HIF-1α-induced β-catenin activation prevents prion-mediated neurotoxicity

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Received April 30, 2013; Accepted July 24, 2013

DOI: 10.3892/ijmm.2013.1457

Abstract. Previous studies have shown that hypoxic preconditioning attenuates prion-mediated neurotoxicity by upregulating hypoxia inducible factor-1α (HIF-1α). However, the mechanisms behind the HIF-1α-mediated neuroprotective effects in neurodegenerative disorders, including prion diseases, are unclear. It is well known that HIF-1α regulates Wnt/β-catenin signaling and that β-catenin protects neurons against misfolded protein-mediated disorders, including Alzheimer's and Parkinson's disease by preventing mitochondrial malfunction. Thus, we hypothesized that the mechanisms responsible for HIF-1α-mediated neuroprotection are associated with β-catenin activation induced by the regulation of mitochondrial function. We used the SH-SY5Y human neuroblastoma cell line and treated the cells with melatonin and then exposed them to the prion protein, PrP, or the β-catenin inhibitor, ICG-001. TUNEL assay was used to measure apoptosis. β-catenin expression measured by western blot analysis. The results revealed that HIF-1α prevented prion protein (PrP) (106-126)-induced neurotoxicity by activating β-catenin. Moreover, HIF-1α-induced β-catenin activation prevented the PrP (106-126)-induced mitochondrial damage under hypoxic conditions, as evidenced by the higher mitochondrial transmembrane potential values in the cells exposed to hypoxic conditions. These results indicate that the regulation of β-catenin activation by HIF-1α may be a therapeutic strategy for prion-mediated disorders.

Introduction

Transmissible spongiform encephalopathies (TSEs) or prion diseases are a family of neurodegenerative disorders caused by the accumulation of the pathological prion protein, PrPSc (1,2). PrPSc is a β-sheet rich structure protein resistant to proteinase K and is derived from the normal cellular prion protein, PrPc, which is α-helix rich structure protein and is sensitized to proteinase K (3-5).

The neuronal aggregation of PrPSc or neuronal cells exposed to the prion protein (PrP) fragment [PrP (106-126)] induces mitochondrial malfunctions which have been reported as major hallmarks of neurodegenerative diseases, including Huntington's disease and Alzheimer's disease (6-10). Previously it has been shown that treatment with gingerol prevents PrP (106-126)-mediated mitochondrial neurotoxicity through the regulation of hypoxia-inducible factor-1α (HIF-1α) activation (11). Xie *et al* (12) also demonstrated that exposing neurons to low oxygen activated HIF-1α and inhibited the activation of the mitochondrial apoptotic pathway induced by nerve growth factor (NGF) deprivation. These observations suggest that regulators of mitochondrial homeostasis, including HIF-1α, may be key factors for the protection against prion-related diseases.

Hypoxic conditions regulate gene transcription in cellular responses to promote metabolism and angiogenesis through adaptive processes (13-16). Accordingly, modulation of gene transcription through HIF-1 plays an important role in hypoxia for cell survival (17-22). It was previously suggested that hypoxia protects neuronal cells against PrP (106-126)-mediated neurotoxicity and that this prevention is associated with hypoxia-mediated HIF-1α signals (22).

HIF-1 is a heterodimeric transcription factor comprising the subunits, α and β, of which HIF-1α is widely expressed in mammalian tissues under hypoxic conditions (19-21,23,24). Recent studies have indicated that hypoxia-mediated HIF-1α exerts neuroprotective effects (22,23,25). According to a previous study, the accumulation of HIF-1α attenuates PrP (106-126)-mediated neurotoxicity by regulating AKT signaling and correlates with the overexpression of the *PRNP* gene (22). Cunningham *et al* (26) also suggested that the upregulation of HIF-1α protects neural stem/progenitor cells (NSPCs) against brain injury and stroke through the modulation of Wnt/β-catenin signals. These data suggest that the upregulation of HIF-1α may have therapeutic benefits for neuronal damage by promoting survival signals, such as Wnt/β-catenin pathways.

β-catenin is a protein that constitutes the cadherin protein complex (27,28). It plays an important role in various aspects of neurobiology, including neuronal development, regeneration and neuronal differentiation (29,30). It has been suggested that β-catenin/Wnt signaling regulates neural stem cell differ-

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Key words: β-catenin, hypoxia, hypoxia inducible factor-1α, mitochondrial dysfunction, prion diseases

entiation by interacting with HIF-1α (31). These data indicate that HIF-1α may regulate the cellular response to adaptive processes through Wnt/β-catenin signaling (31).

Furthermore, β-catenin has been shown to play pivotal roles associated with mitochondrial functions under pathophysiological conditions (27,32,33). Wei *et al* (32) showed that exogenous Wnt1 prevents 6-hydroxydopamine (6-OHDA)-mediated mitochondrial damage through the activation of Wnt/β-catenin signaling pathways in cellular models of Parkinson's disease. It has been demonstrated that the modulation of mitochondrial functions may be a key mechanism behind the neuroprotective effects of β-catenin signaling against neurodegenerative diseases.

It has been suggested that HIF-1α prevents neuronal cells from prion-induced neuronal damage through the activation of β-catenin signals. Previous studies have shown that prion-mediated neurotoxicity is blocked by regulating mitochondrial homeostasis (6,7). However, there is limited knowledge regarding the neuroprotective mechanisms of HIF-1α activation associated with mitochondrial homeostasis induced by the activation of β-catenin. In this study, we examined whether HIF-1α-induced β-catenin signals protect neuronal cells against PrP (106-126)-induced neurotoxicity. We also investigated whether the protective effects are associated with mitochondrial homeostasis through the regulation of β-catenin signals.

Materials and methods

Cell culture. The SH-SY5Y human neuroblastoma cell line was obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). Cells were cultured in minimum essential medium (MEM; Gibco-Invitrogen, Carlsbad, CA, USA) that contained 10% fetal bovine serum (FBS) (Sigma-Aldrich, St. Louis, MO, USA) and gentamycin (0.1 mg/ml) in a humidified incubator which was maintained at 37˚C and 5% CO_2. The cells were treated with melatonin (Sigma-Aldrich) for 12 h and then exposed to 50 μM of PrP (106-126) with or without 10 μM of the β-catenin inhibitor, ICG-001 (Axon Medchem BV, Groningen, The Netherlands) for 24 h. Melatonin was dissolved in ethanol with the final ethanol concentration in the culture medium not exceeding 0.5%. In addition, ICG-001 dissolved in dimethylsulfoxide (DMSO) was added to the culture medium to a final concentration of 0.1% DMSO.

PrP (106-126) treatment. Synthetic PrP (106-126) peptides with the sequence, Lys-Thr-Asn-Met-Lys-His-Met-Ala-Gly-Ala-Ala-Ala-Ala-Gly-Ala-Val-Val-Gly-Gly-Leu-Gly, were synthesized by Peptron (Seoul, Korea). The peptides were dissolved in sterile DMSO at a stock concentration of 10 mM and stored at -80˚C.

Annexin V assay. Apoptosis was assessed in the detached cells using the Annexin V assay kit (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) according to the manufacturer's instructions. Annexin V measurement was performed by measuring the fluorescence at excitation (488 nm) and at emission (525 nm) using a Guava EasyCyte™ HT Flow Cytometer (Millipore, Billerica, MA, USA).

Terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay. TUNEL analysis was performed to measure the degree of cellular apoptosis using an *in situ* ApoBrdU DNA fragmentation assay kit (Sigma-Aldrich) following the manufacturer's instructions. Cells were washed with phosphate-buffered saline (PBS) and fixed with paraformaldehyde for 15 min. The cells were pre-incubated with 50 μl DNA-labeling solution (10 μl TdT reaction buffer, 0.75 μl TdT enzyme, 8 μl BrdUTP) for 1 h at 37˚C. The cells were then incubated with 5 μl Alexa Fluor 488-labeled-anti-5-bromodeoxyuridine (BrdUrd)-antibody for 0.5 h at room temperature (20˚C). Finally, the cells were mounted with DakoCytomation fluorescent medium (Dako, Carpintena, CA, USA) and were visualized under a fluorescence microscope. The cells were then counterstained with propidium iodide to show all cell nuclei.

Immunocytochemistry (ICC). The SH-SY5Y neuroblastoma cells were cultured on glass coverslips. The cells were washed with PBS and fixed with cold acetone for 90 sec at room temperature. The cells were washed again with PBS, blocked with 5% FBS in Tris-buffered saline and Tween-20 (TBST) and incubated with anti-β-catenin (2 μg/ml) monoclonal antibody for 48 h at room temperature. The unbound antibody was removed by an additional PBS wash and the cells were then incubated with labeled anti-mouse FITC (for anti-β-catenin antibody) IgG antibodies (4 μg/ml) for 2 h at room temperature. Finally, the cells were mounted with DakoCytomation fluorescent medium (Dako) and visualized under a fluorescence microscope.

Western blot analysis. The SH-SY5Y cells were lysed in buffer comprising 25 mM HEPES, 100 mM NaCl, 1 mM EDTA, 5 mM $MgCl_2$, 0.1 mM dithiothreitol and protease inhibitor mixture, at pH 7.4. Equal amounts of protein lysates were dissolved in 10-15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred onto a nitrocellulose membrane. Immunoreactivity was detected through sequential incubation with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminescence (ECL) reagents. The antibodies used for immunoblotting were β-catenin (Cell Signaling Technology, Inc., Beverly, MA, USA), survivin (Santa Cruz Biotechnology, Inc.), caspase-3 (Cell Signaling Technology, Inc.), Bax (Santa Cruz Biotechnology, Inc.), cytochrome *c* (BD Bioscience, Franklin Lakes, NJ, USA), HIF-1α (Santa Cruz Biotechnology, Inc.) and β-actin (Sigma-Aldrich). The images were examined using a Fusion-FX7 imaging system (Vilber Lourmat, Marne-la-Vallée, France).

Cellular fractionation. The SH-SY5Y cells were re-suspended in mitochondrial buffer (210 mM sucrose, 70 mM mannitol, 1 mM EDTA and 10 mM HEPES), broken by a 26-guage needle and subjected to centrifugation at 700 x g for 10 min. The post-nuclear supernatant was centrifuged at 10,000 x g for 30 min. The pellet was then used as a mitochondrial fraction and the supernatant was used as a cytosolic fraction. Total proteins were obtained and subjected to western blot analysis.

Mitochondrial transmembrane potential (MTP) assay. Alterations in MTP were evaluated using the cationic fluorescent indicator, JC-1 (Molecular Probes, Eugene, OR, USA). J-aggregates in intact mitochondria are evident as red fluorescence with emission at 583 nm, indicating high or normal MTP

and as green fluorescence with emission at 525 nm, indicating low MTP when JC-1 remains in the monomeric form in the cytoplasm. The SH-SY5Y cells were incubated in MEM containing 10 μM JC-1 at 37˚C for 15 min, washed with PBS and then transferred to a clear 96-well plate. The Guava EasyCyte HT System (Millipore) was used to measure the JC-1 aggregate fluorescence emission at 583 nm with an excitation wavelength of 488 nm, while the JC-1 monomer fluorescence intensity was measured with an excitation and emission wavelength of 488 and 525 nm, respectively. The SH-SY5Y cells were cultured on coverslips in a 24-well plate, incubated in MEM containing 10 μM JC-1 at 37˚C for 15 min, and then washed with PBS. Finally, the cells were mounted with DakoCytomation fluorescent medium (Dako) and visualized under a fluorescence microscope.

Statistical analyses. Data are expressed as the means ± standard deviation (SD) and were compared using the Student's t-test, ANOVA and Duncan's test using SAS statistical software. P-values <0.05 <0.01 were considered to indicate statistically significant differences.

Results

Neuroprotective effects of HIF-1α against prion-induced neuronal apoptosis are dependent on β-catenin activation. Studies have suggested that exposure to low oxygen conditions activates the Wnt/β-catenin signaling pathways (26,31). A previous study also showed that HIF-1α plays a pivotal role as a neuroprotective factor in prion-mediated neuronal cell death (22). Thus, in this study, we examined whether HIF-1α induces β-catenin activation and protects SH-SY5Y neuronal cells against prion peptide-mediated neurotoxicity. The changes in β-catenin and β-catenin target gene survivin expression levels in the SH-SY5Y cells following exposure to hypoxia with or without the HIF-1α inhibitor, doxorubicin, were examined. It was found that the SH-SY5Y cells exposed to hypoxic conditions for 12, 24 and 36 h had increased protein expression levels of β-catenin and the β-catenin target gene, survivin (Fig. 1A). Consistent with this, the ICC images revealed that the expression level of β-catenin was increased under hypoxic conditions (Fig. 1B). However, the HIF-1α inhibitor, doxorubicin, inhibited HIF-1α, β-catenin and β-catenin target gene survivin protein levels (Fig. 1A). These data indicate that HIF-1α activates the β-catenin signaling pathway in neuronal cells.

To determine whether the HIF-1α-mediated neuroprotective effects on PrP (106-126)-induced neuronal cell damage are associated with the activation of β-catenin signals, the cells were exposed to hypoxiac conditions for 12 h and then treated with 50 μM of PrP (106-126) for 24 h with or without the β-catenin inhibitor, ICG-001 (10 μM). The results revealed that the apoptotic cell population (Annexin V-positive) increased in the PrP (106-126)-treated cells compared with the control groups, whereas exposure to hypoxic conditions decreased the PrP (106-126)-mediated apoptotic cell population (Fig. 2A and B). However, the neuroprotective effects of hypoxia against PrP (106-126)-mediated neuronal cell death were inhibited by the β-catenin inhibitor, ICG-001 (Fig. 2A and B). These results were confirmed by measuring Alexa Fluor 488-labeled anti-BrdUrd with the use of microscopic methods in TUNEL assay microscopic images (Fig. 2C). Collectively, these results suggest that HIF-1α prevents neurotoxicity caused by PrP (106-126) through the inhibition of β-catenin signals.

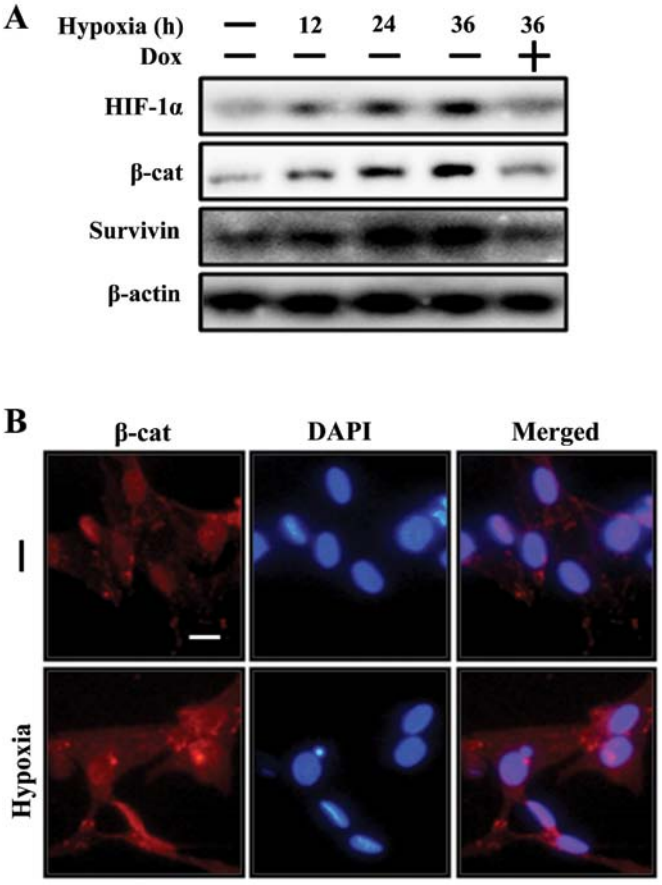

Figure 1. Hypoxia inducible factor-1α (HIF-1α) increases β-catenin signals. (A) The SH-SY5Y neuronal cells were exposed to hypoxic conditions for various periods of time with or without doxorubicin (Dox). The cells exposed to hypoxic conditions were assessed for HIF-1α, β-catenin and survivin production by western blot analysis. The results were normalized with β-actin. (B) The SH-SY5Y cells were incubated under hypoxic conditions for 24 h. The cells were immunostained with DAPI (blue) and β-catenin antibody (red) and fluorescence was examined. Magnification, x400; scale bar, 40 μm.

HIF-1α-induced activation of β-catenin pathway prevents prion-mediated mitochondrial neurotoxicity under hypoxic conditions. As hypoxia increases β-catenin activation and β-catenin regulates mitochondrial functions (27,34), in this study, we investigated whether hypoxia-induced β-catenin signals exert neuroprotective effects against PrP (106-126)-induced mitochondrial dysfunction under hypoxic conditions. The cells were exposed to hypoxic conditions for 12 h and then treated with 50 μM PrP (106-126) with or without ICG-001 (10 μM). Exposure to PrP increased the cell population in which JC-1 existed in its monomeric form, a marker of lower MTP values, while exposure of the cells to hypoxia blocked the PrP (106-126)-induced JC-1 monomeric appearance (Fig. 3A). However, the protective effects of hypoxia on the neuronal cells against PrP (106-126)-mediated mitochondrial dysfunction were inhibited by the β-catenin inhibitor, ICG-001 (Fig. 3A). This result was confirmed by measuring the MTP values using fluoroscopic methods (Fig. 3B). Similarly,

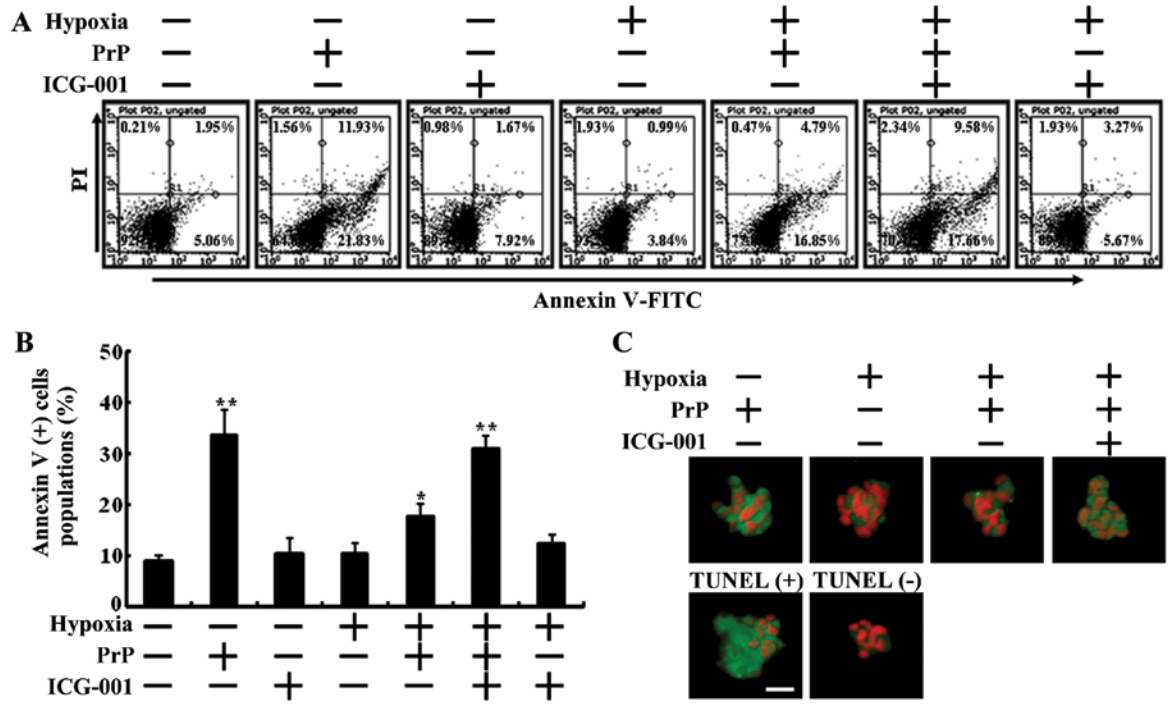

Figure 2. Hypoxia inducible factor-1α (HIF-1α) inhibits prion protein (PrP) (106-126)-induced apoptosis by activating β-catenin. (A) The SH-SY5Y cells were exposed to hypoxic conditions for 12 h and then exposed to 50 μM of PrP (106-126) with or without β-catenin inhibitor (50 μM ICG-001) for 24 h. Cell viability was measured by Annexin V assay. (B) The bar graph indicates the average number of Annexin V-positive cells. There were significant differences between the control (no treatment) and each treatment group (*P<0.05 and **P<0.01). (C) Representative immunofluorescence images of TUNEL-positive (green) SH-SY5Y cells exposed to hypoxic conditions and then treated with PrP (106-126) with or without ICG-001. The cells were counterstained with propidium iodide (red) to show all cell nuclei. Magnification, x400; scale bar, 100 μm.

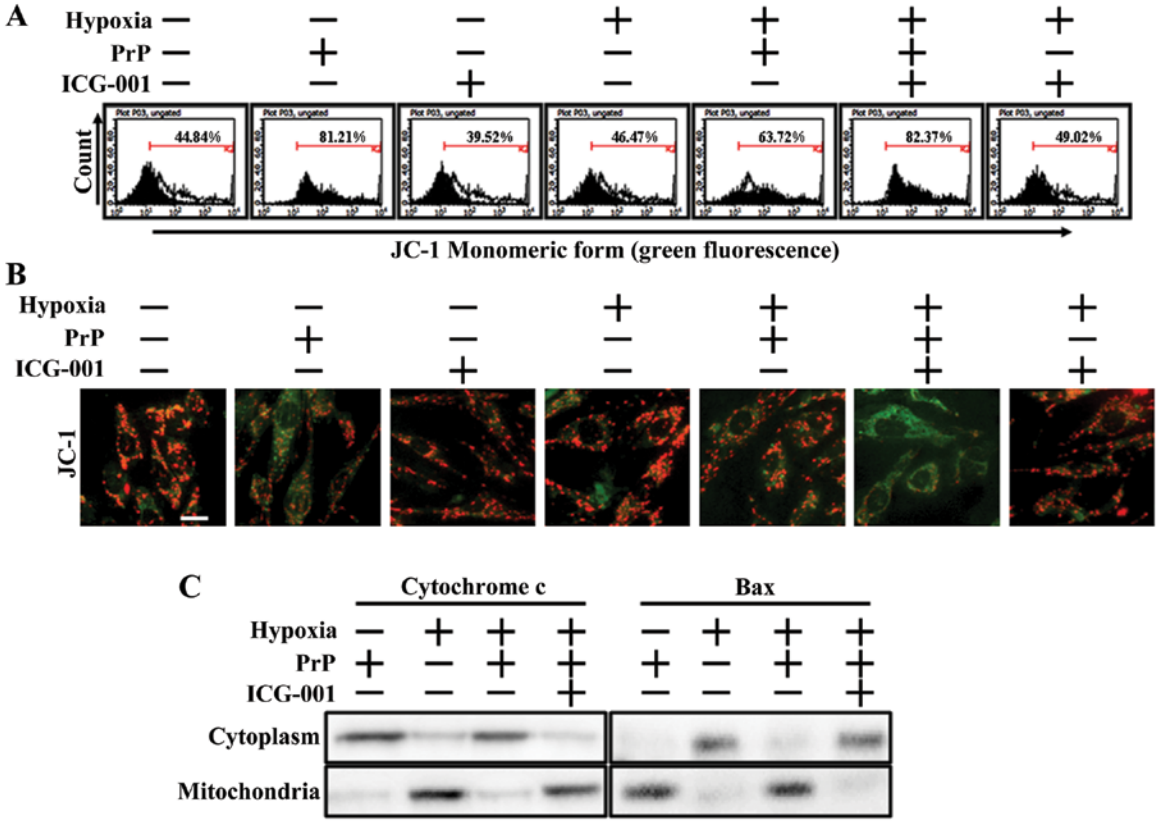

Figure 3. Hypoxia protects neuronal cells against prion protein (PrP) (106-126)-induced mitochondrial damage by regulating β-catenin signals. (A) The SH-SY5Y cells were exposed to hypoxic conditions (12 h) and then treated with 50 μM PrP (106-126) with or without ICG-001 for 24 h. R1 represents the population of JC-1 monomeric cells. (B) Representative images of J aggregate formation in the SH-SY5Y cells treated as described in (A). (C) Scale bar denotes 100 μm. (C) The cells were homogenized in mitochondrial buffer. The separation of the cytosol and the mitochondrial extracts was analyzed by western blot analysis using antibodies against cytochrome *c* and Bax protein. Western blots show the levels of cytochrome *c* and Bax translocation in the SH-SY5Y cells.

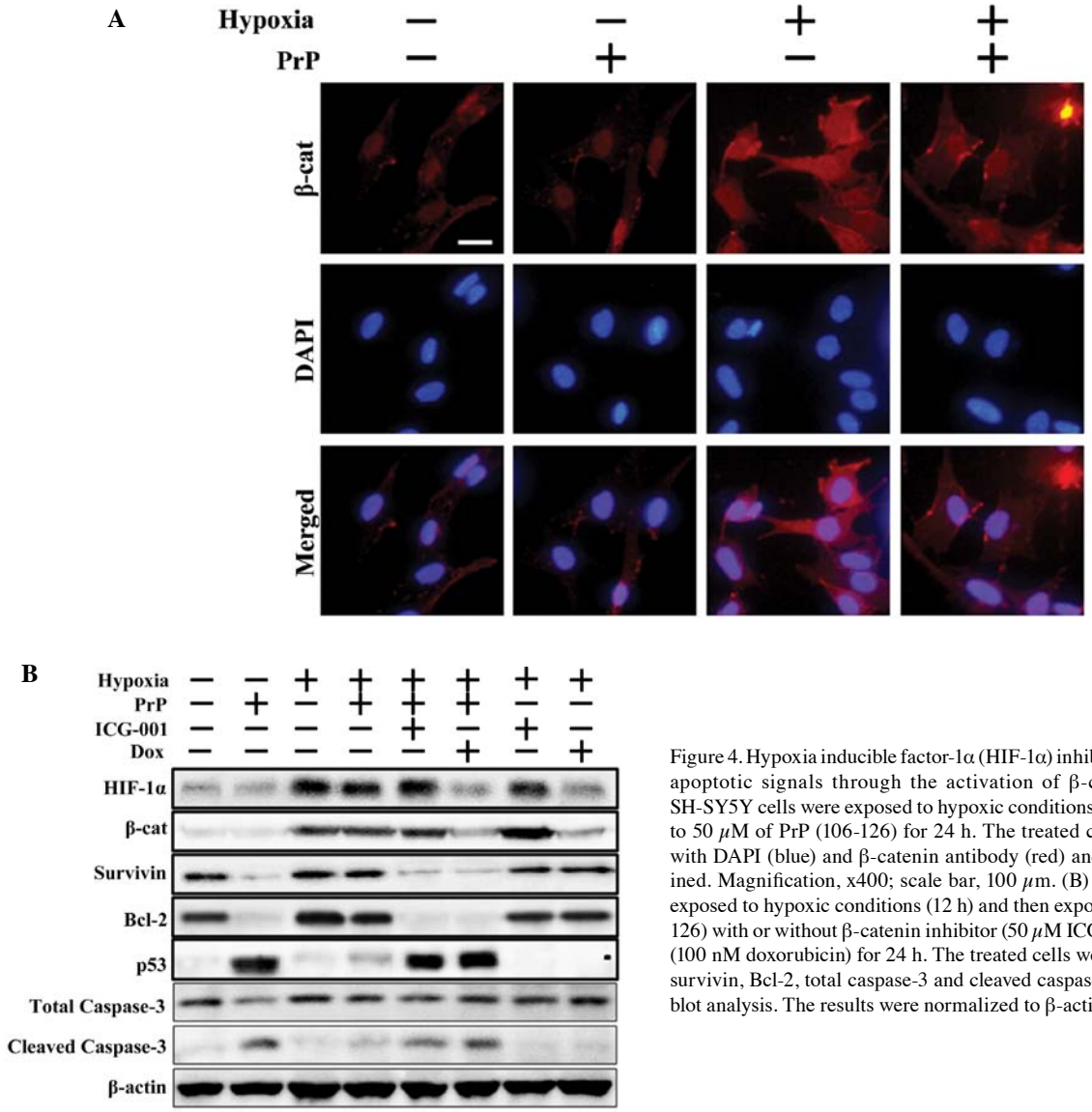

Figure 4. Hypoxia inducible factor-1α (HIF-1α) inhibits PrP (106-126)-induced apoptotic signals through the activation of β-catenin signals. (A) The SH-SY5Y cells were exposed to hypoxic conditions for 12 h and then exposed to 50 μM of PrP (106-126) for 24 h. The treated cells were immunostained with DAPI (blue) and β-catenin antibody (red) and fluorescence was examined. Magnification, x400; scale bar, 100 μm. (B) The SH-SY5Y cells were exposed to hypoxic conditions (12 h) and then exposed to 50 μM of PrP (106-126) with or without β-catenin inhibitor (50 μM ICG-001) or HIF-1α inhibitor (100 nM doxorubicin) for 24 h. The treated cells were assessed for β-catenin, survivin, Bcl-2, total caspase-3 and cleaved caspase-3 production by western blot analysis. The results were normalized to β-actin.

mitochondrial damage induced by exposure to PrP (106-126) resulted in the translocation of Bax and in the blockage of cytochrome *c* release by hypoxic exposure, whereas ICG-001 blocked the protective effects of hypoxia against the PrP (106-126)-induced mitochondrial apoptotic signals (Fig. 3C). Collectively, these results confirm the hypothesis that hypoxia prevents PrP (106-126)-mediated mitochondrial neurotoxicity by upregulating β-catenin signals.

It has been shown that hypoxia regulates β-catenin activation through the modulation of HIF-1α stabilization (26,31) and that HIF-1α protects neuronal cells against PrP (106-126)-induced mitochondrial damage (11). Therefore, in this study, we assessed whether HIF-1α has an effect on PrP (106-126)-mediated mitochondrial toxicity by activating β-catenin signals. Initially, β-catenin expression levels were examined in the SH-SY5Y cells following treatment with PrP (106-126) with or without the β-catenin inhibitor, ICG-001, under hypoxic conditions. ICC images obtained using fluoroscopic methods revealed that hypoxia-mediated β-catenin protein upregulation was decreased following treatment with PrP (106-126) (Fig. 4A). Consistent with these results, immunoblot assay revealed that the downregulation of HIF-1α, β-catenin and survivin proteins, induced by PrP (106-126) treatment, was reversed following exposure to hypoxic conditions. Furthermore, ICG-001 blocked survivin expression levels (a marker of β-catenin activation), although the β-catenin and HIF-1α expression levels were not altered (Fig. 4B). In addition, exposure to hypoxic conditions increased the levels of the anti-apoptotic protein, Bcl-2, decreased those of the pro-apoptotic protein, p53, and cleaved caspase-3 levels in the PrP (106-126)-treated cells. However, the inhibitory effects of hypoxia against the PrP (106-126)-induced apoptotic signals were obstructed following treatment with the β-catenin inhibitor, ICG-001, or the HIF-1α inhibitor, doxorubicin. Collectively, these data suggest that HIF-1α prevents PrP (106-126)-induced apoptotic signals by upregulating β-catenin signals.

Discussion

A previous study showed that hypoxia protects neuronal cells against prion peptide-induced neurotoxocity by upregulating HIF-1α (24). It has also been demonstrated that the protective

effects of HIF-1α are associated with the regulation of mitochondrial functions (11,35). In this study, we demonstrate that HIF-1α prevents prion-mediated mitochondrial neurotoxicity by regulating β-catenin signaling pathways.

It has previously been demonstrated that gingerol protects neuronal cells against PrP (106-126)-induced neurotoxicity; the protective effects were associated with HIF-1α activation and correlated with the regulation of mitochondrial homeostasis (11). The activation of β-catenin signals has been suggested to protect neuronal cells from 6-OHDA-induced mitochondrial damage (32). In addition, a recent study suggested that HIF-1α stabilization, induced by exposure to hypoxic conditions, activates the Wnt/β-catenin signaling pathways (31). However, the effects of HIF-1α on the activation of β-catenin signals has not been reported as a neuroprotective effect against prion disease. Therefore, in the current study, we focused on the correlation between hypoxia-induced HIF-1α and β-catenin signals in prion-mediated neurotoxicity.

Studies have shown that the activation of β-catenin prevents neurodegenerative diseases, while several lines of evidence support a protective role for β-catenin in neurodegenerative diseases, including Alzheimer's and Parkinson's disease (36,37). L'Episcopo *et al* (36) suggested that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) inhibits the activation of Wnt/β-catenin signaling pathways, whereas manipulation of β-catenin signaling with the inactivation of glycogen synthase kinase-3 (GSK-3)β decreases MPTP-induced neurotoxicity in a mouse model of Parkinson's disease. Exogenous Wnt1 has been reported to protect neuronal cells from 6-OHDA (a dopaminergic neurotoxin), commonly used to generate an experimental model of Parkinson's disease, through the upregulation of the Wnt/β-catenin signaling pathways (32). Consistent with this, the present study demonstrated that the treatment of cells with PrP (106-126) induced neuronal apoptosis (Fig. 2) and decreased β-catenin protein levels (Fig. 4B). However, exposure of the cells to hypoxic conditions inhibited the effects of PrP (106-126) (Figs. 2 and 4). Hypoxia also increased the Bcl-2 protein expression levels and decreased cleaved caspase-3 protein expression levels (Fig. 4B). Consequently, these anti-apoptotic effects of hypoxia were blocked by the β-catenin inhibitor, ICG-001, or the HIF-1α inhibitor, doxorubicin (Figs. 2 and 4). These results suggest that the regulation of β-catenin pathways may be a key mechanism behind the neuroprotective effects of HIF-1α against prion-mediated neuronal cell death.

On the other hand, the protective role of HIF-1α in the cellular response to oxidative damage, including hypoxia has been supported by several lines of evidence (31,38). Mazumdar *et al* (31) showed that hypoxia-HIF-1α stimulates Wnt/β-catenin signaling through β-catenin activation and the expression of the downstream effectors, lymphoid enhancer-binding factor-1 (LEF-1) and T cell factor-1 (TCF-1). In addition, a recent study suggested that HIF-1α upregulates neuronal stem cell differentiation through the regulation of the Notch and Wnt/β-catenin signaling pathways (26), while other studies have found that β-catenin exerts protective effects against mitochondrial malfunctions in metabolic diseases (27,33). Lehwald *et al* (27) reported that Wnt/β-catenin signaling regulates liver metabolism by maintaining mitochondrial homeostasis. However, the correlation between the protective effects of hypoxia-induced HIF-1α and β-catenin on mitochondrial dysfunction in prion-mediated neurotoxicity remains to be elucidated. Thus, the effects of hypoxia-induced β-catenin on PrP (106-126)-mediated mitochondrial damage were also investigated in this study. The results revealed that hypoxia-induced β-catenin protected the SH-SY5Y neuronal cells from PrP (106-126)-mediated mitochondrial damage (Fig. 3). Exposure to hypoxic conditions blocked the PrP (106-126)-induced reduction in MTP values (Fig. 3A and B) and inhibited Bax translocation and cytochrome *c* release (Fig. 3C). However, these effects of HIF-1α were blocked by ICG-001 or doxorubicin (Fig. 3). For this reason, this study supports the hypothesis that the upregulation of β-catenin, caused by HIF-1α, prevents PrP (106-126)-mediated neurotoxicity through the modulation of mitochondrial functions.

Collectively, the results from the present study suggest that HIF-1α prevents the PrP (106-126)-induced activation of mitochondrial apoptotic pathways in neuronal cells by activating β-catenin signaling. Our results also suggest that regulators of Wnt/β-catenin signaling, including HIF-1α or exposure to hypoxic conditions, may be a potential neurotherapeutic target for the prevention of prion diseases.

Acknowledgements

This study was supported by the Cooperative Research Program for Agriculture Science and Technology Development (PJ907116) of the Rural Development Administration (RDA) and by the National Research Foundation of the Korea Grant funded by the Korean Government (2012R1A1B3000463).

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