Biochemical and Biophysical Research Communications 429 (2012) 93–98

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Transcriptional regulation of specific protein 1 (SP1) by hypoxia-inducible factor 1 alpha (HIF-1α) leads to *PRNP* expression and neuroprotection from toxic prion peptide

Jae-Kyo Jeong, Sang-Youel Park *

Bio-Safety Research Institute, College of Veterinary Medicine, Chonbuk National University, Jeonju, Jeonbuk 561-756, South Korea

ARTICLE INFO

Article history:
Received 20 October 2012
Available online 3 November 2012

Keywords:
Specific protein 1
Prp(106–126)
HIF-1α
Prion disease

ABSTRACT

Our previous study demonstrated that hypoxia-inducible factor-1 (HIF-1)-mediated neuroprotective effects are related to cellular prion protein (PrPc) gene (*PRNP*) regulation under hypoxic conditions. However, the mechanism of HIF-1α-mediated *PRNP* gene regulation in prion-mediated neurodegenerative disorders is not clear. Transcription factor specific protein 1 (SP1) is necessary for *PRNP* transcription initiation, and SP1 gene expression is regulated through HIF-1α activation under hypoxic conditions. Thus, we hypothesized that HIF-1α-mediated neuroprotection is related to the SP1 transcription pathway as a result of *PRNP* gene regulation. Inhibition of SP1 expression blocked the HIF-1α-mediated protective effect against prion-mediated neurotoxicity. Also, knockdown of HIF-1α induced downregulation of SP1 expression and sensitivity to prion-mediated neurotoxicity, whereas upregulation of SP1 transcriptional activity lead to protection against prion-mediated neuron cell death and *PRNP* gene expression even in HIF-1α depleted cells. This report is the first study demonstrating that HIF-1α-mediated SP1 expression regulates PrPc transcription, and upregulation of SP1 induced by HIF-1α plays a key role in protection from prion-mediated neurotoxicity. These studies suggest that transcription factor SP1 may be involved in the pathogenesis of prion diseases and also may be a potential therapeutic option for neurodegeneration caused by the pathological prion protein, PrPsc.

1. Introduction

Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric transcriptional activator that consists of two subunits, alpha and beta, among which HIF-1α is broadly expressed in mammalian tissues [1]. Recent studies have shown that HIF-1α has neuroprotective properties [2–4]. Wu et al. reported that accumulation of HIF-1α attenuated rotenone-induced apoptosis in a cell model of Parkinson's disease [4]. Also, our previous study suggested that prion protein fragment [PrP(106–126)]-mediated neurotoxicity was blocked by overexpression of HIF-1α [2]. These observations suggest that regulation of HIF-1α activation may have therapeutic benefits in neurodegenerative disorders, including prion disease.

Transmissible spongiform encephalopathies, or prion diseases, are a group of progressive neurodegenerative disorders characterized by accumulation of the misfolded prion protein PrPsc [5–8]. PrPsc, a protease-resistant prion protein, is converted from the normal prion protein PrPc, which is naturally expressed in neurons [9].

PrP(106–126), containing the amino acid residues 106–126 of human PrPc, is different from PrPsc but is found to have some similar characteristics of PrPsc, including neurotoxicity and the ability to form amyloid fibrils *in vivo* and *in vitro* [10–13]. These properties are useful for the *in vitro* examination of prion-mediated neurotoxicity.

Some reports have shown that modulation of PrPc expression protects against neurodegenerative diseases, including Alzheimer's disease and Huntington's disease [14,15]. Upregulation of PrPc inhibited β-secretase cleavage of the amyloid precursor protein and reduced amyloid beta (Aβ) formation [15]. On the other hand, Aβ levels in scrapie-infected mice were significantly increased [15]. Also, overexpression of PrPc in neuronal cells transfected with huntingtin fragments (Htt) decrease Htt-mediated neurotoxicity, whereas PrPsc-infected cells showed increased Htt-mediated neurotoxicity [14]. These findings indicate that upregulation of PrPc expression may be a key factor in the prevention of neurodegenerative disorders.

Our previous study showed that overexpression of PrPc caused by exposure to hypoxic conditions or upregulation of HIF-1α prevents prion peptide-mediated neurotoxicity [16,17]. However, the regulatory mechanism of hypoxia-inducible HIF-1α-mediated PrPc expression is not clear.

It is well known that specific protein 1 (SP1), belonging to the SP/KLF family of transcription factors, regulates gene expression

* Corresponding author. Fax: +82 63 270 3780.
E-mail address: sypark@chonbuk.ac.kr (S.-Y. Park).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.086

in the development of an organism [18–20]. Transcription factor SP1 contains zinc finger motifs that directly bind to DNA and enhance gene transcription [21]. Some reports have shown that SP1 transcription factors are key factors for manipulating prion gene (*PRNP*) expression [22,23]. Bellingham et al. suggested that *PRNP* expression is regulated through the interaction of transcription factor SP1 and metal transcription factor-1 [23]. Also, a recent study showed that HIF-1 binds to hypoxia response elements on the SP1 promoter to stimulate the SP1 gene transcription activity [24]. It is proposed that upregulation of HIF-1α expression protects against prion-mediated neurotoxicity through an increase in SP1-mediated *PRNP* expression. Indeed, some studies demonstrated that HIF-1α-mediated transcriptional activity is regulated by the SP1 transcription factor under hypoxic conditions [25,26]. For *PRNP* expression, however, the relationship between SP1 and HIF-1α is not yet identified.

Thus, this study focused on the influence of SP1 transcriptional activity on hypoxia-inducible HIF-1α-mediated *PRNP* expression and analyzed the influence of SP1-mediated *PRNP* expression during prion-mediated neurotoxicity. Our research showed that upregulation of HIF-1α expression increased SP1 protein levels. Also, depletion of SP1 in neuronal cells by siRNA led to prion peptide-mediated neuron cell death and decreased transcriptional activity of *PRNP*, in spite of HIF-1α activation. The present results demonstrated that regulation of hypoxia-inducible HIF-1α affects the transcriptional activity of SP1-mediated *PRNP* expression, which consequently regulates PrP(106–126)-induced neurotoxicity.

2. Materials and methods

2.1. Cell culture and reagents

The neuroblastoma cell line SH-SY5Y was obtained from the American Type Culture collection (ATCC, Rockville, MD, USA). Mouse neuronal cell lines ZW 13–2 and Zpl 3–4 established from the hippocampus of ICR ($PRNP^{+/+}$) and Zürich I ($PRNP^{-/-}$) mice, respectively, were kindly provided by Professor Yong-Sun Kim (Hallym University, Chuncheon, Kangwon-do, South Korea). SH-SY5Y cells were cultured in minimum essential medium (MEM; Hyclone Laboratories, Logan, UT, USA), whereas ZW 13–2 and Zpl 3–4 cells were grown in DMEM (Hyclone Laboratories) that contained 10% fetal bovine serum (Invitrogen-Gibco, Grand Island, NY, USA) and gentamycin (0.1 mg/ml) in a humidified incubator maintained at 37 °C and 5% CO_2. Deferoxamine, doxorubicin and insulin-like growth factor-1 (IGF-1) were purchased from Sigma.

2.2. Construction of HIF-1α shRNA plasmid

The shRNA against the HIF-1α gene was a kind gift of Dr. Yong-Nyun Kim (National Cancer Research, Goyang, Gyeonggi-do, South Korea). The shRNA plasmid constructs for HIF-1α (shHIF-1α) were constructed in the pL-UGIP vector. The shRNA for HIF-1α was obtained using the oligonucleotide sequences 5′-CTGATGACCAG-CAACTTGA-3′ and 5′-TCAAGTTGCTGGTCATCAG-3′ as the forward and reverse primers, respectively. SH-SY5Y cells were transfected with shHIF-1α, and stable transfectants were selected in puromycin after 24 h recovery in standard growth medium. SH-SY5Y cells transfected with a mock vector were used as a control.

2.3. RNA interference

SH-SY5Y cells were transfected with SP1 small interfering RNA (siRNA; Stealth RNAi, *VHS40867*; Invitrogen, Carlsbad, CA, USA) using Lipofectamine 2000 according to the manufacturer's instructions. After a 48 h culture, knockdown efficiency was typically measured at the protein level by immunoblot. Stealth RNAi Negative Control (Invitrogen, Carlsbad, CA, USA) was used as a control.

2.4. PrP(106–126) treatment

Synthetic PrP(106–126) (sequence: Lys-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-Ala-Ala-Gly-Ala-Val-Val-Gly-Gly-Leu-Gly) was synthesized by Peptron (Seoul, Korea). The peptides were dissolved in sterile dimethyl sulfoxide at a 12.5 mM concentration and stored at −80 °C.

2.5. Annexin V assay

Apoptosis was assessed in detached cells using an Annexin V assay kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA) according to the manufacturer's protocol. Annexin V levels were determined by measuring the fluorescence at 488 nm excitation and 525/530 nm emission wavelengths using a Guava easyCyte HT System (Millipore, Billerica, MA, USA).

2.6. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

TUNEL analysis was performed to measure the degree of cellular apoptosis using the *in situ* Apo-BrdU DNA Fragmentation Assay Kit (BioVision, San Francisco, CA, USA) following the manufacturer's instructions. Cells were washed with phosphate buffered saline, fixed with paraformaldehyde for 15 min, preincubated with 50 μL of DNA-labeling solution (10 μL TdT reaction buffer, 0.75 μL TdT enzyme, 8 μL Br-dUTP) for 1 h at 37 °C, and then incubated with 5 μL anti-BrdU-fluorescein isothiocynate antibody for 0.5 h at room temperature. Finally, cells were mounted with DakoCytomation fluorescent medium and visualized using fluorescence microscopy. Cells were counterstained with propidium iodide (PI) to show all cell nuclei.

2.7. Western Blot

After SH-SY5Y cells were lysed in buffer [25 mM HEPES (pH 7.4), 100 mM NaCl, 1 mM EDTA, 5 mM $MgCl_2$, 0.1 mM dithiothreitol, and protease inhibitor mixture], proteins were electrophoretically resolved by 10–15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a nitrocellulose membrane. Immunoreactivity was detected through sequential incubation with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence reagents. The antibodies used for immunoblotting were anti-SP1 (Millipore, Billerica, MA, USA), anti-HIF-1α (BD Bioscience, San Diego, CA, USA), anti-PrPc (Millipore, Billerica, MA, USA), anti-murine PrP (provided by Professor Yong-Sun Kim), and anti-ß-actin (Sigma–Aldrich). Images were examined using a Fusion FX7 imaging system (Vilber Lourmat, Marne-la-Vallée, France).

2.8. Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from SH-SY5Y cells using the Hybrid-RTM kit (GeneAll Biotechnology, Seoul, Korea). cDNA was synthesized using the TaKaRa Prime Script™ 1st strand cDNA synthesis kit (Takara Bio Inc., Tokyo, Japan) following the manufacturer's instructions. The following primers were designed:

HIF-1α: forward 5□-CGC AAG TCC TCA AAG CAC AG-3□; reverse 5□-TGG TAG TGG TGG CAT TAG CA-3□;
PRNP: forward, 5□-GTG CAC GAC TGC GTC AAT-3□; reverse, 5□-CCT TCC TCA TCC CAC TAT CA-3□;