RESEARCH ARTICLE

# Upregulation of HIF-1α by Hypoxia Protect Neuroblastoma Cells from Apoptosis by Promoting Survivin Expression

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## Abstract

**Apoptosis is one of main types of neural cell death and is reversible and is a major target of therapeutic interventions. However, detailed apoptotic cascades still need to be recognized. In present study, we determined the promotion of HIF-1α and survivin in brain samples of a mouse model of hypoxic-ischemia and in neuroblastoma SH-SY5Y cells post hypoxia treatment. Then gain-of-function and loss-of-function strategies were adopted to manipulate the HIF-1α in SH-SY5Y cells, and hypoxia-induced survivin upregulation and cell apoptosis were determined. Results demonstrated that the HIF-1α and survivin were significantly promoted in a mouse model of hypoxic-ischemia or in SH-SY5Y cells post hypoxia *in vitro*. Manually upregulated HIF-1α could promote the hypoxia-induced survivin upregulation and improve the hypoxia-induced SH-SY5Y cell apoptosis. On the other hand, the HIF-1α knockdown by RNAi reduced the hypoxia-induced survivin upregulation and cell apoptosis. Therefore, the present study confirmed the protective role of HIF-1α and survivin in the hypoxia-induced SH-SY5Y cell apoptosis, and the survivin upregulation by hypoxia is HIF-1α-dependent. Promotion of HIF-1α and survivin might be a valuable stragegy for therapeutic intervention for hypoxic-ischemic encephalopathy.**

**Keywords:** Hypoxic-ischemic encephalopathy - HIF-1α - survivin - apoptosis

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## Introduction

Stroke is one of the most common causes of death worldwide (Bonita et al., 2004), and is estimated to be responsible for about 50% of the patients hospitalized for acute neurologic disorders (Nakka et al., 2008). In particular, stroke is currently the leading cause of death in China (He et al., 2005), with a rapid increasing incidence of hypertension (Gu et al., 2002; Sun et al., 2007; Du et al., 2014) which is the most important modifiable risk factor for stroke (McBride et al., 2014; Savitz et al., 2014). Usually, the local reduced or completely interrupted cerebral blood supply can not be restored in time, and leads to the short supply of oxygen and glucose and damages to that area of brain tissue, and veven results in cerebral infarction (Whiteley et al., 2008). During stroke-promoted brain damage, two major processes lead to neural cell death: necrosis and apoptosis. In the core area of the ischemic stroke, where blood flow is most severely restricted, necrotic cell death is dominant and occurs shortly after stroke. In the periphery of the ischemic area, where collateral blood flow can buffer the degree of the damage, which may start hours or even days after transient ischemia, apoptosis mainly develops (Graham et al., 2001; Northington et al., 2001). Evidence suggests that activation of apoptotic pathways occur in the periphery cells of the ischemic area, both in the caspase-dependent and caspaseindependent manner that may contribute to delayed ischemic cell death (Linnik et al., 1993; Ferrer et al., 2003). And in the early stages of cerebral infarction, neurons in the necrotic core also display apoptotic characteristic (Benchoua et al., 2001). It appears that necrosis is a more complex phenomenon, which can be linked to apoptosis. Therefore, the apoptotic cascades during brain damage are reversible and are a major target of therapeutic interventions (Kato et al., 1999; Newcomb et al., 2006).

A wide array of transcription factors has been reported to be implicated in regulating genes responsible for the metabolic changes under hypoxia-ischemia (Cummins et al., 2005; Licausi et al., 2011). A key component of these factors is hypoxia-inducible factor 1 (HIF-1), existing as a heterodimer composed of a constitutively expressed HIF-1β subunit and an oxygen sensitive HIF-1α subunit. The HIF-1α/HIF-1β dimmer (Semenza et al., 2003) binds to a conserved DNA consensus on the promoters of its target genes known as hypoxia-responsive element (Wang et al., 1995; Pouyssegur et al., 2006; Christofk et al., 2008) post activation and induces a group of gene products crucial for hypoxic adaptation (Kaelin et al., 2008; Ping et al., 2013).

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HIF-1 and/or hypoxia have been shown to be either anti-apoptotic or pro-apoptotic in cerebral hypoxia-ischemia. Severe or prolonged hypoxia rather induces apoptosis, at least in part. Other wise, HIF-1α, along with other molecules, protects neural cells from apoptosis (Piret et al., 2002). HIF-1α is involved in hypoxia induced apoptosis. Hypoxia in combination with hypoglycaemia reduces proliferation and increases apoptosis (Greijer et al., 2004), via increasing the stability of the product of the tumour suppressor gene p53 (Chen et al., 2003), or via inducing the BNIP3 expression (Kothari et al., 2003). On the other side, HIF-1α also prevents apoptosis by activating the PI3K/Akt pathway (Akakura et al., 2001) in acute hypoxia or via increasing the expression of glycolytic enzymes, p21 and erythropoietin. Thus, more molecules implicated in the pro- or anti-apoptosis of HIF-1α in the cerebral hypoxia-ischemia.

Survivin is a protein that inhibits apoptosis and regulates cell division (Altieri et al., 1999; Karami et al., 2013). As a member of inhibitor of apoptosis protein (IAP) family, survivin binds to caspase 9 via its baculovirus inhibitor of apoptosis repeat (BIR) domain and inhibits caspase 9 activity (O'Connor et al., 2000). Survivin has also shown to indirectly supress another key caspase in apoptosis, caspase 3 via binding to and sequestering Smac/DIABLO (Tarnawski et al., 2001), or directly binds to caspase-3 and inhibits its activity (Shin et al., 2001; Rubio et al., 2012). The expression and function of survivin is regulated by various molecules. Nuclear factor-κB, which can be activated indirectly by growth factors via the phosphatidylinositol 3-kinase/Akt pathway upregulates the survivin expression (Van Antwerp et al., 1998). And the insulinlike growth factor I/mTOR signaling has also been reported to upregulate survivin expression (Vaira et al., 2007). Besices, the alternative splicing of survivin mRNA yields different expression patterns and abilities to prevent apoptosis (Noton et al., 2006). In addition, survivin degradation is also regulated by heat shock protein 90 (Zhao et al., 2000; Fortugno et al., 2003). Moreover, It has been indicated that survivin and HIF-1α were implicated in the anti-apoptotic effect of 2ME2, an estradiol derivative and a known HIF-1-alpha inhibitor treated following global ischemia in rat brain (Li et al., 2011), implying a regulatory role of HIF-1α/hypoxia in the survivin-mediated apoptosis.

In present study, we determined the promotion of HIF-1α and survivin in brain samples of a mouse model of hypoxic-ischemia and in neuroblastoma SH-SY5Y cells post hypoxia treatment. Then gain-of-function and loss-of-function strategies were adopted to manipulate the HIF-1α in SH-SY5Y cells, and the hypoxia-induced survivin upregulation and cell apoptosis were determined. Results demonstrated the protective role of HIF-1α and survivin in the hypoxia-induced SH-SY5Y cell apoptosis, and the survivin upregulation by hypoxia is HIF-1α-dependent. Thus, present study confirmed the key role of survivin in the apoptosis inhibition by HIF-1α, and implyed that the promotion of HIF-1α and survivin might be a valuable stragegy for therapeutic intervention to hypoxic-ischemic encephalopathy.

## Materials and Methods

*Reagents, cell culture and treatment*

SH-SY5Y (human neuroblastoma cell line) cells were gifted by the National platform of experimental cell resources for sci-tech in China and were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) containing 10% FBS (Invitrogen, Carlsbad, CA, USA) at 37°C under 5% CO2. The DMEM supplemented with 2% FBS was used for the cells maintaining. For hypoxia treatment, cells were placed in a hypoxia incubator infused with a gas mixture of 5% $CO_2$ and nitrogen to obtain 3% oxygen concentration. Oxygen concentration was monitored continuously (Forma 3130; Thermo Scientific, Rockford, IL, USA). To overexpress HIF-1α in SH-SY5Y cells, the wild HIF-1α coding sequence was amplified and cloned into the pcDNA3.1 (+) vector. And the HIF-1α-pcDNA3.1 (+) or control CAT-pcDNA3.1 (+) plasmid was transfected into SH-SY5Y cells by lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The positive cell clones were selected in the presence of 500μg/ml G418, and maintained in medium containing G418 at 300μg/ml. The 40nM siRNA-HIF-1α or siRNA-Control (Sangon, Shanghai, China) was transfected with lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) to abrogate the HIF-1α expression.

*RNA Isolation, Reverse Transcription, quantitative real-time PCR*

Total cellular RNA was isolated with PureLink® RNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to manuals. The expression of HIF-1α and survivin in mRNA was quantified by the real-time RT-PCR method with Takara One Step RT-PCT kit (Takara, Dalian, China). mRNA samples were amplified using primer/probe sets specific for the genes of interest on a Lightcycler 480 II (Roche, Mannheim, Germany). Relative quantification was determined using the ΔΔCt method using tubulin as reference gene (Livak et al., 2001). The primers used were available upon a request.

*Western blot analysis*

Aproximately $1\text{-}5 \times 10^5$ SH-SY5Y cells were collected and lyzed with the cytoplasmic protein extraction Kit (ZmTech Scientific Inc, San Jose, CA, USA) and supplemented with protease inhibitor cocktail (Roche, Basel, Switzerland) according to manuals. All protein samples were quantified using Bradford Reagent (Bio-Rad, Hercules, CA, USA) and then separated by a 8-12% gradient SDS-PAGE gel, transferred to PVDF membrane and blocked in 5% skimmed milk. Rabbit polyclonal antibodies to HIF-1α (1:500), survivin (1:800) and tubulin (1:2000) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and were used to quantify the protein level of each molecule. Goat anti-rabbit IgG conjugated to horseradish peroxidase (1:2000; Pierce, Rockford, IL, USA) and ECL detection systems (Super Signal West Femto; Pierce, Rockford, IL, USA) were used for detection.

*Apoptosis assay Caspase 3 assay*

Apoptosis of SH-SY5Y cells was examined with an annexin V-FITC apoptosis detection kit (Sigma-Aldrich, St. Louis, Missouri). Briefly, 2-6 × $10^5$ cells were stained with annexin V-FITC and propidium iodide and detected by a FACScan flow cytometer (Bio-Rad, Hercules, CA, USA) to analyze cellular apoptosis. The results were calculated using the CellQuestTM Pro software (Bio-Rad, Hercules, CA, USA) and expressed as the percentage of apoptotic cells from the total cells. The caspase 3 activity was examned with a caspase 3 activity assay kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manual. And the activity was expressed as a relative value to control.

*Immunohistochemical staining for HIF-1α and survivin in mice brains with HIE*

All mice experiments were approved by the Institutional Animal Care and Use Committee at Shandong University and were performed according to the guidelines of Chinese Animal Protection law. The neonatal HIE mouse model was produced by following the method of Sheldon et al (Sheldon et al., 1998). Briefly, neonatal mice were anesthetized with halothane and the left common carotid artery was permanently ligated, 7 days post birth. The ligated neonatal mice were recuperated for 12h and then were placed in a hypoxic chamber maintained at 37°C through which humidified 8% oxygen and balanced nitrogen flowed. After the mice had remained in the hypoxic environ-ment for 30min. Then mice were sacrificed for analysis of HIF-1α and survivin expression. Fresh mice brain specimens were resected and fixed with 10% formalin, then were embedded with paraffin. Sliced tissue was transferred to slides before proceeding with the staining protocol. HIF-1α or survivin expression was determined by immunohistochemical staining. A polyclonal rabbit anti-HIF-1α (Abcam, Cambridge, UK) or anti-sunvivin antibody (Abcam, Cambridge, UK) and horseradish peroxidase (HRP)-coupled goat anti-rabbit immunoglobulin G (IgG) antibodies (Sino Biological, Beijing, China) were utilized to detect the HIF-1α or survivin antigens in the mice brain, and the HRP substrate 3, 3'-diaminobenzidine (DAB) (Abcam, Cambridge, UK) was utilized for staining.

*Cell Immunocytochemistry*

Cells were cultured on poly-lysine-coated coverslips for 24 h followed by treatment with various reagents and/or transfection. After fixation in 4% paraformaldehyde for 15min at room temperature, cells were washed twice in PBS at room temperature for 5min and permeabilized with 0.25% Triton X-100 for 10min, and blocked with 1% normal goat serum, 20mg/ml BSA, 0.25% Triton X-100 in PBS for 3h at room temperature. Cells were incubated with primary antibodies for α-Syn (Sigma-Aldrich, St. Louis, MO, USA) which was diluted 1:1000 in blocking solution overnight at 4°C and then washed with PBS containing 0.1% Triton X-100 three times for 5min each, then were incubated with anti-rabbit Alexa Fluor 488-conjugated secondary antibody (Danvers, MA USA) diluted at 1:400 in blocking solution. Cells were then washed with PBS containing 0.1% Triton X-100 three times for 5 min each. Signals were analyzed on a fluorescence microscope (Axiovert 200M, Zeiss, Germany). And the α-Syn aggregation dots were counted with Image J software.

*Statistical Analysis*

Statistical analyses were performed using SPSS16.0 software (IBM SPSS, Armonk, NY, USA). The HIF-1α and survivin expression in mRNA level and in protein level, the percentage of apoptotic cells, the caspase 3 activity between two groups were analyzed by Student's t test. A $p$ value <0.05 or less was considered statistically significant.

## Results

*HIF-1α and survivin are upregulated by acute hypoxic-ischemia in vivo or in vitro*

To determine whether HIF-1α and survivin are overexpressed in brains under hypoxic-ischemia *in vivo*, we examined HIF-1α and survivin protein expression in brain samples of a mouse model of hypoxic-ischemia and normal brain samples by immunohistochemistry. Results revealed that eight of the ten (80%) hypoxic-ischemic brain samples exhibited significant high level of HIF-1α (Figure 1A) and survivin (Figure 1C) expression in both nuclei and cytoplasm of neurons or neuroglial cells. While the normal mice brain demonstrated weak or non expression of HIF-1α and survivin (Figure 1B and 1D). Although staining intensity varied among samples, it was relatively homogeneous within same brain sample. To further confirm the promotion of HIF-1α and survivin overexpression by hypoxia, we treated neuroblastoma SH-SY5Y cells by hypoxia and determined the expression of two molecules in both mRNA and protein levels. Figure 2A demonstrated that there was no significant difference in HIF-1α mRNA level in SH-SY5Y cells post hypoxia

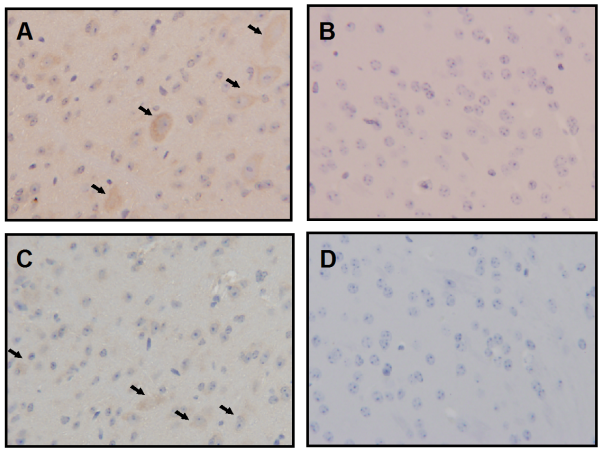


**Figure 1. Overexpression of HIF-1α and Survivin in Mice Brains Under Hypoxic-ischemia.** Immunohistochemical staining results for HIF-1α and survivin overexpression in mice brains with HIE. **A:** Overexpression of HIF-1α in hypoxic-ischemic mice brains. **B:** Immunohistochemical staining for HIF-1α in normal mice brains. **C:** Upregulation of survivin in hypoxic-ischemic mice brains. **D:** Immunohistochemical staining for survivin in normal mice brains. Positive staining cells were labeled with arrows

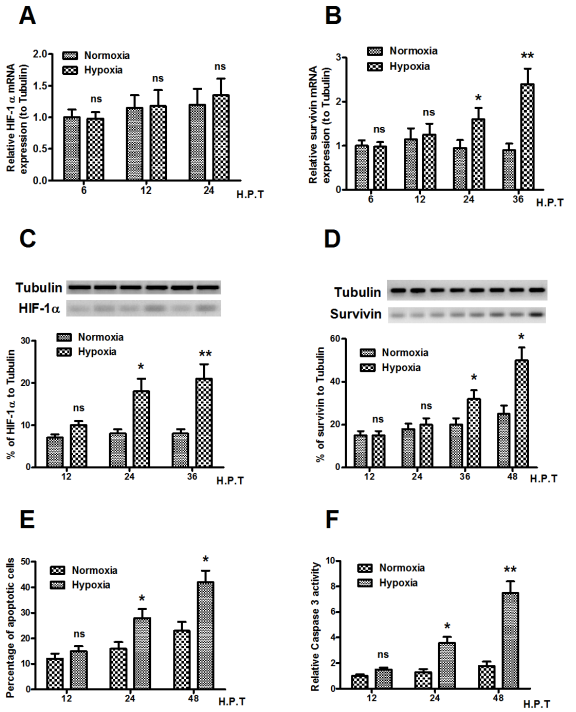


**Figure 2. HIF-1α and Survivin are Upregulated by Acute Hypoxic-Ischemia in Neuroblastoma SH-SY5Y Cells. A** and **B:** Expression of HIF-1α and survivin in SH-SY5Y cells under hypoxia (for 6, 12 or 24h), which was examined by RT-qPCR. **C** and **D:** Upregulation of HIF-1α and survivin in protein level, in SH-SY5Y cells under hypoxia (for 12, 24 or 48h), which was assayed by western blotting. **E:** Apoptosis of SH-SY5Y cells promoted by hypoxia treatment. Apoptosis of SH-SY5Y cells was examined with an annexin V-FITC apoptosis detection kit, and was expressed as the percentage of apoptotic cells to total cells. **F:** Upregulation of caspase 3 activity in SH-SY5Y cells under hypoxia, which was expressed as a relative value to control. All experiments were performed in triplicate. And statistical significance was showed as *$p$<0.05, or **$p$<0.01, ns: no significance

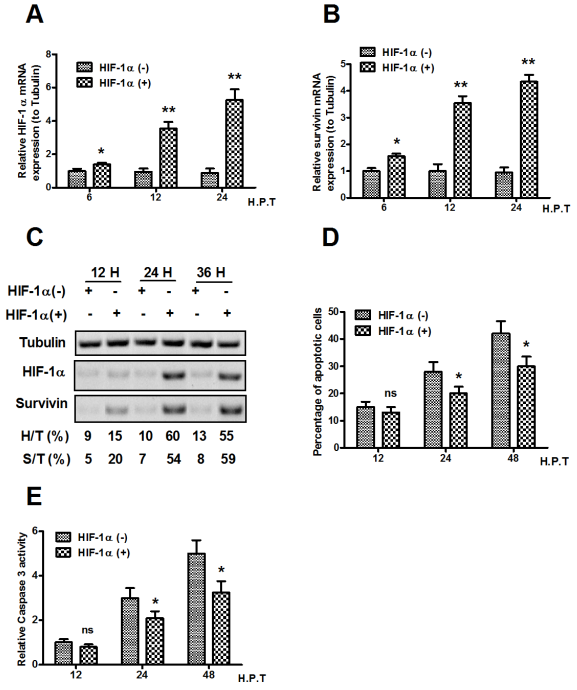


**Figure 3. Overexpression of HIF-1α Upregulated The Survivin Expression and Inhibits The Hypoxia-induced Apoptosis of SH-SY5Y Cells. A:** HIF-1α-pcDNA3.1(+) transfection significantly promoted the HIF-1α **(A)** and survivin **(B)** mRNA level in SH-SY5Y cells, compared to CAT-pcDNA3.1(+) (From 6 to 24h post transfection). **C:** HIF-1α-pcDNA3.1(+) transfection significantly upregulated the protein level of HIF-1α and survivin in SH-SY5Y cells, compared to CAT-pcDNA3.1(+) (From 12 to 36h post transfection). **D:** HIF-1α upregulation reduced the hypoxia-induced apoptotic SH-SY5Y cells (from 24 to 48h post transfection). **E:** HIF-1α upregulation reduced the hypoxia-induced caspase 3 activity in SH-SY5Y cells (from 24 to 48h post transfection). All experiments were performed in triplicate. And statistical significance was showed as *$p$<0.05, or **$p$<0.01, ns: no significance

or normoxia treatment. While 24 or 36h post hypoxia treatment, the protein level of HIF-1α in SH-SY5Y cells post hypoxia was significantly promoted, compared to the normaxia-treated cells ($p$<0.05 for 24 H.P.T and $p$<0.01 for 36 H.P.T.; Figure 2B). Interestingly, the survivin was significantly promoted in both mRNA and protein levels in SH-SY5Y cells from 24h (for mRNA) or from 36h (for protein) post hypoxia, compared to the normaxia-treated cells ($p$<0.05 or $p$<0.01; Figure 2C and 2D). Taken together, The HIF-1α and survivin were significantly promoted in a mouse model of hypoxic-ischemia or in SH-SY5Y cells post hypoxia *in vitro*.

*Upregulation of HIF-1α and survivin by hypoxia protect SH-SY5Y cells from apoptosis*

It has been well known that hypoxia induces apoptosis via HIF-1α (Carmeliet et al., 1998; Moritz et al., 2002; Greijer et al., 2004), in various tumor cells. The most direct induction of hypoxia induced apoptosis is the inhibition of the electron transport chain at the inner membrane of the mitochondria. To recognize the influence of hypoxia on the SH-SY5Y cells *in vitro*, we next determine the apoptosis of the SH-SY5Y cells under hypoxia. Figure 2E indicated that there were more apoptotic cells developed in the hypoxia group than in the normoxia group since 24h post treatment ($p$<0.05 respectively). And the casapase 3 results also confirmed such difference: A significantly higher level of relative caspase 3 activity was induced by hypoxia than by normoxia ($p$<0.05 or $p$<0.01; Figure 2F).

To explore the regulatory role of HIF-1α and survivin in the adaptation to hypoxia of SH-SY5Y cells, particularly in the hypoxia-induced apoptosis, we then overexpressed the HIF-1α in SH-SY5Y cells via gain-of-function strategy. The HIF-1α coding sequence was amplified and cloned into an eukaryotic expression vector, pcDNA3.1 (+). Positive recombinant HIF-1α-pcDNA3.1 (+) or CAT-pcDNA3.1 (+) plasmid, as a negative control, were transfected into SH-SY5Y cells to manipulate the HIF-1α level. It was demonstrated a significant upregulation of HIF-1α in mRNA level ($p$<0.05 or $p$<0.01; Figure 3A) or in protein level (Figure 3B) from 6h (for HIF-1α mRNA) or 12h (for HIF-1α protein level) post HIF-1α-pcDNA3.1 (+) transfection in SH-SY5Y cells under hypoxia. Moreover, we also determined the survivin level in the SH-SY5Y cells post HIF-1α upregulation.

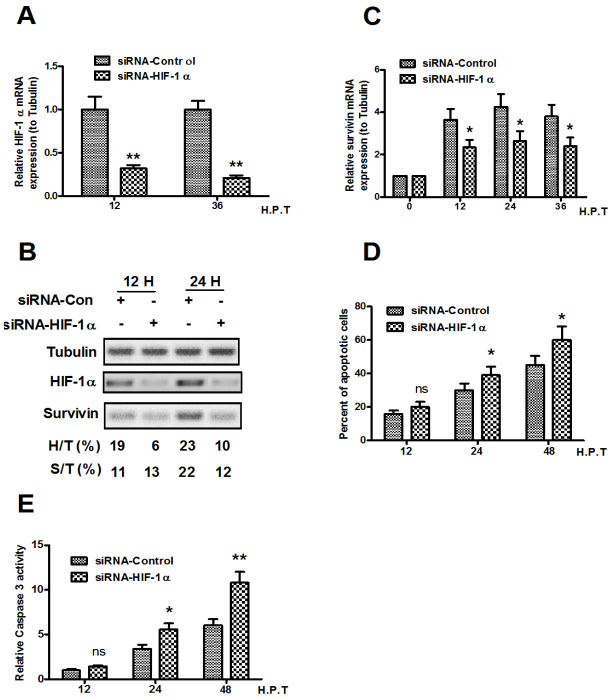


**Figure 4. HIF-1α Knockdown Downregulated the Survivin Expression and Aggravated the Hypoxia-induced Apoptosis of SH-SY5Y Cells. A** and **B:** HIF-1α specific siRNA transfection significantly downregulated the HIF-1α in both mRNA **(A)** and protein **(B)** levels in hypoxia-induced SH-SY5Y cells, compared to control siRNA. **C:** HIF-1α knockdown by siRNA downregulated the hypoxia-induced survivin expression in both mRNA **(C)** and protein **(B)** levels in SH-SY5Y cells. compared to control siRNA. **D:** HIF-1α knockdown by siRNA deteriorated the hypoxia-induced apoptosis in SH-SY5Y cells. **E:** HIF-1α knockdown by siRNA aggravated the hypoxia-promoted caspase 3 activity in SH-SY5Y cells. All experiments were performed in triplicate. And statistical significance was showed as *$p$<0.05, or **$p$<0.01, ns: no significance

Interestingly, compared to the pcDNA3.1 (+)-transfection cells, the HIF-1α upregulation by HIF-1α-pcDNA3.1 (+) transfection also promoted the survivin expression under hypoxia in both protein (Figure 3B) and mRNA ($p$<0.05 or $p$<0.01; Figure 3C) levels. As above-mentioned, the apoptotic cells and relative caspase 3 activity were also assayed, Figure 3D revealed a less apoptosis in the HIF-1α-upregulated SH-SY5Y cells than in the control cells (either $p$<0.05 for the 24 and 48h results). And a less casapase 3 activity was induced in the HIF-1α-upregulated SH-SY5Y cells by hypoxia (either $p$<0.05 for the 24 and 48h results; Figure 3E).

*HIF-1α knockdown inhibits the survivin promotion and deteriorates the apoptosis of SH-SY5Y cells under hypoxia*

In order to further identify the regulatory role of HIF-1α on apoptosis and survivin promotion by hypoxia, we then adopted a loss-of-function strategy to knockdown the HIF-1α and re-evaluated the hypoxia-promoted SH-SY5Y cell apoptosis and survivin upregulation. Firstly, Figure 4A showed that siRNA against HIF-1α, siRNA-HIF-1α, significantly blocked the HIF-1α expression in mRNA level (either $p$<0.01 for the 12 and 36h post siRNA transfection). And the HIF-1α upregulation by hypoxia was also blocked in protein level by the siRNA targeting HIF-1α (Figure 4B). Then we re-evaluated the survivin promotion by hypoxia, and it was shown in Figure 4B and 4C that both protein and mRNA levels of survivin were significantly lower in hypoxia-treated SH-SY5Y cells post siRNA-HIF-1α transfection than post siRNA control transfection ($p$<0.05 respectively). Then we evaluated the apoptotic cells and relative caspase 3 activity in siRNA-HIF-1α-or siRNA control-transfected SH-SY5Y cells post hypoxia. It was shown in Figure 4D and 4E that the siRNA-HIF-1α transfection deteriorated the hypoxia-promoted cell apoptosis and casapase 3 activity promotion ($p$<0.05 or $p$<0.01). Thus, it was confimed that the HIF-1α mediated the hypoxia-promoted survivin upregulation and apoptosis in SH-SY5Y cells.

## Discussion

Survivin was firstly discovered in 1997 by hybridization screening to the effector cell protease receptor-1 (EPR-1) (Ambrosini et al., 1997), and was the smallest member of the inhibitor of apoptosis proteins family (IAP; reviewed by Schimmerin (Schimmer et al., 2004). Survivin over-expression *in vivo* increases cell resistance to apoptosis (Grossman et al., 2001). Conversely, inhibition of survivin expression *in vitro*, by antisense survivin oligonucleotide or by chemical, increased the susceptibility to apoptosis of cervical cancer cell line and human neural tumor cell lines (Shankar et al., 2001; Karami et al., 2013; Li et al., 2013; Al-Astani et al., 2014). The synthesis and degradation of survivin is cell cycle-dependent. Survivin transcription increases during G1, and reaches a peak in G2-M (Kobayashi et al., 1999). The expression of survivin is up-regulated at a transcriptional level by the nuclear factor-ϰB, which, in turn, can be activated indirectly by growth factors via the phosphatidylinositol 3-kinase/Akt pathway (Van Antwerp et al., 1998). Additionally, insulinlike growth factor I/mTOR signaling and other factors have been reported to up-regulate survivin (Sommer et al., 2003; You et al., 2004; Vaira et al., 2007). On the other hand, survivin is repressed at the transcriptional level by wild-type p53 and p75 (Hoffman et al., 2002). And the alternative splicing of survivin mRNA yields isoforms with different expression patterns and abilities to prevent apoptosis (Noton et al., 2006). Besides, survivin degradation has a regulation on the survivin level (Fortugno et al., 2003).

Recently, survivin expression has been indicated to be upregulated following the induction of HIF-1α by hypoxia resulting from tumor formation, possibly leading to tumor progression of cervical cancers (Bai et al., 2013). The participation of HIF-1α in hypoxia-induced survivin expression was also confirmed in non-small cell lung cancer cells (Chen et al., 2012). And the HIF-1α-dependence was also found in the epidermal growth factor (EGF)-stimulated survivin upregulation in breast cancer cells under hypoxia. The EGFR signaling activation subsequently increased the level of HIF-1α, which then activated survivin gene transcription through direct binding to the survivin promoter (Peng et al., 2006). Thus, the HIF-1α-dependent survivin promotion might be

a novel adaptive strategy for IHE.

Because the apoptosis during brain damage is reversible and is regarded as major target of therapeutic interventions (Kato et al., 1999; Newcomb et al., 2006). In present study, to recognize the detailed apoptotic cascades in apoptosis in hypoxic-ischemic encephalopathy caused by Reduced or completely interrupted cerebral blood supply, we determined the promotion of HIF-1α and survivin in brain samples of a mouse model of hypoxic-ischemia by immunochemistry and in the neuroblastoma SH-SY5Y cells post hypoxia treatment by RT-qPCR and western blot assay. Then we adopted the gain-of-function and loss-of-function strategies to enlarge or block the promotion of HIF-1α and survivin by hypoxia in SH-SY5Y cells; also the influence of the HIF-1α manipulation on the hypoxia-induced cell apoptosis was determined. Results demonstrated that both HIF-1α and survivin were significantly promoted in a mouse model of hypoxic-ischemia or in SH-SY5Y cells post hypoxia treatment *in vitro*. And a manually upregulated HIF-1α could promote the hypoxia-induced survivin upregulation and improve the hypoxia-induced SH-SY5Y cell apoptosis. On the other hand, the HIF-1α knockdown by RNAi reduced the hypoxia-induced survivin upregulation and cell apoptosis. Therefore, present study confirmed the protective role of HIF-1α and survivin in the hypoxia-induced SH-SY5Y cell apoptosis, and the survivin upregulation by hypoxia is HIF-1α-dependent. Promotion of HIF-1α and survivin might be a valuable stragegy for therapeutic intervention to hypoxic-ischemic encephalopathy.

In conclusion, HIF-1α and survivin are upregulated by acute hypoxic-ischemia in an IHE mouse model or in hypoxic neuroblastoma cells. And the upregulation of HIF-1α and survivin by hypoxia protect SH-SY5Y cells from apoptosis, in particular, the survivin-mediated apoptosis protection is HIF-1α-dependent. Therefore, upregulation of HIF-1α by hypoxia protect neuroblastoma cells from apoptosis by promoting survivin expression.

## Acknowledgements

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