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Regulation of hemeoxygenase-1 gene expression by Nrf2 and c-Jun in tertiary butylhydroquinone-stimulated rat primary astrocytes

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ABSTRACT

Hemeoxygenase-1 (HO-1) is a phase II antioxidant enzyme that is primarily involved in detoxification and cytoprotection in a variety of tissues. However, the mechanism underlying *HO-1* gene expression remains unclear. In the present study, we investigated the regulation of HO-1 expression in primary cultured astrocytes by using the natural antioxidant compound tertiary butylhydroquinone (tBHQ). We found that tBHQ increased HO-1 mRNA and protein levels. Promoter analysis revealed that tBHQ enhanced *HO-1* gene transcription in an antioxidant response element (ARE)-dependent manner. In addition, tBHQ increased the nuclear translocation and DNA binding of Nrf2 and c-Jun to ARE. Small interfering RNA (siRNA) experiments demonstrated that Nrf2 and c-Jun are involved in the differential modulation of HO-1 expression. Thus, Nrf2 knockdown reduced the basal level of HO-1 expression but did not affect the fold induction by tBHQ. On the other hand, knockdown of c-Jun diminished tBHQ-mediated induction of HO-1 without affecting basal expression. The data suggest that Nrf2 generally modulates the basal expression of HO-1, while c-Jun mediates HO-1 induction in response to tBHQ. The results of co-immunoprecipitation assays demonstrated a physical interaction between Nrf2 and c-Jun in tBHQ-treated astrocytes. The results suggest that Nrf2 and c-Jun regulate HO-1 expression via their coordinated interaction in tBHQ-treated rat primary astrocytes.

1. Introduction

Astrocytes play an essential role in the brain by interacting with surrounding neurons to provide metabolic, structural, and trophic support; they also actively participate in the regulation of chemical neurotransmission [1,2]. In addition, astrocytes control oxidative stress and maintain a homeostatic environment for neurons. Various antioxidant genes are preferentially expressed in astrocytes, facilitating more efficient detoxification and defense systems compared to neurons [1,3,4].

Hemeoxygenase (HO), the rate-limiting enzyme in heme degradation, catalyzes the oxidation of heme to carbon monoxide (CO), biliverdin, and ferrous ion. HO-1, HO-2, and HO-3 have been isolated and characterized in mammals [5,6]. Under oxidative stress, HO-1 plays a role as an antioxidative defense factor in different cell types in the brain and other organs [7–9]. Many recent studies have investigated the diversity of chemicals that could up- or downregulate HO-1 activity and expression. HO-1 expression is upregulated following cellular stimulation with cytotoxic agents, including arsenite, ultraviolet (UV) radiation, quinones, and hydrogen peroxide. A number of therapeutic compounds, such as curcumin, resveratrol, and ginsenosides, have also been shown to induce HO-1 [10]. On the other hand, HO-1 is often highly upregulated in tumor tissues, and its overexpression can inhibit tumor cell apoptosis and promote angiogenesis, tumor growth, and metastasis [11–14]. Therefore, therapeutic approaches that up- or downregulate HO-1 expression are of clinical interest.

The regulation of HO-1 expression occurs primarily at the level of transcription [5]. The *HO-1* gene contains two inducible enhancers, E1 and E2. Each enhancer region contains three copies of a *cis*-acting element termed the antioxidant response element (ARE). An analysis of ARE-nuclear protein complexes have identified several nuclear transcription factors, including Nrf1, Nrf2, c-Jun, c-Fos, Fra1, MafK, aromatic hydrocarbon receptor, and the estrogen receptor. After Nrf2 translocates to the nucleus, it usually heterodimerizes with other transcription factors to regulate ARE-

Abbreviations: AP-1, activator protein-1; ARE, antioxidant response element; EMSA, electrophoretic mobility shift assay; HO-1, hemeoxygenase-1; Nrf2, nuclear factor-erythroid 2 (NF-E2)-related factor 2; siRNA, small interfering RNA; tBHQ, tertiary butylhydroquinone.

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mediated antioxidant gene expression [5]. However, the detailed roles of these transcription factors in HO-1 gene expression have not been clearly demonstrated.

In the present study, we analyzed the possible roles of Nrf2 and c-Jun and their interaction in HO-1 expression in rat primary astrocytes stimulated with tertiary butylhydroquinone (tBHQ), a well-known antioxidant that is widely used in mechanistic studies of antioxidant effects [15]. Here we demonstrate that Nrf2 and c-Jun modulate HO-1 transcription by coordinately binding to AREs and via their physical interaction with each other. Furthermore, we identified differential roles of Nrf2 and c-Jun in tBHQ-mediated HO-1 upregulation in rat primary astrocytes.

2. Materials and methods

2.1. Reagents

All reagents used for cell culture were purchased from Gibco BRL (Grand Island, NY, USA). tBHQ and antibodies against β-actin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Antibodies against c-Jun, c-Fos, HO-1, lamin B, Nrf1, and Nrf2 were purchased form Cell Signaling Technology (Danvers, MA, USA). All reagents used for reverse transcription polymerase chain reaction (RT-PCR) and oligonucleotides for electrophoretic mobility shift assay (EMSA) were purchased from Promega (Madison, WI, USA).

2.2. Rat primary astrocyte cell culture

Primary astrocyte cultures were prepared from mixed glial cultures using a previous method with modifications [16]. In brief, after cortices were dissected from 2-day-old rats, cells were dissociated by pipetting and resuspended in minimal essential medium containing 10% fetal bovine serum, streptomycin (10 μg/mL), penicillin (10 U/mL), 2 mM glutamine, and 10 mM HEPES. Cell suspensions were plated on poly-D-lysine (1 μg/mL)-coated T75 flasks and incubated for 7–10 days. After the primary cultures reached confluence, the culture flasks were shaken at 280 rev/min for 16 h to remove microglia and oligodendrocytes. The purity of the astrocyte-enriched cultures (>95%) were confirmed by staining with antibodies against the astrocyte-specific marker glial fibrillary acidic protein.

2.3. Western blot analysis

Total cell lysates and nuclear extracts were prepared as described previously [17]. The proteins (20–100 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membranes (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA). The membranes were blocked with 5% bovine serum albumin in 10 mM Tris–HCl containing 150 mM NaCl and 0.5% Tween-20 (TBST), then incubated with primary antibodies (1:1000) that recognized HO-1, Nrf1, Nrf2, c-Jun, or c-Fos. After thoroughly washing with TBST, horseradish peroxidase-conjugated secondary antibodies (1:2000 dilution in TBST; Amersham, Little Chalfont, UK) were applied, and the blots were developed using an enhanced chemiluminescence detection kit (Thermo Scientific, Waltham, MA, USA).

2.4. RT-PCR

To analyze HO-1 mRNA expression, primary astrocytes were treated with tBHQ, and the total RNA was isolated with TRI reagent (Ambion, Austin, TX, USA). For RT-PCR, total RNA (1 μg) was reverse transcribed in a reaction mixture containing 1 U of RNase inhibitor, 500 ng of random primers, 3 mM $MgCl_2$, 0.5 mM dNTP,

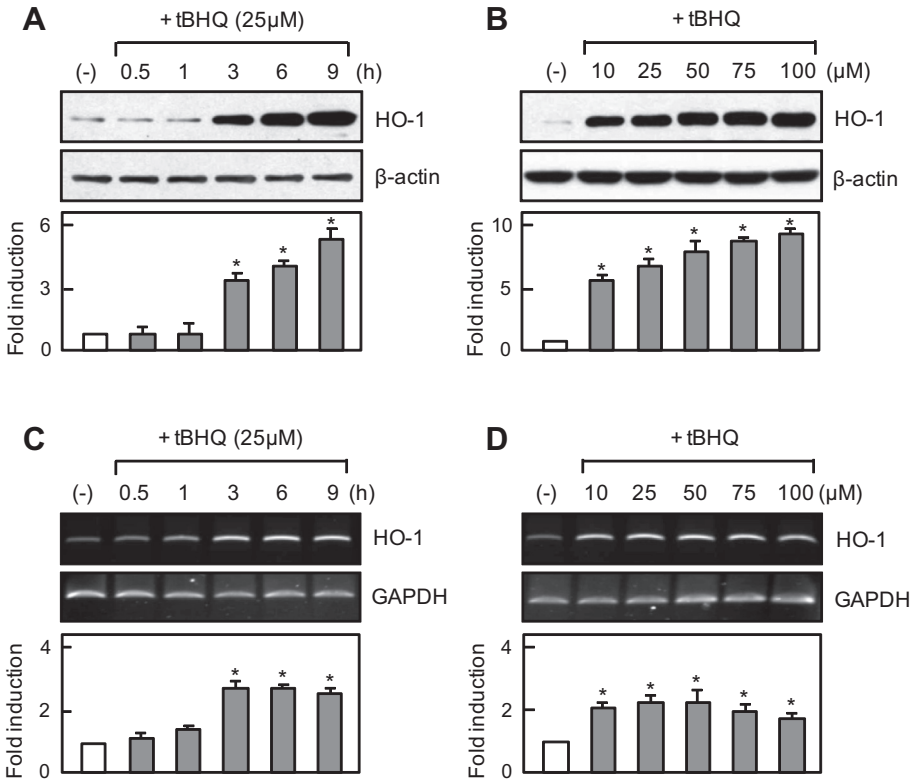

Fig. 1. Effect of tBHQ on HO-1 expression in rat primary astrocytes. (A) Cells were incubated with 25 μM tBHQ for the indicated times, and Western blot analyses were performed with an HO-1 antibody. (B) HO-1 protein expression was detected in cells treated with various concentrations of tBHQ for 9 h. (C and D). HO-1 mRNA levels were determined by RT-PCR analysis. The data are representative of three independent experiments. Quantification data are shown at the bottom of each panel. Values are the mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$ compared with the control group.

1 × RT buffer, and 10 U reverse transcriptase (Promega). The synthesized cDNA was used as a template for the PCR reaction using GoTaq polymerase (Promega) and the following primers: 5′-ATACCCGCTACCTGGGTGAC-3′ (sense) and 5′-TGTCACCCTGTGC TTTGACCT-3′ (anti-sense) for HO-1 and 5′-ACAGTCTTCTGAGTG GCAG TCA-3′ (sense) and 5′-GTGCTGAGTATGTCGTGGAGTC-3′ (anti-sense) for glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2.5. Transient transfection and luciferase assays

The wild-type (E1 and E2) and ARE site-mutated (E1-M739 and E2-M45) HO-1 promoter/luciferase reporter plasmids were kindly provided by Dr. Alam (Ochsner Clinic Foundation, New Orleans, LA, USA) [18]. E1 and E2 are mouse HO-1 promoters containing three ARE sequences, and E1-M739 and E2-M45 have mutations in three ARE core sequences. Transfection of the reporter plasmids into primary astrocyte cells was performed using Convoy™ transfection reagent (ACTGene, Inc., Piscataway, NJ, USA). After 48 h, cells were harvested, and luciferase assays were performed as previously described [19]. Transfection of Nrf2 or c-Jun-specific siRNA into astrocyte cells was performed using siRNA transfection reagent according to the manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.6. EMSA

Nuclear extracts were prepared from astrocytes as previously described [19]. The double-stranded DNA oligonucleotides containing the ARE consensus sequences (Promega) were end-labeled by [γ-32P]ATP. Five micrograms of the nuclear proteins were incubated with ^{32}P-labeled ARE probes on ice for 30 min and resolved on a 5% acrylamide gel as previously described [19]. For the supershift assay, antibodies against Nrf1, Nrf2, c-Jun, or c-Fos were co-incubated with the nuclear extract mixture for 30 min at 4 °C before adding the radiolabeled probe.

2.7. Co-immunoprecipitation assay

Nuclear extracts prepared as described for EMSA were incubated with anti-Nrf2 or anti-c-Jun antibodies for 4 h at 4 °C on a rotator. PureProteome™ Protein A magnetic beads (Millipore, Billerica, MA, USA) were added, and the precipitated proteins were subjected to 12% SDS–PAGE followed by Western blot analyses.

2.8. Statistical analysis

Unless otherwise stated, all experiments were performed with triplicate samples and were repeated at least three times. The data

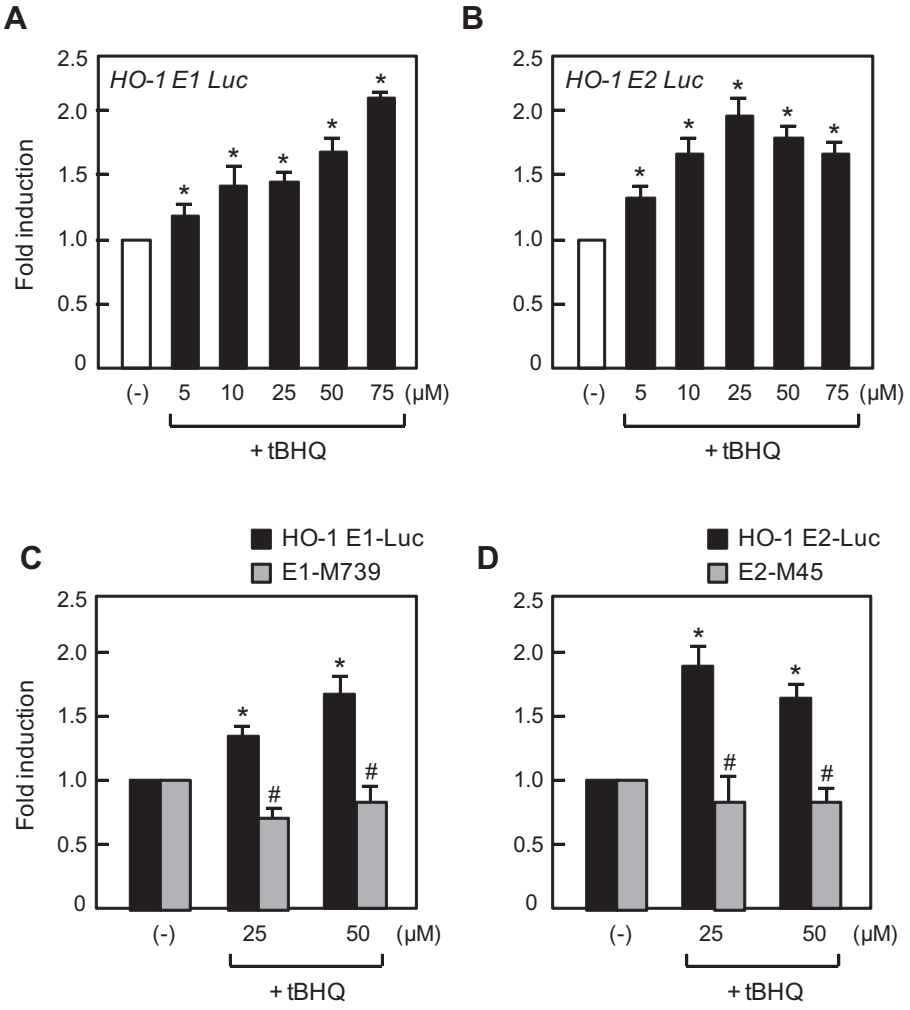

Fig. 2. tBHQ increased HO-1 promoter activity in an ARE-dependent manner. (A and B) Primary astrocytes were transfected with HO-1 E1 and E2 reporter plasmids and treated with tBHQ. After 24 h, cells were harvested, luciferase assays were performed. Values correspond to the mean ± S.E.M of three independent experiments. $^{*}P < 0.05$ compared with control cells. (C and D) Primary astrocytes were transfected with wild-type or ARE triple mutants of HO-1 reporter plasmids (E1-M739 and E2-M45) prior to tBHQ treatment. After 24 h of incubation, cells were harvested, and luciferase assays were performed. Values correspond to the mean ± S.E.M of three independent experiments performed in duplicate. $^{*}P < 0.05$ compared with the control group. $^{\#}P < 0.05$ compared with wild-type HO-1 E1 or E2 promoter activity.

are presented as mean ± S.E.M., and statistical comparisons between the groups were performed using one-way analysis of variance (ANOVA), followed by Student's *t*-tests. A P value <0.05 was considered significant.

3. Results

3.1. tBHQ increased HO-1 expression in rat primary astrocytes

Western blot and RT-PCR analyses were performed to examine the effect of tBHQ on HO-1 expression. As shown in Fig. 1A, HO-1 was increased 3 and 9 h after tBHQ treatment. In addition, we found that 9 h of tBHQ treatment upregulated HO-1 expression in a concentration-dependent manner (Fig. 1B). Maximal HO-1 induction was observed at 50 μM. Similarly, tBHQ increased HO-1 mRNA expression as shown by RT-PCR analysis (Fig. 1C and D).

3.2. tBHQ enhanced HO-1 promoter activity in an ARE-dependent manner

Next, we examined the effect of tBHQ on the activities of HO-1 promoter, which contains enhancer E1 and E2. Each enhancer contains three copies of ARE. We found that tBHQ increased HO1-E1 and E2-luc activities (Fig. 2A and B). To address whether or not the increment in HO-1 promoter activities by tBHQ is mediated through ARE, the cells were transfected with ARE site-mutated E1-M739 and E2-M45 plasmid constructs, and transcriptional activities were compared with those of the wild-type promoter. tBHQ treatment increased HO1-E1 and -E2 promoter activity; however, luciferase activity in cells transfected with E1-M739 and E2-M45 was not increased (Fig. 2C and D). The results suggest that tBHQ enhanced HO-1 promoter activity in an ARE-dependent manner.

3.3. tBHQ increased nuclear translocation of Nrf2 and c-Jun and their binding to ARE

We performed gel shift assays to identify transcription factors bound to ARE. As shown in Fig. 3A, tBHQ increased nuclear protein binding to ARE (Fig. 3A). Because Nrf1, Nrf2, and AP1 complexes are known to bind to the composite sequence of ARE, we examined nuclear translocation and performed antibody supershift assays for these transcription factors. We found that tBHQ increased the nuclear translocation of Nrf1, Nrf2, c-Jun, and c-Fos in a time-dependent manner (Fig. 3B). However, the antibody supershift assay revealed that the transcription factors bound to ARE were Nrf2 and c-Jun; the DNA-protein complexes were diminished by antibodies against Nrf2 or c-Jun but not by Nrf1 or c-Fos antibodies (Fig. 3C).

3.4. The interaction between Nrf2 and c-Jun plays a critical but differential role in HO-1 expression in BHQ-treated primary astrocytes

Because Nrf2 usually forms heterodimers with other transcription factors to regulate ARE-mediated gene expression [20,21], we examined whether Nrf2 and c-Jun mediate HO-1 expression. Astrocytes were transfected with Nrf2- or c-Jun-specific siRNA prior to tBHQ treatment. We confirmed that Nrf2 or c-Jun protein expression was significantly inhibited by their specific siRNA but not by control siRNA (Fig. 4A). Intriguingly, we found that Nrf2 knockdown reduced the basal level of HO-1 expression by 70% compared with control siRNA-transfected cells (Fig. 4B). However, Nrf2 siRNA did not affect the fold induction by tBHQ (approximately 1.9 fold), even though the absolute HO-1 expression level was reduced to basal levels. In contrast, knockdown of c-Jun abolished tBHQ-mediated HO-1 induction without affecting its basal expression (Fig. 4B). Similar expression change patterns were observed in HO-1 E1-luc reporter gene assays (Fig. 4C). Thus, the data collectively suggest that Nrf2 modulates basal HO-1 expression, whereas c-Jun mediates HO-1 induction in response to tBHQ. Concomitant knockdown of these two transcription factors abolished both the basal and induced expression of HO-1 (Fig. 4D).

To address the possible interaction between Nrf2 and c-Jun, coimmunoprecipitation assays was performed using nuclear extracts from primary astrocytes. The data confirmed an interaction between Nrf2 and c-Jun, which was increased by tBHQ treatment (Fig. 4E). The results suggest that Nrf2 and c-Jun regulate HO-1 expression by coordinated interaction after binding to ARE in tBHQ-treated rat primary astrocytes.

4. Discussion

Astrocytes are enriched with antioxidant enzymes that protect them and surrounding neuronal cells against oxidative stress

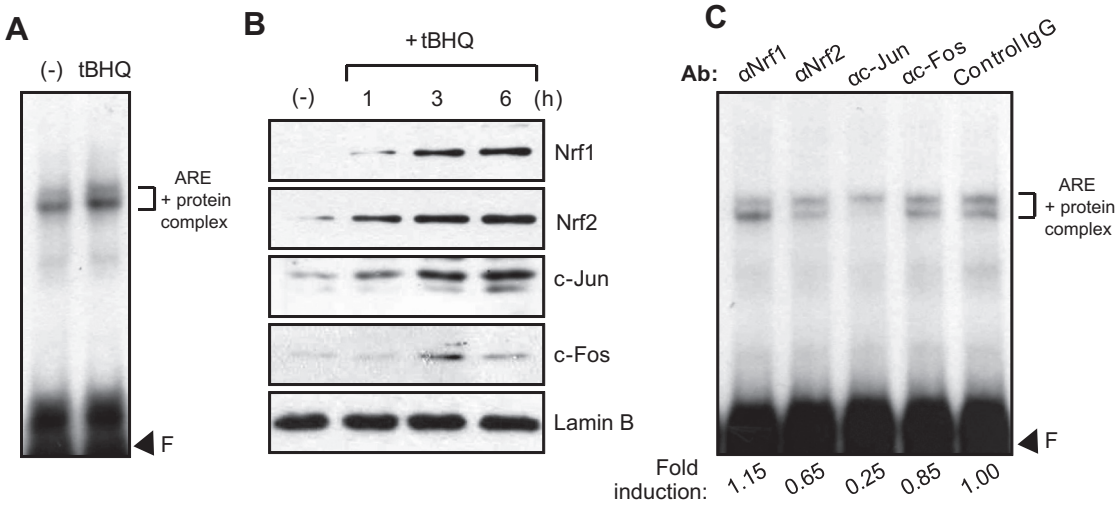

Fig. 3. Effect of tBHQ on nuclear protein binding to ARE and nuclear translocation of transcription factors (A) nuclear extracts were prepared from primary astrocytes after treatment with tBHQ (25 μM) for 3 h and incubated with a ^{32}P-labeled ARE probe. The bracket indicates the ARE-nuclear protein complex. 'F' indicates the free probe. (B) The nuclear extracts used for EMSAs were transferred to nitrocellulose membranes and incubated with antibodies against Nrf1, Nrf2, c-Jun, or c-Fos. tBHQ significantly increased the nuclear translocation of Nrf1, Nrf2, c-Jun, and c-Fos. (C) An antibody supershift assay showed that the DNA-protein complex contained Nrf2 and c-Jun but not Nrf1 or c-Fos. The data are representative of three independent experiments.

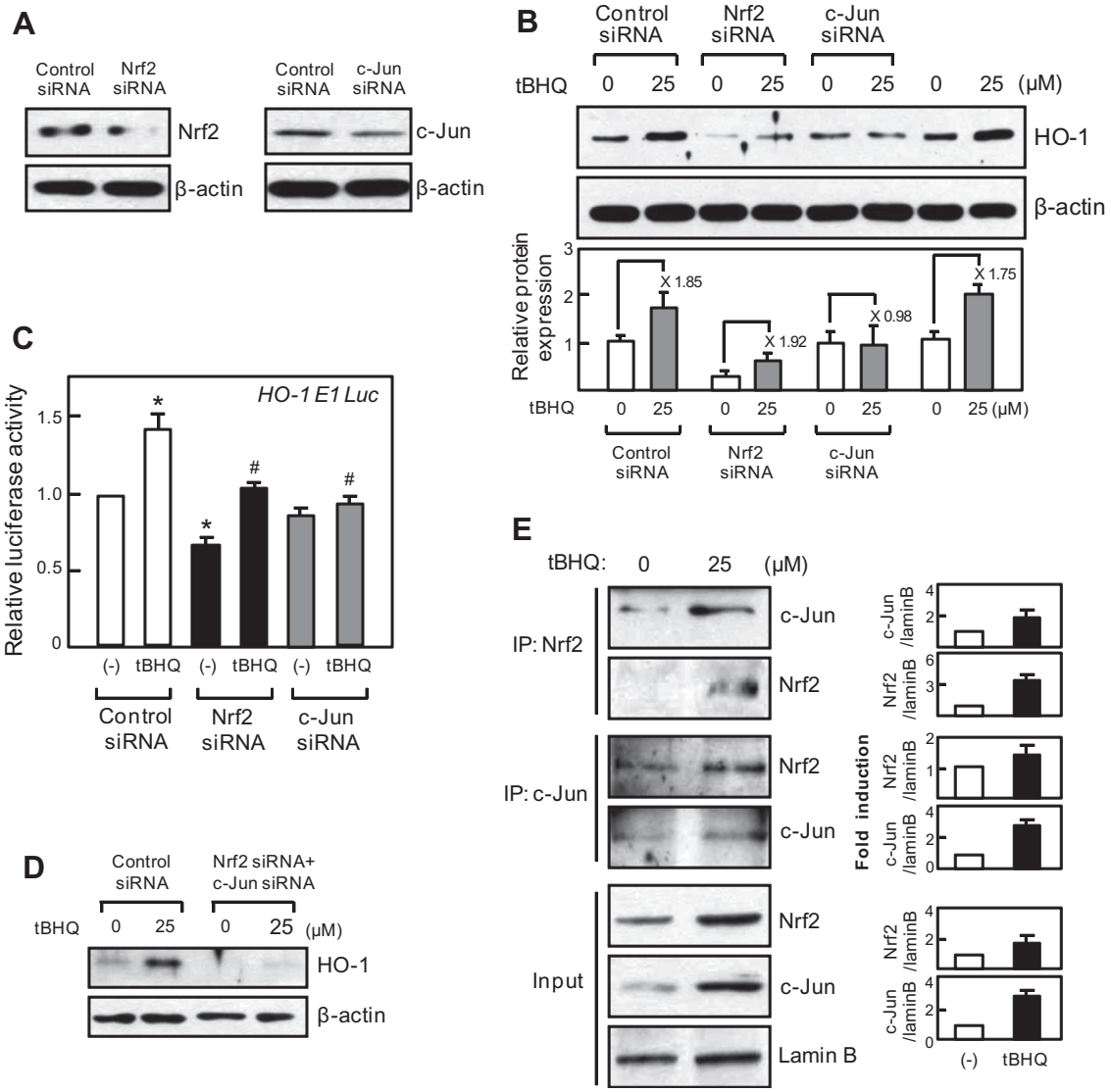

Fig. 4. Differential roles of Nrf2 and c-Jun in basal and tBHQ-induced HO-1 expression. (A) Astrocytes were transfected with Nrf2 or c-Jun siRNA for 24 h, and whole cell lysates were subjected to Western blot analyses using antibodies against Nrf2 or c-Jun. (B) Cells were transfected with Nrf2 or c-Jun siRNA and treated with tBHQ (25 μM) for 6 h. The cell lysates were subjected to Western blot analyses using an antibody against HO-1. The inserted values indicate relative protein expression in comparison with control siRNA-treated samples, the basal level of which was arbitrarily set to 1.0. Values are the mean ± S.E.M. of three independent experiments. The fold inductions in response to tBHQ are indicated for each group. (C) Cells were co-transfected with HO-1 E1-Luc reporter plasmid and siRNA against Nrf2, c-Jun, or control siRNA. Luciferase activity was then assayed after the cells were stimulated with tBHQ (25 μM) for 24 h. Relative luciferase activities are shown compared with the control siRNA-transfected group, which was arbitrarily set to 1.0. Values are the mean ± S.E.M. of three independent experiments. $^{*}P < 0.05$ compared with control siRNA-transfected cells in the absence of tBHQ. $^{\#}P < 0.05$ compared with control siRNA-transfected cells in the presence of tBHQ. (D) Astrocytes co-transfected with Nrf2 + c-Jun siRNA were stimulated with tBHQ for 6 h, and HO-1 expression was measured with Western blot analyses. The data are representative of three independent experiments. (E) Protein–protein interactions between Nrf2 and c-Jun were examined in tBHQ-treated astrocytes by coimmunoprecipitation assays. The nuclear proteins were immunoprecipitated with an anti-Nrf2 antibody, and then Western blot analyses were performed using antibodies against c-Jun and vice versa. Amounts of the precipitated proteins were determined with antibodies against Nrf2 or c-Jun, and input controls before immunoprecipitation were also assessed. Representative data from three independent experiments are shown in the left panel, and the densitometry quantifications are shown in the right panel.

[22,23]. Phase II antioxidant enzymes, such as HO-1, NAD(P)H:quinone oxidoreductase 1 (NQO1), and manganese superoxide dismutase are mainly involved in cytoprotective mechanisms of astrocytes, and their expressions are under the control of Nrf2-ARE signaling pathways [5,24]. However, the regulatory mechanisms were previously unclear.

In the present study, we analyzed the gene regulation mechanism of HO-1 in tBHQ-stimulated astrocytes. We found that tBHQ induced HO-1 expression and increased HO-1 promoter activity in an ARE-dependent manner. Because the ARE sequences usually overlap with AP-1 consensus sequences, AP-1 family transcription factors (c-Jun, Jun B, and c-Fos) are known to bind to ARE/AP1-like sequences in the promoter regions of *HO-1* and regulate gene expression in coordination with Nrf2 [18,20,25]. In agreement with these findings, our results showed that Nrf2 and c-Jun bind to ARE sequences on the HO-1 promoter and regulate *HO-1* gene expression in tBHQ-treated astrocytes. Interestingly, we found differential roles of Nrf2 and c-Jun in *HO-1* expression regulation. The knockdown experiments revealed that Nrf2 modulates the basal expression of HO-1, while c-Jun mediates the induction of HO-1 by tBHQ. Furthermore, we demonstrated a physical interaction between Nrf2 and c-Jun in the nucleus of tBHQ-treated astrocytes. Collectively, the data suggest that Nrf2 and c-Jun coordinately but differentially regulate *HO-1* expression by binding to composite ARE sequences.

A previous study described the differential roles of Nrf2 and c-Jun in arsenite-induced upregulation of HO-1 expression in murine

embryonic fibroblasts [26]. The authors showed that Nrf2 plays a crucial role in the early activation of HO-1 transcription, while c-Jun/JNK plays a role in the late phase of HO-1 expression in an Nrf2-independent manner. This is somewhat different from our present data showing that Nrf2 modulates the basal expression of HO-1, whereas c-Jun mediates the tBHQ-induced HO-1 expression in a coordinated fashion. Thus, the roles of Nrf2 and c-Jun in HO-1 expression may depend on different stimulants or cell types.

Because Nrf2 is generally known as a key transcription factor modulating HO-1 expression, most studies have focused on Nrf2 when investigating *HO-1* gene expression. Along with Nrf2, AP-1 is emerging as an important regulatory molecule in the expressions of HO-1 and other phase II antioxidant enzymes. Ectopic expression of a c-Jun dominant-negative mutant abrogates arsenite-mediated *HO-1* gene expression in chicken hepatoma cells [27]. In mouse photoreceptor-derived 661 W cells, the activation of AP-1 binding to ARE induced HO-1 upregulation by 4-hydroxynonenal [28]. c-Jun is also involved in regulating ethanol-mediated HO-1 expression *in vivo*, as livers derived from ethanol-fed c-Jun$^{flox/flox}$ mice exhibit attenuated levels of HO-1 mRNA compared with wild-type mice [29]. Moreover, c-Jun phosphorylation was reported to be associated with HO-1 expression in CA1 pyramidal neurons after transient forebrain ischemia [30]. In addition to HO-1, the coordinated modulation by Nrf2/c-Jun has also been observed for *NQO1*, glutathione S-transferase Ya subunit, and γ-glutamylcysteine synthetase gene expressions induced by various xenobiotics or antioxidants [20,25,31].

In conclusion, the present results provide the first evidence for differential roles of Nrf2 and c-Jun in HO-1 expression in rat primary astrocyte cells. We demonstrate that Nrf2 plays a key role in basal HO-1 expression, and c-Jun coordinately modulates HO-1 induction by interacting with Nrf2. These findings provide a better understanding of the gene regulation mechanism of *HO-1* and other phase II antioxidant enzymes that are under the control of AREs.

Acknowledgments

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References

[1] Y. Chen, R.A. Swanson, Astrocytes and brain injury, J. Cereb. Blood Flow Metab. 23 (2003) 137–149.
[2] G. Dallérac, O. Chever, N. Rouach, How do astrocytes shape synaptic transmission, insights from electrophysiology, Front. Cell Neurosci. 7 (2013) 159.
[3] H.E. de Vries, M. Witte, D. Hondius, A.J. Rozemuller, B. Drukarch, J. Hoozemans, J. van Horssen, Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease, Free Radical Biol. Med. 45 (2008) 1375–1383.
[4] M.R. Vargas, J.A. Johnson, The Nrf2-ARE cytoprotective pathway in astrocytes, Expert Rev. Mol. Med. 11 (2009) e17.
[5] S.W. Ryter, J. Alam, A.M. Choi, Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications, Physiol. Rev. 86 (2006) 583–650.
[6] S. Hayashi, Y. Omata, H. Sakamoto, Y. Higashimoto, T. Hara, Y. Sagara, M. Noguchi, Characterization of rat heme oxygenase-3 gene. Implication of processed pseudogenes derived from heme oxygenase-2 gene, Gene 336 (2004) 241–250.
[7] A. Jazwa, A. Cuadrado, Targeting heme oxygenase-1 for neuroprotection and neuroinflammation in neurodegenerative diseases, Curr. Drug Targets 11 (2010) 1517–1531.
[8] D.D. Haines, I. Lekli, P. Teissier, I. Bak, A. Tosaki, Role of haeme oxygenase-1 in resolution of oxidative stress-related pathologies: focus on cardiovascular, lung, neurological and kidney disorders, Acta Physiol. (Oxf) 204 (2012) 487–501.
[9] E.O. Farombi, Y.J. Surh, Heme oxygenase-1 as a potential therapeutic target for hepatoprotection, J. Biochem. Mol. Biol. 39 (2006) 479–491.
[10] N.G. Abraham, A. Kappas, Pharmacological and clinical aspects of heme oxygenase, Pharmacol. Rev. 60 (2008) 79–127.
[11] M.T. Do, H.G. Kim, T. Khanal, J.H. Choi, D.H. Kim, T.C. Jeong, H.G. Jeong, Metformin inhibits heme oxygenase-1 expression in cancer cells through inactivation of Raf-ERK-Nrf2 signaling and AMPK-independent pathways, Toxicol. Appl. Pharmacol. 271 (2013) 229–238.
[12] A. Jozkowicz, H. Was, J. Dulak, Heme oxygenase-1 in tumors: is it a false friend, Antioxid. Redox Signal. 9 (2007) 2099–2117.
[13] M. Sunamura, D.G. Duda, M.H. Ghattas, L. Lozonschi, F. Motoi, J. Yamauchi, S. Matsuno, S. Shibahara, N.G. Abraham, Heme oxygenase-1 accelerates tumor angiogenesis of human pancreatic cancer, Angiogenesis 6 (2003) 15–24.
[14] H. Was, J. Dulak, A. Jozkowicz, Heme oxygenase-1 in tumor biology and therapy, Curr. Drug Targets 11 (2010) 1551–1570.
[15] R. Yu, T.H. Tan, A.N. Kong, Butylated hydroxyanisole and its metabolite tert-butylhydroquinone differentially regulate mitogen-activated protein kinases. The role of oxidative stress in the activation of mitogen-activated protein kinases by phenolic antioxidants, J. Biol. Chem. 272 (1997) 28962–28970.
[16] K.J. Min, M.S. Yang, S.U. Kim, I. Jou, E. Joe, Astrocytes induce hemeoxygenase-1 expression in microglia: a feasible mechanism for preventing excessive brain inflammation, J. Neurosci. 26 (2006) 1880–1887.
[17] J.S. Park, E.M. Park, D.H. Kim, K. Jung, J.S. Jung, E.J. Lee, J.W. Hyun, J.L. Kang, H.S. Kim, Anti-inflammatory mechanism of ginseng saponins in activated microglia, J. Neuroimmunol. 209 (2009) 40–49.
[18] J. Alam, J.L. Cook, Transcriptional regulation of the heme oxygenase-1 gene via the stress response element pathway, Curr. Pharm. Des. 9 (2003) 2499–2511.
[19] J.S. Park, M.S. Woo, D.H. Kim, J.W. Hyun, W.K. Kim, J.C. Lee, H.S. Kim, Anti-inflammatory mechanisms of isoflavone metabolites in lipopolysaccharide-stimulated microglial cells, J. Pharmacol. Exp. Ther. 320 (2007) 1237–1245.
[20] A.K. Jaiswal, Nrf2 signaling in coordinated activation of antioxidant gene expression, Free Radical Biol. Med. 36 (2004) 1199–1207.
[21] S.K. Niture, J.W. Kaspar, J. Shen, A.K. Jaiswal, Nrf2 signaling and cell survival, Toxicol. Appl. Pharmacol. 244 (2010) 37–42.
[22] J. Tanaka, K. Toku, B. Zhang, K. Ishihara, M. Sakanaka, N. Maeda, Astrocytes prevent neuronal death induced by reactive oxygen and nitrogen species, Glia 28 (1999) 85–96.
[23] G. Trendelenburg, U. Dirnagl, Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning, Glia 51 (2005) 307–320.
[24] P.J. Syapin, Regulation of heme oxygenase-1 for treatment of neuroinflammation and brain disorders, Br. J. Pharmacol. 155 (2008) 623–640.
[25] R. Venugopal, A.K. Jaiswal, Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes, Oncogene 17 (1998) 3145–3156.
[26] H. Harada, R. Sugimoto, A. Watanabe, S. Taketani, K. Okada, E. Warabi, R. Siow, K. Itoh, M. Yamamoto, T. Ishii, Differential roles for Nrf2 and AP-1 in upregulation of HO-1 expression by arsenite in murine embryonic fibroblasts, Free Radical Res. 42 (2008) 297–304.
[27] K.K. Elbirt, A.J. Whitmarsh, R.J. Davis, H.L. Bonkovsky, Mechanism of sodium arsenite-mediated induction of heme oxygenase-1 in hepatoma cells. Role of mitogen-activated protein kinases, J. Biol. Chem. 273 (1998) 8922–8931.
[28] M. Tanito, M.P. Agbaga, R.E. Anderson, Upregulation of thioredoxin system via Nrf2-antioxidant responsive element pathway in adaptive-retinal neuroprotection in vivo and in vitro, Free Radical Biol. Med. 42 (2007) 1838–1850.
[29] S.M. Yeligar, K. Machida, V.K. Kalra, Ethanol-induced HO-1 and NQO1 are differentially regulated by HIF-1alpha and Nrf2 to attenuate inflammatory cytokine expression, J. Biol. Chem. 285 (2010) 35359–35373.
[30] Y. Matsuoka, M. Okazaki, H. Zhao, S. Asai, K. Ishikawa, Y. Kitamura, Phosphorylation of c-Jun and its localization with heme oxygenase-1 and cyclooxygenase-2 in CA1 pyramidal neurons after transient forebrain ischemia, J. Cereb. Blood Flow Metab. 19 (1999) 1247–1255.
[31] J. Jeyapaul, A.K. Jaiswal, Nrf2 and c-Jun regulation of antioxidant response element (ARE)-mediated expression and induction of γ-glutamylcysteine synthetase heavy subunit gene, Biochem. Pharmacol. 59 (2000) 1433–1439.