Lamotrigine decreases MRP8 and IL-7 in rat models of intractable epilepsy secondary to focal cortical dysplasia

JIANPING WEI[1], QINGMEI NIE[1] and FENG LI[2]

Departments of [1]Emergency and [2]Health Care, Weifang People's Hospital, Weifang, Shandong 261000, P.R. China

Received May 10, 2016; Accepted October 3, 2016

DOI: 10.3892/etm.2016.3806

Abstract. The aim of the present study was to examine the effect of lamotrigine and the expression of myeloid-related protein 8 (MRP8) and interleukin-7 (IL-7) in the treatment of focal cortical dysplasia with secondary intractable epilepsy. In this study, rats with focal cortical dysplasia with secondary intractable epilepsy (constructed by our laboratory) were selected and used for experimentation, 21-day Sprague-Dawley rats were randomly divided into the control group (38 rats), the observation group I (39 rats), and the observation group II (38 rats). Rats in the observation group I received daily intraperitoneal injection of 0.02 mg/kg lamotrigine, and those in the observation group II and the control group received daily intraperitoneal injection of 0.02 mg/kg normal saline. Expression quantities of MRP8 and IL-7 in the hippocampus sample tissues of mice in the control group, observation group I, and observation group II were measured via fluorescence quantitative polymerase chain reaction assay, western blot analysis, enzyme-linked immunosorbent assay, and immunohistochemistry 48 h later. *MRP8* and *IL-7* gene mRNA levels of the control group, the observation group I and the observation group II had no significant differences (P>0.05). The expression quantity on the protein level of MRP8 and IL-7 showed no significant differences (P>0.05) between the observation group I (7.91±1.3, 3.86±0.38) and the control group (7.52±1.03, 3.62±0.29). The expression quantity of MRP8 and IL-7 showed significant differences (P<0.05) between observation group II (27.47±1.13, 19.45±0.48) and observation group I (7.91±1.3, 3.86±0.38). It was found that MRP8 and IL-7 were focused on the nerve cell membrane of hippocampus of rats in the observation group by immunohistochemistry experiments. In conclusion, the results from the present study show that lamotrigine can be used to treat rats with focal cortical dysplasia with secondary intractable epilepsy by reducing the expression levels of MRP8 and IL-7 in the body, providing a new therapeutic target to the follow-up treatment of focal cortical dysplasia with secondary intractable disease.

Correspondence to: Dr Feng Li, Department of Health Care, Weifang People's Hospital, 151 Guangwen Street, Weifang, Shandong 261000, P.R. China
E-mail: li_feng1212@163.com

Key words: myeloid-related protein 8, interleukin-7, lamotrigine, focal cortical dysplasia, secondary intractable epilepsy

Introduction

Focal cortical dysplasia is a type of intractable epilepsy-related congenital malformation of cortical development (MCDs) in children and adults (1). The statistical results of epilepsy in focal cortical dysplasia from Sloan and Barres showed that the incidence of intractable epilepsy in MCDs was as high as 32.5% (2). Study results from Canpolat *et al* (3) showed that, MCDs was most common in children with epilepsy and physical retardation or neurological deficits limitations, thus, MCDs may be considered a predisposing factor for adults and children with intractable epilepsy. Zhang suggested that epilepsy is a type of chronic disease featured by transient brain dysfunction caused by sudden abnormal discharge from brain neurons, with an incidence of approximately 7% in China and nearly 40 million new cases are reported each year in China (4). Myeloid-related protein 8 (MRP8), an endogenous ligand of low molecular weight calcium-binding protein, has been demonstrated to be associated with various autoimmune diseases such as asthma (5), arthritis and inflammatory bowel disease and other autoimmune deficiencies (6). The study results from Pellegrini *et al* have shown that the extremely important cytokine in human body, interleukin-7 (IL-7) was closely associated with many immunoreactions in the body (7). According to Xu *et al* (8), it has been shown that IL-7 plays important roles in the aspects of messaging, activation and regulation of immune cells, and intracellular and intercellular immune response in the body, mediated activation of T and B cells, proliferation and differentiation and inflammation treatment. Although MRP8 and IL-7 have certain therapeutic effects on the associated immune overreaction in the body, the studies on the effect of these proteins on treating focal cortical dysplasia with secondary intractable epilepsy are rarely reported.

In the present study, the effect of lamotrigine in treating focal cortical dysplasia with secondary intractable epilepsy is expected to provide a theoretical and experimental basis for the sequential treatment of the disease cortical dysplasia with secondary intractable epilepsy.

Materials and methods

Animal treatment. The rat model of focal cortical dysplasia with secondary intractable epilepsy used in this experiment was constructed and preserved in our laboratory. In the present study, 21 healthy Sprague-Dawley rats were selected as the subjects and randomly divided into the control group normal mice (n=38), observation group I mice having the disease and treated (n=39), and observation group II mice with the disease but not treated (n=38). The observation group I was given intraperitoneal injection of 0.02 mg/kg lamotrigine (Sigma-Aldrich, St. Louis, MO, USA) each day, and the observation II and the control group were given intraperitoneal injection of the same amount of normal saline each day.

RNA extraction. RNA extraction was conducted in accordance with the Axygen kit instructions (Axygen Biosciences, Union City, CA, USA) with modifications (9).

Fluorescence quantitative polymerase chain reaction (qPCR). In this study, fluorescence qPCR kit was purchased from Takara Bio (Dalian, China). The experiment was carried out in three steps, and the specific scheme was carried out in accordance with the instructions and improved. The primers used are shown in Table I.

Table I. Primers used for the PCR reaction.

Gene	Primer sequence
MRP8	F: 5'-CGACATGGCAACTGAACTGGA-3'
	R: 5'-ACGCCCACCCTTATCACCAAC-3'
IL-7	F: 5'-CGTCGGGTTAGCTAGCATAGC-3'
	R: 5'-TGCTGACGCCTAGCATCGATAC-3'
GAPDH	F: 5'-TCATGGGTGTGAACCATGAGAA-3'
	R: 5'-GGCAGGACTGTGGTCATGAG-3'

PCR, polymerase chain reaction; MRP8, myeloid-related protein 8; IL-7, interleukin-7; F, forward; R, reverse.

Expression of MRP8 and IL-7 in the hippocampus by enzyme-linked immunosorbent assay (ELISA). The double antibody sabdwich method was employed to detect the expression of *TAG1/APP* gene. The specific methods were:

i) Coating: phosphate-buffered saline (PBS), pH 9.0 buffer was used to appropriately dilute the antibody protein, and the concentration was 1-10 μg/ml. Then 0.1 ml was added into the 96-well plate, leaving it overnight at the temperature of 4˚C, then the liquid was discarded the following day and the plate was washed 5 times, each time 2 min, with washing liquid.

ii) The sample: The treated serum samples of 0.1 ml were added into the 96-well plate, leaving it for 1 h at the temperature of 37˚C. Then the plate was washed 5 times, each time 2 min, with washing buffer (the blank well, the negative control, and the positive control were prepared).

iii) Secondary antibody: The secondary antibody of 0.1 ml was added into the 96-well plate after washing, leaving it for 0.5-1.2 h at the temperature of 37˚C, then washing 5 times, each time 2 min, with washing buffer after dyeing red.

iv) Chromogenic substrate: Newly prepared chromogenic substrate was added into the 96-well plate after washing, and then incubated for 30 min at the temperature of 37˚C.

v) Terminating solution: 0.2 M of sulfuric acid termination solution of 0.005 ml was added into the plate on termination.

vi) Qualitative detection: The 96-well plate was quantitatively observed for the depth of color, and the deeper the color, the stronger the positive degree was; and a higher content of GAG1/APP indicated the negative control well was colorless. Quantitative detection involved placing a 96-well plate on the enzyme standard instrument for quantitative detection with a wavelength of 450 nm. Zero adjustment was conducted by the blank well. If the level of OD was 1.2-fold greater than the level of negative control, it was recorded as positive.

Expression quantity of MRP8 and IL-7 in the hippocampus by western blot analysis. The experimental operation of western blot analysis was conducted according to the Molecular Cloning Manual with modifications. Primary rabbit monoclonal MRP8 antibody (dilution, 1/500; cat. no. ab92331), rabbit polyclonal IL-7 antibody (dilution, 1/500; cat. no. ab9732), and secondary goat anti-rabbit (HRP) IgG antibody (dilution, 1/2,000; cat. no. ab6721) were all purchased from Abcam (Cambridge, MA, USA).

Expression quantity of MRP8 and IL-7 in the hippocampus by the immune group. According to the experimental methods of Xiang *et al* (10), the experimental rats were treated and the tissue samples were obtained. The samples were soaked for 10 min with 3% hydrogen peroxide after the conventional dewaxing hydration treatment, and then placed in the microwave oven with gentle heat for 1 min. The experimental samples were removed, the first antibody was added when cooled (MRP8, 1:400 and 1:350, both from Roche Diagnostics), and the samples were incubated for 4 h at 20˚C. Subsequently, the samples were washed 5 times with 0.1 mol/l PBS, each time for 5 min, the antibodies were detected in accordance with the two steps of the instructions of ELISA kit [Tiangen Biotech (Beijing) Co., Ltd., Beijing, China], and then a polymer assistant was added and incubated for 30 min at 37˚C. Treatment samples were washed 5 times with PBS, each time for 5 min, and then goat anti-rabbit IgG [Tiangen Biotech (Beijing) Co., Ltd.] with horseradish peroxidase was added, incubated for 2 h at room temperature, and washed 5 times with 0.1 ml PBS, each time for 5 min. The samples were stained with DAB for 10 min, and washed fully with running water. Subsequently, the nucleus was stained with hematoxylin, and conventionally mounted. The above experiments were repeated 3 times.

Data analysis. Data were presented as mean ± SD, the mean among various samples were analyzed by one-way ANOVA, the mean comparison between two groups was tested using a t-test, and the inter-group comparison was tested using a q test. SPSS 2.0 software (Chicago, IL, USA) was used to conduct

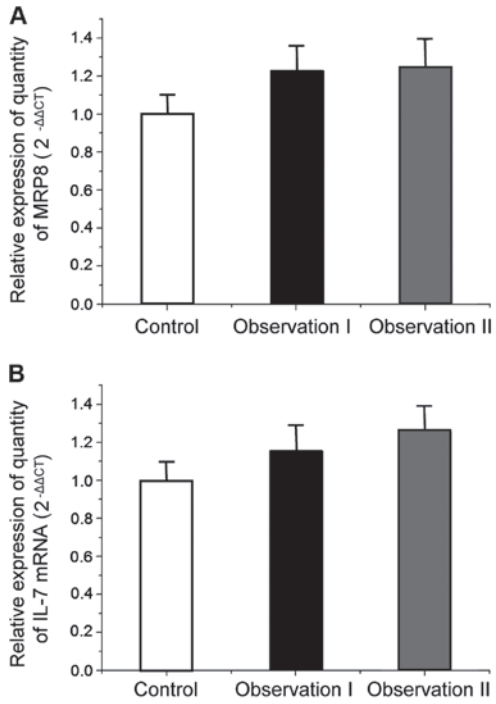

Figure 1. (A) Test on mRNA relevant expression of MRP8 and (B) IL-7 in the control group, the observation group I and the observation group II by RT-PCR. MRP8, myeloid-related protein 8; IL-7, interleukin-7.

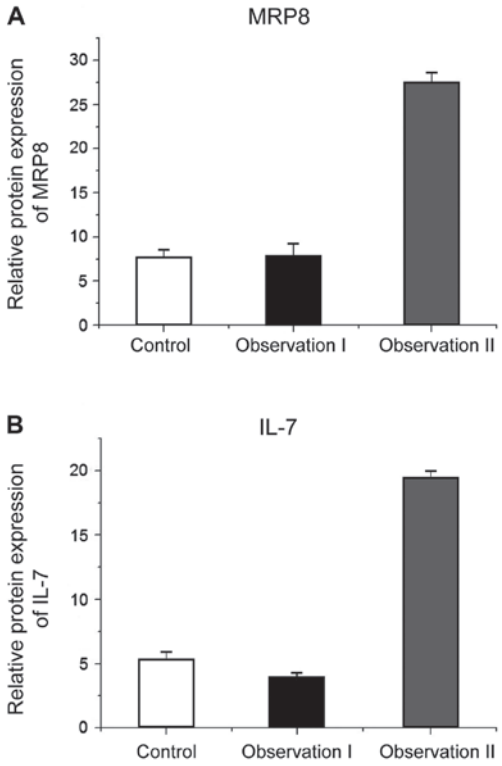

Figure 2. (A) Test on expression quantity of MRP8 and (B) IL-7 in the control group, the observation group I and the observation group II using ELISA. MRP8, myeloid-related protein 8; IL-7, interleukin-7.

statistical analysis, P<0.05 was set as the statistically significant difference.

Results

Expression quantity of MRP8 and IL-7 in the control group, the observation group I and the observation group II measured by RT-PCR. According to the study results from Schroten-Loef *et al* (12), taking lamotrigine can treat focal cortical dysplasia with secondary intractable epilepsy to a certain extent, but the involved mechanism was not clear. By quantitative PCR method on gene mRNA relative expression quantity of MRP8 1 and IL-7 in the samples of rat hippocampus with different treatments in the control group, in the observation group I and the observation group II, it was found that MRP8 and IL-7 gene mRNA had no significant difference between the observation group I and the control group (P<0.05) (Fig. 1); and there were no significant differences in *MRP8 1* and *IL-7* gene mRNA expression between the observation group I treated by lamotrigine and the expression in the observation group I (P<0.05), which showed that lamotrigine did not affect the transcription of *MRP8* and *IL-7* genes.

Expression quantity of MRP8 and IL-7 in the control group, the observation group I and the observation group II by ELISA. There was no significant difference (P<0.05) of MRP8 and IL-7 in the control group (7.52±1.03, 3.62±0.29) and the observation group I (7.91±1.3, 3.86±0.38) treated with lamotrigine, whereas MRP8 and IL-7 content had a significant difference (P<0.05) between the observation group II (27.47±1.13, 19.45±0.48) and the observation group I (7.91±1.3, 3.86±0.38) treated with lamotrigine. This indicated that taking lamotrigine can treat focal cortical dysplasia with secondary intractable epilepsy by lowering MRP8 and IL-7 content in rats to some extent (Fig. 2).

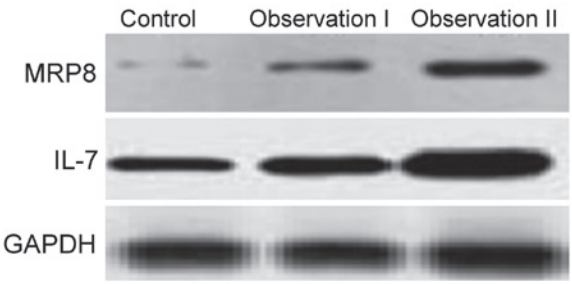

Figure 3. Expression quantity of MRP8 and IL-7 in the control group, the observation group I and the observation group II using western blot analysis. MRP8, myeloid-related protein 8; IL-7, interleukin-7.

Expression quantity of MRP8 and IL-7 in the control group, the observation group I and the observation group II by western blotting. Results of western blotting (Fig. 3) mirrored the above results that the protein contents of MPR8 and IL-7 did not show differences, but those in the observation group I with lamotrigine injection were significantly lower than those in the observation group II without amotrigine injection. This result was consistent with the results of ELISA, which indicated that the treatment of lamotrigine for focal cortical dysplasia with secondary intractable epilepsy treatment was mainly due to reducing MRP8 and IL-7 protein content in the body.

Expression quantity of MRP8 and IL-7 in the control group, the observation group I and the observation group II by

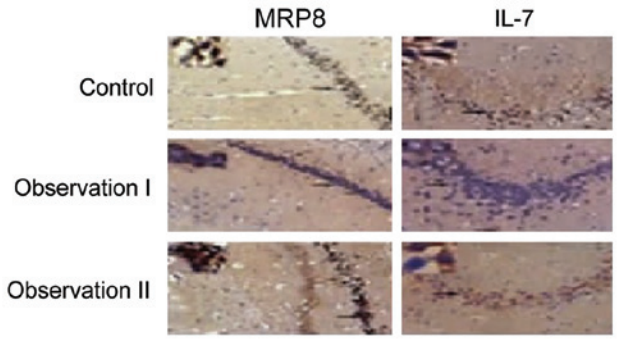

Figure 4. Tests on expression quantity of MRP8 and IL-7 in the control group, the observation group I and the observation group II by immunohistochemistry. MRP8, myeloid-related protein 8; IL-7, interleukin-7.

Table II. MRP8 expression result in immunohistochemical hippocampus tissue of rats.

Groups	Paraffin section no.	Positive cell no.	Negative cell no.	P-value
Control	38	9	29	<0.05
Observation I	39	11	28	
Observation II	38	29	9	

MRP8, myeloid-related protein 8. P<0.05 indicates a statistically significant difference.

Table III. IL-7 expression results in immunohistochemical hippocampus tissue of rats.

Groups	Paraffin section no.	Positive cell no.	Negative cell no.	P-value
Control	38	10	28	<0.05
Observation I	39	9	30	
Observation II	38	32	6	

IL-7, interleukin-7. P<0.05 indicates a statistically significant difference.

immunohistochemistry. From the immunohistochemical staining on hippocampal tissue of the samples in the control group and in the observation group I and group II, it was found that the stained positive cells of MRP8 and IL-7 were less in the sample tissues of the control group and the observation group I than those in the observation group. In addition, from the observation of the immunohistochemical staining image, it was found that the cells presenting positive after staining were mostly irregular in shape, with larger cell size and with cytoplasmic vacuoles and in disorder, in addition, from the statistical results of the quantities of positive cells and invisible cells in the samples of the control group, the observation group I and II, it was found that the positive cell quantity in the observation group II was significantly higher than that in the observation group I, and there was a significant difference (P<0.05) (Fig. 4; Tables II and III).

Discussion

As a spontaneous severe convulsion behavior caused by the internal neuron erethism of the body, the incidence and disability rate of epilepsy increased significantly (11-13). Related study results showed that the main pathogens of epilepsy are the anaphylactic reactions of the nervous system and dominated relevant tissues, which were caused by neurons and associated nerve tissue structural abnormalities or functional abnormalities, thus far the main effect target of the treatment on epilepsy is associated with neuron cells (14,15). However, statistical data indicate that in the current treatment of epilepsy, nearly one-third of patients with epilepsy have related drug resistance (16). In recent years, the relevant study results indicate that MRP8 protein can mediate the inflammatory response of endothelial cells and inflammation of neurons in the body, and as one of internal nervous system diseases of the body, epilepsy has been proven to be related closely with neuron structural abnormalities and function disorders (17,18). As a class of cytokines, IL-7 plays an essential role in regulating activation of immune system in the body itself, proliferation of relevant immune cells and function (19). For example, the previous findings showed that IL-7 can stimulate myeloid precursor cells and megakaryocytes to produce colonies and form units and platelets to aid the body recover from the immunosuppressive effect of cyclic amide phospholipids (20).

In this study, fluorescence quantitative PCR, enzyme immunoassay, western blotting and immunohistochemical staining methods were used to explore the effect of lamotrigine on expression of MRP8 and IL-7 in rat models of focal cortical dysplasia with secondary refractory epilepsy. Results indicated that despite significant effects on treating focal cortical dysplasia with secondary refractory epilepsy, the MRP8 and IL-7 protein levels of the observation group II after lamotrigine treatment showed no significant differences with normal mice in the control group, but its content was much lower than that in the observation group II (rats without the lamotrigine treatment), which indicates that lamotrigine may be used to treat focal cortical dysplasia with secondary intractable epilepsy by reducing MRP8 and IL-7 protein levels in the body. However, since we found that lamotrigine does not reduce mRNA content of MRP8 and IL-7 within the tissues, it can be considered that lamotrigine potentially acts on the protein translation process rather than the transcription process to achieve the regulation of MRP8 and IL-7 content in a different manner, which requires further investigation.

References

1. Ashhab MU, Omran A, Kong H, Gan N, He F, Peng J and Yin F: Expressions of tumor necrosis factor alpha and microRNA-155 in immature rat model of status epilepticus and children with mesial temporal lobe epilepsy. J Mol Neurosci 51: 950-958, 2013.
2. Sloan SA and Barres BA: Mechanisms of astrocyte development and their contributions to neurodevelopmental disorders. Curr Opin Neurobiol 27: 75-81, 2014.
3. Canpolat M, Per H, Gumus H, Yikilmaz A, Unal E, Patiroglu T, Cinar L, Kurtsoy A and Kumandas S: Rapamycin has a beneficial effect on controlling epilepsy in children with tuberous sclerosis complex: results of 7 children from a cohort of 86. Childs Nerv Syst 30: 227-240, 2014.

4. Zhang CL: Study on the relationship and mechanism between dynamin-1 and mesial temporal lobe epilepsy (unpublished PhD thesis). Central South University, China, 2014.
5. Kong H, Omran A, Ashhab MU, Gan N, Peng J, He F, Wu L, Deng X and Yin F: Changes in microglial inflammation-related and brain-enriched MicroRNAs expressions in response to in vitro oxygen-glucose deprivation. Neurochem Res 39: 233-243, 2014.
6. Kwon CH, Moon HJ, Park HJ, Choi JH and Park Y: S100A8 and S100A9 promotes invasion and migration through p38 mitogen-activated protein kinase-dependent NF-κB activation in gastric cancer cells. Mol Cells 35: 226-234, 2013.
7. Pellegrini M, Calzascia T, Toe JG, Preston SP, Lin AE, Elford AR, Shahinian A, Lang PA, Lang KS, Morre M, *et al*: IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. Cell 144: 601-613, 2011.
8. Xu YY, Wang Y, Li YP, Xu XX, Zhu SB and Zhang XG: Interleukin-7 influences the development of thymic T lymphocytes and thymic dendritic cells. Chinese J Immunol 26: 108-112, 2010 (In Chinese).
9. Holtman L, van Vliet EA, Aronica E, Wouters D, Wadman WJ and Gorter JA: Blood plasma inflammation markers during epileptogenesis in post-status epilepticus rat model for temporal lobe epilepsy. Epilepsia 54: 589-595, 2013.
10. Xiang QL, Zhang CL, Peng J, He F, Wu LW, Ou M and Yin F: Expression of TLR4 and MRP8 in the developing immature rats with mesial temporal lobe epilepsy. J Apoplexy Nervous Diseases 7: 580-584, 2012 (In Chinese). http://www.cnki.com.cn/Article/CJFDTotal-ZFSJ201207002.htm.
11. Mohammad G, Siddiquei MM, Othman A, Al-Shabrawey M and Abu El-Asrar AM: High-mobility group box-1 protein activates inflammatory signaling pathway components and disrupts retinal vascular-barrier in the diabetic retina. Exp Eye Res 107: 101-109, 2013.
12. Schroten-Loef C, de Ridder CM, Reneman S, Crezee M, Dalgleish A, Todryk SM, Bangma CH and Kraaij R: A prostate cancer vaccine comprising whole cells secreting IL-7, effective against subcutaneous challenge, requires local GM-CSF for intra-prostatic efficacy. Cancer Immunol Immunother 58: 373-381, 2009.
13. Buchler T, Bortlicek Z, Poprach A, Kubackova K, Kiss I, Zemanova M, Fiala O, Dusek L, Vyzula R and Melichar B; Czech Renal Cancer Cooperative Group: Efficacy of everolimus in second- and third-line therapy for metastatic renal cell carcinoma: a registry-based analysis. Urol Oncol 32: 569-575, 2014.
14. Hille A, Grüger S, Christiansen H, Wolff HA, Volkmer B, Lehmann J, Dörr W and Rave-Fränk M: Effect of tumour-cell-derived or recombinant keratinocyte growth factor (KGF) on proliferation and radioresponse of human epithelial tumour cells (HNSCC) and normal keratinocytes in vitro. Radiat Environ Biophys 49: 261-270, 2010.
15. Yucel AF, Kanter M, Pergel A, Erboga M and Guzel A: The role of curcumin on intestinal oxidative stress, cell proliferation and apoptosis after ischemia/reperfusion injury in rats. J Mol Histol 42: 579-587, 2011.
16. Wu W, Huang Q, He F, Xiao M, Pang S, Guo X, Brunk UT, Zhao K and Zhao M: Roles of mitogen-activated protein kinases in the modulation of endothelial cell function following thermal injury. Shock 35: 618-625, 2011.
17. Lan L, Tao J, Chen A, Xie G, Huang J, Lin J, Peng J and Chen L: Electroacupuncture exerts anti-inflammatory effects in cerebral ischemia-reperfusion injured rats via suppression of the TLR4/NF-κB pathway. Int J Mol Med 31: 75-80, 2013.
18. Schelbergen RF, Blom AB, van den Bosch MH, Slöetjes A, Abdollahi-Roodsaz S, Schreurs BW, Mort JS, Vogl T, Roth J, van den Berg WB, *et al*: Alarmins S100A8 and S100A9 elicit a catabolic effect in human osteoarthritic chondrocytes that is dependent on Toll-like receptor 4. Arthritis Rheum 64: 1477-1487, 2012.
19. Wang FL, Jiang JM, Wang F, Fu ZZ and Zhang ZF: Expressions of interleukin 18 and prostaglandin E2 and their correlation in the synoviocytes of patients with osteoarthritis. J Southern Med University 30: 731-733, 2010 (In Chinese).
20. Peng J, Omran A, Ashhab MU, Kong H, Gan N, He F and Yin F: Expression patterns of miR-124, miR-134, miR-132, and miR-21 in an immature rat model and children with mesial temporal lobe epilepsy. J Mol Neurosci 50: 291-297, 2013.