The HMGB1-RAGE Pathway Contributes to Abnormal Migration of Endogenous Subventricular Zone Neural Progenitors in an Experimental Model of Focal Microgyria

Yi-Wen Mei
College of Medicine, Southwest Jiaotong University; Department of Neurosurgery, General Hospital of Western Theater Command of PLA

Tian-Lan Huang
College of Medicine, Southwest Jiaotong University; Department of Neurosurgery, General Hospital of Western Theater Command of PLA

Xin Chen
Department of Neurosurgery, General Hospital of Western Theater Command of PLA

Si-Xun Yu
College of Medicine, Southwest Jiaotong University; Department of Neurosurgery, General Hospital of Western Theater Command of PLA

Jie Li
College of Medicine, Southwest Jiaotong University; Department of Neurosurgery, General Hospital of Western Theater Command of PLA

Zhi Zhang
Department of Neurosurgery, General Hospital of Western Theater Command of PLA

Yang He
Key Laboratory of Advanced Technologies of Materials, Ministry of Education School of Materials Science and Engineering, Southwest Jiaotong University

Da-qing Guo
The Clinical Hospital of Chengdu Brain Science Institute, MOE Key Lab for NeuroInformation, Center for Information in Medicine, School of Life Science and Technology, University of Electronic Science and Technology of China

Haifeng Shu (shuhaifeng@swjtu.edu.cn)
General Hospital of Western Theater Command of PLA https://orcid.org/0000-0002-0193-1881

Research Article

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Abstract

Abnormal migration of subventricular zone (SVZ)-derived neural progenitor cells (SDNPs) is involved in the pathological and epileptic processes of focal cortical dysplasia (FCD), but the underlying mechanisms are still unclear. Recent studies have indicated that high mobility group box 1 (HMGB1)/receptor for advanced glycation end products (RAGE) is widely expressed in epileptic specimens of FCD, suggesting that the HMGB1-RAGE pathway may be involved in the pathological and/or epileptic processes of FCD. In the present study, by using Nestin-GFP$^{tg/+}$ transgenic mice, we established a model of freezing lesion (FL), as described in our previous report. A “migrating stream”, which was composed of GFP-Nestin^{+} SDNPs, was derived from the SVZ region and migrated to the cortical FL area. We found that translocated HMGB1 and RAGE were expressed in cortical lesion in a clustered distribution pattern, which was especially obvious in the early stage of FL compared with the sham group. Interestingly, the number of GFP-Nestin^{+} SDNPs within the migrating stream was significantly decreased when the HMGB1-RAGE pathway was blocked by an antagonist of RAGE or deletion of the *RAGE* gene. Moreover, the absence of RAGE also decreased the activity of pentylenetetrazol-induced cortical epileptiform discharge. In summary, this study provided experimental evidence that in the early stage of FL, the levels of extranuclear HMGB1 and its receptor RAGE were increased in cortical lesion in the FL model. Activation of the HMGB1-RAGE pathway may contribute to the abnormal migration of SDNPs and the hyperexcitability of cortical lesion in the FL model.

Introduction

Focal cortical dysplasias (FCDs) are a group of heterogeneous developmental disorders that are caused by germline or somatic mutations and characterized by abnormal cortical lamination, cell morphology (e.g., cell enlargement), and cell polarity (Cepeda et al. 2006; Cepeda et al. 2005). FCDs are highly associated with drug-resistant epilepsy and the most common cause of neocortical epilepsy in children (Iffland and Crino 2017; Represa 2019). Previous studies have shown that abnormal cells in epileptogenic lesions of FCD are mainly derived from the abnormal migration and differentiation of subventricular zone-derived neural progenitor cells (SDNPs) (Lamparello et al. 2007). Our previous research suggests that the abnormal neurogenesis of SDNPs is involved in the pathological and epileptic processes of FCD (Shu et al. 2014). However, the mechanisms involved in the regulation of abnormal SDNP migration still need to be explored.

High mobility group box 1 (HMGB1) is a nuclear protein with multiple functions, and these functions depend on its location (Deng et al. 2019). It is well known that under normal conditions, HMGB1 is mainly located in the nucleus as a nonacetylated and thiol form but released from dead and dying cells after tissue injury and can be further converted to disulfide HMGB1 (Venereau et al. 2016). In addition, after cell activation or injury, translocation of HMGB1 from the nucleus to the cytoplasm may occur, followed by inflammasome activation and pyroptosis (Xu et al. 2014). In addition, growing evidence suggests that HMGB1 can regulate cell proliferation, migration and differentiation (Fages et al. 2000; Fang et al. 2012; Lei et al. 2013). As one of the receptors of HMGB1, receptor for advanced glycation end products (RAGE)

can promote the migration of many kinds of cells, including neural stem cells (Rouhiainen et al. 2013) (Xue et al. 2018). Evidence suggests that the expression of RAGE is restricted in the undifferentiated neural stem/progenitor cells of the mouse adult subventricular zone (SVZ) neurogenic region and adult SVZ-derived neurospheres, and the HMGB1-RAGE pathway can promote the proliferation and differentiation of SDNPs (Meneghini et al. 2010). Furthermore, the mRNA and protein levels of HMGB1 and RAGE were significantly upregulated in FCD cortical epileptic foci (Zurolo et al. 2011). Therefore, we proposed that the HMGB1-RAGE pathway is involved in the pathophysiological process of abnormal SDNP migration in FCD. In this study, we established a freezing lesion (FL) model to observe the expression of HMGB1 and RAGE in the cortical lesion of FL animal models, its potential role in regulating the migration of SDNPs, and its influence on cortical epileptiform discharge in mice.

Materials And Methods

Animals

The newborn (P0, day of birth) Nestin-GFP$^{tg/+}$ (N-G) transgenic mice and RAGE knockout (RAGE$^{-/-}$) mice used in this study were purchased from Guangzhou Saiye Biotechnology Co., Ltd. All mice were maintained in cages with a 12-h light/dark cycle and free access to food and drinking water. This study was approved by the Ethics Committee of the General Hospital of the Western Theater Command of China and followed the international animal care guidelines set forth by the Declaration of Helsinki. Every effort was made to reduce the pain and discomfort of the animals.

RAGE$^{-/-}$ mice

Intercrossed heterozygous targeted mice were used to generate homozygous targeted RAGE KO mice. Genotypes were confirmed via PCR using primers F1 (forward, 5'-GAGGTCTCCATTCTTTCTCCAGGTG-3'), R1 (reverse, 5'-GAGACTTAGAAACGCTGTGCATG-3), and R2 (reverse, 5'-CTGGGATTGACTCTTGCCTCCCTC-3'); the 901 bp PCR product was used for sequencing confirmation (homozygotes, 901 bp; heterozygotes, 901 bp/414 bp/1098 bp; wild-type allele, 414 bp/1098 bp).

Mouse neonatal freeze-lesion (FL) model

The experimental microgyri were induced in the mouse pups using a focal FL procedure as previously described (Shu et al. 2014). Neonatal mice at P0 were deeply anesthetized by immersion in ice water for 2 min. The mice were fixed under an anatomical microscope, and a liquid nitrogen-cooled copper cylinder with a diameter of 1.5 mm was placed on the right surface of the skull near the midline for 5–8 s. To create a longitudinal FL, we used the L-shaped tip of the copper needles. These lesions resulted in an approximately 3 mm-long microsulcus in the rostro-caudal direction. Sham-operated mice (sham group) were treated in the same way without cooling the copper cylinder. After the procedure, pups in some groups were injected daily or not with PFS-ZM1 (1 mg/kg, i.p.) (Wang et al. 2018), which is a RAGE-specific antagonist that specifically antagonizes the HMGB1-RAGE pathway, before the pups were returned to their mothers and analyzed after 5 (P5), 15 (P15), and 30 (P30) days.

Immunofluorescence (IF) staining

Mice were anesthetized with an intraperitoneal injection of 4% chloral hydrate (350 mg/kg) and transcardially perfused with a short prerinse of 20-40 ml of ice-cold physiological saline, followed by 20-40 ml of 4% paraformaldehyde (PFA). Whole tissues were then postfixed in 4% PFA at 4°C overnight. After dehydration with 15% and 30% sucrose for 48 h, coronal 30-μm sections were cut using a sliding freezing microtome. The slices were incubated at room temperature for 100 minutes in goat serum, followed by a 24 h incubation with the following primary antibodies: anti-doublecortin (DCX, Abcam, ab18723, 1:500), anti-HMGB1 (GeneTex, GTX101277, 1:600), and anti-RAGE (Abcam, ab3611, 1:300). Proteins were visualized by detection with CY3 secondary antibodies (Servicebio, GB21303, 1:400) after adding the secondary antibody after 1 h at room temperature. The slices were mounted with DAPI (Servicebio, G1012) before images were captured with a Nikon A1R confocal microscope (Nikon, Japan). The fluorescence data were analyzed using ImageJ software.

Western blotting (WB)

The protein levels of HMGB1 and RAGE in the FL and sham cortices were assessed by Western blotting. β-Tubulin was used as a loading control. Samples were selected from the operated side of the cortex, and total protein was extracted from three animals on P5, P15 and P30. The tissues were homogenized in lysis buffer and centrifuged at 4°C. Then, the supernatants were resolved via 12% SDS-PAGE, electrotransferred to a 0.45 μm PVDF membrane, and blocked for 2 h at room temperature with 5% nonfat dry milk in TBST. After overnight incubation with primary antibodies anti-HMGB1 mAb (GeneTex, GTX101277, 1:3000), anti-β-tubulin (Servicebio, GB11017, 1;3000) and anti-RAGE (Servicebio, GB11278, 1:1000)), the PVDF membrane was washed in TBST 3 times and incubated for 1 h with goat anti-rat IgG HRP (ANR02-1, 1:10000). Proteins were visualized by chemiluminescence with an ECL Western Blotting Substrate Kit (Merck-Millipore, WBKLS0100) according to the manufacturer's instructions. Densitometric quantification was performed with ImageJ software.

Electroencephalogram (EEG) analysis of seizures

FL mice were anesthetized with 4% chloral hydrate (350 mg/kg) at 6-8 weeks, and the skulls were exposed. Six silver wire electrodes (0.05 inches in diameter) insulated to within 0.5 mm of their cut ends were soldered to a microminiaturized connector. Channels 1 and 3 of the electrodes represented the FL side, channels 2 and 4 represented the side opposite the FL, and channels 5 and 6 were defined as the ground and reference electrodes, respectively. Small pieces of Gelfoam were used to cover each hole, and dental cement was used to fasten the electrodes to the skull. The mice were put back in the cage and allowed to recover for one week. The electrode was then connected to the instrument using a digital electroalograph (RM6250, Scchengyi, China). Mice were tested for 10 minutes at baseline and then tested for one hour after injection with pentylenetetrazol (PTZ, 30 mg/kg, i.p.). Electroencephalogram (EEG) signal acquisition was filtered at 15 Hz, and sampling was set at 800 Hz. The occurrence of epileptiform discharge was characterized using EEG analysis (*via* the software of the multichannel physiological acquisition and processing system, RM6240). Rhythmic sharp waves were selected when the amplitude

was more than 2 times the baseline, and spike activity with more than a 5-second duration was considered a seizure or epileptiform discharge (Wang et al. 2020). Epileptiform discharges were analyzed by using the coastline bursting index, which calculates the total length of the discharge waveform of multiple population spikes. This analysis thus provides an objective and sensitive measure of the burst intensity, which can reflect the extracellular currents generated by nearly synchronous firing of many pyramidal neurons, as well as the synchrony and firing frequency of neurons participating in the discharge, and is well suited for statistical analysis (Jahromi et al. 2000; Korn et al. 1987; Polc et al. 1996; Thomas et al. 2005). We calculated the coastline bursting index for the baseline and each 10-minute period after PTZ injection (30 mg/kg, i.p.). EEG power was analyzed with MATLAB scripts (MathWorks) and Brainstorm software.

Statistics

Statistical comparisons were performed using Prism 8 software (GraphPad Software Inc.) and detailed information is provided in the figure legend. All data are presented as the mean ± s.e.m. An unpaired t test was used for differences between two groups, and ANOVA followed by Tukey's multiple comparisons test or Games-Howell's multiple comparisons test was used for three or more groups. Statistical significance was set as follows: *p <0.05, **p <0.01, and ***p <0.001.

Results

Expression of HMGB1 and RAGE in cortical lesion of FL

Consistent with previous reports (Shu et al. 2014), we observed a large number of GFP-Nestin$^+$ SDNPs within the cortical lesion that decreased over time (Supplementary Fig. 1c, 1e, 1g); these cells are derived from SVZ region (Supplementary Fig. 2a, 2d, 2g), migrate to the cortical FL area and look like a "migrating stream" (Supplementary Fig. 1c, 1e, 1g, the area indicated by the arrow symbol), whereas no such phenomenon was observed in the sham group (Supplementary Fig. 1b, 1d, 1f). To clarify the potential role of HMGB1/RAGE in SDNP migration, we first investigated the protein levels of HMGB1 and RAGE in cortical lesion after FL. As illustrated in Supplementary Fig. 1a, FL and sham mice were decapitated at three time points (P5, P15, and P30). The protein expression of HMGB1 in the cortex gradually decreased over time in both the FL and sham groups (Fig. 1a). However, compared with the sham group (Fig. 1b), there was no significant difference in the total protein expression level of HMGB1 in the FL cortex at P5 (0.82±0.087 vs 0.94±0.12, P=0.48, n=3), P15 (0.49±0.06 vs 0.61±0.03, P= 0.14, n=3) and P30 (0.23±0.077 vs 0.33±0.029, P=0.29, n=3). Interestingly, the protein level of RAGE in the cortex gradually increased over time in both the FL and sham groups (Fig. 1c). Compared with the sham group (Fig. 1d), there was no significant change in the RAGE protein expression level in the FL cortex at P5 (0.08±0.015 vs 0.08±0.013, P=0.88, n=3), P15 (0.85±0.067 vs 0.75±0.037, P=0.25, n=3) or P30 (1.91±0.23 vs 1.83±0.36, P=0.87, n=3).

Distribution and translocation of HMGB1 in FL cortical lesion

Because the function of HMGB1 is related to its distribution state, we further investigated the distribution pattern of HMGB1 by IF analysis in FL cortical lesion. Interestingly, the localization of HMGB1-positive cells within the FL cortex converged more strongly in the cortical lesion area than in the corresponding cortex of the sham group (Fig. 2a1-f1). We counted the area of HMGB1-positive fluorescence within the FL cortex and the corresponding cortex of the sham group (Fig. 2g), and the statistical data showed that the area of HMGB1-positive fluorescence in the center of the FL cortex was significantly increased compared with that in the sham cortex at P5 (14.43±1.94 vs 23.23±2.23, P=0.04, n=3); however, there is no significant difference between FL and sham groups at P15 (12.30 ±1.54 vs 17.10 ±1.026, P=0.061, n=3) or P30 (7.038±1.132 vs 11.95±1.90, P=0.09, n=3) (Fig. 2g). In addition, the localization of HMGB1 expression was transferred to cells around the FL lesion and the “migrating stream” (Fig. 2a2-f2). HMGB1 was mostly present in the cytoplasm, while it was mostly expressed in the nucleus in the sham cortex. We calculated the proportion of HMGB1 IF signals in the nuclei and cytoplasm. The ratio of cytoplasmic/nuclear HMGB1-positive cells was significantly higher in the FL group than in the sham group at P5 (sham, 0.057±0.02 vs 0.74±0.055, P=0.0003, n=3; FL, 0.79±0.061 vs 0.13±0.049, P=0.0011, n=3) (Fig 2h). The increased ratio started to decrease at P15 (sham, 0.23±0.03 vs 0.63±0.065, P=0.0047, n=3; FL, 0.78±0.04 vs 0.21±0.05, P=0.0009, n=3), and there was no significant difference between the two groups until P30 (sham, 0.18±0.08 vs 0.48±0.12, P=0.12, n=3; FL, vs 0.45±0.045 vs 0.37±0.041, P=0.24, n=3).

Distribution of RAGE in FL cortical lesion

IF analysis also showed that the localization of RAGE-positive cells within the FL cortex converged more strongly in the cortical lesion area than in the corresponding cortex of the sham group (Fig. 3a-3f). The difference was the largest at P5 (Fig. 3a, 3b) but gradually decreased at P15 (Fig. 3c, 3d) and P30 (Fig. 3e, 3f). We evaluated the area of RAGE-positive fluorescence within the FL cortex and corresponding cortex of the sham group (Fig. 3g). The statistical data showed that the percentage of RAGE-positive area was significantly increased compared with that in the sham cortex at P5 (12.33±3.48 vs 30.33±0.88, P=0.0074, n=3) and P15 (10.00±1.00 vs 22.67±2.73, P=0.012, n=3), and there was no difference at P30 (5.00±0.58 vs 9.67±2.40, P=0.13, n=3). We observed the expression of RAGE on a few GFP-Nestin^{+} cells in the cortex of the sham group and a large number of GFP-Nestin^{+} cells in the cell migration stream (Fig. 3h, 3i).

HMGB1-RAGE may regulate the migration of SDNPs

To explore the role of RAGE in SDNP migration after FL, we used a specific antagonist of RAGE (FPS-ZM1, 1 mg/kg, i.p.) to block the HMGB1-RAGE pathway in N-G mice. In addition, Nestin-GFP $^{Tg/+}$/RAGE$^{-/-}$ hybrid mice (N-G/RAGE$^{-/-}$ mice), which are crossbred from Nestin-GFP$^{Tg/+}$ mice and RAGE$^{-/-}$ mice, were also used. The breeding strategy for the hybrid mice is briefly shown in the figure (Fig. 4a). N-G mice without intervention were used as the control group, and a brief operation process is shown in Fig. 4b. The confocal images showed that compared with that in the N-G group (Fig. 4c, 4f, 4i), the density of GFP-Nestin positive cells in the N-G/RAGE$^{-/-}$ mouse group was significantly decreased at P5 (Fig. 4e, 4l;

1072.00±43.67 vs 502.70±81.14, P=0.013, n=3) but significantly increased at P15 (Fig. 4h, 4l; 265.70±62.55 vs 789.30±75.98, P=0.014, n=3), and there was no significant difference between the two groups until P30 (Fig. 4k, 4l; 196.70±33.71 vs 77.67±18.10, P=0.10, n=3). In addition, the change in GFP-Nestin^{+} cell density in the N-G/FPS-ZM1 group was similar to that in the N-G/RAGE$^{-/-}$ group (Fig. 4d, 4g, 4j), but there was no significant difference between the N-G/FPS-ZM1 group and the N-G group at each time point (Fig. 4l; P5, 1072.00±43.67 vs 717.70±87.37, P=0.061, n=3; P15, 265.70±62.55 vs 539.30±44.43, P =0.058, n=3; P30, 196.70±33.71 vs 288.30±40.01, P=0.30, n=3).

To assess the migratory ability of SDNPs, we stained for a marker of migrating neuroblasts, DCX, and determined the colocalization density of GFP-Nestin- and DCX-positive cells. The density of GFP-Nestin^{+} and DCX^{+} colocalized cells in P5 N-G/FPS-ZM1 group (Fig. 4d; 748.30±55.64 vs 242.30±47.70, P=0.0055) and N-G/RAGE$^{-/-}$ group (Fig. 4e; 748.30±55.64 vs 422.00±25.81, P=0.03, n=3) was significantly reduced (Fig. 4m). Interestingly, this difference was variable until P15 and P30 (Fig. 4g, 4h, 4j, 4k). the density of GFP-Nestin^{+} and DCX^{+} colocation cells in the N-G/FPS-ZM1 group and the N-G/RAGE$^{-/-}$ group was slightly higher than that in the N-G group, but the difference was not statistically significant (Fig. 4m), N-G/FPS-ZM1 group (Fig. 4g, 4j; P15, 232.00±39.59 vs 358.30±49.15,P=0.21, n=3; P30, 51.33±12.47 vs 149.30±38.02, P=0.20, n=3), N-G/RAGE$^{-/-}$ group (Fig. 4h, 4k; P15, 232.00±39.59 vs 400.7±48.04, P=0.089, n=3; P30, 51.33±12.47 vs 47.33±13.45, P=0.97, n=3). At the same time, no significant difference was observed between the N-G/FPS-ZM1 group and the N-G/RAGE$^{-/-}$ group at each time point (Fig. 4m; P5, 242.30±47.70 vs 422.00±25.81, P=0.087, n=3; P15, 358.30±49.15 vs 400.7±48.04, P=0.80, n=3; P30, 149.30±38.02 vs 47.33±13.45, P=0.19, n=3).

Activation of HMGB1-RAGE may affect the PTZ-induced epileptiform discharge in the FL model

The electrode implantation schematic diagram and operation process are shown in the figure (Fig. 5a, 5b). Combining the behavioral and EEG findings, we found that RAGE KO reduced the level (Fig. 5c; 4.75±0.25 vs 2.59±0.29, P=0.0011, n=4) and duration (Fig. 5d; 16.00±2.48 vs 8.75±1.32, P=0.042, n=4) of epileptiform discharge after PTZ induction at a subconvulsive dose (30 mg/kg, i.p.) (Szyndler et al. 2018; Taiwe et al. 2016; Van Erum et al. 2019). The coastline bursting index showed that there was no significant difference at baseline between N-G mice and N-G/RAGE$^{-/-}$ mice before PTZ injection (Fig. 5e; 1212.00±55.35 vs 819.8±185.6, P=0.09). However, RAGE KO reduced the PTZ-induced coastline bursting index after PTZ injection, suggesting decreased cortical discharge excitability. The results showed that at 0-10 min (Fig. 5e; 1378.00±203.00 vs 675.50±157.10, P=0.033, n=4) and 40-50 min (Fig. 5e; 1044.00±120.70 vs 646.8±92.16, P=0.04, n=4) after PTZ injection, the difference was the most significant. At other time periods, although the N-G/RAGE$^{-/-}$ group had lower values than N-G group, the difference was not statistically significant (Fig. 5e; 10-20 min, 1030.00±215.30 vs 786.30±238.70, P=0.48, n=4; 20-30 min, 873.30±147.6 vs 671.30±172.50, P=0.41, n=4; 30-40 min, 1186.00±260.2 vs 756.00±211.40, P=0.25, n=4; 50-60 min, 908.80±166.30 vs 666.30±88.15, P=0.25, n=4). Epileptiform EEG events were present in the recordings from the electrodes implanted on both the FL side and opposite side of the FL cortex in the N-G and N-G/RAGE$^{-/-}$ mice (Fig. 5f, 5h). The corresponding time-frequency

analysis results showed that the energy of the epileptiform discharge in the N-G mice was higher than that in the N-G/RAGE$^{-/-}$ mice (Fig. 5g, 5i).

Discussion

In the present study, by using Nestin-GFP$^{tg/+}$ transgenic mice, in which nestin-positive SDNPs were marked by GFP, we established a model of FL as described in our previous report (Shu et al. 2014). A “migrating stream”, which was composed of GFP-Nestin^{+} SDNPs, was derived from the SVZ region and migrated to the cortical FL area. We found that translocated HMGB1 was expressed in cortical lesion in a clustered distribution pattern, which was especially obvious in the early stage of FL compared with the sham group. In addition, one of the HMGB1 receptors, RAGE, was also increased in the cortical lesion of FL. Interestingly, the number of GFP-Nestin^{+} SDNPs within the migrating stream was significantly decreased when the HMGB1-RAGE pathway was blocked by an antagonist of RAGE or deletion of the *RAGE* gene. Moreover, the absence of RAGE also decreased the activity of PTZ-induced cortical epileptiform discharges.

Our previous study showed that the abnormal migration of endogenous SDNPs contributes to the formation and hyperexcitability of an experimental model of focal microgyria (Shu et al. 2014), but the molecular mechanisms involved are not yet clear. Recent studies have indicated that HMGB1/RAGE is widely expressed in pathological specimens of FCD and that translocated HMGB1 is mostly located in dysplastic cells, suggesting the important role of the HMGB1-RAGE pathway in the epileptogenesis of FCD (Zhang et al. 2018; Zurolo et al. 2011). The biological role of HMGB1 varies and depends on its cytoplasmic/nuclear distribution (Deng et al. 2019). In this study, although the overall protein level of HMGB1 showed no significant difference between the FL and sham cortex, the proportion of translocated HMGB1 in the cytoplasm was significantly increased in the FL cortex compared with the sham group. HMGB1 is a nonhistone chromosome binding protein that is abundant in the nucleus of eukaryotic cells. In response to cell damage or stress, HMGB1 is released from the nucleus of damaged or necrotic cells into the extracellular membrane in the cytoplasm (Kang et al. 2014). Extranuclear HMGB1 is considered a danger signal from damaged or stressed cells to alert the tissue to an acute or persistent state of injury. It has been reported that extranuclear HMGB1 interacts with RAGE to play a key role in the pathological process of neurological diseases, such as FCD, Alzheimer's disease, and multiple sclerosis (Andersson et al. 2008; Meneghini et al. 2013; Zhang et al. 2018). In this study, we observed that translocated HMGB1 and RAGE increased within the cortical lesion of FL, suggesting that the HMGB1-RAGE pathway may contribute to the formation of FL-induced microgyria.

Existing literature has demonstrated that in addition to mediating inflammatory pathways, HMGB1 and RAGE are also involved in a variety of cell migration processes (Hayakawa et al. 2013; Rouhiainen et al. 2013). HMGB1/RAGE signaling regulates cell growth, proliferation, and migration *via* activation of mitogen-activated protein kinase and nuclear factor kappa-lightchain-enhancer of activated B cells (NF-κB) pathways (Ding et al. 2017). In the early stage of brain development or in adulthood, restricted expression of RAGE can be observed on undifferentiated neural progenitor cells (NPCs) in the SVZ region.

Moreover, the activation of HMGB1/RAGE under the action of its endogenous ligands can promote the proliferation and differentiation of SVZ-NPCs (Meneghini et al. 2010). In addition, it was observed *in vitro* that the HMGB1-RAGE pathway could affect the migration of neural stem cells, which could be attenuated by the RAGE antagonist FPS-ZM1 (Xue et al. 2018). We first established an FL model in Nestin-GFP$^{tg/+}$ mice, in which NPCs within the SVZ are marked by GFP. As an intermediate filament protein, nestin is transiently expressed only in neuroepithelial stem cells and radial glia during the neurulation and migratory stages of cortical development (Dell'Albani 2008). In the adult mammalian brain, nestin expression is restricted to a select group of subventricular and endothelial cells (Migaud et al. 2010). Therefore, by using Nestin-GFP$^{tg/+}$ mice, we directly observed SDNP migration induced by FL, while the total number of GFP-Nestin^{+} cells within the migration stream decreased with development. The current data showed that the number of GFP-Nestin^{+} cells within the migration stream was significantly reduced when the HMGB1-RAGE pathway was blocked. In addition, the number of GFP-Nestin- and DCX-colocalized cells, which is a key gene involved in neuronal migration and neuronal differentiation processes (Liu 2011), was also reduced if RAGE was blocked or absent. Accordingly, we hypothesized that the activation of the HMGB1-RAGE pathway may be involved in the abnormal migration of SDNPs induced by FL.

Increasing evidence indicates that the intrinsic activation of proinflammatory signaling pathways in FCD may be involved in the pathogenesis of high epileptogenesis (Boer et al. 2006; Iyer et al. 2010; Zhang et al. 2018). HMGB1 is one of the most influential proinflammatory cytokines and activates inflammatory pathways by stimulating two principal receptors, RAGE and TLR4 (Weber et al. 2015); activation of these receptors has been implicated in epileptogenesis (Iori et al. 2013; Maroso et al. 2010; Shi et al. 2018). In this study, we observed that the absence of RAGE reduced the energy and duration of PTZ-induced epileptiform discharge in the FL model, suggesting that the activation of HMGB1-RAGE may be involved in the hyperexcitability of cortical lesions. First, extracellular HMGB1 activates RAGE and/or TLR4 and leads to the phosphorylation of the NR2B subunit and the potentiation of NMDA-mediated Ca^{2+} influx into neurons (Paudel et al. 2018; Vezzani et al. 2012), which may play an important role in the cortical hyperexcitability of the FL model (Bandyopadhyay and Hablitz 2006). On the other hand, previous reports have demonstrated that the abnormal migration of SDNPs may contribute to the development of hyperexcitability (Shu et al. 2014). Therefore, we suspected that the activation of HMGB1-RAGE affects the hyperexcitability of FL cortical lesion through the regulation of SDNP migration.

In summary, this study provided experimental evidence that in the early stage of FL, extranuclear HMGB1 and its receptor RAGE were increased in cortical lesion in the FL model. Activation of the HMGB1-RAGE pathway may contribute to the abnormal migration of SDNPs and the hyperexcitability of cortical lesion in an FL model. Intervening in the HMGB1-RAGE pathway may be a strategy to prevent FCD-related epileptogenesis.

Declarations

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Authors' contributions All authors have contributed significantly. All authors contributed to the study conception and design. Material preparation and data collection were performed by Yi-Wen Mei, Tian-Lan Huang, Zhi Zhang, Jie Li, Yang He, and Daqing Guo. Yi-Wen Mei, Xin Chen and Si-Xun Yu participated in the data analysis. The first draft of the manuscript was written by Yi-Wen Mei and Hai-Feng Shu commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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