Neuregulin-1 Regulates the Conversion of M1/M2 Microglia Phenotype via ErbB4-dependent Inhibition of the NF-κB Pathway

Yuqi Ma
Xinxiang Medical University
Peixia Fan
Xinxiang Medical University
Rui Zhao
Xinxiang Medical University
Yinghua Zhang
Xinxiang Medical University
Xianwei Wang
Xinxiang Medical University
Weigang Cui (cuiweigang1978@126.com)
Xinxiang medical university https://orcid.org/0000-0002-3318-6708

Research Article

Keywords: Neuregulin-1, Microglia phenotype, Neuroinflammation, Alzheimer's disease, NF-κB

Posted Date: December 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1117291/v1

Abstract

Background

The inflammatory response caused by microglia in the central nervous system plays an important role in Alzheimer's disease. Neuregulin-1 (NRG1) is a member of the neuregulin family and has been demonstrated to have anti-inflammatory properties. The relationship between NRG1, microglia phenotype and neuroinflammation remains unclear.

Materials and Methods

BV2 cells were used to examine the mechanism of NRG1 in regulating microglia polarization. Neuronal apoptosis, inflammatory factors TNF-α and iNOS, microglia polarization, ErbB4 and NF-κB p65 expression were assessed.

Results

We found that exogenous NRG1 treatment or overexpression improved microglial activity and reduced the secretion of the inflammatory factors TNF-α and iNOS in vitro. The expression of Bax in SH-SY5Y neuron cells incubated with medium collected from the NRG1 treatment group decreased. Additionally, our study showed that NRG1 treatment reduced the levels of the M1 microglia markers CD120 and iNOS and increased the levels of the M2 microglia markers CD206 and Arg-1. Furthermore, we observed that NRG1 treatment attenuated Aβ-induced NF-κB activation and promoted the expression of p-ErbB4 and that knockdown of ErbB4 abrogated the effects of NRG1 on NF-κB, Bax levels and M2 microglial polarization.

Conclusion

NRG1 inhibits the release of inflammatory factors in microglia and regulates the switching of the M1/M2 microglia phenotype, most likely via ErbB4-dependent inhibition of the NF-κB pathway.

Introduction

The number of people with dementia worldwide is expected to reach 1, 315 million in 2050[1]. Among the various types of dementia, Alzheimer's disease (AD) is the most well-known and the most difficult to manage. The microglia-mediated inflammatory response in the central nervous system plays an important role in the development of AD[2]. Some scholars categorize microglia as “classically activated” and “alternatively activated” microglia[3]. Classically activated microglia, namely, M1 microglia[4], secrete the inflammatory factors CD120, iNOS, and TNF-α to aggravate the inflammatory response. Alternatively activated microglia, or M2 microglia, express Arg-1 and CD206 and exert anti-inflammatory effects [5]. Hence, regulating the activation state of microglia, that is, promoting the switch from the M1 to M2 phenotype, might hinder the progression of AD.

Neuregulin-1 (NRG1) is a member of the neuregulin family, the members of which contain epidermal growth factor (EGF)-like domains. Studies have shown that the expression of NRG1 in AD mice is reduced[6]and that the NRG1 signaling pathway plays a beneficial role in neuroinflammation[7, 8]. ErbB family tyrosine kinases are the main receptors of NRG1, and ErbB4 has tyrosine kinase activity and high affinity for ligands[9]. NRG1/ErbB4 signaling regulates several basic functions, such as cognitive processes[10] and neuronal migration[11], and has anti-inflammatory effects[12]. NRG1 has been found to regulate the activity of resident astrocytes and microglia in spinal cord injury and to promote the preservation and functional recovery of white matter after spinal cord injury[13]. NRG1 also indirectly protects neurons by reducing microglial activation[14]. Intrathecal injection of NRG1 activates ErbB2 receptors and causes microglia to proliferate and adopt an activated state[11, 15]. The above evidence indicates that NRG1 is involved in neuroinflammation and the regulation of microglia.

Although studies have highlighted the important role of NRG1 in regulating the inflammatory response, its possible effects on the microglial phenotype and the role of NRG1 in inflammatory signaling in AD have not been assessed. In the present study, we revealed that NRG1 inhibited the release of inflammatory factors in microglia and regulated the switching of M1/M2 microglia through ErbB4-dependent inhibition of the NF-κB pathway.

Materials And Methods

Cell culture

The BV2 microglial cell line and SH-SY5Y neuronal cell line were obtained from Shanghai Institute of Cell Research (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS, Gibco, USA) in a 5% CO_2 incubator at 37°C. When the BV2 cells reached approximately 80% confluence, 0.25% trypsin (trypsin, Gibco, USA) was used to detach the cells, and the cells were inoculated in 6-well or 24-well plates.

Lentiviral transfection of BV2 cells

BV2 cells were detached with trypsin and seeded in a 6-well plate at a density of 5×10^4 cells/well. Transfection was performed when the cell density reached 30%-50%. NRG1 lentiviral particles (sequences: 5'-CTGGGACCAGCCATCTCATAA-3', 5'-CGTGGAATC AAACGAGATCAT-3', and 5'-GCCTCAACTGAAGGAGCATAT-3'), LV-ErbB4-RNAi lentiviral particles (sequences: 5'-GCTTTGACATTTGCTGTCTAT-3', 5'GCACCCAATCAAGCTCAACTT -3', and 5'- GCAACCTGTGTTATTACCATA -3') and their negative controls (sequence: 5'-TTCTCCGAA CGTGTCACGT-3') were constructed and synthesized by Gene Pharma Co. (Shanghai, China). One milliliter of medium, and an appropriate volume of virus (NRG1-MOI=50, ErbB4-MOI=10) and Hitrans G A or P (Gene, Shanghai, China) were added to each well. The 2 stably expressing cell lines were designated OVER-N and $ER4^{KD}$. The lentiviral vector carries the green fluorescent protein (GFP).

Cell viability test

Cell viability was analyzed by the Cell Counting Kit-8 (CCK-8, Dojindo, Rockville, MD) method. Cells were inoculated in 96-well plates at a density of 1×10^4 cells/well and cultured in a 37°C incubator for 24 h prior to analysis. A total of 5 wells were analyzed. The cells were stimulated with $A\beta_{1\text{-}42}$ for 4 h and then treated with 0, 5, 20, 50, or 100 ng/ml NRG1 for 4 h. CCK-8 reagent (10 μmol/L) and 100 μl culture medium were added to each well, and the cells were incubated for 1 h. A microplate reader was used to measure the absorbance at 450 nm. Cell viability was calculated as follows: (OD value of experimental group－OD value of blank group)/(OD value of control group－OD value of blank group).

Enzyme-linked immunosorbent assay

After different treatments, the supernatant of each group was collected and centrifuged at 4°C and 3000 r/min for 5 min. The content of iNOS and TNF-α in the supernatants was measured with a sandwich enzyme-linked immunosorbent assay kit (ELISA, CUSABIO, Wuhan, China, CSB-E04741 m/96T, CSB-E08326 m/96T). The absorbance was measured with a microplate reader at 570 nm, and the concentrations of iNOS and TNF-α in the sample were determined according to the standard curve.

SH-SY5Y cell induction

The supernatants of cultured BV2 cells were collected after different treatments. The supernatants were filtered through a 0.45-μm filter and stored in a refrigerator at -80°C. The collected medium was used as conditioned medium, and SH-SY5Y cells were incubated in this medium for 24 h. The Bax levels in SH-SY5Y cells were measured by Western blotting and immunofluorescence.

Western blotting

A cell scraper was used to quickly collect cells from each group, and nuclear and cytoplasmic proteins were extracted according to the instructions of a Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Shanghai, China, P0028). The protein concentrations were determined by the BCA method. Thirty micrograms of protein was added to a 10% SDS-PAGE gel and then transferred to polyvinylidene fluoride membranes. The membranes were incubated with fast blocking solution and then incubated with the following primary antibodies overnight at 4°C: rabbit anti-NF-κB p65 (1:2000, Beyotime, Shanghai, China, AF0246), rabbit anti-ErbB4 (1:500, Abcam, United Kingdom, ab32375), rabbit anti-p-ErbB4 (1:500, Abcam, United Kingdom, ab109273), mouse anti-Bax (1:1000, SANTA, Shanghai, China sc-7480), mouse anti-NRG1 (1:1000, SANTA, Shanghai, China, sc-393006), rabbit anti-histone H3 (1:1000, Beyotime, Shanghai, China, AF5614), rabbit anti-GAPDH (1:5000, Abcam, United Kingdom, AF1186), and rabbit anti-β-actin (1:5000, Proteintech, Chicago, USA, 66009-1-lg). The membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Pierce, Rockford, IL, USA) for 2 h at room temperature. The bands were detected by enhanced chemiluminescence (ECL). ImageJ software was used to assess the optical density values.

Immunofluorescence

BV2 cells and SH-SY5Y cells were detached with trypsin and seeded in a 24-well culture plate at a density of 10^4 cells/well. Polylysine-coated glass slides were placed in the bottom of the 24-well plate in advance. The cells were fixed with 4% paraformaldehyde for 20 min at room temperature and washed 3 times with PBS. Triton X-100 (0.1%) was used to permeabilize the cell membrane for 20 min at room temperature. The cells were incubated with rabbit anti-NF-κB p65 (1:400, Beyotime, Shanghai, China, AF0246) and mouse anti-Bax (1:100, SANTA, Shanghai, China sc-7480) primary antibodies overnight at 4°C, incubated with secondary antibody (Beyotime, Shanghai, China, A0216, A0208) and 4', 6-diamidino-2-phenylindole (DAPI, Beyotime, Shanghai, China, C1006) for 1 h at room temperature.

Flow cytometry

The cells were resuspended in 1 ml of precooled PBS. The number of cells in each tube was adjusted to 1×10^6. Surface staining was performed using PE-conjugated anti-mouse CD120 (Biolegend, CA, USA, 113003) and Brilliant Violet 421™-conjugated anti-mouse CD206 (Biolegend, CA, USA, 141717) antibodies. For intracellular staining, the cells were fixed with 2% paraformaldehyde (Solarbio, Beijing, China) and lysed with 1× Intracellular Staining Perm Wash Buffer (Biolegend, CA, USA). Then, the cells were incubated with PE-conjugated iNOS (Biolegend, CA, USA, 696805) and APC-conjugated Arg-1 (R&D, MN, USA, IC5868A) antibodies. All samples were evaluated with a BD FACSC Anto flow cytometer, and the data were analyzed with CytExpert software.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 6 (GraphPad Software, Inc.), and all data are presented as the mean ± SEM. When there were three or more groups, one-way analysis of variance (ANOVA) was used to analyze the data. Each experiment was independently repeated three times, and mean values used as results. $P<0.05$ indicates statistical significance.

Results

NRG1 increased the activity of Aβ-stimulated BV2 microglia, reduced the expression levels of iNOS and TNF-α

To study whether NRG1 can ameliorate the decrease in microglial activity caused by Aβ, we conducted a CCK-8 assay. The results of the CCK-8 assay showed that the cell activity of the Aβ-treated group was significantly reduced compared with that of the control group($P<0.05$). The cell viability of the groups treated with 20, 50, and 100 ng/ml NRG1 was increased compared with that of the Aβ-treated group ($P<0.05$, Fig. 1A). The most effective concentration of NRG1 was 20 ng/ml. Therefore, we chose 20 ng/ml NRG1 for the following experiments.

To investigate the role of NRG1 in inhibiting neuroinflammation, lentivirus transfection was used to upregulate NRG1 expression in BV2 cells. The lentiviral vector carries the green fluorescent protein (GFP). Compared with the control group, the protein expression of NRG1 in the OVER-N group was

increased (P<0.001, Fig. 1B, C). The flow cytometry results showed that compared with that in the control group, the number of positive cells in the lentivirus-transfected group was higher (P<0.01, Fig. 1D, E), which suggested that high expression of NRG1 in BV2 cells was successfully achieved.

Once activated under pathological conditions, microglia release inflammatory factors. We used ELISA kits to measure the expression of the inflammatory factors TNF-α and iNOS in the cell supernatants. Compared with those in the control group, the contents of TNF-α and iNOS in the Aβ-treated group were significantly increased (P<0.01). Compared with those in the Aβ-treated group, the levels of TNF-α and iNOS in the Aβ+N group and the Aβ+OVER-N group were significantly reduced (P<0.01, Fig. 1F, G). The results suggested that NRG1 reduced the inflammatory response caused by the activation of microglia. TNF-α and iNOS are also surface marker molecules of M1 microglia. We speculated that NRG1 may promote the conversion of microglia from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype.

NRG1 switched microglia from the M1 to M2 phenotype

We used flow cytometry to assess the expression of the M1 microglial surface markers iNOS and CD120 and the M2 microglial surface markers CD206 and Arg-1. The results of FACS phenotype analysis showed that compared with those in the control group, the percentages of cells positive for CD120 (Fig. 2A) and iNOS (Fig. 2B) in the Aβ-treated group were higher (P<0.001). Compared with those in the Aβ-treated group, the percentages of cells positive for CD120 and iNOS in the Aβ+N group and the Aβ+OVER-N group were lower (P<0.001). In addition, the expression of Arg-1 (Fig. 2C) and CD206 (Fig. 2D) increased in the Aβ+N group and the Aβ+OVER-N group (P<0.05). The above results indicated that NRG1 promoted the transformation of microglia from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype.

NRG1 inhibited the neuronal damage caused by microglial activation

To test whether NRG1 alleviate the neuronal damage caused by the activation of microglia, immunofluorescence and Western blotting were carried out to evaluate the expression of Bax in SH-SY5Y cells cultured with conditioned medium. Compared with that in the control group, the expression of Bax in the Aβ-treated group was increased (P<0.001). Compared with that in the Aβ-treated group, the expression of Bax in the Aβ+N group and the Aβ+OVER-N group was decreased (P<0.001, Fig. 3A-C). The above results proved that NRG1 inhibited neuronal apoptosis caused by the activation of microglia.

NRG1 inhibited NF-κB p65 nuclear translocation caused by BV2 microglial activation by activating the ErbB4 receptor

To further explore the mechanism underlying the changes described above, the effect of NRG1 on ErbB4 was further analyzed by Western blotting. There was no significant difference in the protein expression of ErbB4 in any group. However, compared with that in the Aβ-treated group, the expression of p-ErbB4 in the Aβ+N group and the Aβ+OVER-N group was significantly increased (P<0.001, Fig. 4A, B).

Compared with that in the control group, the protein expression of the p65 subunit in the nucleus in the Aβ-treated group was significantly increased (P<0.01). Compared with that in the Aβ-treated group, the protein expression of the p65 subunit in the nucleus in the Aβ+N group and the Aβ+OVER-N group was significantly reduced (P<0.05, Fig. 4A, C). The change in the protein expression of p65 in the cytoplasm was opposite that observed in the nucleus (Fig. 4A, D). The immunofluorescence results were consistent with the Western blotting results (Fig. 4E). These results indicated that Aβ-induced microglial activation led to the activation of the NF-κB signaling pathway and that NRG1 treatment inhibited the activation of the NF-κB signaling pathway.

Silencing ErbB4 reversed the anti-inflammatory response induced by NRG1 treatment

To investigate the role of ErbB4 in the anti-inflammatory effect of NRG-1, ErbB4 expression was specifically inhibited by siRNA transfection in NRG1-pretreated BV2 cells. The Western blotting results showed that the protein expression of ErbB4 in the $ER4^{KD}$ group was reduced (P<0.01, Fig. 5A, B). Consistent with this blockade of ErbB4 expression, downregulation of the expression of the inflammatory factors TNF-α and iNOS (P<0.001, Fig. 5C, D) and the M1 microglia markers CD120 and iNOS was abolished (P<0.001, Fig. 6G-J). As expected, ErbB4 siRNA transfection diminished the protective effects of NRG1 against Aβ-induced expression of Bax (P<0.001, Fig. 5E-F). Interestingly, we found that the downregulation of NF-κB-p65 subunit expression in the nuclei of NRG1-treated BV2 cells was also blocked by ErbB4 siRNA transfection (P<0.05, Fig. 5K-M). All these results strongly suggested that the ErbB4 and NF-κB signaling pathways are closely related to the anti-inflammatory effects of NRG-1.

Discussion

Glial cells account for 90% of cells in the brain, and microglia are the main effectors of neuroinflammatory reactions[16]. Accumulating data show that NRG1 is closely related to microglial activity[17]. However, the role of NRG1 in microglial phenotype polarization remains unclear. We demonstrated that the overexpression of NRG1 was closely related to inflammation and microglial activity. In particular, NRG1 acts as a microglial modulator to reverse microglial phenotype polarization from M1 to M2, and suppress NF-κB p65 nuclear translocation. However, with the knockdown of the ErbB4 receptor, the effect of NRG1 on microglia was reversed.

Polarizing M1 microglia to M2 type microglia improves the neuroinflammatory response and pathological damage of AD[18]. However, the connection between NRG1 and microglia polarization has never been reported. We demonstrated that NRG1 promotes the transformation of microglia from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype. Switching of M1 microglia to the M2 microglial phenotype may be a potential target against neuroinflammation. Indeed, inhibition of TLR4 receptors promotes the polarization of M2-type microglia against inflammation in APP/PS1 mice[19]. Magnesium lithospermate B promotes the phenotypic transition of lipopolysaccharide-induced microglia from the M1 phenotype to the M2 phenotype in the hippocampus of adult mice to reduce

neuroinflammation[20]. IRF5/4 signaling can regulate the activation of M1/M2 microglia and affect the prognosis of stroke[21].

The polarization state of microglia is different in different periods of AD. The polarization state of microglia is affected by many factors, such as the exposed pathological environment[22], the age of microglia[23], and the connection with surrounding cells[24]. Activated microglia act on synapses and change the activity of neuronal cells through phagocytosis and the release of different immune inflammatory factors[25]. Excessive neuroinflammation releases toxic substances such as NO and proinflammatory cytokines to cause neuronal damage[26]. We cultured SH-SY5Y cells in conditioned medium and Aβ-containing medium to induce neuronal apoptosis. These results indicate that NRG1 may have neuroprotective functions by inhibiting the activation of microglia and inhibiting the release of cytotoxic substances.

NF-κB has been shown to be an important transcription factor that regulates cell differentiation, proliferation and survival[27]. The main effect of enhanced inflammation is upregulated expression of the nuclear transcription factor NF-κB, leading to a series of uncontrollable long-term autoregulatory processes and a vicious cycle of impaired autotransmission, thereby exacerbating the inflammatory response[28, 29]. Studies have shown that the activity of NF-κB in astrocytes in patients with amyotrophic lateral sclerosis is significantly enhanced[30]. Autopsies of Parkinson's patients have revealed that the number of dopaminergic neurons containing NF-κB in the nucleus is significantly higher in patients than in normal people[31]. In neuroinflammation in the context of AD, hypoxia regulates the M1/M2 polarization of microglia through the NF-κB signaling pathway[32]. Our study revealed that Aβ caused the activation of the NF-κB signaling pathway. After NRG1 treatment, the nuclear transcription of p65 in microglia decreased, indicating that NRG1 inhibited the activation of the NF-κB signaling pathway.

ErbB4 has been associated with inflammation. Increaseing the expression of ErbB4 in pro-inflammatory macrophages reduce the symptoms of colitis[33]. Neuroinflammation-mediated destruction of NRG1-ErbB4 signaling transduction in parvalbumin interneurons may cause abnormal gamma oscillations and lead to cognitive impairment in Sepsis-Associated Encephalopathy mouse model[34]. The mice with increased excitability of 5-HT neurons in the dorsal raphe nucleus mediated by ErbB4 deficiency showed a high degree of anxiety-like behavior and impaired fear resolution[35]. NRG1/ErbB4 signaling has important anti-inflammatory effects[36, 37]. Studies have shown that after ErbB4 is introduced into mesenchymal stem cells, it promotes the synthesis and secretion of NRG1 and myocardial repair after myocardial infarction[36]. Inhibition of NRG1-ErbB4 signaling prevents the recovery of spinal cord function and neuroplasticity[38]. We demonstrated that ErbB4 deficient microglia not effectively cooperate with NRG1 to play an anti-inflammatory role.

We initially revealed the role of NRG1 in microglial polarization and inflammation. In the future, we should focus on regulating the ratio of M1/M2 microglia rather than inhibiting the activation of microglia. Changing the ratio of M1/M2 microglia to regulate neuroinflammation is a promising treatment option for AD.

Conclusion

NRG1 reduced the expression of the inflammatory factors TNF-α and iNOS and inhibited the neuroinflammatory response mediated by microglia. The underlying mechanism may be related to inhibition of the NF-κB signaling pathway through activation the ErbB4 of receptor, which promotes the transformation of microglia from the proinflammatory M1 phenotype to the anti-inflammatory M2 phenotype to exert neuroprotective effects.

Declarations

Data Availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Authors' contributions Weigang Cui conceived and designed the experiments. Yuqi Ma, Peixia Fan, Rui Zhao, Yinghua Zhang performed the research and analyzed the data. Yuqi Ma and Weigang Cui wrote the paper. Xianwei Wang gave the informative advice. All authors read and approved the final manuscript.

Funding This study was supported by funding support from the National Natural Science Foundation of China (U1804185, 81873459, U1804166), and the support project for the Disciplinary Group of Psychology and Neuroscience, Xinxiang Medical University (2016PN-KFKT-11).

Declarations

Ethical Approval and Consent to participate All of the procedures and animal care were approved by Xinxiang Medical University Ethics Committee (Xinxiang, China, permit number: XYLL-2018-B009) and were conducted in accordance with the National Institutes of Health guidelines for the care of laboratory animals.

Consent for Publication Written informed consent was obtained from all participants at the time of obtaining consent to participate.

Competing Interests The authors declare that they have no competing interests.

References

1. Sengoku R (2020) Aging and Alzheimer's disease pathology. Neuropathology 40(1):22-29

2. Matcovitch-Natan O, Winter DR, Giladi A, Vargas Aguilar S, Spinrad A, Sarrazin S, Ben-Yehuda H, David E, Zelada González F, Perrin P, Keren-Shaul H, Gury M, Lara-Astaiso D, Thaiss CA, Cohen M, Bahar Halpern K, Baruch K, Deczkowska A, Lorenzo-Vivas E, Itzkovitz S, Elinav E, Sieweke MH, Schwartz M, Amit I (2016) Microglia development follows a stepwise program to regulate brain homeostasis. Science 353(6301):aad8670

3. Tang Y, Le W (2016) Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases. Mol Neurobiol 53(2):1181-1194

4. Orihuela R, McPherson CA, Harry GJ (2016) Microglial M1/M2 polarization and metabolic states. Br J Pharmacol 173(4):649-665

5. Wang J, Xing H, Wan L, Jiang X, Wang C, Wu Y (2018) Treatment targets for M2 microglia polarization in ischemic stroke. Biomed Pharmacother 105:518-525

6. Cui W, Tao J, Wang Z, Ren M, Zhang Y, Sun Y, Peng Y, Li R (2013) Neuregulin1beta1 antagonizes apoptosis via ErbB4-dependent activation of PI3-kinase/Akt in APP/PS1 transgenic mice. Neurochem Res 38(11):2237-2246

7. Baik TK, Kim YJ, Kang SM, Song DY, Min SS, Woo RS (2016) Blocking the phosphatidylinositol 3-kinase pathway inhibits neuregulin-1-mediated rescue of neurotoxicity induced by Aβ1-42. J Pharm Pharmacol 68(8):1021-1029

8. Xu J, Hu C, Chen S, Shen H, Jiang Q, Huang P, Zhao W (2017) Neuregulin-1 protects mouse cerebellum against oxidative stress and neuroinflammation. Brain Res 1670:32-43

9. Zhao WJ (2013) The expression and localization of neuregulin-1 (Nrg1) in the gastrointestinal system of the rhesus monkey. Folia Histochem Cytobiol 51(1):38-44

10. Xu J, de Winter F, Farrokhi C, Rockenstein E, Mante M, Adame A, Cook J, Jin X, Masliah E, Lee KF (2016) Neuregulin 1 improves cognitive deficits and neuropathology in an Alzheimer's disease model. Sci Rep 6:31692

11. Calvo M, Zhu N, Tsantoulas C, Ma Z, Grist J, Loeb JA, Bennett DL (2010) Neuregulin-ErbB signaling promotes microglial proliferation and chemotaxis contributing to microgliosis and pain after peripheral nerve injury. J Neurosci 30(15):5437-5450

12. Solomon W, Wilson NO, Anderson L, Pitts S, Patrickson J, Liu M, Ford BD, Stiles JK (2014) Neuregulin-1 attenuates mortality associated with experimental cerebral malaria. J Neuroinflammation 11:9

13. Alizadeh A, Santhosh KT, Kataria H, Gounni AS, Karimi-Abdolrezaee S (2018) Neuregulin-1 elicits a regulatory immune response following traumatic spinal cord injury. J Neuroinflammation 15(1):53

14. Yoo SY, Yoo JY, Kim HB, Baik TK, Lee JH, Woo RS (2019) Neuregulin-1 Protects Neuronal Cells Against Damage due to CoCl2-Induced Hypoxia by Suppressing Hypoxia-Inducible Factor-1α and P53 in SH-SY5Y Cells. Int Neurourol J 23(Suppl 2):S111-118

15. Alizadeh A, Dyck SM, Kataria H, Shahriary GM, Nguyen DH, Santhosh KT, Karimi-Abdolrezaee S (2017) Neuregulin-1 positively modulates glial response and improves neurological recovery following

traumatic spinal cord injury. Glia 65(7):1152-1175

16. Kreutzberg GW (1995) Microglia, the first line of defence in brain pathologies. Arzneimittelforschung 45(3A):357-360

17. Xiang Y, Liu T, Yang H, Gao F, Xiang H, Manyande A, Tian Y, Tian X (2015) NRG1-ErbB signalling promotes microglia activation contributing to incision-induced mechanical allodynia. Eur J Pain 19(5):686-694

18. Yao K, Zu HB (2020) Microglial polarization: novel therapeutic mechanism against Alzheimer's disease. Inflammopharmacology 28(1):95-110

19. Cui W, Sun C, Ma Y, Wang S, Wang X, Zhang Y (2020) Inhibition of TLR4 Induces M2 Microglial Polarization and Provides Neuroprotection via the NLRP3 Inflammasome in Alzheimer's Disease. Front Neurosci 14:444

20. Tai Y, Qiu Y, Bao Z (2018) Magnesium Lithospermate B Suppresses Lipopolysaccharide-Induced Neuroinflammation in BV2 Microglial Cells and Attenuates Neurodegeneration in Lipopolysaccharide-Injected Mice. J Mol Neurosci 64(1):80-92

21. Al Mamun A, Chauhan A, Yu H, Xu Y, Sharmeen R, Liu F (2018) Interferon regulatory factor 4/5 signaling impacts on microglial activation after ischemic stroke in mice. Eur J Neurosci 47(2):140-149

22. Kumar A, Stoica BA, Sabirzhanov B, Burns MP, Faden AI, Loane DJ (2013) Traumatic brain injury in aged animals increases lesion size and chronically alters microglial/macrophage classical and alternative activation states. Neurobiol Aging 34(5):1397-1411

23. Caldeira C, Cunha C, Vaz AR, Falcão AS, Barateiro A, Seixas E, Fernandes A, Brites D (2017) Key Aging-Associated Alterations in Primary Microglia Response to Beta-Amyloid Stimulation. Front Aging Neurosci 9:277

24. Xie L, Zhang N, Zhang Q, Li C, Sandhu AF, Iii GW, Lin S, Lv P, Liu Y, Wu Q, Yu S (2020) Inflammatory factors and amyloid β-induced microglial polarization promote inflammatory crosstalk with astrocytes. Aging (Albany NY) 12(22):22538-22549

25. Zhou J, Yu W, Zhang M, Tian X, Li Y, Lü Y (2019) Imbalance of Microglial TLR4/TREM2 in LPS-Treated APP/PS1 Transgenic Mice: A Potential Link Between Alzheimer's Disease and Systemic Inflammation. Neurochem Res 44(5):1138-1151

26. Berköz M, Krośniak M, Özkan-Yılmaz F, Özlüer-Hunt A (2020) Prophylactic effect of Biochanin A in lipopolysaccharide-stimulated BV2 microglial cells. Immunopharmacol Immunotoxicol 42(4):330-339

27. Schnappauf O, Aksentijevich I (2020) Mendelian diseases of dysregulated canonical NF-κB signaling: From immunodeficiency to inflammation. J Leukoc Biol 108(2):573-589

28. Jha NK, Jha SK, Kar R, Nand P, Swati K, Goswami VK (2019) Nuclear factor-kappa β as a therapeutic target for Alzheimer's disease. J Neurochem 150(2):113-137

29. Sivandzade F, Prasad S, Bhalerao A, Cucullo L (2019) NRF2 and NF-κB interplay in cerebrovascular and neurodegenerative disorders: Molecular mechanisms and possible therapeutic approaches. Redox Biol 21:101059

30. D'Erchia AM, Gallo A, Manzari C, Raho S, Horner DS, Chiara M, Valletti A, Aiello I, Mastropasqua F, Ciaccia L, Locatelli F, Pisani F, Nicchia GP, Svelto M, Pesole G, Picardi E (2017) Massive transcriptome sequencing of human spinal cord tissues provides new insights into motor neuron degeneration in ALS. Sci Rep 7(1):10046

31. Singh SS, Rai SN, Birla H, Zahra W, Rathore AS, Singh SP (2020) NF-κB-Mediated Neuroinflammation in Parkinson's Disease and Potential Therapeutic Effect of Polyphenols. Neurotox Res 37(3):491-507

32. Zhang F, Zhong R, Li S, Fu Z, Cheng C, Cai H, Le W (2017) Acute Hypoxia Induced an Imbalanced M1/M2 Activation of Microglia through NF-κB Signaling in Alzheimer's Disease Mice and Wild-Type Littermates. Front Aging Neurosci 9:282

33. Janssen MJ, Leiva-Salcedo E, Buonanno A (2012) Neuregulin directly decreases voltage-gated sodium current in hippocampal ErbB4-expressing interneurons. J Neurosci 32(40):13889-13895

34. Gao R, Ji MH, Gao DP, Yang RH, Zhang SG, Yang JJ, Shen JC (2017) Neuroinflammation-Induced Downregulation of Hippocampacal Neuregulin 1-ErbB4 Signaling in the Parvalbumin Interneurons Might Contribute to Cognitive Impairment in a Mouse Model of Sepsis-Associated Encephalopathy. Inflammation 40(2):387-400

35. Zhang SR, Wu JL, Chen H, Luo R, Chen WJ, Tang LJ, Li XW, Yang JM, Gao TM (2020) ErbB4 knockdown in serotonergic neurons in the dorsal raphe induces anxiety-like behaviors. Neuropsychopharmacology 45(10):1698-1706

36. Liang X, Ding Y, Zhang Y, Chai YH, He J, Chiu SM, Gao F, Tse HF, Lian Q (2015) Activation of NRG1-ERBB4 signaling potentiates mesenchymal stem cell-mediated myocardial repairs following myocardial infarction. Cell Death Dis 6:e1765

37. Liang X, Ding Y, Lin F, Zhang Y, Zhou X, Meng Q, Lu X, Jiang G, Zhu H, Chen Y, Lian Q, Fan H, Liu Z (2019) Overexpression of ERBB4 rejuvenates aged mesenchymal stem cells and enhances angiogenesis via PI3K/AKT and MAPK/ERK pathways. FASEB J 33(3):4559-4570

38. Cardin JA, Carlén M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI (2009) Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 459(7247):663-667

Figures

Figure 1

A BV2 cell viability was measured by the CCK-8 assay. B, C Western blot analysis showing the expression level of NRG1 in the control group, OVER-N group, and OVER-N negative control group. D, E The number of positive cells determined by flow cytometry was used to verify the success of lentivirus transfection. The expression of F iNOS and G TNF-α in the supernatant was measured using an ELISA kit. TNFα, tumor necrosis factor α, iNOS, inducible NOS.

Figure 2

Cells from each group were collected and stained with anti-mouse CD120, iNOS, Arg-1, and CD206 monoclonal antibodies. Flow cytometry was used to analyze the expression profiles of microglia from each group. A CD120-positive cells. B iNOS-positive cells. C CD206-positive cells. D Arg-1-positive cells.

Figure 3

NRG1 decreased the expression of Bax in SH-SY5Y cells. A, B The levels of Bax were determined by Western blot analysis. β-Actin was used as an internal control. C Translocation of Bax was visualized by immunofluorescence staining. Bax (green), DAPI (blue). Scale bar, 20 μm.

Figure 4

Effect of NRG1 on the ErbB4 and NF-κB pathways in Aβ-stimulated BV2 microglia. A Western blot analysis of NF-κB p65 expression in nuclear and cytoplasmic extracts. His H3 and GAPDH were used as internal controls. Representative immunoblot of p-ErbB4. ErbB4 were used as internal controls. B Quantitative analysis of p-ErbB4 expression. C Quantitative analysis of nuclear p65 and D cytoplasmic p65 expression. E Translocation of the NF-κB p65 subunit in BV2 cells was visualized by double immunofluorescence staining. NF-κB p65 (red), DAPI (blue). Scale bar, 20 μm.

Figure 5

A, B Western blot analysis showing the expression level of ErbB4 in the control group, ER4KD group, and ER4KD negative control group. The expression of C iNOS and D TNF-α in the supernatant was measured using ELISA kits. E-F the levels of Bax were determined by Western blot analysis. β-Actin was used as an

internal control. G-J FACS was used to analyze the expression profiles of microglia in each group. K-M Western blot analysis of NF-κB p65 expression in nuclear and cytoplasmic extracts.