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Modulatory Potential of LncRNA Zfas1 for Inflammation and Neuronal Apoptosis in Temporal Lobe Epilepsy

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Purpose: This study aimed to elucidate whether lncRNA ZFAS1 is involved in neuronal apoptosis and inflammation in temporal lobe epilepsy (TLE).

Materials and Methods: Ninety-six TLE patients were recruited, and their peripheral venous blood was gathered to determine Zfas1 expression with polymerase chain reaction. Neurons were separated from hippocampal tissue of newborn SD rats, and si-Zfas1 or pcDNA3.1-Zfas1 was transfected into the neurons. Inflammatory cytokines released by neurons were determined, and neuronal activities were evaluated through MTT assay, colony formation assay, and flow cytometry.

Results: Serum levels of Zfas1 were higher in TLE patients than in healthy controls ($p<0.05$). Furthermore, Zfas1 expression in neurons was raised by pcDNA3.1-Zfas1 and declined after silencing of Zfas1 ($p<0.05$). Transfection of pcDNA-Zfas1 weakened the viability and proliferation of neurons and increased neuronal apoptosis ($p<0.05$). Meanwhile, pcDNA3.1-Zfas1 transfection promoted lipopolysaccharide-induced release of cytokines, including tumor necrosis factor-α, interleukin (IL)-1, IL-6, and intercellular adhesion molecule-1 ($p<0.05$), and boosted NF-κB activation by elevating the expression of NF-κB p65, pIκBα, and IKKβ in neurons ($p<0.05$).

Conclusion: Our results indicated that lncRNA ZFAS1 exacerbates epilepsy development by promoting neuronal apoptosis and inflammation, implying ZFAS1 as a promising treatment target for epilepsy.

Key Words: Epilepsy, LncRNA Zfas1, LPS, neuronal apoptosis, neuronal inflammation

INTRODUCTION

Among all nervous system diseases, epilepsy has become the second most serious threat to human health,[1] and annually, there are up to 2.4 million newly developed cases of epilepsy around the globe.[2] According to statistics, half of all epilepsy sufferers are diagnosed in childhood or adolescence, and unfortunately, they are more likely to die prematurely than healthy children and adolescents.[2] Temporal lobe epilepsy (TLE), an intractable epilepsy accounting for 40% of all epilepsy cases,[1] is characterized by distorted structuring of the medial temporal lobe (amygdala and hippocampus), which has also been described for focal cortical dysplasia, vascular/ischemic lesions, and others.[3] Owing to the hippocampal damage and mental illness, TLE patients are predisposed to memory deficits.[4] Although two-thirds of epilepsy patients are able to successfully keep seizures under control with antiepileptic drugs, the remaining patients, especially those with TLE, are unable to recover with only taking these drugs.[5] Making matters worse, surgery, the recommended treatment for TLE, fails to benefit all.[6-9] Considering the poor prognosis of TLE and shortage of efficacious treatments, in-depth exploration of TLE pathogenesis is required.

LncRNAs, abundant in brain tissues, have been shown to be important in the development of the nervous system,[10] and

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methylation or loss of certain lncRNAs can result in nervous system abnormality and thus TLE deterioration.[11,12] For instance, the methylation rates of lncRNA UCA1, lncRNA ADARB2-AS1, lncRNA LINC324, and lncRNA MAP3K12-AS1 in the hippocampus have been shown to differ among TLE patients with and without hippocampal sclerosis.[13] Additionally, research has indicated that lncRNA Zasf1 is markedly over-expressed in the hippocampus of TLE rats;[14] however, whether gain and loss of Zasf1 leads to TLE onset remains on unknown. Interestingly, silencing of Zasf1 has been found to block Notch signaling,[15] which participates in inhibiting neuronal differentiation and in promoting proliferation of glial cells and astrocytes.[16] Moreover, activation of astrocytes, which is closely related to neuronal damage and abnormal function of synapses,[17] has been described as a major feature of tissue reconstruction in the brain of TLE mammals,[18,19] Taken together, Zasf1 could potentially be involved in TLE etiology by affecting downstream pathways important in neuron development and function, although this has rarely been studied.

Therefore, to investigate the role of Zasf1 in TLE etiology, clinical and in vitro experiments were conducted to clarify whether Zasf1 is associated with inflammatory aberration and neuronal activity and, thus, a potential target in epilepsy treatment.

MATERIALS AND METHODS

Recruitment of TLE patients

TLE patients were admitted to the neurology department of Jiangsu-Shengze Hospital Affiliated to Nanjing Medical University from December 2017 to August 2019, and 82 healthy volunteers were recruited as controls. All epilepsy patients had experienced ≥1 seizure per month within 3 months before enrollment, and they were treated by drugs consisting of carbamazepine, oxcarbazepine, lamotrigine, sodium valproate, topiramate, levetiracetam, and clonazepam. Furthermore, the epilepsy patients all met diagnostic criteria proposed by International League Against Epilepsy in 2017.[20]

The subjects all underwent examinations of cerebral-vascular angiography, computed tomography, electroencephalograph, and magnetic resonance imaging (MRI), which included spin-echo T1-weighted imaging, fast spin-echo T2-weighted imaging, transverse T1 and T2-weighted imaging, and coronal T2-weighted fast fluid-attenuated inversion recovery imaging on a superconducting MRI instrument (model: 1.5T Signa Excite, GE, Milwaukee, WI, USA). Patients were excluded if 1) they revealed severe dysfunctions in liver, kidney, hematopoietic system, and endocrine system; 2) their immunological function was deficient; 3) they received immuno-suppressive treatments, such as glucocorticoid; 4) they were cognitively impaired before epileptic seizure; and 5) their clinical information was incomprehensive. All participants provided signed informed consent, and this study was approved by Jiangsu-Shengze Hospital Affiliated to Nanjing Medical University and the ethics committee of Jiangsu-Shengze Hospital Affiliated to Nanjing Medical University.

Quantitation of inflammatory cytokines and apoptins

Around 5 mL of fasting blood was collected from each TLE patient within 12 h after an epileptic seizure and from each control. After centrifugation of peripheral blood at a speed of 3000 r/min for 15 min, supernatants were gathered and stored at -80°C. Then, the levels of interleukin-2 (IL-2) (Sangon, Shanghai, China), tumor necrosis factor-α (TNF-α) (Sangon), Interferon-γ (IFN-γ) (eBioscience, San Diego, CA, USA), high mobility group box protein 1 (HMGB-1) (Sangon), S100B (Boster, Wuhan, China), neuron specific enolase (NSE) (R&D systems, Minneapolis, MN, USA), glial fibrillary acidic protein (GFAP) (Boster, Wuhan, China), calcitonin gene related peptide (CGRP) (R&D systems), Bcl-2 (MyBioSource, San Diego, CA, USA), Bax (MyBioSource), and Caspase-3 (MyBioSource) were determined using respective ELISA kits.

Cell culture

Newborn SD rats, provided by the Animal Experimental Center of Nanjing Medical University, were decapitated, and the retrieved tissue was immersed in pre-cooled 75% ethanol solution. Hippocampal tissues were separated from brain tissues and preserved in a sterile petri dish after being cut into pieces. The tissues were completely digested into neurons after addition of trypsin solution (Life technology, Gaithersburg, MD, USA), after which neuron number was counted in DMEM/F12 medium (Gibco, Carlsbad, CA, USA). The neurons were cultured in the petri dish for 7 days.

Cell transfection

The isolated neurons, adjusted to the concentration of 3×10^5/mL, were incubated in 10% FBS-containing DMEM (Gibco). After cultivation in 5% CO_2 at 37°C for 1 h, the neurons were transfected with si-Zfas1-1 (5'-CUGGCUGAACCAGUUCCACAAGGUU-3'; GenePharma, Shanghai, China), si-Zfas1-2 (5'-CCCTGTGCTTTCATGAAAGTGAAGA-3'; GenePharma), si-NC (5'-CCAAAACCAGGCUUUGAUUGA-3'; GenePharma), pcDNA3.1-Zfas1 (GenePharma) or pcDNA3.1 (GenePharma) for 48 h, with the assistance of Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA).

RT-PCR

Total RNA was extracted from blood samples and neurons using TRIzol reagent (Invitrogen), and RNA sediments were dissolved after addition of diethyl pyrocarbonate (Invitrogen). The concentration and purity of RNAs were measured with an ultraviolet spectrophotometer (Thermo Scientific, Wilmington, DE, USA) at the wavelength of 260 nm and 280 nm. After being synthesized from RNA with the assistance of reverse transcrip-

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tion kits (TAKARA, Shiga, Japan), cDNAs were amplified by referring to instructions of SYBR Green kit (TAKARA), following procedures of 1) 95°C for 3 min and 2) 40 cycles of 95°C for 12 s and 62°C for 35 s. Primers for Zfas1 (forward, 5'-AAGC CACGTGCAGACATCTA-3', reverse, 5'-CTACTTCCAACACCC GCATT-3') and GAPDH (forward, 5'-GATTCCACCCATG GCAAATTC-3', reverse, 5'-CTGGAAGATGGTGATGGGATT-3') were provided by GenePharma.

Western blotting

The neurons were lysed, pulverized, and centrifuged at 15000 r/min for 15 min. Protein samples in the supernatant of neurons were separated by sodium lauryl sulfate-polyacrylamide gels (Beyotime, Shanghai, China) and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blockage of the PVDF membranes for 1 h, protein samples were incubated with rabbit-anti-mouse primary antibodies against Bcl-2 (1:2000, Catalog No.: ab182858, Abcam, Cambridge, MA, USA), Bax (1:1000, Catalog No.: ab32503; Abcam), Caspase-3 (1:500, Catalog No.: ab13847; Abcam), Caspase-9 (1:2000, Catalog No.: ab202068; Abcam), p53 (1:1000, Catalog No.: ab131442; Abcam), Fas (1:1000, Catalog No.: ab82419; Abcam), NF-κB p65 (1:2000, Catalog No.: ab32536; Abcam), IκBα (1:4000, Catalog No.: ab32518; Abcam), pIκBα (1:1000, Catalog No.: 2859, Cell Signaling Technology, Danvers, MA, USA), IKKβ (1:5000, Catalog No.: ab32135; Abcam) and GAPDH (1:2000, Catalog No.: ab8245; Abcam) at 4°C overnight and then with goat anti-rabbit IgG II antibodies (1: 5000, Catalog No.: ab6721; Abcam).

ELISA assay

TNF-α, IL-1, IL-6, and intercellular adhesion molecule-1 (ICAM-1) levels in the culture medium of neurons were detected utilizing ELISA kits (Boster).

MTT assay

Neurons adjusted to the concentration of 3×10^5 cells/mL were incubated at 37°C for 48 h, and then they were successively blended by 5 mg/mL of MTT and 150 μL of dimethyl sulfoxide solution (all purchased form Sigma, St Louis, MO, USA). Until complete dissolution of crystals after 10-min gentle shaking, absorbance (A) value of neurons was monitored at the wavelength of 490 nm utilizing a microplate reader (model: 550, Bio-Rad, Hercules, CA, USA).

Flow cytometry assay

Neurons digested by trypsin to 2×10^6/mL were centrifuged at 1000 rpm for 10 min. After being suspended in 200 μL of binding buffer, neurons were evenly mixed with 5 μL of PI and Annexin-V (all purchased from Becton Dickinson, Franklin Lakes, NJ, USA). After being left in the dark for 15 min, apoptosis of neurons was examined with flow cytometry (Becton Dickinson).

Statistical analyses

All data analyzed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Measurement data (mean±standard deviation) were analyzed with the LSD-t test or single factor analysis of variance. Statistical significance was set at $p<0.05$.

RESULTS

Comparison of baseline characteristics between TLE patients and healthy controls

In total, we recruited 96 TLE patients, including four generalized tonic-clonic seizure patients, three clonic seizure patients, 87 complex partial seizure patients, and two simple partial seizure patients, and their disease course lasted for 9.31±6.22 years (Table 1). According to examination results of head MRI, obvious lesions were located in 86 TLE patients, including hippo-

Table 1. Comparison of Baseline Characteristics between Epileptic Patients and Healthy Controls

Features	Epileptic patients n=96	Healthy control n=82	χ^2/z	*p* value
Age (yr)				
Average	53.96±15.62	55.84±11.42	0.168	0.875
Range	8–88	12–76		
Sex				
Female	36	31		
Male	60	51	0.04	0.967
Disease course (yr)	9.31±6.22			
Types of epilepsy				
Generalized tonic clonic seizure	4			
Clonic seizure	3			
Complex partial seizures	87			
Simple partial seizure	2			
Inflammatory cytokines				
IL-2 (ng/mL)	13.54±1.47	5.42±0.68	45.98	<0.001
TNF-α (ng/mL)	5.97±0.64	1.48±0.27	59.17	<0.001
IFN-γ (pg/mL)	36.28±5.31	10.74±1.66	41.83	<0.001
HMGB-1 (ng/mL)	8.93±1.02	3.28±0.67	42.87	<0.001
Neurotrophic factors				
S100B (ng/mL)	4.87±0.62	1.36±0.27	47.54	<0.001
NSE (ng/mL)	28.37±3.46	12.76±1.67	37.3	<0.001
GFAP (pg/mL)	3.63±0.67	1.49±0.33	26.32	<0.001
CGRP (pg/mL)	80.43±11.98	177.85±22.06	37.32	<0.001
Apoptotic biomarkers				
Bcl-2 (ng/mL)	3.41±0.98	8.34±1.62	24.95	<0.001
Bax (ng/mL)	3.15±0.42	1.07±0.18	41.68	<0.001
Caspase-3 (ng/mL)	8.06±1.22	3.12±0.51	34.19	<0.001

IL, interleukin; TNF, tumor necrosis factor; IFN, Interferon; HMGB, high mobility group box protein; NSE, neuron specific enolase; GFAP, glial fibrillary acidic protein; CGRP, calcitonin gene related peptide.

campal sclerosis (n=61), temporal lobe softening (n=14), atrophy of the temporal lobe (n=5), temporal lobe tumor (n=3), and arachnoid cysts of the temporal lobe (n=3). In line with electroencephalographs, 63 TLE patients exhibited epileptiform discharge, and epileptic waves were detectable in 31 TLE patients. Also, 68 TLE patients showed unilateral abnormal discharge, and 26 patients revealed bilateral aberrant discharge.

Additionally, TLE patients were detected with higher levels of cytokines (IL-2, TNF-α, IFN-γ, and HMGB-1), neurotrophic factors (S100B, NSE, and GFAP), and apoptotic molecules (Bax and Caspase-3) than healthy controls (p<0.05), and no significant difference was discerned between the two populations in regards to mean age and sex ratio (p>0.05) (Table 1).

Correlation between lncRNA Zfas1 expression and apoptotic/inflammatory biomarker levels among TLE patients

Serum levels of lncRNA Zfas1 were higher in TLE patients than in healthy controls (p<0.05) (Fig. 1A). Among TLE patients, serum levels of lncRNA Zfas1 were positively correlated with serum levels of Bax (r_s=0.372) and Caspase-3 (r_s=0.384), and were negatively correlated with serum levels of Bcl-2 (r_s=-0.339) (Fig. 1B). Meanwhile, increased serum levels of lncRNA Zfas1 were associated with higher serum levels of IL-2 (r_s=0.397), TNF-α (r_s=0.353), IFN-γ (r_s=0.409), and HMGB-1 (r_s=0.392) in TLE patients (Fig. 1C). Additionally, serum levels of S100B (r_s=0.543), NSE (r_s=0.469), and GFAP (r_s=0.497) were up-regulated, while serum level of CGRP were down-regulated (r_s=-0.378), with increases in lncRNA Zfas1 levels in TLE patients (Fig. 1D).

Influence of lncRNA Zfas1 on viability and apoptosis of hippocampal neurons

LncRNA Zfas1 expression in neurons was heightened after transfection of pcDNA3.1-Zfas1 in comparison to pcDNA3.1 (p<0.05) (Fig. 2A), but reduced when si-Zfas1-1 or si-Zfas1-2 was transfected (p<0.05) (Fig. 2B). Furthermore, the viability of neurons in the pcDNA3.1-Zfas1 group decreased to 60.33% of pcDNA3.1 group (p<0.05), while neuron viability in the si-Zfas1 group was significantly improved when compared with NC group and si-NC group (p<0.05) (Fig. 2C). The multiplicative potential of neurons was also impeded when pcDNA3.1-Zfas1 was transfected (p<0.05), but markedly enhanced in the

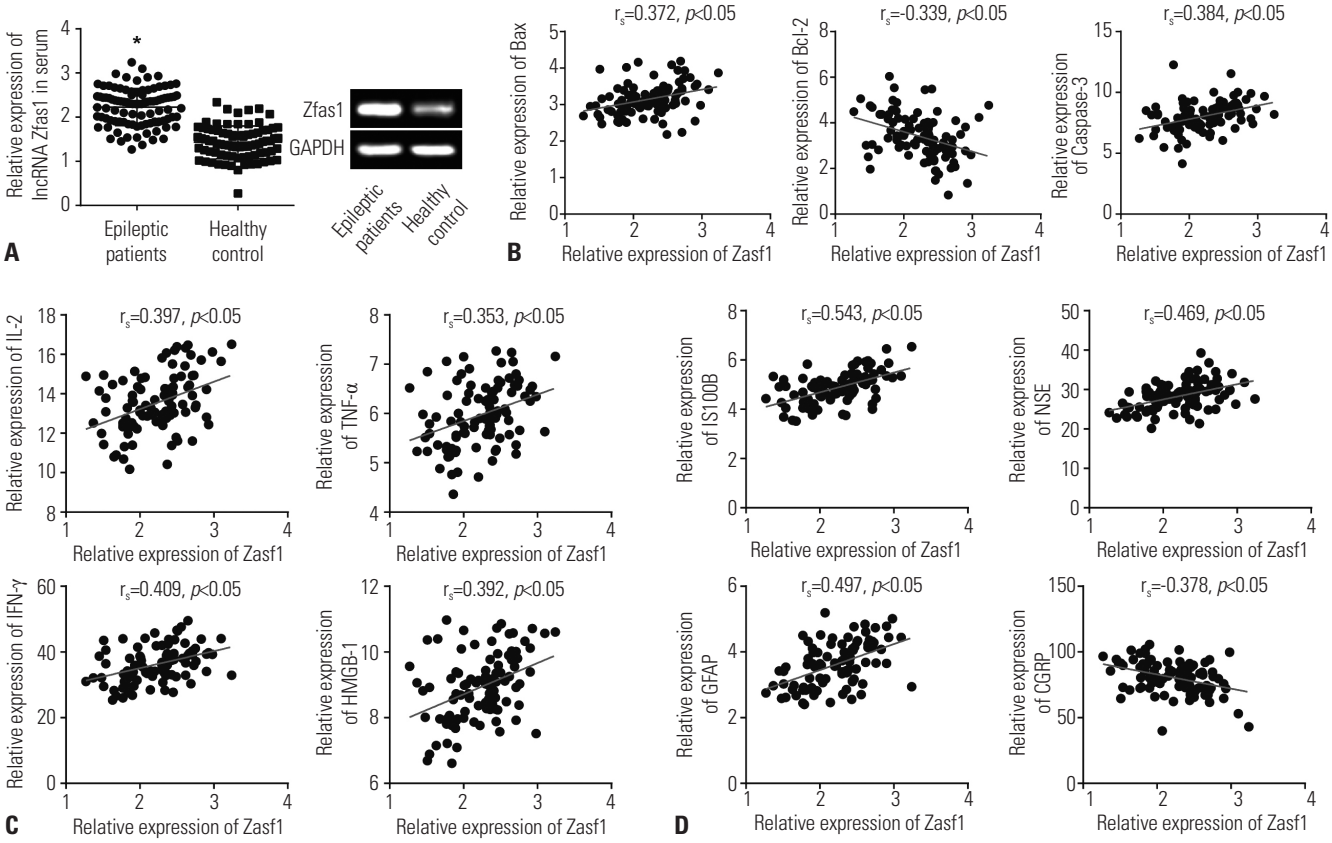

Fig. 1. Evaluation of lncRNA Zfas1 expression in tissues and cell lines. (A) Expression of lncRNA Zfas1 was detected in serum of epilepsy patients and healthy controls. *p<0.05 when compared with NC group. (B) Serum levels of lncRNA Zfas1 were correlative with serum levels of Bax, Bcl-2, and Caspase-3. (C) Serum levels of lncRNA Zfas1 showed an association with IL-2, TNF-α, IFN-γ and HMGB-1 levels. (D) Serum levels of lncRNA Zfas1 were altered with changes in S100B, NSE, GFAP, and CGRP levels. IL, interleukin; TNF, tumor necrosis factor; IFN, Interferon; HMGB, high mobility group box protein; NSE, neuron specific enolase; GFAP, glial fibrillary acidic protein; CGRP, calcitonin gene related peptide; NC, negative control.

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presence of si-Zfas1 (p<0.05) (Fig. 2D). On the contrary, hippocampal neurons in the pcDNA3.1-Zfas1 group were more vulnerable to apoptosis than those in the pcDNA3.1 group (p<0.05), whereas neurons in the si-Zfas1 group were less liable to apoptosis in comparison to the si-NC group (p<0.05) (Fig. 2E). Moreover, Bcl-2 expression was decreased and Bax, Caspase-3, Caspase-9, p53 and Fas expressions were increased in neurons transfected with pcDNA3.1-Zfas1, compared with pcDNA3.1 (p<0.05) (Fig. 3A). In contrast, transfection of si-Zfas1-1 into neurons down-regulated the expressions of Bax, Caspase-3, Caspase-9, p53, and Fas and up-regulated the expression of Bcl-2, compared with si-NC (p<0.05) (Fig. 3B).

Impact of lncRNA Zfas1 on inflammation responses of hippocampal neurons

Compared with the NC group, lipopolysaccharide (LPS) treatment significantly increased the expressions of ICAM-1, IL-1, IL-6, and TNF-α in hippocampal neurons (p<0.05) (Fig. 4). PcDNA3.1-Zfas1 transfection in combination with LPS treatment strongly promoted neuronal release of ICAM-1, IL-1, IL-6, and TNF-α, compared with LPS treatment alone (p<0.05). Conversely, expression of ICAM-1, IL-1, IL-6, and TNF-α decreased in neurons from the si-Zfas1-1+LPS group relative to the LPS group (p<0.05). In addition, NF-κB p65, pIκBα, and IKKβ levels in hippocampal neurons were boosted, while IκBα levels were depressed after LPS treatment (p<0.05) (Fig. 5). Meanwhile, NF-κB p65, pIκBα, and IKKβ expressions were maintained, while IκBα expression decreased in neurons from the pcDNA3.1-Zfas1+LPS group, compared with the LPS group (p<0.05). Silencing of Zfas1 (i.e., si-Zfas1-1+LPS group) could reverse the contributions of LPS to neuronal secretion of NF-κB p65, pIκBα, IκBα, and IKKβ (p<0.05).

DISCUSSION

TLE, pathologically embodied as hippocampal mossy fiber sprouting and synaptic remodeling, has proven difficult to cure due to its tolerance against various anti-epileptic drugs. Meanwhile, although TLE development has been found to result from neuronal loss, gliocyte proliferation, nerve regeneration, axon growth, and abnormal inflammation,[21,22] molecular explanations for TLE progression have remained far from comprehensive.

LncRNAs are intertwined with epilepsy pathogenesis by manipulating neurogenesis, neurotransmitter production, and ion channel and synaptic plasticity.[23] For example, lncRNA BDNF-OS reduced expression of brain-derived neurotrophic factor and promoted neuronal regeneration and remodeling.[24] Here, we discovered that increased serum levels of lncRNA Zfas1 are reflective of disordered neuronal apoptosis and inflammation in TLE patients (Fig. 1), suggesting that Zfas1 is a pronounced

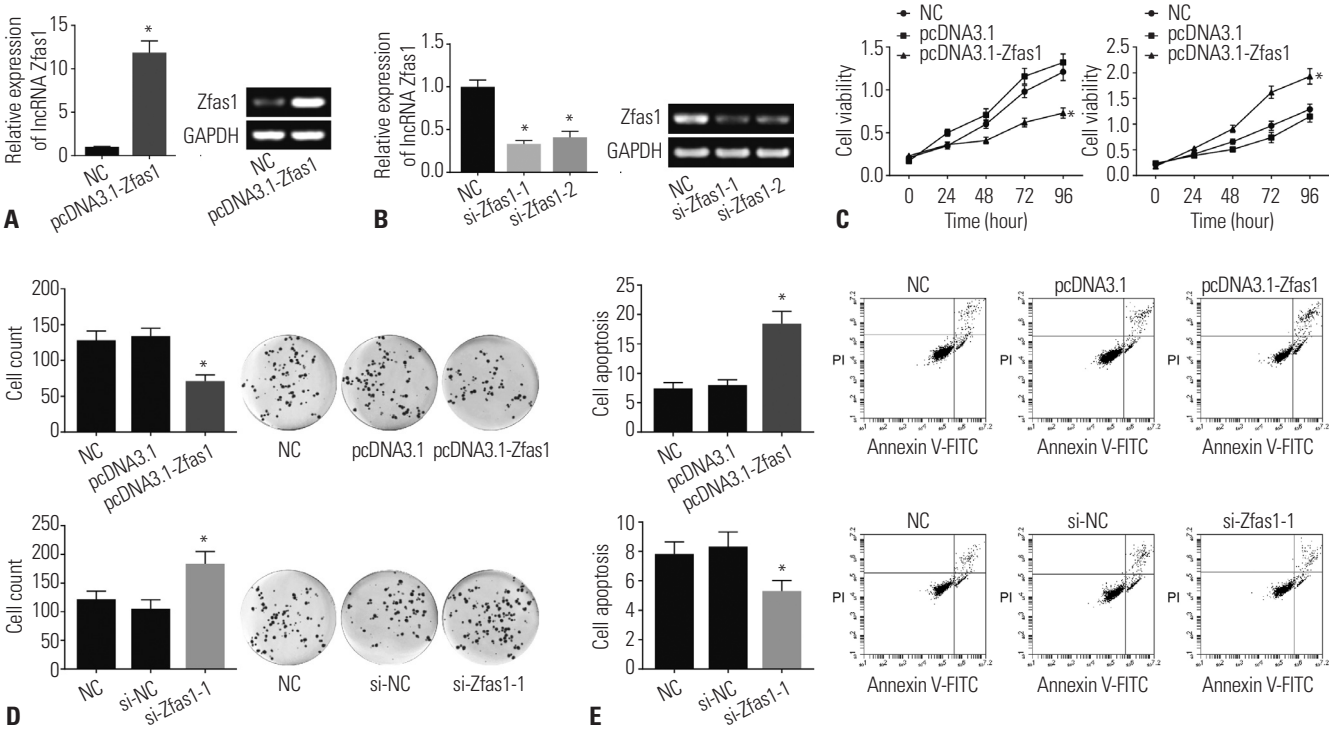

Fig. 2. LncRNA Zfas1 affects the activity of hippocampal neurons. (A) Expression of lncRNA Zfas1 in hippocampal neurons was monitored after transfection of pcDNA-Zfas1. *p<0.05 when compared with NC group. (B) LncRNA Zfas1 expression was evaluated in hippocampal neurons after transfection of si-Zfas1-1 and si-Zfas1-2. *p<0.05 when compared with NC group. (C-E) Viability (C), proliferation (D), and apoptosis (E) of hippocampal neurons were evaluated among NC, pcDNA3.1, pcDNA3.1-Zfas1, si-NC and si-Zfas1-1 groups. *p<0.05 when compared with NC group. NC, negative control.

biomarker for TLE onset and severity.

Research has indicated that immoderate proliferation of astrocytes could dramatically affect synaptic transmission, which then stimulates the occurrence of TLE.[25,26] Another study indicated that TLE onset is accompanied by JNK phosphorylation, which promotes apoptosis of hippocampal neurons.[27] Altogether, we suspect that balanced proliferation and apoptosis of neurons may be indispensable to averting TLE onset, and our study demonstrated that lncRNA Zfas1 is a potent regulator of neuronal proliferation and apoptosis (Figs. 2 and 3). Interestingly, apart from neurons, reduced Zfas1 expression has been found to enable tumor cells (e.g. U87 and U25I cell lines) to stagnate in the G0/G1 phase of the cell cycle,[28] insinuating that lncRNA Zfas1 exerts identical effects in various cell types. In addition, lncRNA Zfas1 has been found to be responsible for elevating expression of b-cell lymphoma protein-2 (bcl-2) (Fig. 3), which plays key roles in antagonizing apoptosis of hippocampal neurons.[29] Moreover, Bax was reported to promote the influx of cytochrome C,[30] which activates Caspase-3 via cascade amplification and finally leads to cell apoptosis

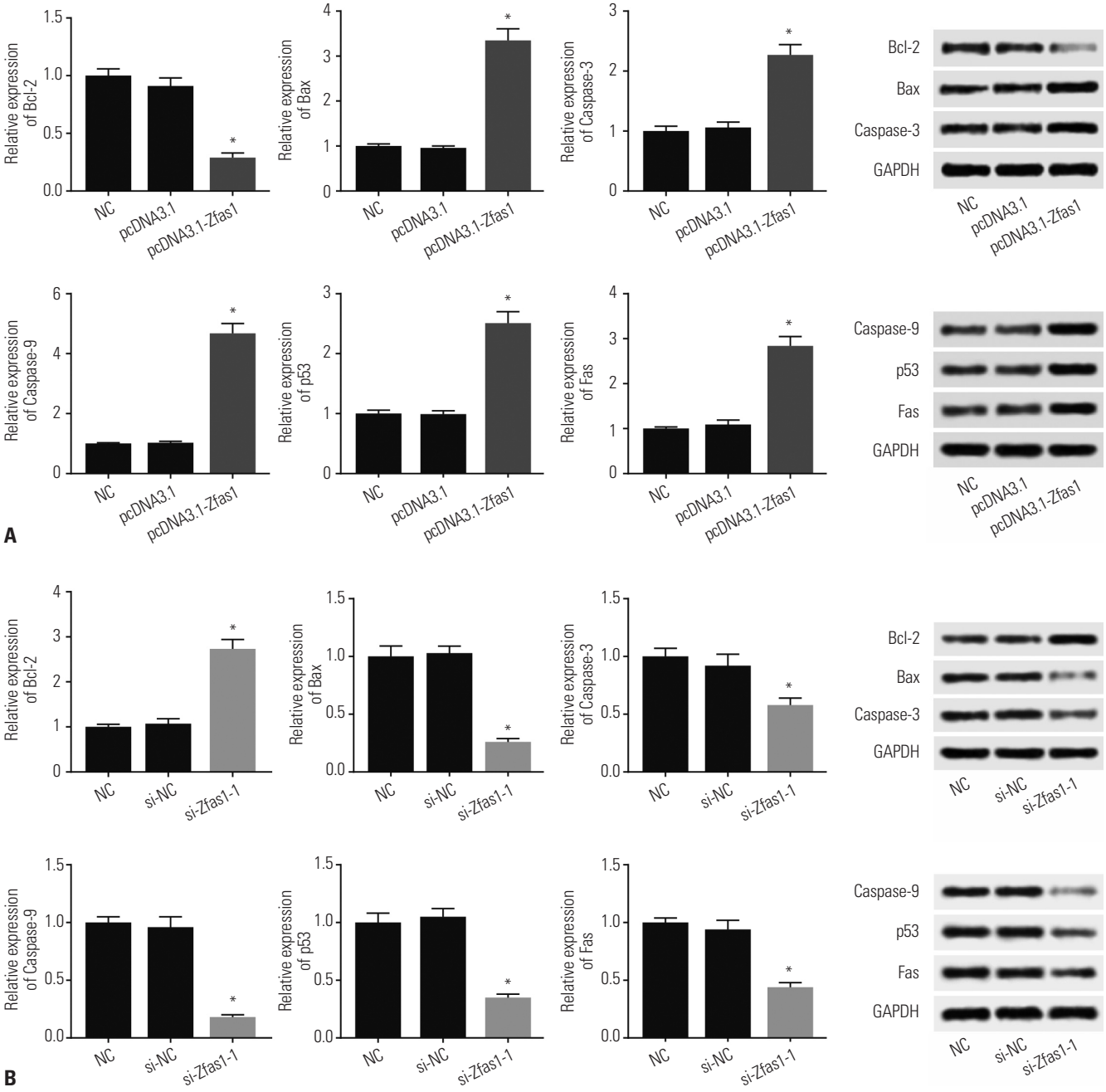

Fig. 3. The protein levels of apoptosis-related proteins were detected. (A) Bcl-2, Bax, Caspase-3, Caspase-9, p53, and Fas, were determined in hippocampal neurons of NC, pcDNA3.1, and pcDNA3.1-Zfas1 group. (B) Bcl-2, Bax, Caspase-3, Caspase-9, p53, and Fas, were determined in hippocampal neurons of NC, si-NC, and si-Zfas1-1 groups. *p<0.05 when compared with NC group. NC, negative control.

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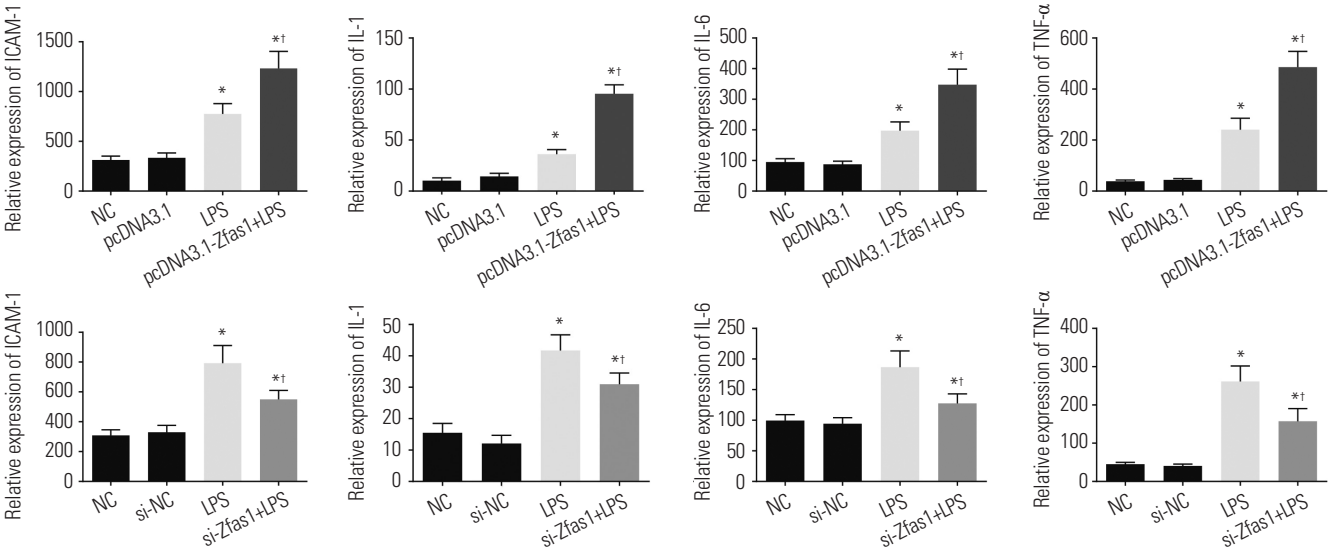

Fig. 4. Inflammatory cytokine levels, including ICAM-1, IL-1, IL-6, and TNF-α, were evaluated in hippocampal neurons of NC, pcDNA3.1, si-NC, LPS, pcDNA3.1-Zfas1+LPS, and si-Zfas1-1+LPS groups. *p<0.05 when compared with NC group, †p<0.05 when compared with LPS group. ICAM, intercellular adhesion molecule; IL, interleukin; TNF, tumor necrosis factor; NC: negative control; LPS: lipopolysaccharide.

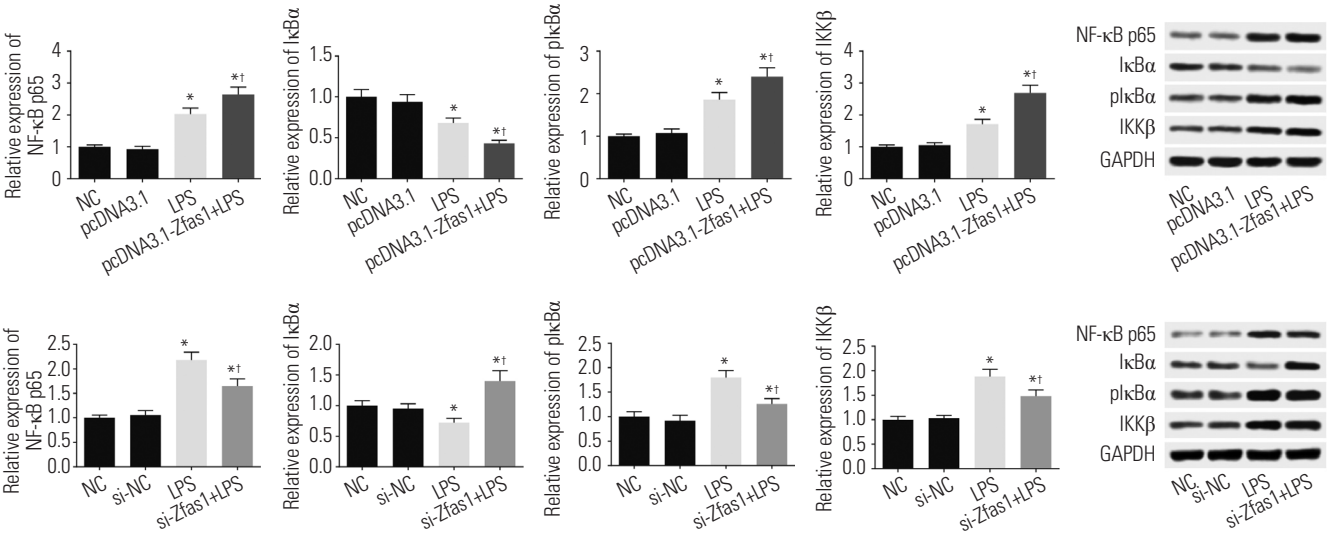

Fig. 5. The protein levels of key components of the NF-κB pathway, including NF-κB p65, IκBα pIκBα, and IKKβ, were evaluated in hippocampal neurons of NC, pcDNA3.1, si-NC, LPS, pcDNA3.1-Zfas1+LPS, and si-Zfas1-1+LPS groups. *p<0.05 when compared with NC group, †p<0.05 when compared with LPS group. NC: negative control; LPS: lipopolysaccharide.

[PMID: 25674005]. Collectively, we discovered that lncRNA Zfas1 restrained neuronal apoptosis by deactivating the Bax/Caspase-3 axis (Fig. 3). Other pro-/anti-apoptosis signaling pathways, such as non-sense-mediated decay, NF-κB pathway and Wnt/β-catenin pathway, have also been found to be modified by lncRNA Zfas1 in cells other than neurons.[31-33] Supporting these results, we showed for the first time that lncRNA Zfas1 regulates proteins of the NF-κB pathway in neurons (Fig. 5) and demonstrated that lncRNA Zfas1 contributes to neuronal damage by strengthening NF-κB signaling.

Inflammation can cause seizures of convulsion and promote chronic self-seizures. Convulsion, in turn, also enhances release of pro-inflammatory cytokines in the brain.[34] In particular, IL-2 has been found to manipulate calcium concentrations and stimulate neuronal excitability,[35] and TNF-α, originating from mono-nuclear macrophages, has been shown to directly give rise to neuronal damage.[36] Moreover, research has shown that IFN-γ and HMGB-1 are adept at modulating the sprouting and migration of neurons, thereby facilitating neuronal discharge.[37] Interestingly, IL-1β, IL-6, and TNF-α have been found to be involved in worsening inflammation in neurons, and anti-inflammatory treatments have proven effective at overcoming intractable epilepsies, such as infantile spasm and acquired epileptic aphasia.[38] Thus, we suggest that since lncRNA Zfas1 elicits over-production of inflammation cytokines in neurons, including ICAM-1, IL-1, IL-6, and TNF-α (Fig. 4), targeting

it might be conducive to hindering epileptic onset.

This study had several limitations. First, the association of lncRNA Zfas1 expression with clinical symptoms of TLE was not explored, and the potential of lncRNA Zfas1 in diagnosing TLE was not estimated. Second, downstream lncRNA Zfas1-related miRNA and genes contributing to the aberrant functions of neurons were not investigated. Finally, animal models were not constructed to simulate in vivo effects of lncRNA Zfas1 on TLE development. All of these shortcomings need to be addressed in future studies.

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AUTHOR CONTRIBUTIONS

Conceptualization: all authors. Data curation: Wentong Zhang. Formal analysis: Qin Zhou, Junjie Yang, and Naixian Shi. Funding acquisition: Chuan He. Investigation: Chuan He, Caixia Su, and Wentong Zhang. Methodology: Wentong Zhang and Qin Zhou. Project administration: Chuan He and Caixia Su. Resources: Xu Shen. Software: Junjie Yang. Supervision: Chuan He. Validation: Caixia Su, Wentong Zhang, and Qin Zhou. Visualization: Xu Shen and Junjie Yang. Writing—original draft: Naixian Shi. Writing—review & editing: Chuan He and Wentong Zhang. Approval of final manuscript: all authors.

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