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Original Article

MiRNA expression profiles in the hippocampi of immature rats following status epilepticus

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Abstract: Status epilepticus (SE) can cause damage to brain regions such as the hippocampus and can result in cognitive deficits and the pathogenesis of epilepsy. Moreover, special attention has been paid to children with epilepsy because of the high risk of impaired mental developmental and physical comorbidities. To date, miRNA expression in the developing hippocampus following SE has not been well characterized. In the present study, we investigated miRNA expression patterns in post-SE immature rats (24 h after status). Through miRNA array and differential analysis, 30 up-regulated miRNAs and 21 down-regulated miRNAs in developing rat hippocampi were identified. Six randomly selected up-regulated (miR-31b, 145-3p and let-7d-3p) and down-regulated (miR-96-5p, 182, 301b-3p) miRNAs were examined by qRT-PCR, and the results proved the accuracy of the miRNA array. Moreover, targets of these deregulated miRNAs were analyzed using the miRWalk database, showing that the identified enriched metabolic pathway and MAPK signaling pathway were involved in the molecular mechanisms underlying epileptogenesis. In conclusion, the present study provides clues for enhancing our understanding of the mechanisms and roles of miRNAs as key regulators of epileptogenesis.

Keywords: MiRNA, status epilepticus, miRNA array, pediatric, metabolic pathway, MAPK signaling

Introduction

Epilepsy is a chronic neurologic disorder characterized by recurrent unprovoked seizures [1], and it affects 1% of all people, with 20-30% of them experiencing more than one seizure per month. Epilepsy often results from abnormal and highly synchronous neuronal discharges within the brain [1]. Epileptogenesis refers to the events occurring between the first spontaneous seizure and the cause of epilepsy. Typically, epileptogenesis is a self-promoted pathological process triggered through an initial insult. Increasing evidence has shown that epileptogenesis results in eventual neuronal death or dysfunction, ion channel dysfunction, mossy fiber sprouting, gliosis, neurogenesis, inflammation, and so on [2, 3]. A prolonged, non-terminating seizure (status epilepticus, SE) is a neurological emergency that has the potential to cause irreversible brain damage. SE can follow drug withdrawal in patients with epilepsy, but it also occurs due to a myriad of other factors, including central nervous system (CNS) infection [4]. The molecular mechanisms underlying the transition from seizure to SE are poorly understood, but they might involve loss of surface receptors of the inhibitory neurotransmitter γ-amino butyric acid [5]. Moreover, special attention has been paid to children with epilepsy because of the high risk of impaired mental development and physical comorbidities. However, the underlying molecular mechanisms of SE in children are poorly understood. Hence, there is an increasing need for novel biomarkers and therapeutic targets in pediatric SE.

MicroRNAs (miRNAs) are short noncoding RNAs of 18 to 22 nucleotides that regulate gene expression at posttranscriptional levels by base pairing with targeted messenger RNA (mRNA) [6]. MiRNAs bind to the 3'-untranslated region of mRNA by perfect base pairing, leading to mRNA cleavage. In contrast, binding with imper-

fect base pairing can cause translational repression or deadenylation [6]. Each miRNA has the potential to suppress the expression of hundreds of genes [7]. Therefore, miRNA-mRNA interactions form a complex gene regulatory network. Disease-associated miRNAs represent a new class of diagnostic marker or therapeutic target [8]. Because the CNS expresses the richest diversities of miRNAs of all human tissues [9], these small non-coding RNAs could provide opportunities for the diagnosis and treatment of damaged nervous systems. To elucidate the functions of miRNAs in CNS disease, global profiling techniques, such as miRNA microarray, miRNA sequencing, real-time PCR, and next generation sequencing of miRNA [10, 11], could be performed to determine the differentially and uniquely expressed miRNAs involved in the diagnosis and treatment of CNS disease. For instance, miRNAs (miR-183, 135a, 125b, 128, 30c and 27a) were dynamically expressed in the rat pilocarpine model and in temporal lobe epilepsy patients [12]. A previous study by Hu et al reported 19 up-regulated miRNAs and 7 down-regulated miRNAs in the adult rat hippocampus following lithium-pilocarpine-induced status epilepticus using rat miRNA array and differential analysis [13]. However, no studies to date have characterized the miRNA expression patterns in developing hippocampi following SE.

In the present study, an SE model was established and identified in 25-day-old immature rats. MiRNA array and qRT-PCR were performed to determine the differential expression of miRNAs in hippocampi of developing rats following SE. Furthermore, the key miRNAs related to biological functions and pathways were analyzed.

Materials and methods

Animal and SE model

All of the animal research was approved by the Sichuan University Committee on Animal Research. Female Sprague-Dawley rats with mixed-sex litters were acquired from the animal center of Sichuan University (Chengdu, China). The mothers were provided food and water and were housed in a temperature- and light-controlled facility until the pups were 25 days old. All experiments were performed in accordance with relevant guidelines and regulations of the Sichuan University Committee on Animal Research.

Lithium chloride (125 mg/kg, Sigma) was intraperitoneally (i.p.) administered 18-20 h prior to pilocarpine injection. Pilocarpine was then i.p. administered (40 mg/kg, Sigma). The severity of convulsions was evaluated by electroencephalography, and only those animals that were defined as showing continuous behavioral seizure activity lasting at least 30 min were used in this study. All of the SE rats were then intraperitoneally injected with diazepam (10 mg/kg) to terminate the seizure attacks. The control rats received an injection of the same amount of normal saline as a replacement for pilocarpine. For the rat miRNA array, 4 randomly selected post-SE rats were included in the experimental group (n = 4), and 4 randomly selected normal rats were included in the control group (n = 4). The animals were euthanized 24 h after SE onset. The hippocampus was quickly removed from the brain after decapitation and then preserved in RNAlater (-20°C, 2 mL, Qiagen, Germany) for microarray analysis.

RNA isolation and quantification

RNA was isolated from the hippocampus with the miRNA Isolation Kit (Qiagen, Germany), in accordance with the manufacturer's instructions. The purity and quantity of RNA were measured by NanoDrop (ND-1000 spectrophotometer, Thermo Scientific, Wilmington, DE, USA). The samples were used immediately or stored at -80°C.

MiRNA array

MiRNA expression profiling was performed using the RiboArray platform (RiboBio, *Guangzhou*, China). In brief, 5 μg of total RNA was labeled with Cy3 using a ULS™ miRNA Labeling Kit (Krea-tech, Amsterdam, Netherlands) and hybridized on the microarray. Based on the Sanger miRBase database, version 19.0, RiboBio designed 795 specific oligos for 765 rat miRNAs, of which 795 were non-redundant sequences. In addition, 54 RiboArray™ internal controls were used as internal controls. We also used some probes for location identify functions. The control probes were replicated between 3 and 40 times.

Cluster analysis using gplots (R software package) was performed. Graphs were generated by R. After data extraction, backgrounds for individual samples were calculated. For the back-

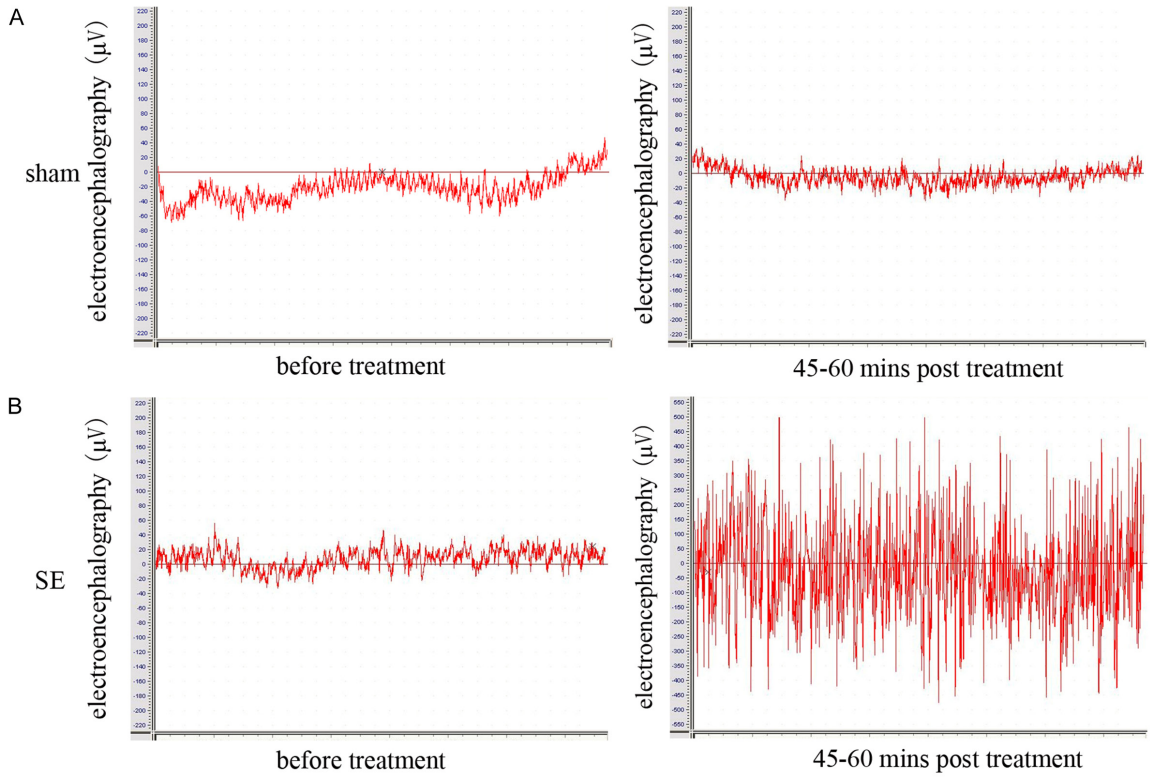

Figure 1. Electrical activity in the brain after lithium-pilocarpine treatment was evaluated by electroencephalography. One and half hours after lithium-pilocarpine treatment, the immature rats were anesthetized by i.p. injection of diazepam.

ground calculation, the median signal intensity that could be used for subtraction was calculated. The microarray data for individual samples were normalized by a quintile normalization, using the probes with signal values greater than zero. A t-test *P*-value of <0.05 and fold-changes >1.5 were used to determine two differentially expressed sets of genes in four experimental samples. We also performed hierarchical clustering based on Euclidean distance measurements of samples, using the normalized significant genes. We examined the patterns of expressed changes for the groups.

qRT-PCR of miRNA

Reverse transcription was performed on the isolated total RNA using a Reverse Transcription kit (Takara Bio, Inc., Otsu, Japan), and PCR was performed using a Real Time PCR kit (Takara Bio, Inc.). Reverse transcription was performed at 65°C for 5 min, 30°C for 10 min, 42°C for 10-30 min and 92°C for 3 min. The PCR conditions were as follows: denaturation at 94°C for 2 min; amplification for 30 cycles of denaturation at 94°C for 0.5 min, annealing at 60°C for 0.5 min, and extension at 72°C for 1 min; followed by a terminal elongation step at 72°C for 10 min. The procedure was performed on a Bio-Rad CFX96 thermal cycler (Bio-Rad Laboratories, Inc.). U6 was amplified as an internal control, the Ct value of each PCR product was calculated, and the fold changes were analyzed. The r-miR-96-5p, 182, 301b-3p, 31b, 145-3p, r-let-7d-3p and r-U6 primers were supplied by RiboBio Technology (Guangzhou, China); the sequences were not supplied due to the rules of the company.

Target prediction

Target mRNAs were predicted by the miRWalk database (http://www.ma.uni-heidelberg.de/apps/zmf/mirwalk/) and by other programs (miRanda, Sanger miRDB, RNAhybrid and Targetscan) on the most used prediction Web site (http://www.umm.uni-heidelberg.de/apps/zmf/mirwalk/predictedmirnagene.html). This module hosts all experimentally verified miRNAs information associated with the genes and

Table 1. Upregulated microRNA in the hippocampal of post-SE immature rat compared with the normal group

microRNA	Fold change	*p*-value
miR-31b	2.3745	0.0039
miR-134-5p	2.0959	0.0489
miR-496-5p	2.0074	0.0077
miR-132	1.9872	0.0091
miR-211-5p	1.9593	0.0418
miR-3559-3p	1.9452	0.0009
miR-153-5p	1.8554	0.0004
miR-3064-5p	1.8411	0.0171
miR-29b-1-5p	1.8132	0.0300
miR-494-5p	1.7840	0.0009
miR-297	1.7404	0.0037
miR-21	1.7329	0.0163
let-7i-5p	1.7212	0.0110
miR-493-3p	1.7156	0.0029
miR-466c-5p	1.7057	0.0002
miR-499-5p	1.7031	0.0130
miR-195-3p	1.6828	0.0019
miR-410-5p	1.6766	0.0340
miR-488-3p	1.6705	0.0027
miR-450b-5p	1.6689	0.0151
miR-346	1.6365	0.0058
miR-489-5p	1.6264	0.0107
let-7d-3p	1.6137	0.0249
miR-7b	1.6042	0.0043
miR-145-3p	1.6034	0.0246
miR-450a-5p	1.5906	0.0074
miR-6323	1.5881	0.0130
miR-298-3p	1.5852	0.0010
miR-30d-3p	1.5356	0.0000
miR-743-5p	1.5202	0.0266

Table 2. Downregulated microRNA in the hippocampal of post-SE immature rat compared with the normal group

microRNA	Fold change	*p*-value
miR-204-5p	3.5092	0.0373
miR-96-5p	2.7843	0.0283
miR-3591	2.6230	0.0302
miR-105	2.4719	0.0052
miR-30c-5p	2.3195	0.0435
miR-3574	2.2610	0.0013
miR-568	2.0939	0.0132
miR-122-3p	2.0846	0.0475
miR-182	2.0391	0.0045
miR-210-5p	2.0327	0.0037
miR-196-3p	1.8800	0.0285
miR-299a-5p	1.8609	0.0063
miR-329-3p	1.8176	0.0191
miR-3084c-5p	1.7814	0.0068
miR-466b-2-3p	1.7751	0.0458
miR-301b-3p	1.6744	0.0004
miR-28-3p	1.6830	0.0259
miR-128-3p	1.6824	0.0063
miR-3594-3p	1.6266	0.0144
miR-6316	1.5975	0.0125
miR-3576	1.5555	0.0169

pathways, as well as information about proteins known to be involved in miRNA processing. The list of targeted mRNAs, in the form of official gene symbols, was extracted from the miRWalk prediction results for further analysis.

Pathway analysis

A pathway prediction analysis was performed using DAVID. Similar to GO analysis, significant pathways were identified based on the input list of predicted genes and corrected *p*-values. The gene lists from the normal and SE groups were considered in the analysis. Only highly significant pathways with *p*-values less than 0.05 were listed as potential pathways for further analysis. The pathway information used in this study was generated from the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg/) online database.

Statistical analysis

Two-way ANOVA was performed for the miRNA profiles using GeneSpring software. Statistical comparisons of the results were analyzed using one-way analysis of variance. Statistical analyses were performed using SPSS software, version 19.0 (IBM SPSS, Armonk, NY, USA). Values are expressed as the mean ± standard error of the mean. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of SE in developing rats

Lithium and pilocarpine were i.p. administered to establish the SE model in developing rats, as described in the Methods section. On a random basis, 12 rats received the lithium-pilocarpine treatment, whereas 6 rats received saline treat-

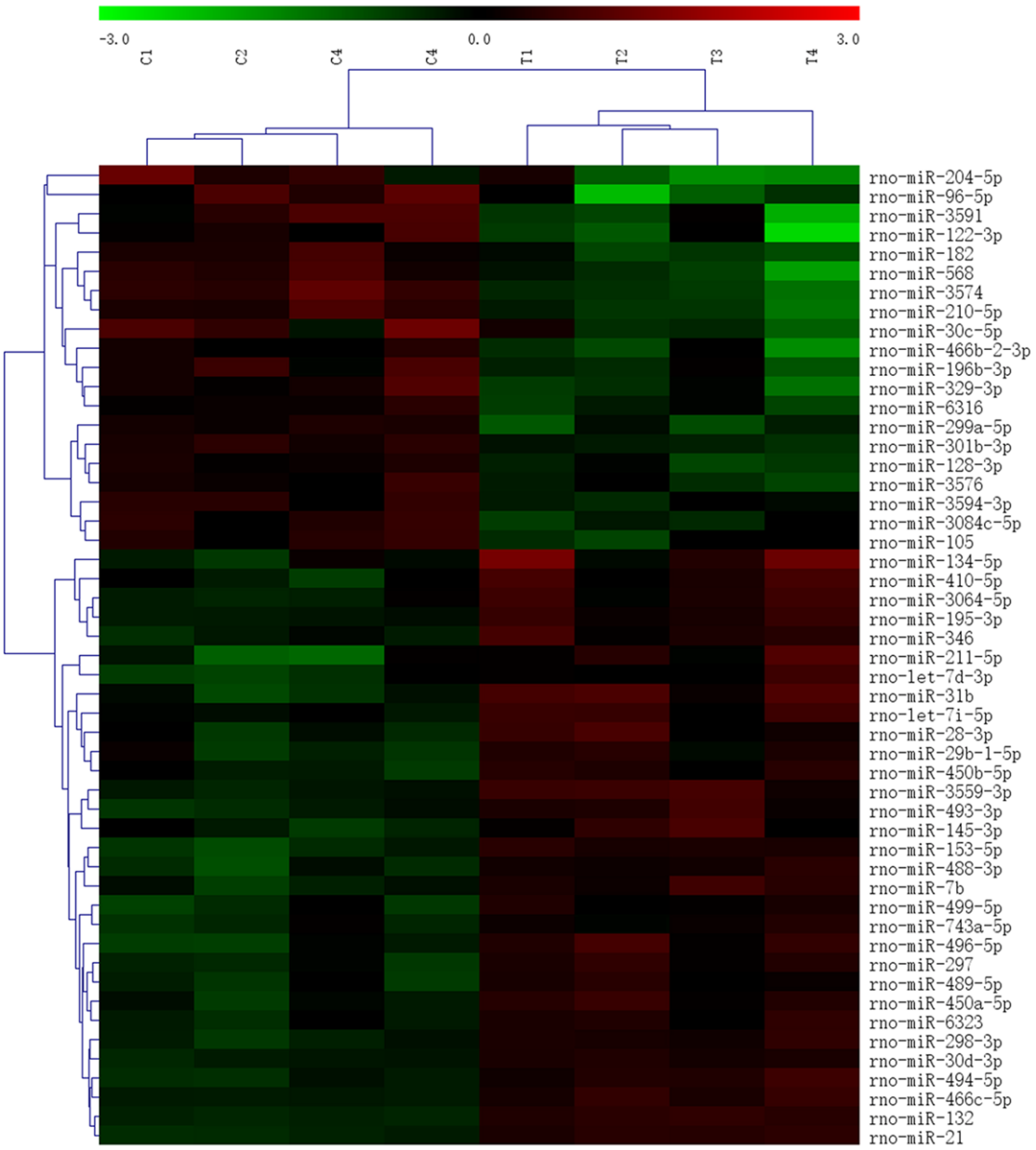

Figure 2. Hierarchal clustering of differentially expressed miRNAs. 51 differentially expressed miRNAs (up- and down-regulated), compared to the sham group in at least one sample, were clustered hierarchically. Each row represents an individual miRNA, and each column represents an individual sample. The expression ratio, represented by color, ranges from green (low) to red (high), as indicated by the scale bar.

ment as a control. After pilocarpine treatment, 9 rats (approximately 75%) successfully developed SE, and 3 rats died after SE onset (mortality rate, 25%). Electroencephalography was performed to evaluate the electrical activity in the brain. As shown in **Figure 1**, continuous electrical activity in the brain was found in the lithium-pilocarpine-treated group. The severity of convulsions was also assessed. Our results indicated that the SE model in immature rats was established after treatment with lithium-pilocarpine. This model should be used in the next study.

Identification of miRNAs by miRNA array

To compare miRNA expression patterns between the normal and epilepsy groups, we analyzed the miRNA expression with RiboArray miRNA arrays. Among the 765 mature miRNAs

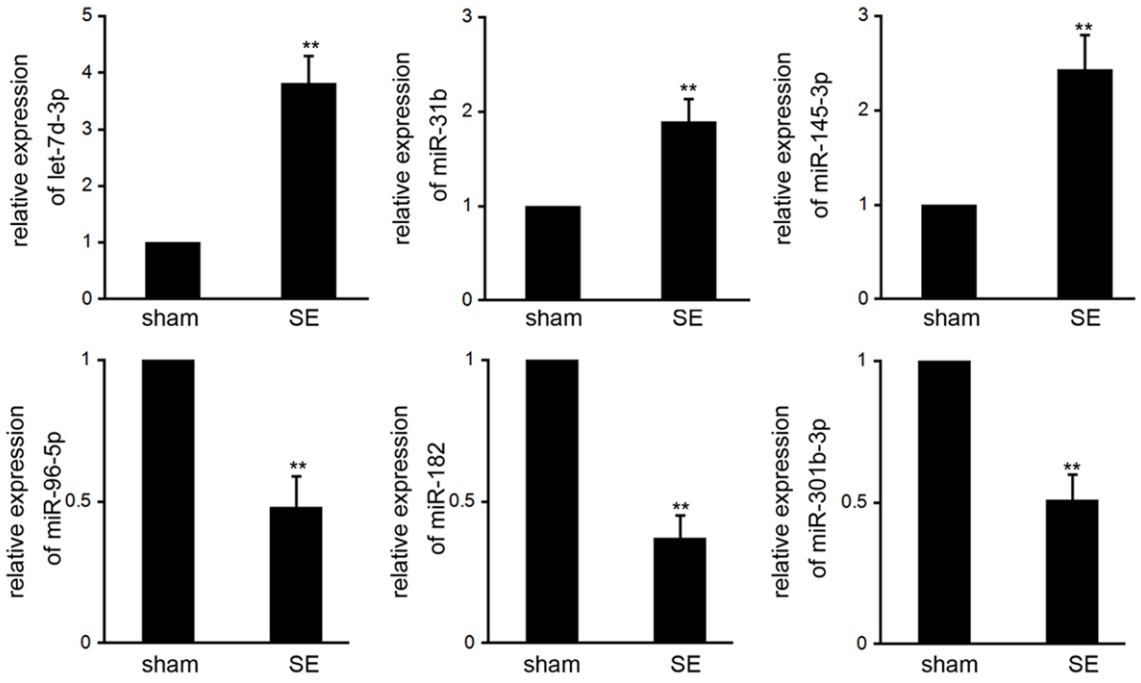

Figure 3. qRT-PCR validation of six differentially expressed miRNAs in the hippocampus. Expression of let-7d-3p, miR-31b, 145-3p, 96-5p, 182 and miR-301b-3p (data are presented as the mean ± SEM, **P<0.01; n = 4 in each group).

present on the array chip, 51 miRNAs were detected to have differential expression in the SE group, including 30 up-regulated miRNAs (miR-31b, 134-5p, 496-5p, 211-5p, 3559-3p, 153-5p, 3064-5p, 29b-1-5p, 494-5p, 297, 493-3p, 466c-5p, 499-5p, 195-3p, 410-5p, 488-3p, 450b-5p, 132, 21, 346, 489-5p, 7b, 145-3p, 450a-5p, 6323, 298-3p, 30d-3p, 743-5p, let-7i-5p and let-7d-3p; **Table 1**) and 21 down-regulated miRNAs (miR-204-5p, 96-5p, 3591, 30c-5p, 3574, 568, 122-3p, 182, 210-5p, 196-3p, 105, 299a-5p, 329-3p, 3084c-5p, 466b-2-3p, 301b-3p, 28-3p, 128-3p, 3594-3p, 6316, and miR-3576; **Table 2**). To better demonstrate the differential expression of these miRNAs, a hierarchical clustering/heatmap of the 51 deregulated rat miRNAs is shown in **Figure 2**.

Quantitative RT-PCR for miRNA validation

To validate the miRNA microarray expression data, a qRT-PCR assay was conducted to confirm the expression levels of six randomly selected miRNAs (miR-96-5p, 182, 301b-3p, 31b, 145-3p and let-7d-3p). As shown in **Figure 3**, miR-31b, 145-3p and let-7d-3p were confirmed by qRT-PCR to be significantly up-regulated in the developing hippocampi of rats following SE, while miR-96-5p, 182, 301b-3p were dramatically down-regulated after SE. The qRT-PCR results were consistent with the results of the miRNA array, thus proving the accuracy of the latter methodology.

Target prediction analysis and GO analysis

To identify miRNA-targeted mRNAs, target prediction for all miRNAs consistently up-regulated or down-regulated in both groups (**Tables 1** and **2**) was performed using the validated target search engine in the miRWalk database [14]. A total of 3306 genes were predicted to be targeted by 30 up-regulated miRNAs, while 2677 genes were targeted by 21 down-regulated miRNAs. The detailed pathway analysis data for top 12 pathways in the groups of up-regulated miRNA targets and down-regulated miRNA targets contained the pathway title, gene numbers in the pathway, the percentage of background and the *p*-value. The top 12 pathways in each group are shown in **Table 3** (up-regulated miRNAs) and **Table 4** (down-regulated miRNAs). The pathway analysis of miRNA targets revealed the

Table 3. Top 12 pathways list in groups of up-regulated microRNA targets

Term	Sample number	% of background	*p*-value
Metabolic pathways	196	15.30	2.2×10^{-15}
MAPK signaling pathway	62	23.85	2.2×10^{-15}
Ras signaling pathway	55	23.91	2.2×10^{-15}
Rap1 signaling pathway	47	21.96	2.2×10^{-15}
cAMP signaling pathway	50	25.38	2.2×10^{-15}
FoxO signaling pathway	47	34.81	2.2×10^{-15}
Neuroactive ligand-receptor interaction	56	19.31	2.2×10^{-15}
Endocytosis	53	21.90	2.2×10^{-15}
mTOR signaling pathway	26	40.63	2.2×10^{-15}
PI3K-Akt signaling pathway	79	23.30	2.2×10^{-15}
AMPK signaling pathway	36	27.90	2.2×10^{-15}
Wnt signaling pathway	45	31.47	2.2×10^{-15}

Table 4. Top 12 pathways list in groups of down-regulated microRNA targets

Term	Sample number	% of background	*p*-value
Metabolic pathways	180	14.05	2.2×10^{-15}
MAPK signaling pathway	64	24.61	2.2×10^{-15}
Ras signaling pathway	47	20.43	2.2×10^{-15}
Rap1 signaling pathway	49	22.90	2.2×10^{-15}
cAMP signaling pathway	41	20.81	2.2×10^{-15}
FoxO signaling pathway	40	29.63	2.2×10^{-15}
Endocytosis	52	21.49	2.2×10^{-15}
PI3K-Akt signaling pathway	71	20.94	2.2×10^{-15}
Hippo signaling pathway	40	25.64	2.2×10^{-15}
Wnt signaling pathway	36	25.17	2.2×10^{-15}
TGF-beta signaling pathway	27	32.53	2.2×10^{-15}
Axon guidance	34	26.15	2.2×10^{-15}

metabolic pathway (**Figure 4**) and the MAPK signaling pathway (**Figure 5**) as important pathways in both up-regulated and down-regulated miRNAs.

Discussion

In this study, 51 deregulated miRNAs were detected by miRNA array in the hippocampi of immature rats following SE. Further qRT-PCR of six randomly selected miRNAs proved the accuracy of miRNA array. Moreover, the targets of these deregulated miRNAs were analyzed using the miRWalk database, and the identified enriched metabolic pathway and the MAPK signaling pathway were involved in the molecular mechanisms underlying epileptogenesis. The present study could provide clues to increase our understanding of the mechanisms and roles of miRNAs as key regulators of epileptogenesis.

Recently, miRNAs have been proposed as potential diagnostic tools for many diseases due to their characteristics of stability in serum, low cost, rapidity and noninvasiveness. Notably, miRNAs have been reported to be promising biomarkers with great accuracy for aging [15], cancer [16, 17], and neurodegenerative disorders, such as Parkinson's disease[18], multiple sclerosis [19], and Alzheimer's disease [20]. Multiple studies have demonstrated that more than 20 miRNAs can be found in the hippocampus, suggesting conserved miRNA responses after SE [13, 21, 22]. Moreover, intracerebral delivery of chemically modified antisense oligonucleotides (antagomirs) has been shown to have potent, specific and long-lasting effects on the brain levels of miRNAs. For instance, targeting miR-34a, miR-132 and miR-184 has been reported to alter seizure-induced neuronal death, whereas targeting miR-134 was neuroprotective, reducing seizure severity during SE and the later emergence of recurrent spontaneous seizures [4, 23]. These studies supported the roles of miRNAs in the pathophysiology of SE, and miRNAs could represent novel therapeutic targets to reduce brain injury and epileptogenesis. Our study examined for the first time the miRNA expression profiles in the hippocampi of immature rats following SE and demonstrated that 30 up-regulated miRNAs and 21 down-regulated miRNAs had differential expression, compared to a normal group. The present study might provide clues to enhance our understanding of the roles of miRNAs as key regulators of epileptogenesis. In the

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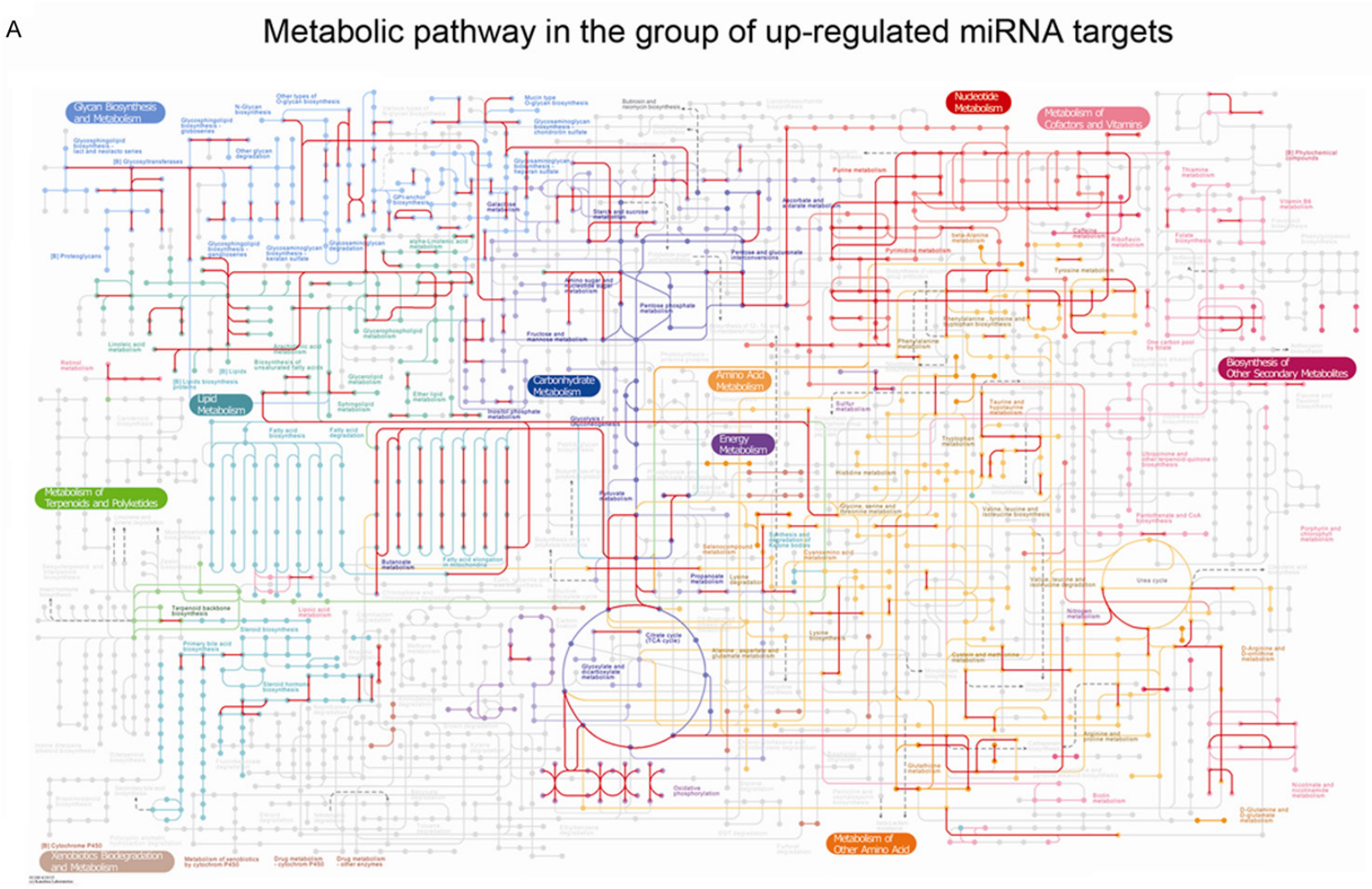
Metabolic pathway in the group of up-regulated miRNA targets
Glycan Biosynthesis and Metabolism
Lipid Metabolism
Carbohydrate Metabolism
Energy Metabolism
Amino Acid Metabolism
Nucleotide Metabolism
Metabolism of Cofactors and Vitamins
Biosynthesis of Other Secondary Metabolites
Metabolism of Terpenoids and Polyketides
Xenobiotics Biodegradation and Metabolism
Metabolism of Other Amino Acid

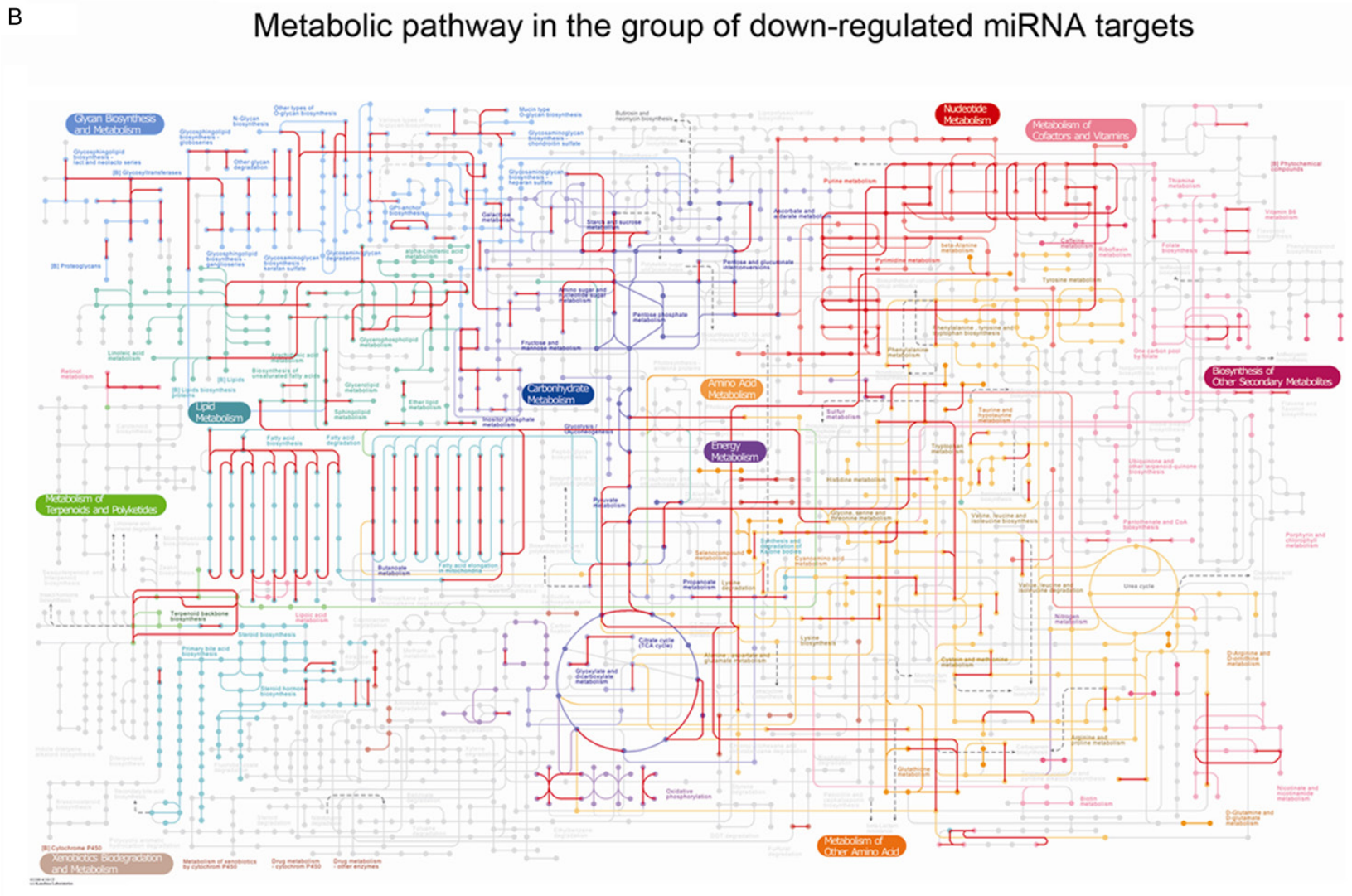

Figure 4. The enriched metabolic pathway was identified using the KEGG pathway database. The target genes were highlighted in red. A: Metabolic pathway in the up-regulated miRNA targets. B: Metabolic pathway in the up-regulated miRNA targets.

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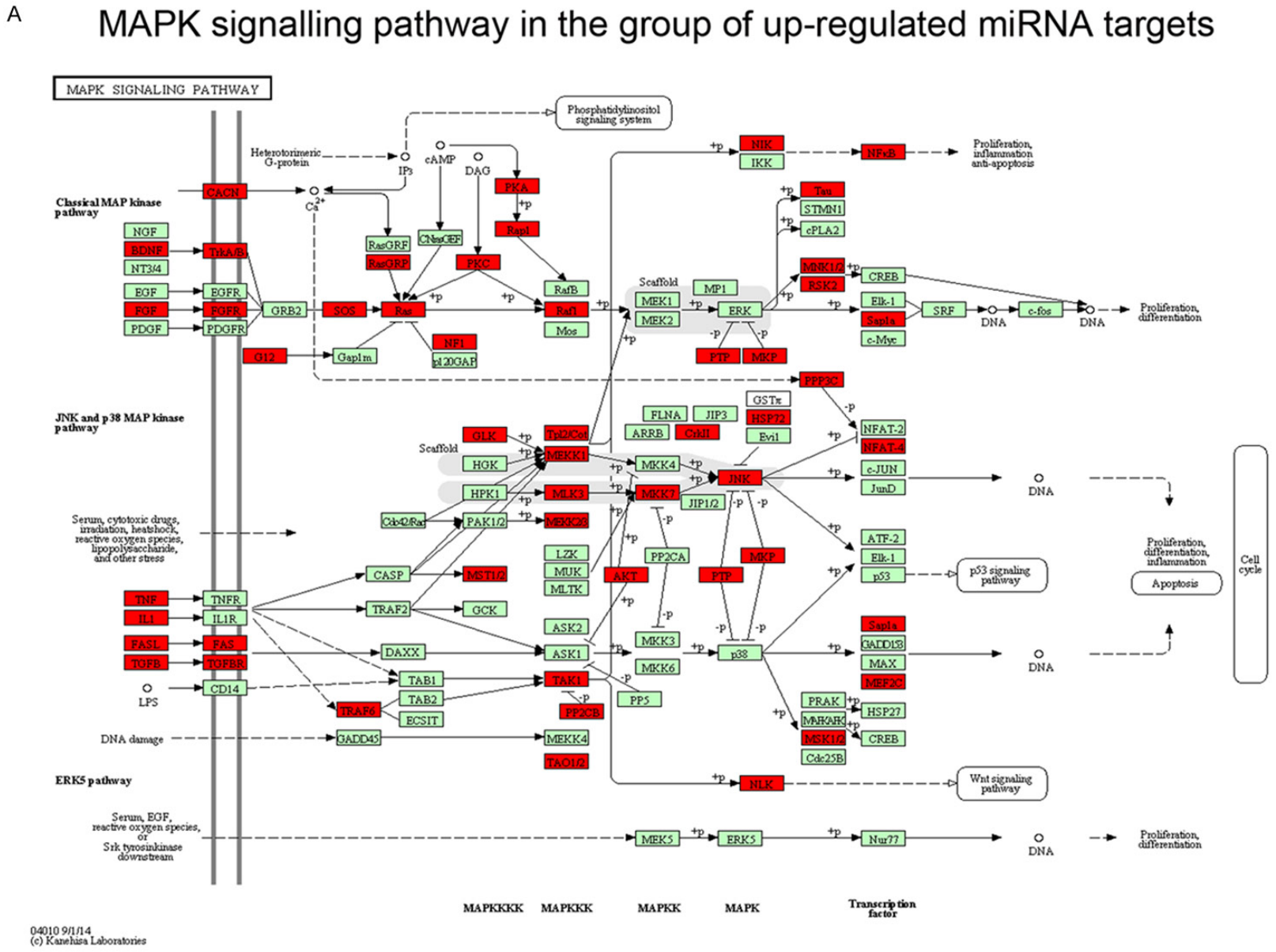
MAPK signalling pathway in the group of up-regulated miRNA targets
MAPK SIGNALING PATHWAY
Classical MAP kinase pathway
JNK and p38 MAP kinase pathway
ERK5 pathway
Phosphatidylinositol signaling system
Heterotorimeric G-protein
cAMP
DAG
IP3
Ca2+
CACN
PKA
Rap1
RasGRF
RasGRP
CNesGEF
PKC
NGF
BDNF
NT3/4
TrkA/B
EGF
FGF
PDGF
EGFR
FGFR
PDGFR
GRB2
SOS
Ras
RafB
Raf1
Mos
G12
Gap1m
NF1
p120GAP
Scaffold
MP1
MEK1
MEK2
ERK
PTP
MKP
NIK
IKK
NFκB
Proliferation, inflammation anti-apoptosis
Tau
STMN1
cPLA2
MNK1/2
RSK2
CREB
Elk-1
Sap1a
c-Myc
SRF
c-fos
DNA
Proliferation, differentiation
PPP3C
GSTπ
FLNA
JIP3
HSP72
ARRB
CrkII
Evi1
GLK
HGK
HPK1
Tpl2/Cot
MEKK1
MLK3
MEKK2/3
MKK4
MKK7
JIP1/2
JNK
NFAT-2
NFAT-4
c-JUN
JunD
ATF-2
Elk-1
p53
p53 signaling pathway
Proliferation, differentiation, inflammation
Apoptosis
Cell cycle
Cdc42/Rac
PAK1/2
Serum, cytotoxic drugs, irradiation, heatshock, reactive oxygen species, lipopolysaccharide, and other stress
CASP
MST1/2
LZK
MUK
MLTK
AKT
PP2CA
PTP
MKP
TNF
IL1
FASL
TGFB
TNFR
IL1R
FAS
TGFBR
CD14
LPS
TRAF2
GCK
DAXX
TAB1
TAB2
ECSIT
TRAF6
TAK1
PP2CB
ASK2
ASK1
MKK3
MKK6
PP5
p38
Sap1a
GADD153
MAX
MEF2C
HSP27
CREB
PRAK
MAPKAPK
MSK1/2
Cdc25B
DNA damage
GADD45
MEKK4
TAO1/2
NLK
Wnt signaling pathway
Serum, EGF, reactive oxygen species, or Src tyrosinkinase downstream
MEK5
ERK5
Nur77
MAPKKKK
MAPKKK
MAPKK
MAP
Transcription factor
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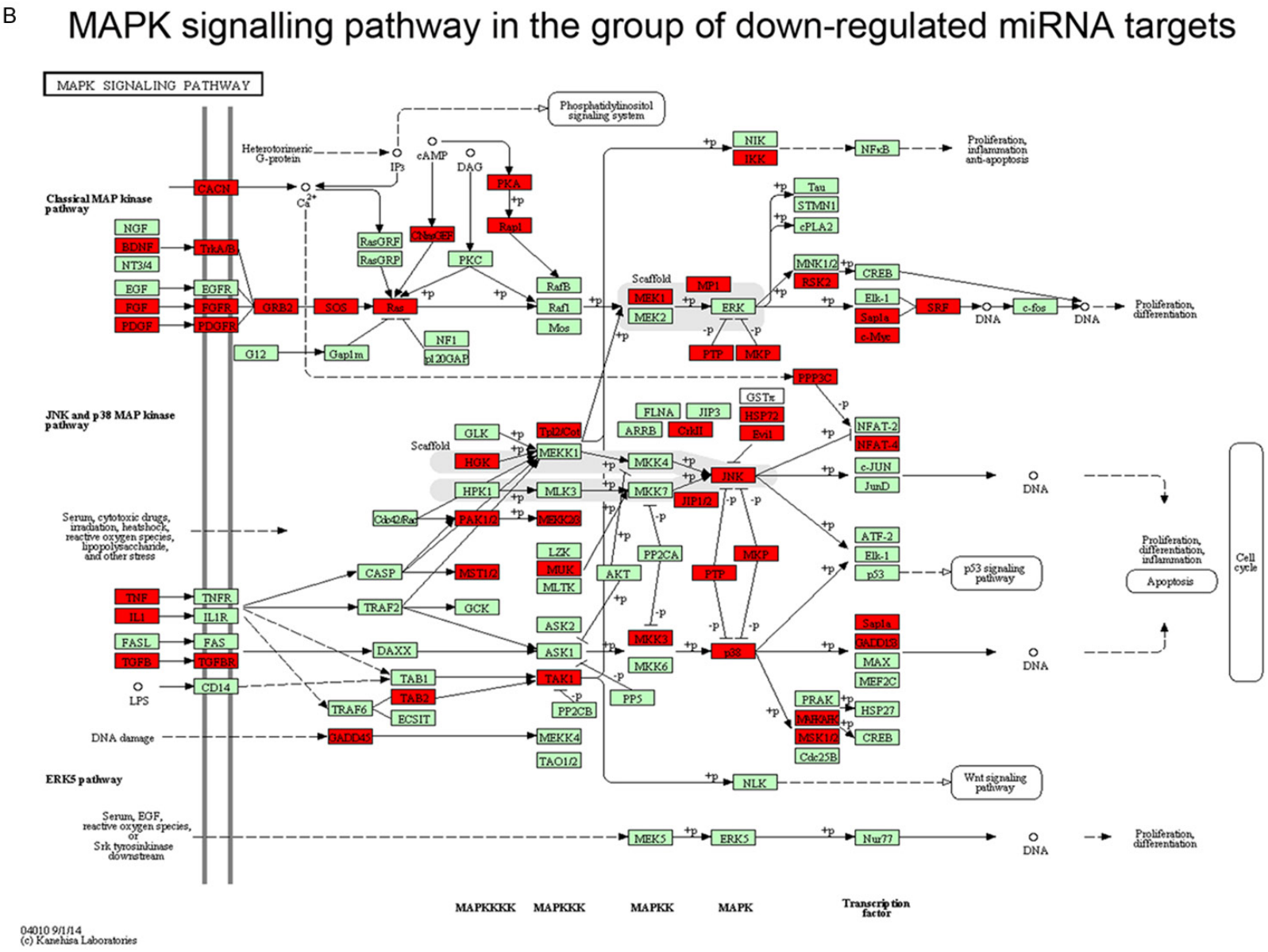

Figure 5. The enriched MAPK signaling pathway was identified using the KEGG pathway database. The target genes were highlighted in red. A: MAPK signaling pathway in the up-regulated miRNA targets. B: MAPK signaling pathway in the up-regulated miRNA targets.

next study, we will attempt to identify novel biomarkers and therapeutic targets for pediatric SE among the 51 deregulated miRNAs.

MiRNA microarray technology is a specific and efficient method to generate miRNA expression profiles. This approach has been applied to study the functional linkages between miRNAs and physiological/pathological processes [24-26]. To analyze miRNA expression systematically in the hippocampi of immature rats following SE, a microarray was employed that included 765 mature miRNAs on the array chip. Among the 765 mature miRNAs, 51 miRNAs were detected to have differential expression in the SE group, including 30 up-regulated miRNAs and 21 down-regulated miRNAs. In the subsequent study, the miRNA microarray accuracy was determined by qRT-PCR. Our results indicated that the qRT-PCR analysis of six randomly selected miRNAs was consistent with the expression changes by miRNA microarray, thus proving the accuracy of the miRNA array.

The MAPK signaling pathway includes the classical MAP kinase pathway, the JNK and p38 MAP kinase pathway and the ERK pathway. These signaling pathways regulate cellular activities, including cellular proliferation, differentiation, survival and death. A previous study by Hu et al indicated that MAPK pathways can be activated by external oxidative stress in disease conditions, and the activation of the JNK and p38 signaling pathway was suggested to mediate neuronal apoptotic/inflammatory processes in post-SE rat hippocampi [13]. In our study, the pathway analysis of miRNA targets revealed the MAPK signaling pathway to be an important pathway in pediatric epileptogenesis, consistent with the findings by Hu et al in adult epileptogenesis [13].

The energy source for the brain switches from glucose to ketone bodies (acetoacetate and β-hydroxybutyrate). Thus, ketone bodies directly regulate neural excitation and seizures via adenosine 5'-triphosphate (ATP)-sensitive K^+ channels (KATP channels) [27, 28] and vesicular glutamate transporters [29]. Ketogenic diets also suppress seizures via adenosine A1 receptors [30], and they act on energy metabolism (for example, glycolysis and the TCA cycle). Notably, seizures and epileptiform activity are reduced by inhibition of the metabolic pathway via lactate dehydrogenase (LDH), a component of the astrocyte-neuron lactate shuttle [31]. An LDH inhibitor significantly suppressed seizures *in vivo* in a mouse model of epilepsy [31]. Our results indicated that metabolic pathways are important pathways in pediatric epileptogenesis. Several metabolic pathways, including energy metabolic, nucleotide metabolic, metabolic of cofactors and vitamin pathways, were predicted to be involved in pediatric epileptogenesis, which might provide clues for further understanding the molecular mechanisms of pediatric epileptogenesis.

In conclusion, our study demonstrated for the first time 51 deregulated miRNAs in the hippocampi of developing rats following SE, which could provide clues to increase our understanding of the roles of miRNAs as key regulators of epileptogenesis. Future studies of the functional linkage between the deregulated miRNAs and their target genes could indicate that the metabolic pathway and MAPK signaling pathway are involved in pediatric epileptogenesis, perhaps providing a better understanding of the molecular mechanisms of pediatric epileptogenesis. Collectively, our study could shed light on novel biomarkers and therapeutic strategies for pediatric SE.

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Disclosure of conflict of interest

None.

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