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

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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 dysplasias (FCDs), but the underlying mechanisms are not clear. Recent studies indicated that high mobility group box 1 (HMGB1)/receptor for advanced glycation end products (RAGE) are widely expressed in epileptic specimens of FCDs, which suggests that the HMGB1-RAGE pathway is involved in the pathological and/or epileptic processes of FCDs. The present study used Nestin-GFPtg/+ transgenic mice, and we established a model of freezing lesion (FL), as described in our previous report. A “migrating stream” 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 to the sham group. Notably, the number of GFP-Nestin + SDNPs within the “migrating stream” was significantly decreased when the HMGB1-RAGE pathway was blocked by a RAGE antagonist or deletion of the RAGE gene. The absence of RAGE also decreased the activity of pentylenetetrazol-induced cortical epileptiform discharge. In summary, this study provided experimental evidence that the levels of extranuclear HMGB1 and its receptor RAGE were increased in cortical lesion in the early stage of 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.

Keywords Freeze lesion · High mobility group box 1 · Receptor for advanced glycation end products · Neural progenitors · Migration · Epileptiform discharge

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, 2005). FCDs are highly associated with drug-resistant epilepsy, and the most common cause of neocortical epilepsy in children (Ilfland and Crino 2017; Represa 2019). Previous studies have shown that abnormal cells in epileptogenic lesions of FCDs are primarily 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 FCDs (Shu et al. 2014). However, the mechanisms involved in the regulation of abnormal SDNP migration require further study.
High mobility group box 1 (HMGB1) is a nuclear protein with multiple functions, and these functions depend on its location (Deng et al. 2019). Under normal conditions, HMGB1 is primarily located in the nucleus in a nonacetylated and thiol form, but it is released from dead and dying cells after tissue injury and may be further converted to disulfide HMGB1 (Venereau et al. 2016). HMGB1 may also translocate from the nucleus to the cytoplasm after cell activation or injury, followed by inflammasome activation and pyroptosis (Xu et al. 2014). 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) is a receptor of HMGB1 that promotes the migration of many types of cells, including neural stem cells (Rouhiainen et al. 2013) (Xue et al. 2018). 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 promotes the proliferation and differentiation of SDNPs (Meneghini et al. 2010). The mRNA and protein levels of HMGB1 and RAGE are significantly upregulated in FCD (Meneghini et al. 2010). The mRNA and protein levels of HMGB1 and RAGE in the FL and sham cortices were assessed using WB. β-Tubulin was used as an internal control. The data were analyzed using ImageJ software.

**Materials and Methods**

**Animals**

Newborn (P0, day of birth) Nestin-GFP<sup>tg/+</sup> (N-G) transgenic and RAGE knockout (RAGE<sup>−/−</sup>) mice 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. The Ethics Committee of the General Hospital of the Western Theater Command of China approved this study, which 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<sup>−/−</sup> Mice**

Intercrossed heterozygous targeted mice were used to generate homozygous targeted RAGE KO mice. Genotypes were confirmed via PCR using the following primers: F1 (forward, 5′-GAGGTCTCCATTCTTCTCCAGGTG-3′); R1 (reverse, 5′-GAGACCTAGAACGCTGTGCATG-3′), and R2 (reverse, 5′-CTGGGATTGACTCTTGCCCTCCCTC-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 Model**

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 via 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 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 manner 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 Staining**

Mice were anesthetized via an intraperitoneal injection of 4% chloral hydrate (350 mg/kg) and transcardially perfused with a short prerinse of 20–40 ml ice-cold physiological saline, followed by 20–40 ml 4% paraformaldehyde (PFA). Whole tissues were postfixed in 4% PFA at 4 °C overnight. After dehydration in 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 min 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 with CY3 secondary antibodies (Servicebio, GB21303, 1:400) after 1 h at room temperature. The slices were mounted using 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**

The protein levels of HMGB1 and RAGE in the FL and sham cortices were assessed using WB. 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. The supernatants were resolved via 12% SDS-PAGE, electro-transferred 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 the anti-HMGB1 mAb (GeneTex, GTX101277, 1:3000), anti-β-tubulin (Servicebio, GB11017, 1:3000) and anti-RAGE (Servicebio, GB11278, 1:1000), and primary antibodies, the PVDF membrane was washed in TBST 3 times and incubated for 1 h with goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) (ANR02-1, 1:10,000). Proteins were visualized via chemiluminescence using the ECL Western Blotting Substrate Kit (Merck-Millipore, WBKLS0100) according to the manufacturer’s instructions. Densitometric quantification was performed using ImageJ software.

**Electroencephalogram 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 1 week. The electrode was then connected to the instrument using a digital electroalograph (RM6250, Scchengyi, China). Mice were tested for 10 min at baseline then for 1 h after injection with pentylenetetrazol (PTZ, 30 mg/kg, i.p.). 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 and 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-s duration was considered a seizure or epileptiform discharge (Wang et al. 2020). Epileptiform discharges were analyzed using the coastline bursting index, which calculates the total length of the discharge waveform of multiple population spikes. This analysis provides an objective and sensitive measure of the burst intensity, which reflects the extracellular currents generated by the nearly synchronous firing of many pyramidal neurons and the synchrony and firing frequency of neurons participating in the discharge, and it is well suited for statistical analyses (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-min period after PTZ injection (30 mg/kg, i.p.). EEG power was analyzed using 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 legends. All data are presented as the means ± s.e.m. An unpaired t test was used to assess differences between two groups, and ANOVA followed by Tukey’s multiple comparisons test or Games-Howell’s multiple comparisons test was used for comparisons of three or more groups. Statistical significance was set as *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, e, g). These cells were derived from the SVZ region (Supplementary Fig. 2a, d, g), migrated to the cortical FL area, and looked like a “migrating stream” (Supplementary Fig. 1c, e, g; the area indicated by the arrow symbol). No such phenomenon was observed in the sham group (Supplementary Fig. 1b, d, f). To clarify the potential role of HMGB1/RAGE in SDNPs migration, we investigated the protein levels of HMGB1 and RAGE in cortical FL. As illustrated in Supplementary Fig. 1a, FL and sham mice were decapitated at 3 time points: P5, P15, and P30. The protein expression of HMGB1 in the cortex gradually decreased over time in the FL and sham groups (Fig. 1a). However, there was no significant difference in the total protein expression level of HMGB1 in the FL cortex compared with the sham group (Fig. 1b) at P5 (sham vs. FL: 0.82 ± 0.087 vs. 0.94 ± 0.12, P = 0.48, n = 3), P15 (sham vs. FL: 0.49 ± 0.06 vs. 0.61 ± 0.03, P = 0.14, n = 3), or P30 (sham vs. FL: 0.23 ± 0.077 vs. 0.33 ± 0.029, P = 0.29, n = 3). Notably, the protein levels of RAGE in the cortex gradually increased over time in the FL and sham groups (Fig. 1c). However, there was no significant change in RAGE protein expression level in the FL cortex compared with the sham group (Fig. 1d) at P5 (sham vs. FL: 0.08 ± 0.015 vs. 0.08 ± 0.013, P = 0.88, n = 3), P15 (sham vs. FL: 0.85 ± 0.067 vs. 0.75 ± 0.037, P = 0.25, n = 3), or P30 (sham vs. FL: 1.91 ± 0.23 vs 1.83 ± 0.36, P = 0.87, n = 3).
Distribution and Translocation of HMGB1 in Cortical FL

Because the function of HMGB1 is related to its distribution state, we further investigated the distribution pattern of HMGB1 using IF analysis in cortical FL. Notably, the localization of HMGB1-positive cells within the FL cortex converged more strongly in the cortical lesion area than the corresponding cortex of the sham group (Fig. 2a1–f1). We measured 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 to the sham cortex at P5 (sham vs. FL: 14.43 ± 1.94 vs. 23.23 ± 2.23, \( P = 0.04, n = 3 \)). However, there was no significant difference between the FL and sham groups at P15 (sham vs. FL: 12.30 ± 1.54 vs. 17.10 ± 1.02, \( P = 0.061, n = 3 \)) or P30 (sham vs. FL: 7.038 ± 1.132 vs. 11.95 ± 1.90, \( P = 0.09, n = 3 \)) (Fig. 2g). The localization of HMGB1 expression changed in cells around the FL and the “migrating stream” (Fig. 2a2–f2).

Distribution of RAGE in Cortical FL 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 the corresponding cortex of the sham group (Fig. 3a–f). The difference was largest at P5 (Fig. 3a, b) then gradually decreased at P15 (Fig. 3c, d) and P30 (Fig. 3e, f). We evaluated the area of RAGE-positive fluorescence within the FL cortex and the corresponding cortex of the sham group (Fig. 3g). The statistical data showed that the percentage of RAGE-positive area of FL group was significantly increased compared to the sham cortex at P5 (sham vs. FL: 12.33 ± 3.48 vs. 30.33 ± 0.88, \( P = 0.0074, n = 3 \)) and P15 (sham vs. FL: 10.00 ± 1.00 vs. 22.67 ± 2.73, \( P = 0.012, n = 3 \)). There was no difference at P30 (sham vs. FL: 5.00 ± 0.58 vs. 9.67 ± 2.40, \( P = 0.13, n = 3 \)). We observed RAGE expression on a few GFP-Nestin+ cells in the cortex of the sham group and a large number of GFP-Nestin+ cells in the “migrating stream” (Fig. 3h, i).
Fig. 2 Distribution and translocation of HMGB1 in cortical FL. (a–f) Representative images showing the overall expression of HMGB1 in the sham and FL cortices (blue, DAPI; green, Nestin; red, HMGB1). Rectangular boxes represent the FL central area and corresponding area in the sham group that were used for statistical analyses. Bar, 100 μm. (a1–f1) Representative images showing the distribution of HMGB1 in the center of the FL cortex and the corresponding region of the sham cortex (blue, DAPI; green, Nestin; red, HMGB1). Bar, 50 μm. (a2–f2) Representative images showing translocation of HMGB1 around the FL cortex and the corresponding region of the sham cortex (blue, DAPI; green, Nestin; red, HMGB1). Cells with nuclear immunoreactivity (arrowheads) and cytoplasmic staining (arrow). Bar, 50 μm. (g) Quantification of the HMGB1-positive area percentage in the center of the FL cortex (n = 3, mice) and the corresponding region of the sham cortex (n = 3, mice). *P < 0.05 by unpaired t test. (h) Quantification of the proportion of HMGB1 IF signal in nuclei and cytoplasm around the FL cortex (n = 3, mice) and the corresponding region of the sham cortex (n = 3, mice). Extranuclear staining vs nuclear staining: **P < 0.01, ***P < 0.001 by unpaired t test.
HMGB1-RAGE May Regulate the Migration of SDNPs

To examine the role of RAGE in SDNP migration after FL, we used a specific RAGE antagonist (FPS-ZM1, 1 mg/kg, i.p.) to block the HMGB1-RAGE pathway in N-G mice. Nestin-GFP<sup>Tg</sup>/+RAGE<sup>−/−</sup> hybrid mice (N-G/RAGE<sup>−/−</sup> mice), which were crossbred from Nestin-GFP<sup>Tg</sup>/+ mice and RAGE<sup>−/−</sup> mice, were also used. The breeding strategy for the hybrid mice is briefly shown in Fig. 4a. N-G mice without intervention were used as the control group, and the brief surgical process is shown in Fig. 4b. We observed the beginning of the “migrating stream” where SDNPs converged. A rectangular region with fixed length and width was selected from the upper margin of white matter at distances of 190 μm (P5), 150 μm (P15), and 120 μm (P30). The size of the region was referred to the average size of the “migrating stream” in the middle region from the beginning of the “migrating stream” of N-G mice in P5 to below the lower boundary of the FL region. Two experienced researchers independently counted the number of GFP-Nestin<sup>+</sup> cells and DCX/Nestin<sup>+</sup> cells in the region, and both counters were blind for the group. GFP-Nestin<sup>+</sup> cells were defined as Nestin/DAPI (green/blue) colocalized cells, as shown in Fig. 4c1–k1. DCX/Nestin<sup>+</sup> cells were defined as DAPI/Nestin/DCX (blue/green/red) colocalized cells with an overlap area > 50%, as shown in Fig. 4c2–k2. Confocal images showed that the density of GFP-Nestin<sup>+</sup> cells in the N-G/RAGE<sup>−/−</sup> mouse group was significantly decreased compared to the N-G group (Fig. 4c, f, i) at P5 (Fig. 4e, l); N-G vs. N-G/RAGE<sup>−/−</sup>: 1072.00 ± 43.67 vs. 502.70 ± 81.14, P = 0.013, n = 3) but significantly increased at P15 (Fig. 4h, l); N-G vs. N-G/RAGE<sup>−/−</sup>: 265.70 ± 62.55 vs. 789.30 ± 75.98, P = 0.014, n = 3). There was no significant difference between the two groups at P30 (Fig. 4k, l); N-G vs. N-G/RAGE<sup>−/−</sup>: 196.70 ± 33.71 vs. 77.67 ± 18.10, P = 0.10, n = 3). The change in GFP-Nestin<sup>+</sup> cell density in the N-G/FPS-ZM1 group was similar to the N-G/RAGE<sup>−/−</sup> group (Fig. 4d, g, j), but there was no significant difference between the N-G/FPS-ZM1 group and the N-G group at any time point (Fig. 4d); N-G vs. N-G/FPS-ZM1; 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).

Activation of HMGB1-RAGE May Affect PTZ-Induced Epileptiform Discharge in the FL Model

The electrode implantation schematic diagram and surgical process are shown in Fig. 5a, b. Combining the behavioral and EEG findings, we found that RAGE KO reduced the level (Fig. 5c); N-G vs. N-G/RAGE<sup>−/−</sup>: 4.75 ± 0.25 vs. 2.59 ± 0.29, P = 0.0011, n = 4) and duration (Fig. 5d); N-G vs. N-G/RAGE<sup>−/−</sup>: 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 no significant difference at baseline between N-G mice and N-G/RAGE<sup>−/−</sup> mice before PTZ injection (Fig. 5e); N-G vs. N-G/RAGE<sup>−/−</sup>: 1212.00 ± 55.35 vs. 819.8 ± 185.6, P = 0.09). However, RAGE KO reduced the PTZ-induced coastline bursting index, which suggests decreased cortical discharge excitability. The results showed that the differences were most significant at 0–10 min (Fig. 5c); N-G vs. N-G/RAGE<sup>−/−</sup>: 1378.00 ± 203.00 vs. 675.50 ± 157.10, P = 0.033, n = 4) and 40–50 min (Fig. 5e); N-G vs. N-G/RAGE<sup>−/−</sup>: 1044.00 ± 120.70 vs. 646.8 ± 92.16, P = 0.04, n = 4) after PTZ injection. Although the N-G/RAGE<sup>−/−</sup> group had lower values than the N-G group at the other time periods, the differences were not statistically
Fig. 4 HMGB1-RAGE may regulate the migration of SDNPs. (a) Breeding strategy for hybridization of N-G/RAGE−/− mice. (b) Basic experimental scheme. (c–k) Representative images showing DCX/Nestin+ cells in the migratory flow of N-G, N-G/FPS-ZM1 and N-G/RAGE−/− cells at P5, P15, and P30 (blue, DAPI; green, Nestin; red, DCX). Bar, 100 μm. (cl–k1) Representative image showing GFP-Nestin+ cells. (c–k2) Representative image showing DCX/Nestin+ cells. (I) Quantification of GFP-Nestin+ cell density in the migratory flow of N-G (n = 3, mice), N-G/FPS-ZM1 (n = 3, mice), and N-G/RAGE−/− (n = 3, mice). *P < 0.05. (m) Quantification of colocalization of DCX/Nestin+ cells density in the “migrating stream” of N-G (n = 3, mice), N-G/FPS-ZM1 (n = 3, mice), and N-G/RAGE−/− (n = 3, mice). *P < 0.05, **P < 0.01. ANOVA followed by Tukey’s multiple comparisons test with equal variance or Games-Howell’s multiple comparisons test with unequal variance.

Discussion

The present study used Nestin-GFPtg/+ transgenic mice, in which Nestin-positive SDNPs were marked by GFP, to establish a model of FL as described in our previous report (Shu et al. 2014). A “migrating stream” 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 to the sham group. The HMGB1 receptor RAGE was also increased in the cortical FL. Notably, the number of GFP-Nestin+ SDNPs within the “migrating stream” was significantly decreased when the HMGB1-RAGE pathway was blocked with a RAGE antagonist FPS-ZM1 (Xue et al. 2018). We first established an FL model in Nestin-GFPtg/+ mice, in which NPCs within the SVZ were labeled with GFP. Nestin is an intermediate filament protein that is transiently expressed only in neuroepithelial stem cells and radial glia during the neurulation and differentiation of SVZ-NPCs (Meneghini et al. 2010). The HMGB1-RAGE pathway contributes to the formation of FL-induced microgyria.

Previous studies demonstrated that HMGB1 and RAGE were 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-light-chain-enhancer of activated B cell (NF-κB) pathways (Ding et al. 2017). Restricted RAGE expression is observed in undifferentiated neural progenitor cells (NPCs) in the SVZ region in the early stage of brain development and adulthood. The activation of HMGB1/RAGE by endogenous ligands promotes the proliferation and differentiation of SVZ-NPCs (Meneghini et al. 2010). The HMGB1-RAGE pathway affected the migration of neural stem cells in vitro, which was attenuated by the RAGE antagonist FPS-ZM1 (Xue et al. 2018). We first established an FL model in Nestin-GFPtg/+ mice, in which NPCs within the SVZ were labeled with GFP. Nestin is an intermediate filament protein that is transiently expressed only in neuroepithelial stem cells and radial glia during the neurulation and migratory stages of cortical development (Dell’Albani 2008). Nestin expression in the adult mammalian brain is restricted to a select group of subventricular and endothelial cells (Migaud et al. 2010). Therefore, the Nestin-GFPtg/+ mice allowed us to directly observe FL-induced SDNP migration, while the total number of GFP-Nestin+ cells within the “migrating stream” decreased with development. The current data showed the number of GFP-Nestin+ cells within the “migrating stream” was significantly reduced in RAGE KO mice in the early stage of FL. DCX is a key gene located in dysplastic cells, suggesting an important role of the HMGB1-RAGE pathway in the epileptogenesis of FCDs (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). Although the overall protein level of HMGB1 showed no significant difference between the FL and sham cortices in the present study, the proportion of translocated HMGB1 in the cytoplasm was significantly increased in the FL cortex compared to the sham group. HMGB1 is a nonhistone chromosome-binding protein that is abundant in the nuclei of eukaryotic cells. 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. Extranuclear HMGB1 interacts with RAGE to play a key role in the pathological process of neurological diseases, such as FCDs, Alzheimer’s disease, and multiple sclerosis (Andersson et al. 2008; Meneghini et al. 2013; Zhang et al. 2018). The present study observed an increase in translocated HMGB1, and RAGE increased within the cortical lesion of FL, which suggests that the HMGB1-RAGE pathway contributes to the formation of FL-induced microgyria.
**Fig. 5** Activation of HMGB1-RAGE may affect the PTZ-induced epileptiform discharge in the FL model. a Diagram of cortical electrode implantation. b Diagram of the surgical process. c Racine score of N-G and N-G/RAGE−/− mice. d Duration of epileptiform discharge of N-G and N-G/RAGE−/− mice. e Quantitative analysis of the coastline bursting index of N-G and N-G/RAGE−/− mice for each 10-min period. *P<0.05, by unpaired t test. All error bars are the means±s.e.m. f, g EEG waveform and time–frequency diagram of N-G mice. h, i EEG waveform and time–frequency diagram of N-G/RAGE−/− mice.

Increasing evidence suggests that the intrinsic activation of proinflammatory signaling pathways in FCDs is 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 it activates inflammatory pathways by stimulating two principal receptors, RAGE and TLR4 (Weber et al. 2015). The activation of these receptors is implicated in epileptogenesis (Iori et al. 2013; Maroso et al. 2010; Shi et al. 2018). The present study 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 the HMGB1-RAGE pathway was involved in the FL-induced abnormal migration of SDNPs.

Previous reports demonstrated that the abnormal migration of SDNPs contributed to the development of hyperexcitability (Shu et al. 2014), and activation of the HMGB1-RAGE pathway may be involved in the FL-induced abnormal migration. Therefore, we hypothesized that the secreted HMGB1 bound to RAGE on the surface of SDNPs (Supplementary Fig. 3a, c, e), and activation of HMGB1-RAGE affected the hyperexcitability of FL cortical lesion via the regulation of SDNPs migration. Notably, we also observed that HMGB1 and RAGE were colocalized on both SDNPs (Supplementary Fig. 3a, c, e) and non-SDNPs (Supplementary Fig. 3a, c, e), which suggests that the HMGB1-RAGE pathway directly participate in the regulation of cortical excitability by affecting neuron ion channels, but this hypothesis needs further investigation.

In summary, the present study demonstrated that extranuclear HMGB1 and its receptor RAGE were increased in cortical lesion in the early stage of 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. Intervention in the HMGB1-RAGE pathway may be a strategy to prevent FCDs-related epileptogenesis.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12031-021-01891-x.

**Author Contribution** 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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**Declarations**

**Ethics Approval** All procedures and experiments were conducted under the guidelines approved by the Ethics Committee of this hospital.

**Conflict of Interest** The authors declare no competing interests.

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