Suppression of NLRP3 inflammasome attenuates stress-induced depression-like behavior in NLGN3-deficient mice

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ARTICLE INFO

ABSTRACT

Depression, regulated by central nervous system (CNS), is a significant inflammatory disorder. Neuroligin3 (NLGN3) has been implicated in brain functions. In the study, a chronic unpredictable mild stress (CUMS) model in wild type (WT) or NLGN3-knockout (KO) mice was established to explore the role of NLGN3 in regulating depression and to reveal the underlying molecular mechanism. The results indicated that NLGN3-knockout markedly reversed the loss of body weight, the reduction of sucrose consumption, the decrease of immobile time in the forced swimming tests (FST) and tail suspension tests (TST) induced by CUMS paradigm. CUMS up-regulated corticosterone (CORT) in serum, and downregulated serotonin (5-HT), norepinephrine (NE) and brain-derived neurotrophic factor (BDNF) in hippocampus of mice, which were significantly reversed by NLGN3 deficiency. The results further demonstrated that NLGN3-knockout improved the degenerative neurons in cortex and hippocampus of CUMS-treated mice, accompanied with a significant decrease of ionized calciumbinding adapter molecule 1 (Iba-1) and glial fibrillary acidic protein (GFAP) expressions. Additionally, NLGN3-KO mice challenged with CUMS showed a significant reduction of pro-inflammatory cytokines and chemokine, including tumor necrosis factor-alpha (TNF-α), interleukin-18 (IL-18), interleukin-1 beta (IL-1β), interleukin-4 (IL-4), CC-chemokine ligand-1 (CCL-1) and CXC-chemokine ligand-1 (CXCL-1), in cortex, hippocampus and amygdala tissue samples. Western blot analysis suggested that NLGN3-knockout inhibited the activation of nod-like receptor protein 3 (NLRP3) inflammasome and its adaptor of apoptosis-associated speck like protein (ASC), and reduced the expression of Caspase-1, along with the inactivation of nuclear factor-κB (NF-κB) in CUMS-challenged mice. The role of NLGN3 in regulating depression in mice was confirmed in vitro using astrocytes stimulated by LPS that NLGN3 knockdown reduced LPS-induced inflammation. Importantly, the suppressive effects of NLGN3-knockdown on inflammatory response were reversed by NLRP3 or ASC over-expression in AST exposed to LPS. In sum, our findings indicated that suppressing NLGN3 played a potential antidepressant role in CUMS animal model by inactivating NLRP3 inflammasome, providing a new therapeutic avenue for depression.

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1. Introduction

Depression is a chronic, multi-factorial psychiatric disorder, which could be debilitating and even threaten life [1]. Depression is projected to become the second leading cause of disability in the world by 2030 [2]. Therefore, depression is considered as a serious health concern when there are enduring episodes of low mood, anhedonia, altered pattern of sleep and appetite, retardation or psychomotor agitation, and at its worst, suicidal tendency [3,4].

Inflammation has been reported as an essential molecular mechanism of neuropsychiatric disorders, which include major depression [5]. Increasing studies indicated that there is a close relationship between the expression of inflammatory biomarkers and depression symptoms [6,7]. Patients suffering from major depression showed accelerated release of inflammatory cytokines, including IL-1β, IL-6, IL-18, TNF-α and CCL-1 [8,9]. Although the pathogenesis contributing to depression progression has been widely investigated, further study is still required to fully reveal the underlying molecular mechanism.

Neuroligins are important cell adhesion molecules that localized postsynaptically in glutamatergic synapses, and interact with presynaptic neurexins, contributing to the formation of heterophilic

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complex [10]. The procedure plays an essential role in synaptic transmission and differentiation of synaptic contacts [11]. Neuroligin 3 (NLGN3), a X-linked neuroligin gene, been considered to influence information processing in neuronal networks through changing network architecture and synchrony [12]. NLGN3 is associated with the development of autism spectrum disorders [13]. And up-regulation of NLGN3 impairs spatial memory was reported in the brain of rodents [14]. Therefore, we supposed that NLGN3 might be of potential in regulating major depression induced by CUMS in murine animals.

In the present study, we used the NLGN3 wild type (WT) and knockout (KO) mice to establish major depression model through CUMS. The results showed that NLGN3 deletion attenuated CUMS-induced depression-like phenotype, as evidenced by the increased sucrose consumption and body weight, as well as the reduced immobility time using FST and TST. In addition, NLGN3 knockout reduced the activation of astrocytes and microglia cells. Further, inflammatory response in CNS was alleviated by NLGN3-deletion, supported by the reduced release of inflammatory cytokines or chemokine through inactivating NLRP3 inflammasome and NF-κB pathways. The results provided the evidence that NLGN3 could be considered as a potential target for developing effective therapeutic strategies against major depression development.

2. Materials and methods

2.1. Animals and treatments

Male C57BL6 wild type (WT) mice (18–20 g) were purchased from the Experimental Animal Center of Wenzhou Medical University (Wenzhou, China). The male, NLGN3 knockout (KO) mice (18–20 g) with C57BL6 background were obtained from Cyagen Bioscience (Guangzhou, China). Mice were group housed under standard housing conditions (temperature $25 \pm 2\,°C$; a 12/12 h light/dark cycle). Water and food were provided ad libitum. Animal handling and experimental procedure were carried out in accordance with the National Institutes of Health Guidelines for animal research. All the animal experiment protocols were approved by the Institutional Animal Ethics Committee, Wenzhou Medical University. Mice were randomly divided into 4 groups (n = 12): I, wild type control group (WT/Con); II, wild type CUMS group (WT/CUMS); III, knockout control group (KO/Con); and IV, knockout CUMS group (KO/CUMS). Mice were subjected to the CUMS stimuli for 6 weeks. The CUMS procedure was carried out as followings (Fig. 1A). After 1 week acclimation, CUMS groups of mice were single housed and exposed to mild stressors for six weeks: (1) water deprivation for 24 h, (2) food deprivation for 24 h, (3) cage tilt (45°) for 7 h, (4) overnight illumination, (5) soiled cage, (6) overhang (15 min), (7) light/dark perversion, (8) foreign object exposure, (9) 1 min tail pinch, (10) physical restraint for 4 h, (11) white noise, and (12) oscillation for 5 min. The stressors were randomly subjected to mice to ensure the unpredictability of the experiment. Body weight was measured every week. 24 h after completion of TST, all mice were sacrificed through cervical dislocation. The blood was collected and centrifugated for further measurements. Whole brains were rapidly removed from the mice and cortex, hippocampus and amygdala tissue samples were dissected on a cold plate, and either frozen in liquid nitrogen and kept at −80 °C for further analysis, or fixed in 4% paraformaldehyde for immunohistochemical analysis.

2.2. Astrocytes (AST) isolation, culture and transfection

The isolation of AST from 1 to 3-day old neonatal mice was according to modified methods from previous reports [15]. The

isolated primary AST cells were cultured in uncoated culture flasks containing DMEM/F12 supplemented with 10% FBS, 1×10^5 U/L streptomycin sulfate (GIBCO Corporation, USA) with a concentration 1×10^6/mL at 37 °C, 5% CO_2. The pcDNA3.1-NLRP3 and pcDNA3.1-ASC or pcDNA3.1 empty vector (0, 25, 50 and 100 ng) constructed by Genechem Co.,Ltd. (Shanghai, China) were transfected into AST cells using Lipofectamine 2000 reagent (Invitrogen, USA). All siRNAs, including NLGN3 siRNAs and negative control siRNA (NC) were synthesized by GenePharma (Shanghai, China), and were transfected to cells for 48 h using Lipofectamine 2000 reagent.

2.3. Sucrose preference test (SPT)

The SPT was carried out as described previously [16]. Sucrose preference = sucrose consumption (ml)/(water consumption (ml) + sucrose consumption (ml)) × 100.

2.4. Forced swimming test (FST)

FST was carried out 48 h after the sucrose preference test and the method employed was according to that described previously [17].

2.5. Tail suspension test (TST)

TST, performed 24 h after the FST, was based on the method described previously [18].

2.6. Determination of CORT, 5-HT, NE and BDNF

Serum CORT level was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Abnova Corporation, USA) according to the manufacturer's protocol. Hippocampal BDNF level was calculated using an ELISA kit (Promega Corporation, USA) following the manufacturer's instructions. The contents of 5-HT and NE in hippocampus of mice were measured by HPLC [19].

2.7. Quantitative real-time PCR (RT-qPCR) detection

Total RNA extraction of cortex, hippocampus, amygdala and cells were performed using Trizol reagent (GIBCO Corporation). Total RNA was reverse transcribed using the M-MLV-RT system (Promega, USA). qPCR were conducted using SYBR Green (Bio-Rad, USA) in ABI PRISM 7900HT detection systems (Applied Biosystems, USA). All primers were provided by Invitrogen Corporation and listed as followings:

TNF-α (forwards: TCG GAG GAA CGC AGA GCG G; rewards: GGA GCT AGA TCA CAA AGA CAA); IL-1β (forwards: AAT TTC CAT CCG GTC AGG GA; rewards: AGA CCA CAG CAG ACG CTA CA); IL-6 (forwards: AGA TTC GCC TCA TGC ACG CC; rewards: AGC TTC AAC GAC CTG TTT); IL-4 (forwards: ATC ACG GCC ACG ACC TGA; rewards: CAG ATA GGA ACC ATG ACA A); CCL-1 (forwards: AAA CGA CCT CCG AGC CGA AG; rewards: ATG ATC GAC AAG GAC ACA T); CXCL-1 (forwards: CAG GAG GCC GAG GGG GAT C; rewards: TCG ATC GAC TAT CAC ACG ACG); and GAPDH (forwards: GGC ACA AGA ACC TAA; rewards: GCA GGA CGC AAG CAG TAG CT).

2.8. Western blot analysis

Western blotting was carried out determine protein expressions. All proteins were resolved on 10–12% SDS-PAGE and were then transferred onto a PVDF membrane. Membranes were incubated with blocking buffer for 2 h at room temperature and then incubated with antibodies against NLRP3, ASC, Caspase-1, NF-κB, p-