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Activation of the HMGB1-RAGE axis upregulates TH expression in dopaminergic neurons via JNK phosphorylation

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ABSTRACT

The derangement of tyrosine hydroxylase (TH) activity reduces dopamine synthesis and is implicated in the pathogenesis of Parkinson's disease. However, the extracellular modulator and intracellular regulatory mechanisms of TH have yet to be identified. Recently, high-mobility group box 1 (HMGB1) was reported to be actively secreted from glial cells and is regarded as a mediator of dopaminergic neuronal loss. However, the mechanism for how HMGB1 affects TH expression, particularly through the receptor for advanced glycation endproducts (RAGE), has not yet been investigated. We found that recombinant HMGB1 (rHMGB1) upregulates TH mRNA expression via simultaneous activation of JNK phosphorylation, and this induction of TH expression is blocked by inhibitors of RAGE and JNK. To investigate how TH expression levels change through the HMGB1-RAGE axis as a result of MPP^+ toxicity, we co-treated SN4741 dopaminergic cells with MPP^+ and rHMGB1. rHMGB1 blocked the reduction of TH mRNA following MPP^+ treatment without altering cell survival rates. Our results suggest that HMGB1 upregulates TH expression to maintain dopaminergic neuronal function via activating RAGE, which is dependent on JNK phosphorylation.

1. Introduction

Neuroinflammation in the brain has been regarded to be the pathogenic mechanism of neuronal degeneration in Parkinson's disease (PD) [1]. Environmental factors, including insults with toxins and/or noxious agents, can trigger an inflammatory response and release various molecular mediators, such as cytokines and damage associated molecular patterns (DAMPs), from reactive glial cells in the brain [2]. These cytokines and DAMPs induce neuronal damage or protective signals; however, there are no reports showing a relationship between these molecular mediators and TH expression in dopaminergic neurons [3,4].

TH [EC 1.14.16.2] is a rate-limiting enzyme in the biosynthesis of catecholamine (CA) neurotransmitters, which catalyzes the conversion of L-tyrosine to 3,4-dihydroxy-1-phenylalanine (L-DOPA). Reduction of TH expression depletes dopamine in the nigrostriatal pathway and leads to the motor symptoms attributed to PD. Given the relevance and close association between TH activity and the occurrence of PD motor symptoms the regulatory mechanism of the relationship must to understand the pathogenesis underlying PD. According to a recent report, short-term regulation of TH activity can be controlled by enzymatic phosphorylation and feedback inhibition. In addition to short-term regulation of it, molecules involved in long-term regulation of TH activity have been reported and include the cAMP response element-binding (CREB) protein [5], activator protein 1 (AP-1) [6] and activating transcription factor

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2 (ATF-2) [7], all of which were identified to have binding sites in the TH promoter. Moreover, Nuclear receptor related 1 (Nurr1) was also reported to be necessary to maintain the expression of TH and the synthesis of DA in the later stages of DA neuronal differentiation [8]. However, the roles of cytokines and DAMPs-in mediating TH modulation have not yet been studied.

HMGB1 is one of the most important chromatin proteins, similar to the histones. Although HMGB1 binds to DNA and promotes the interaction of transcription factors with specific DNA elements in the nucleus [9], secreted HMGB1 can act as a cytokine that mediates inflammation by binding to other molecules, such as the toll-like receptor (TLR), RAGE and other cytokines [10]. After secreted HMGB1 binds to TLR or RAGE, the downstream signaling pathway [e.g., AP-1, nuclear factor-κB (NF-κB), IFN regulatory factor-3 (IRF3), and phosphoinositide 3-kinase (PI3K)] is activated, producing a variety of functional responses, such as innate immune cells activation, induction of proinflammatory cytokines, stimulation of cell adhesion, migration, promotion of cell proliferation and angiogenesis [11]. Recently, HMGB1 was found to mediate dopaminergic neuronal loss following release from glial cells in a sub-acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model [12,13]. However, the molecular mechanism responsible for the regulation of HMGB1-mediated TH expression following MPTP administration and prior to neuronal cell loss has not been elucidated in dopaminergic neurons. Here, we studied for correlation between the HMGB1–RAGE axis and TH expression and revealed the underlying mechanism of how the HMGB1-RAGE axis modulates TH expression *in vitro* following MPP^+ treatment.

2. Materials and methods

2.1. Cell lines and culture conditions

The dopaminergic neuronal progenitor cell line (SN4741) was cultured as described previously [14]. SN4741 cells were grown in RF medium containing Dulbecco's Modified Eagle's medium from Gibco (Grand Island, NY) supplemented with 10% fetal bovine serum from Gibco (Grand Island, NY), 1% glucose from Amresco (Solon, OH, USA), 1% penicillin-streptomycin and 2 mM L-glutamine at 33 °C with 5% CO_2 without differentiation, as described previously [14].

2.2. Chemicals, reagents and antibodies

Oligomycin (O4876), carbonyl cyanide *m*-chlorophenyl hydrazine (CCCP, C2759), rotenone (R8875), 1-methyl-4-phenylpyridinium (MPP^+, D048) and the JNK inhibitor (SP600125, S5567) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anti-RAGE (rabbit polyclonal, ab3611) were purchased from Abcam (Cambridge, MA, USA). Anti-phospho JNK (rabbit polyclonal, #9251), anti-total JNK (rabbit polyclonal, #9252), anti-phospho p65 (rabbit monoclonal, #3033) and anti-total p65 (rabbit monoclonal, #8242), were purchased from Cell Signaling Technology (Beverly, MA, USA). β-actin and α-tubulin rabbit polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Recombinant human HMGB1 (1690-HMB) was purchased from R&D system (Minneapolis, MN, USA). RAGE antagonist/inhibitor

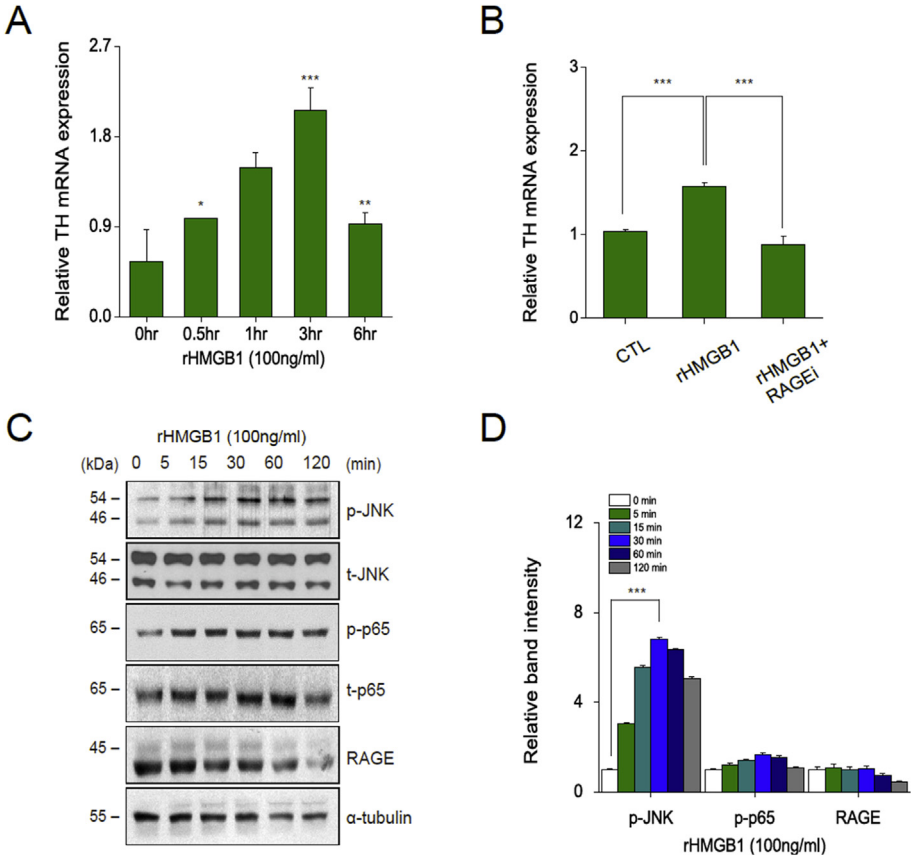

Fig. 1. HMGB1-RAGE axis increases TH expression and increases JNK phosphorylation. (A) The mRNA expression of tyrosine hydroxylase (TH) was analyzed by real-time PCR in SN4741 cells following treatment with HMGB1 100 ng/ml (0–6 h) (n = 5). **(B)** The mRNA expression of TH in dopaminergic neurons, treated with 100 nM of the RAGE inhibitor and HMGB1 (100 ng/ml) for 3 h were assessed by real-time PCR. **(C)** Protein levels and quantification of *p*-JNK and p-p65 were analyzed by Western blotting of cells stimulated with 100 ng/ml of recombinant HMGB1 for up to 120 min in SN4741 cells (n = 5). **(D)** Quantification of band intensity of pJNK, p-p65 and RAGE, presented in panel A. Data are given as the mean and SD from three experiments under the same conditions; ns, not significant; ***p < 0.001 (one-way ANOVA).