Brief report

Gsk-3β aggravates depression symptoms in a chronic stress mouse model

Hong Peng[1], Hong-bin Wang[2,*], Ling Wang[1], Bing Zhou[1], Xiao-yong Li[1], Jian Tan[1]

[1] *Department of Anesthesiology, People's Hospital of Pingxiang City, Jiangxi province, Pingxiang, 337000, P.R. China*
[2] *Department of Rehabilitation Medicine, Shangluo Central Hospital, Shangluo, Shanxi, 726000, P.R. China*

***Correspondence**: hbinwang@slzxyy.cn (Hong-bin Wang)

https://doi.org/10.31083/JIN-170050

Abstract

Depression caused by genetic and environmental factors is acomplicated disease. Here, it is demonstrated that glycogen synthase kinase-3β is highly expressed and phosphorylated in the brain of a chronic stress mouse. Inhibition of glycogen synthase kinase-3β leads to decreased depression-like symptoms which manifest in open-field test, tail-suspension test, forced-swim test, and a novelty suppressed feeding test. It was also found that β-catenin is attenuated, and its target genes Cyclin D1 and c-Myc are down-regulated. Glycogen synthase kinase-3β was also found to inhibit Erk-Creb-BDNF signaling. These results show that glycogen synthase kinase-3β may promote the progression of depression. Therefore, targeting glycogen synthase kinase-3β may be an effective therapeutic strategy.

Keywords

Gsk-3β; depression; chronic stress mouse model; behavioral testing; Wnt pathway; BDNF pathway

Submitted: May 27, 2017; **Accepted:** September 5, 2017

1. Introduction

As one of the complex neuropsychiatric diseases, depression ranks first among all neurological and mental diseases contributing to the worldwide burden of disease and leads to an unnecessary burden for patients and their families. However, current treatments are far from ideal [1]. Animal models [2] and circulating measurements of depression patients [3, 4] are believed by most researchers to understand the development of major depressive disorder. The complex and unique features of depressive disorder increase the necessity for exploration of the particular pathogenesis of depression.

Glycogen synthase kinase (GSK)-3 is a serine threonine kinase involving in many biomedical processes. It was originally identified as one of crucial regulators balancing glucose metabolism [5]. Recent studies report that glycogen synthase kinase-3β (GSK-3β) might play an important role in the pathogenesis of affective and neurodegenerative disorders [6, 7]. The GSK-3β isoform is associated with cell mortality and is shown to activate pro-apoptotic signals in multifarious neurons [8]. Inhibition of GSK-3β using specific inhibitors has neuroprotective effects in many preapoptotic insults [9]. Reports have suggested that GSK-3β might have a broader function in regulation of the behavioral domain. However, there have been contradictory results concerning GSK-3β expression and activity in some studies of depressive models [10–13]. Therefore, a depressive mice model was established to study the role of GSK-3β and related signalling pathways. The schematic representation of the GSK-3β signalling pathway identified through this study is shown in Fig. 1.

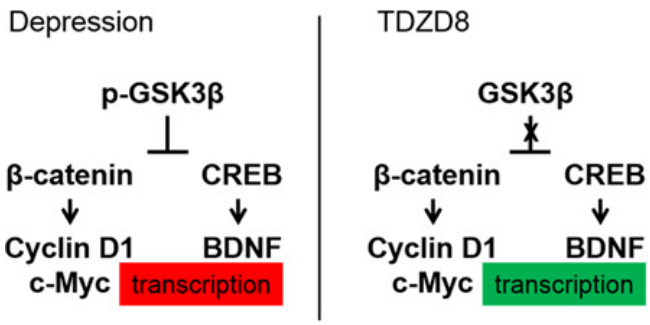

Fig. 1. Schematic representation of the GSK-3β signalling pathway.

2. Materials and methods

2.1. Animals

Adult male C57/B6 mice (two months old) were housed in specific-pathogen free conditions with 12 h light/dark cycles and fed by free access to a standard diet and water unless otherwise noted. Each group contained 10 mice. All animals used were grouped randomly and blind behavioral tests were employed. The animal experiments were reviewed and approved by the Animal Ethics Committee of People's Hospital of Pingxiang City.

2.2. Induction of chronic stress mouse model

The chronic stress mouse model was produced via severe metamorphic stress [14, 15]. The detailed procedure is illustrated in Fig. 2, stimulations were as follows: 1. fasted for 12 h; 2. no water for 12 h; 3. swimming in cold water (10°C) for 6 min; 4. tail clamp (clamp at 1 cm away from the caudal root) for 1 min; 5. housed in

damp for 12 h; 6. noise environment (100 db) for 1 h; 7. high-speed horizontal oscillation (200 r/min) for 45 min; 8. all-night lighting for 12 h; 9. pelmatic shock (1 mA, 30 s/min) for 30 min; 10. cage tilted 45 degrees (no sawdust) for 12 h. 1–2 of the above stresses were executed randomly every day and continued for 30 days.

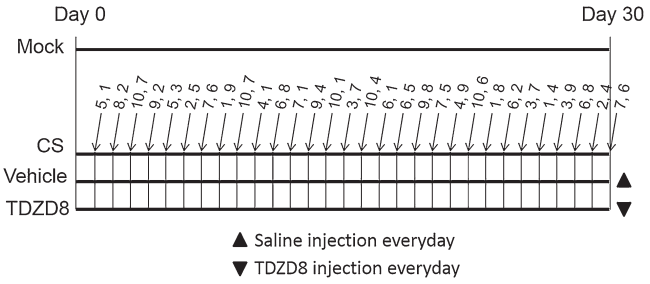

Fig. 2. Flow diagram of inducing chronic stress model.

2.3. *Drug treatment*

Highly stressed male C57/B6 mice were daily intraperitoneally injected with either normal saline (vehicle) or TDZD8 (10 mg/kg, Sigma), a specific inhibitor of GSK-3β. TDZD8 was dissolved in Tween and diluted using saline.

2.4. *Open field test*

The experimental equipment was placed in a soundproof room. Indoor temperature and ventilation were strictly controlled. A black box equipped with infrared positioning and fluorescent lighting was employed as the experimental enclosure. Mice were placed in a corner of the square field (each mouse was positioned in the same place) and they were free to explore for 15 min. Software was employed to analyze the test data and obtain peripheral activity distance/time and the central activity distance/time [16].

2.5. *Forced-swim test*

Experiments were performed in a transparent plexiglass cylinder (diameter 20 cm, height 30 cm). Before the experiment, a tank was filled with water (23∼25°C). First day, mice were placed in the tank and adapted to swimming for five minutes. Second day, mice were placed in the cylinders and swam for 6 min (0–2 min adaptation). The duration mice were immobile in the water during 2–6 min was recorded. Mice were considered to be immobile when they floated on the surface of the water, making no movement, with the exception of tiny movements to keep their head out of the water. At the end of each experiment, mice were removed from the water, dried using towels, and returned to normal temperature in warm air. The total immobility time of mice in the water was analyzed by video recording [14].

2.6. *Tail-suspension test*

The mouse was suspended by its tail from a metal ring 50 cm above the ground using medical adhesive plaster. Activity of mice was recorded by a camera. The duration of the experiment was 6 min, with a 0–2 min adaptation time. When mice stopped struggling during 2–6 min, the time was recorded (i.e. immobility time).

2.7. *Novelty suppressed feeding test*

First, sawdust was spread 1.0 cm over the bottom of a plastic box ($76 \times 76 \times 46$ cm). 12 similarly suzed food pellets were then spread evenly in the middle of the box. Mice were fasted for 24 h, and placed in the experimental apparatus to calculate the incubation period. The criterion for eating was when mice began chewing food rather than just sniffing or playing with food. The incubation period was employed as a measure of the behavioral activity of the drug. In the experiment, the test environment was different from the feeding environment. The light intensity of the test environment was greater than that of the feeding environment. Each time, a mouse was tested, it was placed in the same position and with the same orientation.

2.8. *Western blot*

Mice were anesthetized and decapitated, the whole brain was separated and total protein was extracted with 1 mL lysate added to each 100 mg tissue. Tissue was fractured ultrasonically and ice-incubated for 30 min. Cytosolic and nuclear fractions were separated and the supernatant was centrifuged. The BCA method was used to detect protein concentration. Proteins were separated by SDS-PAGE electrophoresis (10% gel) and transferred onto nitrocellulose membranes. Next, the membranes were blocked using 5% skimmed milk and incubated overnight with corresponding antibodies at 4°C. Membranes were then washed three times with PBS containing 1% tween, incubated with secondary antibodies, and washed and developed. All antibodies were purchased from Cell Signaling Technology. Dilution factors were GSK-3β, p-GSK-3β, PKB, p-PKB, β-catenin (1: 1000); cyclin D1, c-myc, BDNF (1: 500); CREB, p-CREB, ERK, p-ERK, GAPDH (1: 2000); secondary antibodies (1: 5000).

2.9. *Real-time PCR*

Whole brain of the mouse was separated and RNA extracted using Trizol reagent (Takara) according to the manufacturer's instructions. Endogenous DNAs were removed from total RNAs, and RNAs were reverse transcribed into cDNA. Real-time PCR was carried out with a SYBR Green PCR kit (Takara). Amplifications were performed in Roche 9700. Relative transcripts were calculated using the $\Delta\Delta$Ct method with β-actin as the reference gene. Samples were analyzed in triplicate. The primer was 5'-ATGGCAGCAAGGTAACCACAG-3'and 5'-TCTCGGTTCTTAAATCGCTTGTC-3'.

2.10. *Statistical analysis*

Quantified data are presented as mean $\pm$ SD, comparisons between groups were analyzed by one way ANOVA, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. *Gsk-3β was activated in chronic stress mouse brain*

To study the role of Gsk-3β in the development of depression, mice were stressed to produce the chronic stress (CS) model. According to behavioral testing, it was found that CS mice were more depressive than untreated mice after 30 days of stress treatment. CS mice showed longer immobility times in forced swim test and tail-suspension tests, longer latency time in a novelty suppressed feeding test, shorter total and central distance moved and time and frequency staying in the central areas in an open-field test (Fig. 3). All behaviors

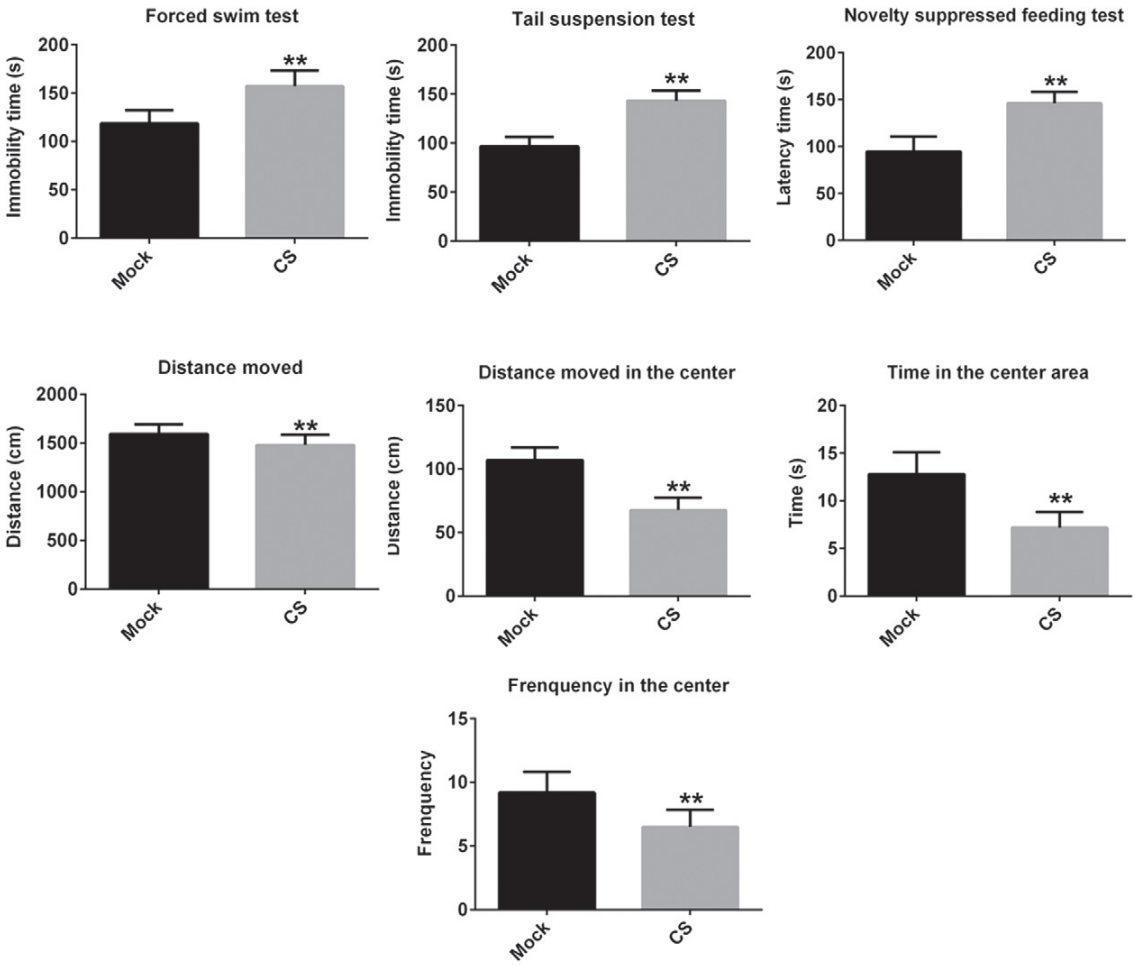

Fig. 3. Gsk-3β is activated in chronic stress-induced depressive brain. Behavioral testing (forced-swim test, tail-suspension test, novelty suppressed feeding test and open field test) of untreated mice (Mock) and chronic stress mice (CS). $N = 15$ per groups. $^{**}p < 0.01$.

of CS model were representative depressive characteristics. These results illustrate that the CS model is more depressive and is successful in producing a chronic stress depression mouse model which provides a useful tool for the study of depression. The expression of Gsk-3β was then tested in CS mouse brains.Gsk-3β was found to be up-regulated in protein level and its activated phosphorylated form also increased in CS brains, accompanied by PKB activation (Fig. 4a). Real-time PCR showed that the RNA level of Gsk-3β was also up-regulated (Fig. 4b). These findings illustrate Gsk-3β is high activated in chronic stress depressed brains and may play a crucial role in depression.

3.2. *Inhibition of Gsk-3β ameliorates depression symptoms in CS model*

To further study the function of Gsk-3β in the development of depression, activation of Gsk-3β was inhibited by TDZD8 during chronic stress treatment. Behavioral testing showed mice injected with TDZD8 were less depressive than those injected with saline. Specifically, the group of mice with Gsk-3β inhibition by TDZD8 showed shorter duration of immobility in a forced-swim test and a tail-suspension test, shorter latency time in a novelty suppressed feeding test, and longer central distance moved and time and frequency spent in central areas during an open-field test (Fig. 5). These results reveal that inhibition of Gsk-3β ameliorates depressive symptoms.

3.3. *β-catenin and BDNF may be involved Gsk-3β-associated depression*

Results reported here show that Gsk-3β plays an important role in the progression of depression, but the mechanisms are obscure. Hence, crucial molecules downstream of Gsk-3β were detected. It was found that β-catenin and its target genes were inhibited in the CS model employed. Protein expression of β-catenin was increased in the nucleus and after TDZD8 treatment its target genes were up-regulated (Fig. 6). Meanwhile, an Erk-Creb-BDNF pathway showed a similar tendency. The pathway was inhibited by Gsk-3β activation and re-activated accompanied by Gsk-3β inhibition (Fig. 7). Obviously, β-catenin and BDNF play an important role in the development of depression due to their roles in the cell cycle and neurotrophy.

4. Discussion

Depressive disorder exhibits a high incidence of mental illness which seriously damages human health. Its features include high morbidity, high recurrence rate, and high suicide rate.

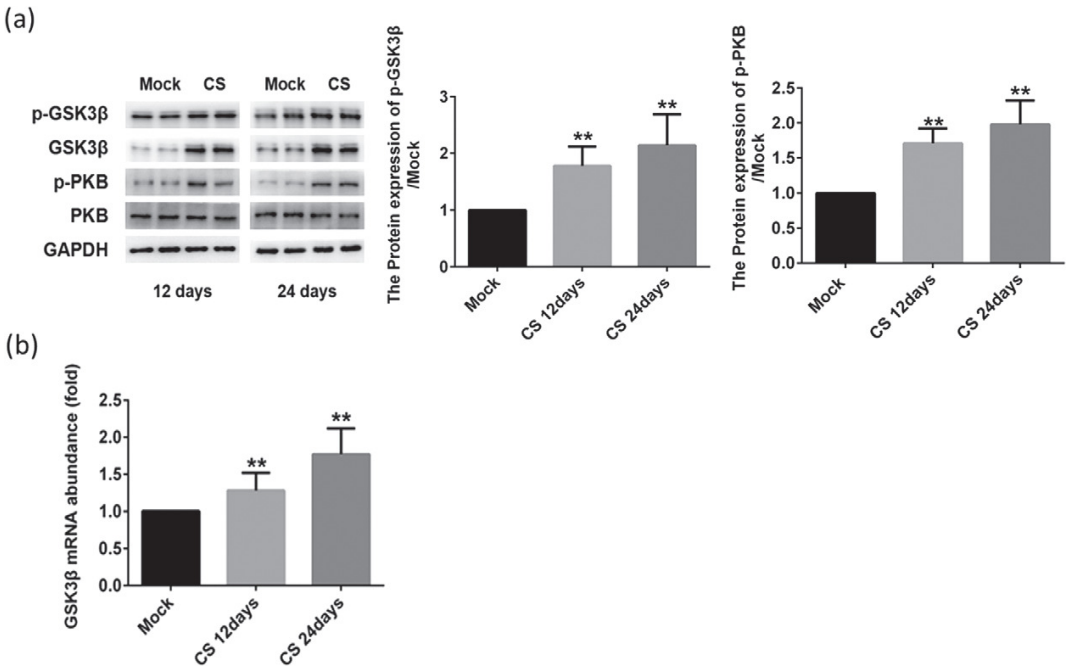

Fig. 4. Upregulation of Gsk-3β in mRNA, protein and phosphorylation levels as well as PKB activation in CS brains. a, Western blot of corresponding brains of two time-points and quantifications of indicated proteins. The data were mean of three scanning intensities. b, Relative RNA level of Gsk3β detected through real-time PCR. $^{**}p < 0.01$.

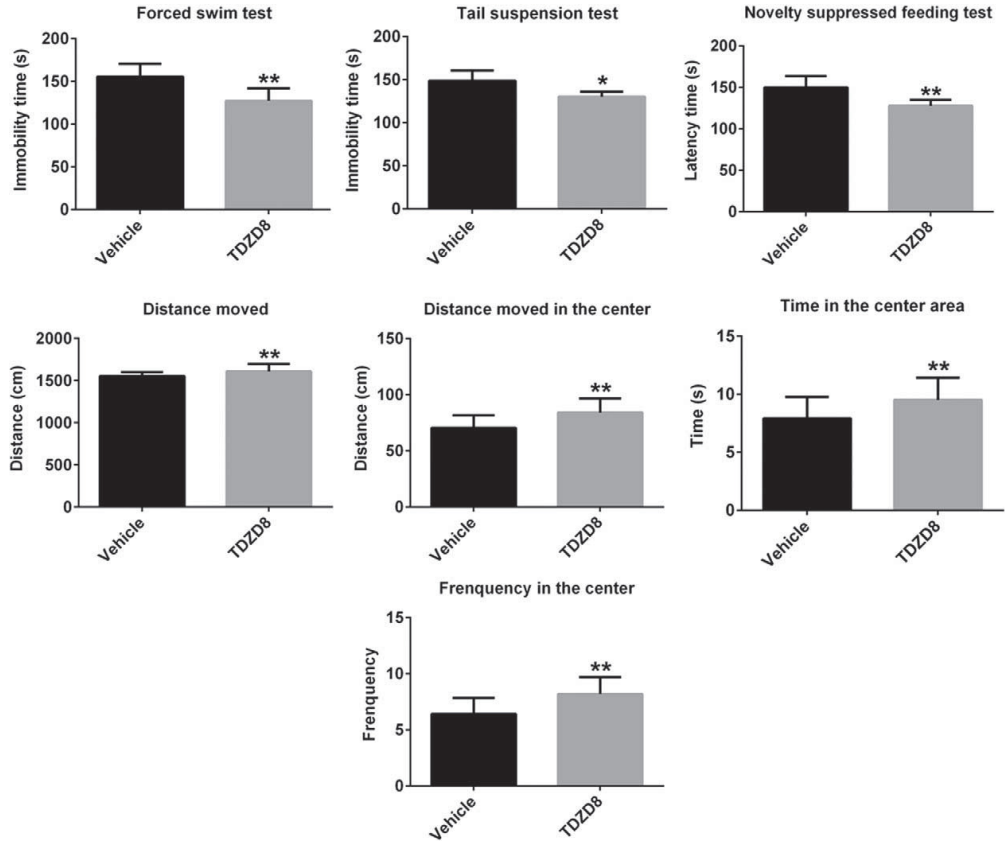

Fig. 5. Inhibition of Gsk-3β alleviates depressive symptom. Behavioral testing (forced-swim test, tail-suspension test, novelty suppressed feeding test and open field test) of chronic stress mice injected with saline or TDZD8. $N = 15$ per groups. $^{*}p < 0.05$; $^{**}p < 0.01$.

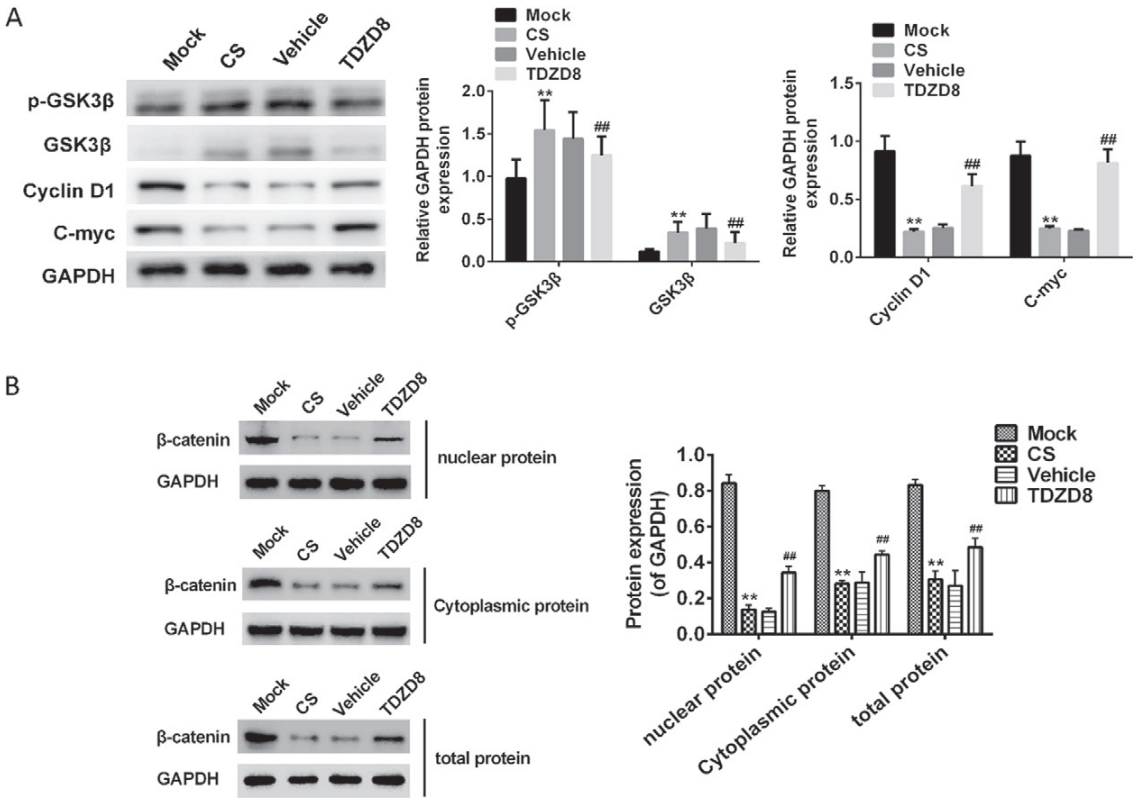

Fig. 6. β-catenin is depressed in chronic stress model. A, Protein expression of Gsk-3β, p-Gsk3β, Cyclin D1 and c-Myc in corresponding brains. B, Western blot of β-catenin in corresponding cytosolic and nuclear fractions. The data were mean of three scanning intensities. $^{**}p < 0.01$.

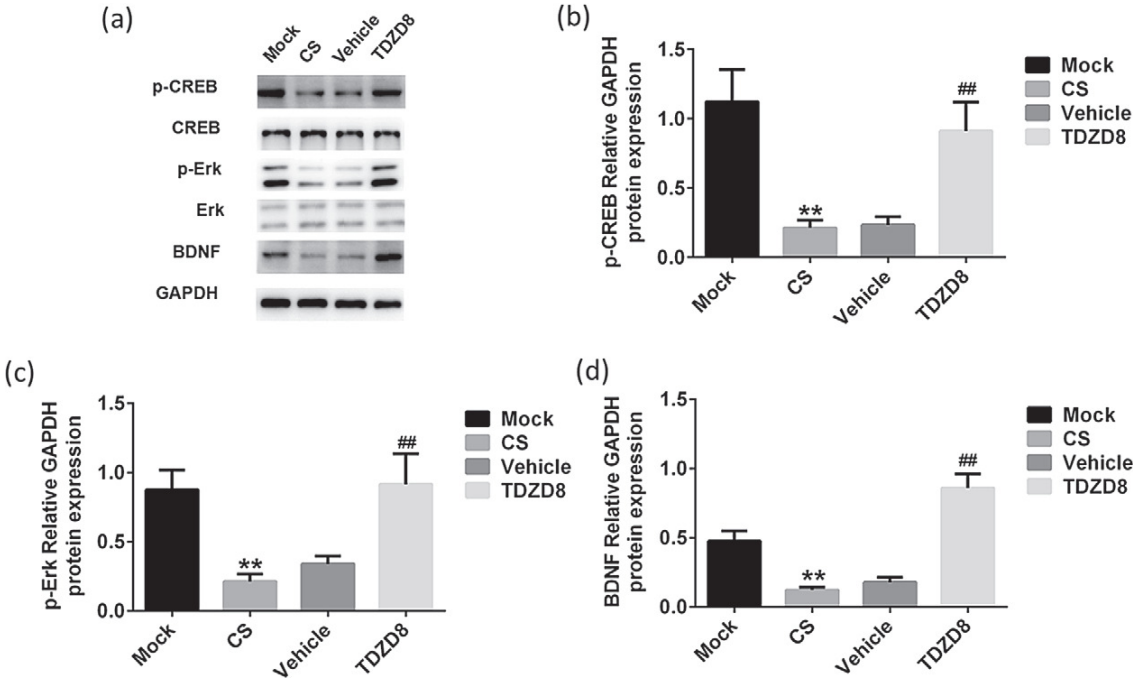

Fig. 7. Erk-Creb-BDNF pathway is inhibited in chronic stress model. a, Western blot of indicated proteins in corresponding brains. b, Quantifications of p-Creb showed in a. c, Quantifications of p-Erk showed in a. d, Quantifications of BDNF showed in a. The data were mean of three scanning intensities. $^{**}p < 0.01$.

So far, the etiology of depression is unclear, although it is certain that many psychological, biological, and social elements are involved in the pathogenesis of depressive disorder [17]. Biological elements are mainly related to heredity, nerve biochemistry, nerve incretion, nerve regeneration, etc. The psychology which is closely related to depression, is susceptible to premorbid personality traits, such as depressive temperament [18]. In adulthood, stressful life events are important triggers for the emergence of clinically significant depressive episodes [19]. However, these factors never work alone. The current emphasise is on environmental and genetic stress factors or interactions. This interactive point likely has an important effect on the development of depressive disorders.

Gsk-3β is involved in neuronal cell development, energy metabolism, and body pattern formation [20]. Several studies have implicated GSK-3β as a potent regulator of cell apoptosis and have demonstrated its contribution to depressive behavior [21, 22]. This has prompted the exploration reported here of how GSK-3β is manipulated in a physiological model of chronic stress, and whether this may affect depressive disorder.It was found that chronic stress influenced neuronal cell development but induced no apoptotic effect on GSK-3c. Results showed a functional role Gsk-3β in the development of chronic stress-induced depression. Furthermore, Gsk-3β may promote the development of depression through inhibition of β-catenin and Erk-Creb pathways due to its direct involvement in the cell cycle or neurotrophy. Besides, Cyclin D1 is a required protein for progression through the G1 phase of the cell cycle. During the G1 phase, Cyclin D1 is synthesized rapidly and accumulates in the nucleus. It is degraded when the cell enters the S phase [23]. Meanwhile, c Myc is important, acting to drive cell proliferation and regulating cell growth [24]. BDNF is a growth factor that acts on certain neurons to support the survival of existing neurons, and promote the growth and differentiation of new neurons and synapses [25]. Therefore, the number of neurons may be decreased or the neurons damaged due to nutritional deficiency when Cyclin D1, c-Myc, and BDNF decrease in the brain, eventually leading to depression.

In summary, it was found that when using chronic stress mouse model, Gsk-3β plays a crucial role in the development of depression. Gsk-3β may inhibit β-catenin and Erk-Creb pathways to reduce the development of depression. In the future, it well may be a valuable target for treatment therapies of depression.

Acknowledgments

None.

Conflict of Interest

All authors declare no conflicts of interest.

References

[1] Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ (2006) Evaluation of outcomes with citalopram for depression using measurement-based care in STAR* D: implications for clinical practice. *American Journal of Psychiatry* **163(1)**, 28-40.

[2] Krishnan V, Nestler EJ (2011) Animal models of depression: molecular perspectives. *Molecular and Functional Models in Neuropsychiatry: Current Topics in Behavioral Neurosciences* **7**, 121-147.

[3] Felger JC, Cole SW, Pace TW, Hu F, Woolwine BJ, Doho GH, Raison CL, Miller AH (2012) Molecular signatures of peripheral blood mononuclear cells during chronic interferon- α treatment: relationship with depression and fatigue. *Psychological Medicine* **42(8)**, 1591-1603.

[4] Raison CL, Borisov AS, Woolwine BJ, Massung B, Vogt G, Miller AH (2008) Interferon- α effects on diurnal hypothalamic–pituitary–adrenal axis activity: relationship with proinflammatory cytokines and behavior. *Molecular Psychiatry* **15(5)**, 535-547.

[5] Seira O, Del Río JA (2014) Glycogen synthase kinase 3 beta (GSK3 β) at the tip of neuronal development and regeneration. *Molecular Neurobiology* **49(2)**, 931-944.

[6] Hye A, Kerr F, Archer N, Foy C, Poppe M, Brown R, Hamilton G, Powell J, Anderton B, Lovestone S (2004) Glycogen synthase kinase-3 is increased in white cells early in Alzheimer's disease. *Neuroscience Letters* **373(1)**, 1-4.

[7] Phiel CJ, Wilson CA, Lee VMY, Klein PS (2003) GSK-3 α regulates production of Alzheimer's disease amyloid-β peptides. *Nature* **423(6938)**, 435-439.

[8] Yin F, Boveris A, Cadenas E (2014) Mitochondrial energy metabolism and redox signaling in brain aging and neurodegeneration. *Antioxidants & Redox Signaling* **20(2)**, 353-371.

[9] Hongisto V, Smeds N, Brecht S, Herdegen T, Courtney MJ, Coffey ET (2003) Lithium blocks the c-Jun stress response and protects neurons via its action on glycogen synthase kinase 3. *Molecular and Cellular Biology* **23(17)**, 6027-6036.

[10] Bian YY, Yang LL, Wang ZL, Wang Q, Zeng L, Xu GH (2015) Repeated three-hour maternal separation induces depression-like behavior and affects the expression of hippocampal plasticity-related proteins in C57BL/6N mice. *Neural Plasticity* **2015**, 627837.

[11] Costemale-Lacoste J, Guilloux J, Gaillard R (2016) The role of GSK-3 in treatment-resistant depression and links with the pharmacological effects of lithium and ketamine: A review of the literature. *L'Encéphale* **42(2)**, 156-164.

[12] Strekalova T, Markova N, Shevtsova E, Zubareva O, Bakhmet A, Steinbusch HM, Bachurin S, Lesch KP (2016) Individual differences in behavioural despair predict brain GSK-3beta expression in mice: The power of a modified swim test. *Neural Plasticity* **2016**, 5098591.

[13] Szymańska M, Suska A, Budziszewska B, Jaworska-Feil L, Basta-Kaim A, Leśkiewicz M, Kubera M, Gergont A, Kroczka S, Kaciński M (2009) Prenatal stress decreases glycogen synthase kinase-3 phosphorylation in the rat frontal cortex. *Pharmacological Reports* **61(4)**, 612-620.

[14] Liu C, Wang J, Xu S, An S, Tang S, He J, Liu Y, Lee RJ, Wang D (2017) Paecilomyces tenuipes extract prevents depression-like behaviors in chronic unpredictable mild stress-induced rat model via modulation of neurotransmitters. *Molecular Medicine Reports* **16(2)**, 2172-2178.

[15] Mu D-z, Huang X (2015) Anti-depression effects of electroacupuncture through up-regulating serum E2 and BDNF and expression of BDNF in hippocampus in chronic depression rats. *Journal of Acupuncture and Tuina Science* **13(3)**, 141-145.

[16] Miyagawa K, Tsuji M, Ishii D, Takeda K, Takeda H (2015) Prenatal stress induces vulnerability to stress together with the disruption of central serotonin neurons in mice. *Behavioural Brain Research* **277**, 228-236.

[17] Lindert J, von Ehrenstein OS, Grashow R, Gal G, Braehler E, Weisskopf MG (2014) Sexual and physical abuse in childhood is associated with depression and anxiety over the life course: systematic review and meta-analysis. *International Journal of Public Health* **59(2)**, 359-372.

[18] Schmidt PJ (2005) Mood, depression, and reproductive hormones in the menopausal transition. *The American Journal of Medicine* **118(12)**, 54-58.

[19] Davey CG, Yücel M, Allen NB (2008) The emergence of depression in adolescence: Development of the prefrontal cortex and the representation of reward. *Neuroscience & Biobehavioral Reviews* **32(1)**, 1-19.

[20] Mandelli L, Serretti A (2013) Gene environment interaction studies in depression and suicidal behavior: an update. *Neuroscience & Biobehavioral Reviews* **37(10)**, 2375-2397.

[21] Chen G, Huang LD, Jiang YM, Manji HK (2000) The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. *Journal of Neurochemistry* **72(3)**, 1327-1330.

[22] Beurel E, Grieco SF, Jope RS (2015) Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacology & Therapeutics* **148**, 114-131.

[23] Xiang Q, Chen W, Ren M, Wang J, Zhang H, Deng DY, Zhang L, Shang C, Chen Y (2014) Cabozantinib suppresses tumor growth and metastasis in hepatocellular carcinoma by a dual blockade of VEGFR2 and MET. *Clinical Cancer Research* **20(11)**, 2959-2970.

[24] Boros K, Lacaud G, Kouskoff V (2011) The transcription factor Mxd4 controls the proliferation of the first blood precursors at the onset of hematopoietic development in vitro. *Experimental Hematology* **39(11)**, 1090-1100.

[25] Huang EJ, Reichardt LF (2001) Neurotrophins: roles in neuronal development and function. *Annual Review of Neuroscience* **24(1)**, 677-736.