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Role of NDEL1 and VEGF/VEGFR-2 in Mouse Hippocampus After Status Epilepticus

Lin Zhu[1,*], Shujuan Dai[1,*], Di Lu[2], Puying Xu[1], Lu Chen[1], Yanbing Han[1], Lianmei Zhong[1], Lvhua Chang[1,*], and Qian Wu[1,*]

Abstract

Nuclear-distribution element-like 1 (NDEL1) is associated with the proliferation and migration of neurons. Vascular endothelial growth factor (VEGF) in combination with VEGF receptor-2 (VEGFR-2) regulates the proliferation and migration of neurons. This study was performed to explore undefined alterations in the expression levels of NDEL1 and VEGF/VEGFR-2 within the hippocampus after status epilepticus (SE). Following the creation of pilocarpine-induced epilepsy models using adolescent male C57BL/6 mice, Western blotting and reverse transcription quantitative polymerase chain reaction were applied to assess the levels of NDEL1, VEGF, and VEGFR-2 expression in whole hippocampi at 1, 2, 3, and 4 weeks post-SE, respectively. Immunofluorescent labeling was also employed to detect the colocalization of NDEL1 and VEGF in the hippocampus. Our results indicated that NDEL1 and VEGF have similar patterns of upregulation throughout the hippocampus. Upregulation of VEGFR-2 occurred only in the early stages, and the expression decreased shortly afterward. NDEL1 and VEGF were coexpressed in the cornu ammonis 3 pyramidal cell, granular, and polymorph layers of the dentate gyrus in the hippocampus. This study revealed that NDEL1, VEGF, and VEGFR-2 may work together and are involved in the pathophysiology in the hippocampus after SE.

Keywords

VEGF/VEGFR-2, NDEL1, hippocampus, status epilepsy

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Status epilepticus (SE) can induce dramatic changes in neurogenesis in the hippocampus dentate gyrus (DG) subgranular zone as well as cell loss and apoptosis in the pyramidal neurons in hippocampal cornu ammonis (CA)1 and CA3 and the interneurons of the hilus. Nuclear-distribution element-like 1 (NDEL1), which is located at chromosome 17q13.1, has received increasing attention due to recent findings regarding its function in neurogenesis in neuropsychiatric diseases, along with several molecules, such as disrupted in schizophrenia 1 (DISC1) and Lissencephaly 1 (LIS1; Moon et al., 2014). Wu et al. reported enhanced expression of NDEL1 in the hippocampus and decreased expression in CA3 and the DG. They also found that detectable levels of NDEL1 can be observed not only in the neurons but also in the capillaries within the hippocampal region (Wu et al., 2014). Not long afterward, Choi, Lee, Hansen, et al. (2016) suggested that NDEL1 is consistently and abundantly expressed in the hippocampus, possibly due to the regulation of the cAMP response element-binding protein/cAMP response element (CREB/CRE) transcriptional pathway.NDEL1 is also considered to be involved in axon regeneration,

[1]Department of Neurology, First Affiliated Hospital, Kunming Medical University
[2]Biomedicine Engineering Research Centre, Kunming Medical University

*These authors contributed equally to the work.

Corresponding Authors:
Lvhua Chang, Department of Neurology, First Affiliated Hospital, Kunming Medical University, 295 Xi Chang Road, Kunming, Yunnan 650032, P. R. China.
Email: changluh@aliyun.com
Qian Wu, Department of Neurology, First Affiliated Hospital, Kunming Medical University, 295 Xi Chang Road, Kunming, Yunnan 650032, P. R. China.
Email: qian.wu.neuro@gmail.com

microtubule assembly, neuronal proliferation and migration, and so forth. Neurofilaments are considered to be involved in axonal regeneration. Nguyen et al. (2004) reported that in a mouse model of neurodegeneration, NDEL1 contributed to the release of newly formed microtubules from the centrosomes and the projection to neurites by regulating NF assembly. In addition, NDEL1 and dynein interact with lamin B to facilitate the accumulation and assembly of neurofilaments on microtubules (Ma et al., 2009). NDEL1 can also bind to paxillin, markedly strengthening its adhesivity and facilitating cell migration (Shan et al., 2009; Kuga et al., 2014). After phosphorylation mediated by Aurora-A (Mori et al., 2009; Yamada et al., 2010; Takitoh et al., 2012; Inaba et al., 2016) and the cyclin-dependent kinase family (Toyo-oka et al., 2008; Pandey and Smith, 2011; Klinman and Holzbaur, 2015; Wynne and Vallee, 2018), p-NDEL1 participates in the regulation of the cytoskeleton, spindle recombination, and chromosome arrangement (Liang et al., 2007; Moon et al., 2014), which underlie neuronal proliferation and migration.

In addition, a number of reports suggested that vascular endothelial growth factor (VEGF) plays a role in both the developing and mature nervous systems and therefore acts as a fundamental modulator in various kinds of neurological diseases. VEGF could prevent amyloid peptide-induced endothelial apoptosis *in vitro*, and neuronal expression of VEGF in a transgenic mouse model of Alzheimer's disease restored memory behavior (Religa et al., 2013). Importantly, Weiner et al. found that VEGF was associated with baseline hippocampal volume, and the level of VEGF in cerebrospinal fluid was associated with brain aging. The neuroprotective effect of VEGF could be enhanced by Alzheimer's disease biomarkers, indicating that VEGF may be particularly beneficial as an early hallmark of the disease (Hohman et al., 2015). In a rat model of Huntington's disease induced by quinolinic acid, VEGF injected into the striatum significantly improved motor impairments and protected against the loss of striatal neurons (Emerich et al., 2010). Moreover, Wada et al. found a high level of VEGF in the striatum of some patients with Parkinson's disease (Wada et al., 2006). In addition, combined administration of VEGF- and glial cell line-derived neurotrophic factor (GDNF)-loaded nanospheres could increase antiparkinsonian efficacy (Herran et al., 2014). VEGF also plays a key role in epilepsy. Blood–brain barrier dysfunction, such as the interruption of endothelial tight cell junctions, increased permeability of blood vessels, and serum protein extravasation into brain parenchyma (Marchi and Lerner-Natoli, 2013), has been reported to be related to epilepsy pathogenesis. VEGF expression was increased, and VEGF receptor-2 (VEGFR-2) was activated in a kainate-induced seizure-like events model with increased vascular density and branching. VEGF prevented these changes, possibly through a relationship with phosphatidylinositol 3-kinase and protein kinase C (Morin-Brureau et al., 2011). Nikitidou et al. (2012) reported that the overexpression of VEGFR-2, also known as FLK-1, could upregulate the elevated threshold of seizure and decrease the duration of focal after-discharges. Lenzer-Fanara et al. (2017) also found that proper VEGF stimulation could rescue the uncontrolled firing caused by the branching complexity and spatial patterning of astrocytes in a Sprague Dawley (SD) rat seizure model. Based on the roles mentioned earlier, VEGF has great potential in novel treatments for microvascular dysplasia following the onset of epilepsy. Hence, VEGF can regulate vascular endothelial cell proliferation to facilitate cellular epileptogenic processes.

VEGFR-2 is widely accepted to be one of the most critical receptors mediating the normal function of the blood–brain barrier due to its close association with the survival and migration of endothelial, neuronal, and Schwann cells, as well as multiple fundamental processes such as neuronal stem cell proliferation, axonal guidance, and angiogenesis. Liu et al. (2018) reported that the LIS1/NDE1 complex is an essential component of fibroblast growth factor (FGF) signaling, primarily by regulating intracellular trafficking of the FGF receptor. Considering the role of VEGF as a growth factor, in addition to regulating homogeneity between NDEL1 and NDE1, we hypothesized that NDEL1 and VEGF/VEGFR-2 are likely to have cross talk between their canonical signaling pathways, especially in terms of their involvement in the pathophysiology of hippocampus after SE. Thus, we aimed to evaluate the dynamic alterations in the coexpression of NDEL1 and VEGF/VEGFR-2 in adolescent mouse hippocampus following SE.

Materials and Methods

Housing and Handling Animals

All animals used in the present study were adolescent C57BL/6 male mice aged 5 to 6 weeks old and weighing between 20 and 22 g. The mice were housed in cages with a standardized control environment, specifically a constant temperature of 22°C to 26°C, humidity of 50% to 60%, 12 hr/12 hr light cycle, and food/water ad libitum. Prior to officially launching the experiments, all protocols were approved by Kunming Medical University in compliance with all regulatory guidelines. All experiments conformed to the official recommendations of the Chinese animal ethics community.

Pilocarpine Treatment

Mice were randomly subdivided into control and experimental groups after 1 week of adjustment to their environment. Briefly, mice were injected intraperitoneally (i.p.) with 20 mg/ml of scopolamine butyl bromide (Yantai Luyin Pharmaceutical Co Ltd., Shandong, China) using a dose of 1 mg per animal weight unit (kg) to reduce the peripheral side effects of pilocarpine. Thirty minutes later, SE was induced by i.p. injection with 260 to 280 mg/kg of pilocarpine (#P6503; Sigma chemical Co., St. Louis, MO, USA). Turski et al. reported that 100 to 400 mg/kg of pilocarpine could successfully induce epilepsy 1 to 2 hr after administration (Turski, Cavalheiro, et al., 1983; Turski, Czuczwar, et al., 1983), whereas Wu et al. (2014) used 320 to 340 mg/kg of pilocarpine to induce SE. Based on the literature, we chose 260 to 280 mg/kg for the experimental group, particularly considering our practical experiences with the related mortality rate during SE induction. Seizures were evaluated using the Racine standard (Racine, 1972). Briefly, only mice classified as Racine Stages 4 to 5 with seizures lasting at least 60 min were used in this study. Two hours after SE, 7.5 mg/kg of diazepam (Shanghai Xudong Haipu Pharmaceutical Co., Ltd., Shanghai; China) with a concentration of 10 mg/ml was injected. The control group was treated similarly but was administered the same volume of sterile saline instead of pilocarpine.

Tissue Preparation

At 1, 2, 3, and 4 weeks after the termination of SE, mice were anesthetized with an i.p. injection of 10% chloral hydrate (0.01–0.02 ml per body weight unit [g]) and transcardially perfused with 0.01 M cold phosphate-buffered saline (PBS, pH 7.2–7.4). After perfusion with 4% paraformaldehyde in PBS (pH 7.2–7.4), brain tissues were dissected from the skull and postfixed in 4% paraformaldehyde for another 12 to 24 hr at 4°C. The brain tissues were rinsed with PBS afterward and dehydrated with 30% sucrose in PBS at 4°C for another 48 to 72 hr until they sank completely. Finally, the brains were embedded in optimal cutting temperature compound and stored at –80°C. Coronal sections with a thickness of 6 μm were obtained with a cryostat (Leica, CM1850, Germany) and transferred onto polylysine-coated slides. The sections were stored at –20°C until further processing. For Western blotting and reverse transcription quantitative polymerase chain reaction (RT-qPCR), hippocampal tissues were harvested after PBS perfusion and stored at –80°C until further processing.

Immunofluorescence

For immunofluorescence, slides containing coronal sections were rinsed with PBS and incubated with 10% normal rabbit serum in 0.1% PBS with Tween 20 (PBST) for 2 hr at room temperature, followed by incubation with NDEL1 primary antibody (goat anti-mouse, 1:50; Santa Cruz, SC-48063, RRID: AB_2149891; Santa Cruz, Dallas, TX, USA) and VEGF primary antibody (mouse monoclonal antibody, 1:100; Santa Cruz, SC–7269, RRID: AB_628430) at 4°C for 24 hr. The sections were rinsed with 0.1% PBST and incubated with goat anti-mouse secondary antibody conjugated with Cy3 (1:300, Bioss, Beijing, China) or rabbit anti-goat secondary antibody conjugated with Alexa Flour 488 (1:300, Bioss, Beijing, China) for 2 hr at room temperature. Both the primary and secondary antibodies were diluted in PBS with 5% rabbit serum plus 0.1% Triton-X100. Finally, the sections were rinsed with 0.1% PBST and mounted using a fluoromount-G solution containing 4′,6-diamidino-2-phenylindole. Images were captured with a digital camera attached to a microscope (BX53, OLYMPUS, Japan). Three different brain slices were analyzed by two blinded independent evaluators, who measured the area of the CA3 and DG in a random region (20×) and calculated the number of cells with NDEL1 and VEGF coexpression in this region. As the slices in our study were only 6 μm, we only compared the number of cells per unit area (N.O./mm^2).

Western Blotting

Tissues from the mouse hippocampus were dissected, suspended, and sonicated using radioimmunoprecipitation assay buffer (Solarbio, Beijing, China) supplemented with phenylmethylsulfonyl fluoride (Solarbio, Beijing, China). After centrifugation (12,400 × g for 20 min), the supernatant of the homogenate was collected. Bicinchoninic acid was used to determine the protein concentration. All samples contained an equal amount of protein diluted in 5× loading buffer (Solarbio, Beijing, China) and were electrophoresed in 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Beyotime, Sanghai, China), followed by transferring to polyvinylidene difluoride membranes (45 μm, Millipore, USA). The polyvinylidene difluoride membranes were blocked in 5% nonfat milk in Tris-buffered saline with 0.1% Tween 20 for 2 hr at room temperature and incubated overnight with the following primary antibodies: NDEL1 antibody (goat anti-mouse, 1:300, Santa Cruz, SC-48063, RRID: AB_2149891), VEGF antibody (mouse monoclonal antibody, 1:500, Santa Cruz, SC-7269, RRID: AB_628430), and VEGFR-2 antibody (mouse monoclonal antibody, 1:1000, Santa Cruz, SC-6251, RRID: AB_628431). All the primary antibodies were diluted in 5% nonfat milk in Tris-buffered saline with 0.1% Tween 20. The membranes were then rinsed and incubated with the following secondary antibodies: rabbit anti-goat secondary antibody conjugated with

peroxidase (1:2000, Proteintech, Wuhan, China) and goat anti-mouse secondary antibody conjugated with peroxidase (1:2000, Proteintech, Wuhan, China) for 2 hr at room temperature. Following rinses, the membranes were incubated with enhanced chemiluminescence reagents (Santa Cruz), followed by visualization using Amersham Imager 600 (GE, Boston, MA, USA). ImageJ software was employed for evaluating the densitometer of the protein bands.

RT-qPCR

Total RNA was isolated and reverse transcribed into complementary DNA with a thermal cycling platform (1000-series, BIO-RAD, USA). Each reaction mixture contained 1.5 μg of all RNA samples. The RT-qPCR reaction was performed using the following primers:

NDEL1: Forward 5′-TGCACAGAGCTACAAGCAG GT-3′, Reverse 5′-CTCGCTCCAGGTCATCATTG-3′;

VEGF: Forward 5′-AGGCGAGGCAGCTTGAGTT A-3′, Reverse 5′-TTCCGGTGAGAGGTCTGGTT-3′;

VEGFR-2: Forward 5′-CTTGGATGAGCGCTGTGA AC-3′, Reverse 5′-CGTCTGCCTCAATCACTTG-3′;

β-actin: Forward 5′-CAACTTGATGTATGAAGGCTT TGGT-3′, Reverse 5′-ACTTTTATTGGTCTCAAGTCA GTGTACA-3′.

The reaction was incubated at 95°C for 15 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 30 s. The process was performed with a real-time qPCR system (Mx3005P, Stratagene, USA).

Statistical Analysis

All results were analyzed using the SPSS 22.0 software. The Western blot, RT-qPCR, and cell N.O. data were analyzed with two-way analysis of variance tests. Differences were considered significant when $p < .05$* and very significant when $p < .01$**. Statistical graphs were created with GraphPad Prism 7.0, and the bars in the graphs corresponded to the mean ± SEM.

Results

NDEL1 and VEGF/VEGFR-2 Expression in Mouse Hippocampi

To investigate the association between NDEL1 and VEGF/VEGFR-2 following SE, we assessed the respective levels of RNA and protein isolated from the tissues of the hippocampus, in addition to analysis using RT-qPCR and Western blotting. Western blotting revealed that the expression of NDEL1 protein in the hippocampus was upregulated at 1, 2, 3, and 4 weeks following SE ($n = 4$, $p < .01$; Figure 1A and D). The expression level of VEGF in the hippocampus was also upregulated following SE ($n = 4$, $p < .01$; Figure 1B and E). However, the protein expression of VEGFR-2 in the hippocampus was increased during the first week ($n = 6$, $p < .05$) and prominently decreased in the last week ($n = 6$, $p < .05$; Figure 1C and F). RT-qPCR further revealed that in the SE group, NDEL1 ($n = 4$) was substantially increased in 2 ($p < .01$), 3, and 4 ($p < .05$) weeks and also increased in 1 ($p < .01$) and 4 ($p < .05$) weeks after SE (Figure 1G and H), whereas VEGFR-2 was slightly increased in the first week only ($n = 4$, $p < .01$; Figure 1I). With regard to the protein expression, NDEL1 and VEGF exhibited no obvious statistical differences at 1, 2, 3, and 4 weeks post-SE, whereas VEGFR-2 was downregulated very quickly at the second week after SE (Figure 1D to F and J to L). Overall, these results suggested that NDEL1, VEGF, and VEGFR-2 participated in the pathophysiology of epilepsy; VEGFR-2 might act in the early stage after SE with NDEL1 and VEGF.

Coexpression of VEGF and NDEL1 in Hippocampus

Using immunofluorescence to assess the relationship between NDEL1 and VEGF showed they were colocalized in the pyramidal cell layer of CA3, as well as in the granular and polymorph layers of the DG across the entire hippocampus in both the SE and control groups (Figure 2A to E). The number of cells with NDEL1 and VEGF coexpression in the CA3 decreased at 1, 2, 3, and 4 weeks following SE (1, 2, and 4 weeks, $p < .05$; 3 weeks, $n = 3$, $p > .05$; Figure 2F). The number of cells with NDEL1 and VEGF coexpression in the DG also decreased at both 1 and 4 weeks post-SE (1 and 4 weeks, $n = 3$, $p < .05$; Figure 2G). Therefore, these results suggested that NDEL1 possibly regulates the pathophysiology of epilepsy in the hippocampus via VEGF signaling.

Discussion

NDEL1 is extensively involved in the regulation of numerous cellular processes. By directly regulating the neurofilament light subunit (Nguyen et al., 2004), α-tubulin, vimentin (Shim et al., 2008), and paxillin lamin B (Ma et al., 2009; Kuga et al., 2014), NDEL1 contributes to the modulation of the structural stability of the cytoskeleton, spindle recombination, and chromosome arrangement. For instance, in combination with dynein and kinase, several substances can be transported by NDEL1 along the microtube assembly. All of these

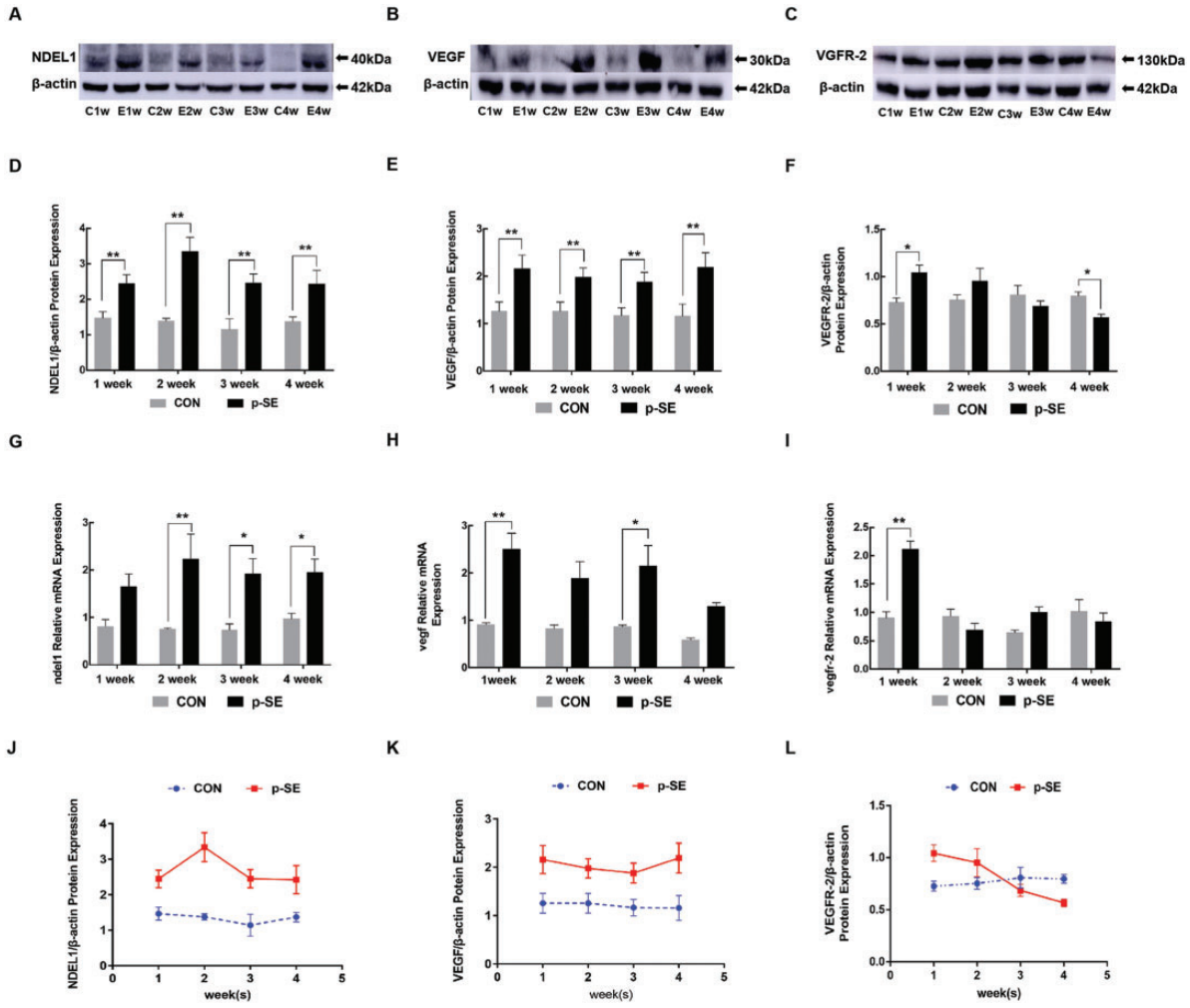

Figure 1. NDEL1 and VEGF Expression in Mouse Hippocampus. (A, D, and G) The results revealed that NDEL1 protein and mRNA were upregulated post-SE after 1, 2, 3, and 4 weeks. (B, E, and H) Both VEGF protein and mRNA were increased after SE. (C, F, and L) The expression of VEGFR-2 protein in the hippocampus increased during the first week and decreased by the fourth week. (I) The expression of VEGFR-2 was only increased in the first week. (D, J, E, and K) Both NDEL1 and VEGF protein expression levels were not significantly different in the post-SE group at different weeks.
NDEL1 = nuclear-distribution element-like 1; VEGF = vascular endothelia growth factor; VEGFR-2 = vascular endothelial growth factor receptor 2; SE = status epilepticus; CON = control.

substances arguably underpin multiple stages of neuronal development such as neuronal proliferation, differentiation, and migration. Wu et al. (2014) proposed that NDEL1 expression is increased across the entire hippocampus, and Choi, Lee, Hansen, et al. (2016) found that in SD rat models, NDEL1 is consistently and abundantly expressed in the hippocampus. In our study, we confirmed the NDEL1 expression in the hippocampus was upregulated at 1, 2, 3, and 4 weeks following SE (Figure 1A and D), which is basically consistent with the observations by Wu and Choi. Nevertheless, Wu et al. (2014) reported that, based on immunohistochemistry results, NDEL1 is expressed not only in neurons but also in the capillary of the hippocampus in mice. However, in our study using immunofluorescence, NDEL1 expression could be solely observed in the neurons and did not appear along the capillaries. This difference may be due to the different morphological methods used and especially the differences in section thickness (we chose 6 μm, while Wu chose 35 μm). In our study, the sections were too thin to display the outline of the capillaries; thus, NDEL1 expression in the capillaries could not be fully captured. The upregulation of NDEL1 hints at its potential role in epilepsy, even though at the present, it is impossible to conclude how an increase in NDEL1 contributes to the pathophysiology of epilepsy or the progression of epilepsy. Future studies using an epilepsy model of knock-in NDEL1 or overexpression of

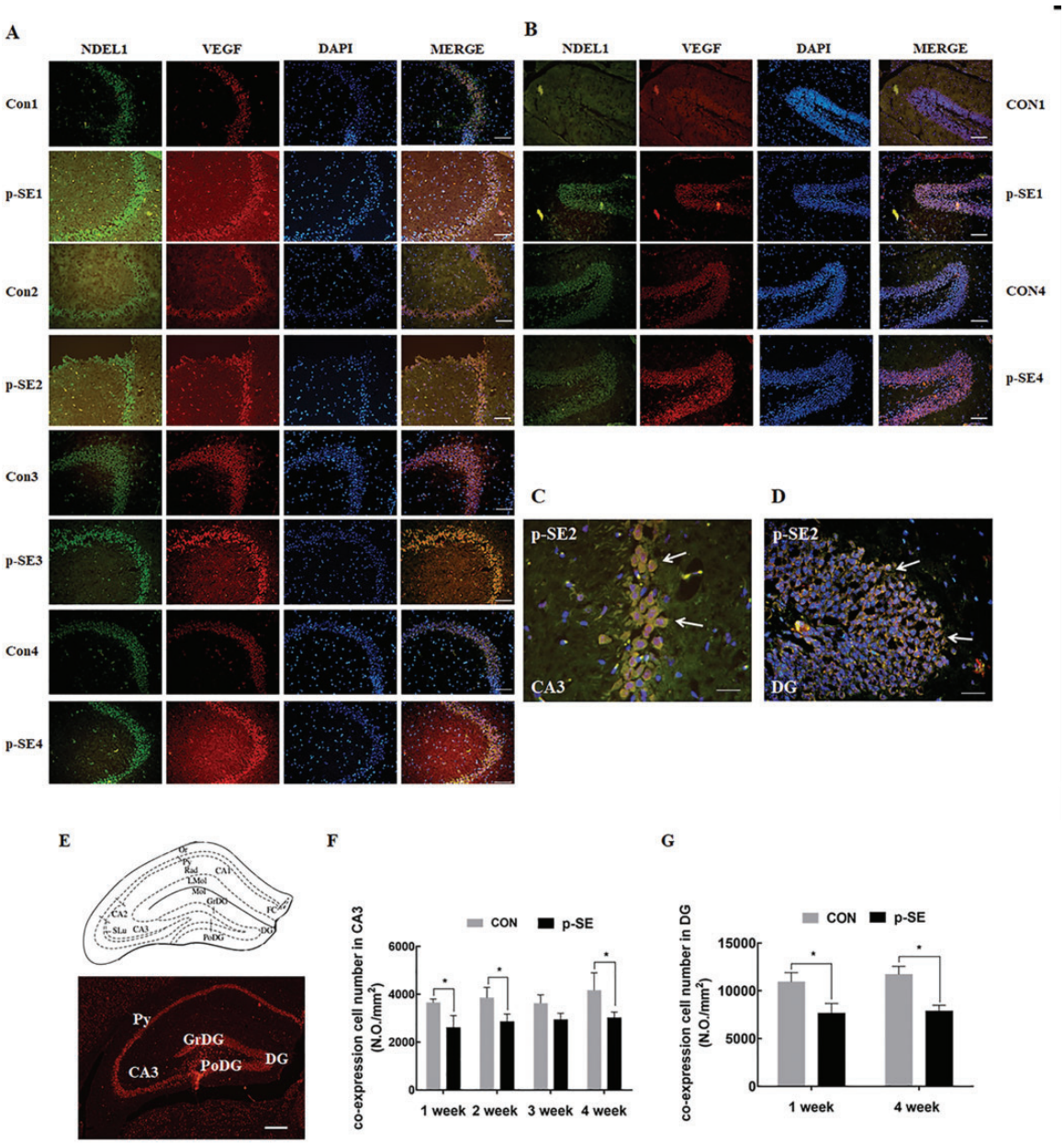

Figure 2. Coexpression of VEGF and NDEL1 in Hippocampus. (A to D) NDEL1 and VEGF were frequently coexpressed in the CA3 pyramidal cell, granular, and polymorph layers of the DG in both the control and SE groups. (E) VEGF staining results showing a section of the hippocampus. (F) The number of cells with NDEL1 and VEGF coexpression in the CA3 was decreased post-SE after 1, 2, 3, and 4 weeks. (G) The number of coexpressed cells in the DG was also decreased post-SE after 1 and 4 weeks. Green fluorescence labeling NDEL1, red fluorescence labeling VEGF, blue fluorescence labeling nucleus; scale bar = 100 μm in A and B, 40 μm in C and D, and 400 μm in E; all sections were 6 μm thick.
NDEL1 = nuclear-distribution element-like 1; VEGF = vascular endothelia growth factor; DAPI = 4′, 6-diamidino-2-phenylindole; SE = status epilepticus; DG = dentate gyrus; CA = cornu ammonis; CON = control.

NDEL1 may lead to more insights in terms of exploring the precise role of NDEL1 and the corresponding dysfunctional effects from its absence.

VEGF alteration in CA1, CA3, and DG in the hippocampus, motor cortex, and cerebellum has been associated with multiple neuropsychiatric diseases, such as cerebral ischemia (Zhou et al., 2017; Song et al., 2019), brain injury (Xu et al., 2016), epilepsy (Choi, Lee, Chang, et al., 2016; Tawfik et al., 2018), and depression (Wang et al., 2014; Seong et al., 2018). Especially, in recent studies on epilepsy, a large body of evidence has emerged suggesting that VEGF not only regulates angiogenesis

and vascular permeability but is also involved in neuronal protection in hippocampus. These functions are performed in several ways. First, VEGF plays a role in promoting the proliferation of neuronal stem cells, regulating the survival and migration of neurons and Schwann cells, stimulating axonal outgrowth, and reducing cell loss in the hippocampus through either the VEGF/VEGFR-2 or phosphatidylinositol 3-kinase/protein kinase C signaling pathways (Morin-Brureau et al., 2011; Park et al., 2013; Han et al., 2017). Consequently, enhanced cell death and apoptosis of pyramidal neurons in hippocampal CA1/CA3 and the interneurons in the hilus are characteristic features that appear after SE. Experiments using SD rats showed that the branching complexity and spatial patterning of astrocytes contributed to uncontrolled firing in seizures, which can be rescued by proper VEGF stimulation (Lenzer-Fanara et al., 2017). VEGF may contribute to the pathophysiology of epilepsy by modulating neural stem cells (NSCs), especially to promote homeostasis in the vascular microenvironment (Marchi and Lerner-Natoli, 2013). VEGF can also regulate the proliferation of vascular endothelial cells related to epileptogenic processes. We tested the levels of VEGF expression at different stages post-SE and found that the expression level in the hippocampus was upregulated following SE (Figure 1B and E). In previous studies, VEGF was regarded as a double-edged sword in epilepsy, and hence, it is difficult to determine what the effects precisely are following SE when the level of VEGF is relatively high. What's more, we observed that both NDEL1 and VEGF were upregulated at all four stages post-SE. Wu et al. found that NDEL1 is expressed in both the neurons and capillaries in mice hippocampus. As previously mentioned, the LIS1/NDE1 complex is essential for FGF signaling as it regulates intracellular trafficking of the FGF receptor (Liu et al., 2018). Considering the role of VEGF as a growth factor, in addition to regulating the homogeneity of NDEL1 and NDE1, we speculated that the presence of NDEL1 was associated with that of VEGF. Thus, we decided to investigate the localization of both NDEL1 and VEGF in the hippocampal areas after SE using immunofluorescence. Surprisingly, we observed a matching pattern in the distribution of NDEL and VEGF not only in the pyramidal cell layer of CA3 but also in the granular and polymorph layers of the DG (Figure 2). Indeed, VEGF was expressed along the capillaries in the hippocampal region, which has been confirmed in several previous studies. However, VEGF expression was observed only in neurons but not in cells within the capillaries. As mentioned, this finding is arguably due to the 6-μm thickness of the sections examined, which is insufficient to fully display the entire architecture of the capillary, strongly implying that we should assess the expression of NDEL1 and VEGF in capillaries within a thicker hippocampal section in the future. As we did not control the exposure time for the sections, we were unable to assess the fluorescence intensity to determine the protein expression in CA3 and the DG. However, quantitative analysis by counting the number of cells with colocalized NDEL1 and VEGF in both CA3 and the DG showed that the number of colocalized cells in CA3 decreased following SE (Figure 2F and G). Despite the upregulation of NDEL1 and VEGF across the entire hippocampus, colocalization of the proteins actually decreased. Furthermore, Wu et al. reported that NDEL1 was decreased in CA3 and DG. In addition, SE usually induced cell loss and apoptosis in the hippocampal pyramidal cells in CA1/CA3 and the interneurons of the hilus. Thus, we speculated that the decrease in coexpressed cells in the SE group was due to the loss of neurons following SE and that some cross talk occurred between NDEL1 and VEGF, which can be demonstrated by a coimmunoprecipitation assay in a future study.

The VEGF/VEGFR-2 signaling pathway has not received sufficient attention despite its role as the most fundamental signal pathway after SE. Sun et al. (2016) reported that VEGFR-2 is highly expressed in reactive astrocytes that contribute to the epileptogenesis of mesial temporal lobe epilepsy. Han et al. reported that in rat models following SE, intracerebroventricular injection of VEGF increased the number of NSCs in the hippocampus, whereas the injection of SU5416, a VEGFR-2 inhibitor, reduced the number of NSCs. Thus, the VEGF/VEGFR-2 signaling pathway may be a novel target for treatment following epilepsy onset (Han et al., 2017). In the electrically stimulated seizure model, the threshold for seizure induction in mice overexpressing VEGFR-2 is twice the value required for wild-type mice (Nikitidou et al., 2012). Blocking VEGFR-2 using SU5416 significantly increased the number of apoptotic, that is, TdT-mediated dUTP Nick-End Labeling (TUNEL)-positive, cells in the hilus, whereas the infusion of VEGF failed to reduce the number of TUNEL-positive cells. This indicated that VEGFR-2 is involved in mediating the cellular death or survival of hilar neurons after brain injury (Lee and Agoston, 2009). If there was a link between NDEL1 and VEGF, VEGFR-2 would be the most likely candidate. Based on these assumptions, we tested the expression of VEGFR-2. We found that VEGFR-2 was upregulated in the SE group during the first and second weeks and was substantially decreased in the third and fourth weeks (Figure 1C, F, and I). However, unlike VEGF, which plays contrasting roles in mice following SE, VEGFR-2 is more frequently reported to display a neuronal protective effect (Lee and Agoston, 2009; Nikitidou et al., 2012). This led us to speculate that the decreased expression of VEGFR-2 in the fourth week may be due to its exhaustion or even decompensation.

In summary, NDEL1 and VEGF may work together and are involved in the pathophysiology in the hippocampus after SE, but accurate understanding of the mechanism requires further studies. The VEGF/VEGFR-2 signaling pathway may participate in early-stage regulation with NDEL1 after SE.

Summary

NDEL1 and VEGF are coexpressed and similarly altered throughout the hippocampus, especially the DG, after SE.

NDEL1, VEGF, and VEGFR-2 may work together and are involved in the pathophysiology in the hippocampus after SE.

Author Contributions

L. Z. and S. D. were responsible for experimentation, data interpretation, and writing of the article. D. L., P. X., and L. C. were responsible for experimentation and data analysis. Y. H. and L. Z. were responsible for data interpretation. Y. H. and Q. W. were responsible for funding. L. C. and Q. W. were responsible for scientific consultation at all stages, the conceptualization of the study, data interpretation, and revision of the article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORCID iD

Qian Wu https://orcid.org/0000-0003-3345-4247

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