitors for hsa-miR-153 (Ambion, Austin, TX) at a concentration of 50 nM were incubated with Lipofectamine 2000 (Invitrogen) in culture medium before addition to cells according to the manufacturer’s protocol. Vehicle treatment only and miRNA mimics or morpholin inhibitors for hsa-miR-328 (not targeting HIF-1α) were incubated under the same condition as controls.

**Luciferase reporter assay.** A 217 fragment of HIF1A 3′-UTR containing two possible miR-153 target sites predicted by TargetScan was cloned using primers: F: 5′-TAGAAAAATTGCCCTGATAT-3′, R: 5′-TAGATC CAACCAACAGAG-3′, and subcloned into TA-vector (Life science). The fragment then subcloned into pmirGLO dual-luciferase reporter vector (Promega,) using BamHI and Xho I restriction enzymes (NEB). The reporter gene constructs were cotransfected into cells containing a miR mimic control or miR-153 mimic for ca
36 h. The dual luciferase system (Promega) was used to measure luciferase activity following manufacturer's protocol. Normalized firefly luciferase activity (firefly luciferase activity/Renilla luciferase activity) was used to compare the expression of miR-153 between different samples. For each transfection, luciferase activity was averaged from three replicates. Mutations were generated using the QuickChange Site-Directed Mutagenesis Kit (Agilent). For MUT-1, the WT sequence of binding site 1 TACAATGTGTTGATTTT was changed to the antisense sequence AAAATCAAACATTGTA that impairs miRNA binding; For MUT-2, the WT sequence of binding site 2 AATATCTTGTTTTTTCTATGTAC was changed to the antisense sequence GTACATAGAAAAACAAGATATT.

Statistical analyses. All statistical analyses were performed by SPSS software 18.0 (SPSS, Inc., Chicago, USA). Relative expression of miRNAs was calculated by using the fold change in miRNAs expression method. Differences in miRNAs expression were determined by student’s t-test. We used ANOVA, multiple logistic regression analysis and ROC analysis to evaluate the diagnostic potential of different plasma miRNAs and its association with different physiological characteristics. P value < 0.05 (two-tailed) was considered statistically significant.

References
1. Guerini, R., Sicca, F. & Parmeggiani, L. Epilepsy and malformations of the cerebral cortex. Epileptic disorders: international epilepsy journal with videotape 5 Suppl 2, 59–26 (2003).
2. de Boer, H. M., Mula, M. & Sander, J. W. The global burden and stigma of epilepsy. Epilepsy & behavior: E&B 12, 540–546, doi: 10.1016/j.ybeh.2007.12.019 (2008).
3. Leonardi, M. & Ustun, T. B. The global burden of epilepsy. Epilepsia 43 Suppl 6, 21–25 (2002).
4. Brandt, C., Bethmann, K., Gastens, A. M. & Loscher, W. The multidrug transporter hypothesis of drug resistance in epilepsy: Proof-of-principle in a rat model of temporal lobe epilepsy. Neurobiology of disease 24, 202–211, doi: 10.1016/j.nbd.2006.06.014 (2006).
5. Kwan, P. & Sander, J. W. The natural history of epilepsy: an epidemiological view. Journal of neurology, neurosurgery, and psychiatry 75, 1376–1381, doi: 10.1136/jnnp.2004.045690 (2004).
6. Jackson, T. C. & Foster, T. C. Regional Health and Function in the hippocampus: Evolutionary compromises for a critical brain tissue and cells. Journal of cancer research and clinical oncology 136, 1697–1707, doi: 10.1007/s00432-010-0828-5 (2010).
7. Ding, Z. et al. Expression and significance of hypoxia-inducible factor-1 alpha and MDRI/P-glycoprotein in human colon carcinoma tissue and cells. Journal of cancer research and clinical oncology 136, 1697–1707, doi: 10.1007/s00432-010-0828-5 (2010).
8. Guntert, T., Gassmann, M. & Ogunshola, O. O. Temporal Rac1-HIF-1 crosstalk modulates hypoxic survival of aged neurons. Brain research, doi: 10.1016/j.brainres.2016.03.025 (2016).
9. Sen, T. & Sen, N. Treatment with an activator of hypoxia-inducible factor 1, DMOG provides neuroprotection after traumatic brain injury. Neuropharmacology 107, 79–88, doi: 10.1016/j.neuropharm.2016.03.009 (2016).
10. Womeldorf, M., Gillespie, D. & Jensen, R. L. Hypoxia-inducible factor-1 dependent regulation of the multidrug resistance (MDR1) gene. Circulation research 106, 1203–1208, doi: 10.1161/01.RES.000072104-1681-0 (2014).
11. Chai, J. et al. Highly Predictive Reprogramming of tRNA Modifications Is Linked to Selective Expression of Codon-Biased Genes. Chemical research in toxicology 28, 978–988, doi: 10.1021/acs.chemrestox.8b00005 (2015).
12. Deng, W. J., Nie, S., Dai, J., Wu, J. R. & Zeng, R. Proteome, phosphoproteome, and hydroxyproteome of liver mitochondria in diabetic rats at early pathogenic stages. Molecular & cellular proteomics: MCP 9, 100–116, doi: 10.1074/mcp.M900020-MCP200 (2010).
13. Marchi, N. et al. Significance of MDR1 and multiple drug resistance in refractory human epileptic brain tissue. European journal of pharmacology 107, 79–88, doi: 10.1016/j.neuropharm.2016.03.009 (2016).
14. Cascio, S. et al. miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells. Circulation research 110, 191–201, doi: 10.1161/CIRCRESAHA.111.247452 (2012).
15. Tishler, D. M. & Aronica, E. Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. Epilepsia 45, 441–451, doi: 10.1111/j.1528-1167.2004.07703.x (2004).
16. Song, X. et al. Hypoxia-induced resistance to cisplatin is linked to silencing of HIF-1alpha gene. Cancer chemotherapy and pharmacology 58, 776–784, doi: 10.1007/s00280-006-0224-7 (2006).
17. Risbud, R. M. & Porter, B. E. Changes in microRNA expression in the whole hippocampus and hippocampal synaptoneurosome fraction following pilocarpine induced status epilepticus. PloS one 8, e53464, doi: 10.1371/journal.pone.0053464 (2013).
18. Liu, L. et al. miR-153 sensitized the K562 cells to AxO3-induced apoptosis. Medicinal oncology 29, 241–247, doi: 10.1007/s12032-010-9807-6 (2012).
19. Chai, J. et al. MicroRNA-494 sensitizes colon cancer cells to fluorouracil through regulation of DPYD. IJMBB life 67, 191–201, doi: 10.1002/121661 (2015).
20. Tijssen, A. J., Pinto, Y. M. & Creemers, E. E. Circulating microRNAs as diagnostic biomarkers for cardiovascular diseases. American journal of physiology. Heart and circulatory physiology 303, H1085–H1095, doi: 10.1152/ajpheart.00191.2012 (2012).
21. Creemers, E. E., Tijssen, A. J., Creemers, E. E. Circulating microRNAs: novel biomarkers and extracellular communicators in cardiovascular diseases? Circulation research 110, 483–495, doi: 10.1161/CIRCRESAHA.111.247452 (2012).
22. Tishler, D. M. et al. MDRI gene expression in brain of patients with medically intractable epilepsy. Epilepsia 36, 1–15 (1995).
23. Aronica, E. et al. Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. Epilepsia 45, 441–451, doi: 10.1111/j.1528-1167.2004.07703.x (2004).
24. Comerford, K. M. et al. Hypoxia-inducible factor-1 dependent regulation of the multidrug resistance (MDR1) gene. Cancer research 62, 3387–3394 (2002).
25. Xu, Y. et al. Hypoxia-inducible factor-1alpha induces multidrug resistance protein in colon cancer. Oncotargets and therapy 8, 1941–1948, doi: 10.2147/ott.s82835 (2015).
26. Esteller, M. Non-coding RNAs in human disease. Nature reviews. Genetics 12, 861–874, doi: 10.1038/nrg3074 (2011).
27. Jimenez-Mateos, E. M. et al. Silencing microRNA-134 produces neuroprotective and prolonged seizure-suppressive effects. Nature medicine 10, 1087–1094, doi: 10.1038/nm.2384 (2012).
28. Roncon, P. et al. MicroRNA profiles in hippocampal granule cells and plasma of rats with pilocarpine-induced epilepsy—comparison with human epileptic samples. Scientific reports 5, 14143, doi: 10.1038/srep14143 (2015).

33. Chan, C. T. et al. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. Nature communications 3, 937, doi: 10.1038/ncomms1938 (2012).

34. Yin, S. et al. Dosage compensation on the active X chromosome minimizes transcriptional noise of X-linked genes in mammals. Genome biology 10, R74, doi: 10.1186/gb-2009-10-7-r74 (2009).

35. Yin, S. et al. Evidence that the nonsense-mediated mRNA decay pathway participates in X chromosome dosage compensation in mammals. Biochemical and biophysical research communications 383, 378–382, doi: 10.1016/j.bbrc.2009.04.021 (2009).

36. Yin, S., Deng, W., Hu, L. & Kong, X. The impact of nucleosome positioning on the organization of replication origins in eukaryotes. Biochemical and biophysical research communications 385, 363–368, doi: 10.1016/j.bbrc.2009.05.072 (2009).

37. Wang, P. et al. Evidence for common short trans sense-antisense pairing between transcripts from protein coding genes. Genome biology 9, R169, doi: 10.1186/gb-2008-9-12-r169 (2008).

38. Lei, H., Zhai, B., Yin, S., Gygi, S. & Reed, R. Evidence that a consensus element found in naturally intronless mRNAs promotes mRNA export. Nucleic acids research 41, 2517–2525, doi: 10.1093/nar/gks1314 (2013).

39. Liu, D. Z. et al. Brain and blood microRNA expression profiling of ischemic stroke, intracerebral hemorrhage, and kainate seizures. Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism 30, 92–101, doi: 10.1038/jcbfm.2009.186 (2010).

40. Wei, C. et al. miR-153 regulates SNAP-25, synaptic transmission, and neuronal development. PLoS one 8, e57080, doi: 10.1371/journal.pone.0057080 (2013).

41. Sempere, L. F. et al. Expression profiling of mammalian microRNAs uncovers a subset of brain-expressed microRNAs with possible roles in murine and human neuronal differentiation. Genome biology 5, R13, doi: 10.1186/gb-2004-5-3-r13 (2004).

42. Tsai, P. C. et al. MiR-153 targets the nuclear factor-1 family and protects against teratogenic effects of ethanol exposure in fetal neural stem cells. Biology open 3, 741–758, doi: 10.1242/bio.20147765 (2014).

43. Dokaik, E. Post-transcriptional regulation of alpha-synuclein expression by mir-7 and mir-153. The Journal of biological chemistry 285, 12726–12734, doi: 10.1074/jbc.M109.086827 (2010).

44. Guarneri, D. J. & DeLeon, R. J. MicroRNAs: a new class of gene regulators. Annals of medicine 40, 197–208, doi: 10.1080/07853890701771823 (2008).

45. Esquela-Kerscher, A. & Slack, F. J. Oncomirs-microRNAs with a role in cancer. Nature reviews. Cancer 6, 259–269, doi: 10.1038/nrc1840 (2006).

46. Engel, J. Jr. Surgery for seizures. The New England journal of medicine 334, 647–652, doi: 10.1056/nejm19960333341008 (1996).

47. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔC(T) Method. Methods (San Diego, Calif.) 25, 402–408, doi: 10.1006/meth.2001.1262 (2001).

48. Yang, T. H. et al. Suppression of the multidrug transporter P glycoprotein using RNA interference in cultured rat astrocytes induced by coriaria lactone. Neurological research 31, 1084–1091, doi: 10.1179/174313208x319134 (2009).

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Author Contributions

Dr. Li takes full responsibility for the data, analyses, interpretation and the conduct of the research, as well as drafting of the manuscript. Dr. Huang participated in experiment conducting, data analyses and manuscript drafting. Dr. Feng contributed in experiment design and conducting, literature review and manuscript revision. Dr. Li, Dr. Huang and Dr. Feng contributed equally to this manuscript. Dr. Liang and Dr. Wang participated in sample collection and experiment conducting. Dr. Zhou supported for clinical diagnosis and supervising of the research. Dr. Chen served as the corresponding author for providing study design, data analysis and interpretation and final approval of the manuscript.

Additional Information

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