Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

YY1 promotes IL-6 expression in LPS-stimulated BV2 microglial cells by interacting with p65 to promote transcriptional activation of IL-6

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ARTICLE INFO

Article history:
Received 15 May 2018
Accepted 23 May 2018
Available online xxx

Keywords:
Neuroinflammation
Lipopolysaccharide
Yin-Yang 1
Interleukin-6
Nuclear factor kappa B

ABSTRACT

Neuroinflammation plays a critical role in the process of neurodegenerative disorders, during which microglia, the principal resident immune cells in the central nervous system, are activated and produce proinflammatory mediators. Yin-Yang 1 (YY1), a multi-functional transcription factor, is widely expressed in cells of the immune system and participate in various cellular processes. However, whether YY1 is involved in the process of neuroinflammation is still unknown. In the present study, we found that YY1 was progressively up-regulated in BV2 microglial cells stimulated with lipopolysaccharide (LPS), which was dependent on the transactivation function of nuclear factor kappa B (NF-κB). Furthermore, YY1 knockdown notably inhibited LPS-induced the activation of NF-κB signaling and interleukin-6 (IL-6) expression in BV-2 cells, but not mitogen-activated protein kinase (MAPK) signaling. Moreover, YY1 strengthened p65 binding to IL-6 promoter by interacting with p65 but decreased H3K27ac modification on IL-6 promoter, eventually increasing IL-6 transcription. Taken together, these results for the first time uncover the regulatory mechanism of YY1 on IL-6 expression during neuroinflammation responses and provide new lights into neuroinflammation.

1. Introduction

Neuroinflammation plays a critical role in initiation and development of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, stroke and traumatic brain injury and so on [1–3]. Microglia, the principal resident macrophages in the central nervous system, are the first line of defense against pathological infection and act important role in regulating neuroinflammation [4]. Microglia are rapidly activated by exogenous or endogenous stimuli such as pathogens and brain injury, and exposure to lipopolysaccharide (LPS), interferon gamma (IFN-γ), or β-amyloid [5,6]. Activated microglia produce a number of proinflammatory mediators, including inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and so on, which mediate neuroinflammatory processes and consequently contribute to neuronal death, disorders and degeneration [7,8]. Since microglia-mediated neuroinflammatory is closely involved in the development of neurodegenerative disorders [9], understanding the underlying mechanism of neuroinflammation and developing effective therapeutic targets for inhibition of microglial activation may provide potential therapeutic approaches against neurodegenerative disorders.

Numerous studies indicate that nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling including p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase (ERK), are the major pathways involved in the process of inflammation associated with microglial activation [10,11]. Under normal conditions, NF-κB subunit p65 is resident in the cytoplasm as an inactivation form. Upon stimulation by inflammatory signals, p65 is phosphorylated, and then translocates into the nucleus to trigger transcription of pro-inflammatory mediators, such as TNF-α, IL-6, IL-1β and so on [12]. While, MAPKs are critical regulators of cellular processes such as apoptosis, oxidative stress response and immune response [13,14]. More and more studies show that natural products display anti-neuroinflammatory

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https://doi.org/10.1016/j.bbrc.2018.05.159
0006-291X/

effects by suppressing the NF-κB and MAPK signaling pathways [15,16].

Yin-Yang 1 (YY1), an ubiquitously expressed zinc-finger DNA-binding transcription factor, acts multiple roles in different cellular processes, including cellular proliferation, apoptosis, differentiation, development and tumorigenesis [17,18]. YY1 has two N-terminal transactivation domains, while the C-terminal domain is required for direct DNA binding and for repression of some promoters [19]. YY1 could directly or indirectly initiate, activate or repress gene transcription, dependent on interacting partners, promoter context and chromatin structure [20–22]. Previous study showed that YY1 regulates effector cytokine gene expression and Th2 immune responses by regulating chromatin remodeling and chromosomal looping of the Th2 cytokine locus [23]. Besides, YY1, which is regulated by NF-κB through promoter binding in undifferentiated myoblasts, inhibits skeletal myogenesis through transcriptional silencing of myofibrillar genes [24]. However, whether YY1 is involved in the process of neuroinflammation and its underlying mechanism are still unknown.

In the present study, the role of YY1 on LPS-triggered neuroinflammation in BV2 microglial cells was investigated. We found YY1 was progressively up-regulated in BV2 cells stimulated with LPS, which was dependent on the transactivation function of NF-κB. YY1 knockdown suppressed LPS-induced the activation of NF-κB signaling and IL-6 expression in BV-2 cells. Moreover, YY1 strengthened p65 binding to IL-6 promoter by interacting with p65 but decreased H3K27ac modification on IL-6 promoter, eventually increasing IL-6 transcription. These results for the first time uncover the regulatory mechanism of YY1 on IL-6 expression during neuroinflammation responses and provide new lights into neuroinflammation.

2. Materials and methods

2.1. Cells and reagents

BV2 and HEK293T cells, obtained from ATCC, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, Penicillin/Streptomycin at 37 °C in 5% CO2. LPS (1 μg/mL) and the NF-κB inhibitor PDTC (10 μM) were purchased from Beyotime Biotechnology.

2.2. siRNA and plasmids

siRNA against YY1 (si-YY1) and scrambled siRNA (si-NC) were synthetized by Shanghai GenePharma Co., Ltd, as described previously [25]. Full-length mouse YY1 gene, YY1n (N-terminal domain, 1-200aa), and YY1c (C-terminal domain, 201-414aa) were amplified with following primers: 5′-GCCATGGAGGCCCGAATTCGGATGGCCTCGGGCGACACCCTC-3′ and 5′-GGCCGCGGTACCTCGAGTCACTGGTTGTTTTTTGGC-3′, 5′-GCCATGGAGGCCCGAATTCGGATGGCCTCGGGCGACACCCTC-3′ and 5′-GGCCGCGGTACCTCGAGGGTCGGCGCCGCCGCCGCCC-3′, and 5′-GCCATGGAGGCCCGAATTCGGCGGGGAATAAGAAGTGGGAG-3′ and 5′-GGCCGCGGTACCTCGAGTCACTGGTTGTTTTTTGGC-3′, respectively, and then cloned into pCMV-flag-N-vector. Mouse YY1 promoter (−774 to +69) and IL-6 (−1500 to +36) promoter were amplified using following primers: 5′-AAGACATGGAATTTTCAAAGAGC-3′ and 5′-GAAGGCAGAGGGAGGAACAGCGGG-3′, and 5′-CAAGGCCGACTACACTCTTCATCCCACAATA-3′ and 5′-GACTCAGAACACATCCCAATCATGCCA-3′, respectively, and then cloned into pGL3 basic plasmid. A mutant version of YY1 reporter plasmid containing a mutant NF-κB p65 binding site (YY1-MUT-Luc) was generated using a Mut Express II Fast Mutagenesis Kit (Vazyme Biotech, China). All constructs were confirmed by DNA sequencing. Plasmids and siRNAs were transfected using Exfect Transfection Reagent (Vazyme Biotech, China) in the indicated cells, as described previously [25].

2.3. Western blotting

After treatment, BV2 cells were collected and then lysed with NP40 lysis buffer (Beyotime, China). Whole cell lysates were subjected to SDS-PAGE and immunoblotting. Primary antibodies against YY1 (ab109237, Abcam), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ab8245, Abcam), p38 (ab170099, Abcam), phosphorylated p38 (p-p38) (ab47363, Abcam), JNK (ab208035, Abcam), phosphorylated JNK (*p*-JNK) (ab219584, Abcam), ERK1/2 (ab196883, Abcam), phosphorylated ERK1/2 (*p*-ERK1/2) (ab214362, Abcam), NF-κB p65 (6956, Cell Signal Technology), phosphorylated NF-κB p65 (p-p65) (ab86299, Abcam), flag (ab18230, Abcam), HDAC1 (ab19845, Abcam) were used. Co-immunoprecipitation was performed as previously described [25,26].

2.4. Quantitative real-time PCR (qRT-PCR)

qRT-PCR was performed as previously described [25,26]. qRT-PCR primers were as follows: YY1, 5′-GTGGTTGAAGAGCAGATCATTGG-3′ and 5′-TTGCTTAGGGTCTGAGAGGTC-3’; TNF-α, 5′-ACAAGCCTGTAGCCCACGTC-3′ and 5′-AAGACTCCTCCCAGGTATATGG-3’; IL-6, 5′-GATTTACATAAAATAGTCCTTCCTACC-3′ and 5′-GGTTTGCCGAGTAGATCTCAAAGTG-3’; IL-1β, 5′-ACGATGCACCTGTACGATCA-3′ and 5′-TCTTTCAACACGCAGGACAG-3’; GAPDH, 5′-TCAACAGCAACTCCCACTCTTCCA-3′ and 5′-ACCCTGTTGCTGTAGCCGTATTCA-3’.

2.5. Immunofluorescent

Immunofluorescent was performed as previously described [26,27].

2.6. ELISA

TNF-α and IL-6 in the cultural supernatants were measured by TNF alpha Mouse ELISA Kit (BMS607-3TEN, ThermoFisher Scientific) and IL-6 Mouse ELISA Kit (BMS603-2TWO, ThermoFisher Scientific), respectively, according to the manufacturer's instructions.

2.7. Luciferase assay

HEK293T cells were co-transfected with the mixture of indicated luciferase reporter plasmid, pRL-TK-Renilla-luciferase plasmid, and pCMV-flag-p65 plasmid, pCMV-flag-YY1 plasmid or its truncated plasmid. Total amounts of plasmid DNA were equalized via empty control vector. After 24 h, the cells were then treated with or without LPS for 12 h. Luciferase activities were measured with Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Data are normalized for transfection efficiency by dividing Firefly luciferase activity with that of Renilla luciferase.

2.8. Chromatin immunoprecipitation (ChIP) assays

ChIP assays were performed with an Agarose ChIP Kit (Thermo Scientific), according to the manufacturer's instructions. The cells were lysed, and the chromatin was mainly fragmented to 320 bp by prior digestion with micrococcal nuclease. DNA/protein complexes were precipitated by overnight incubation with 4 μg antibodies against NF-κB p65 (6956, Cell Signal Technology), YY1 (ab38422, Abcam), H3K4me3 (ab8580, Abcam), H3K27ac (ab177178, Abcam) or with IgG (ab171870, Abcam), and then incubated with Protein A/

Please cite this article in press as: X.-C. Zhang, et al., YY1 promotes IL-6 expression in LPS-stimulated BV2 microglial cells by interacting with p65 to promote transcriptional activation of IL-6, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.159