Neuroscience Letters 481 (2010) 154–158

Contents lists available at ScienceDirect

Neuroscience Letters

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

Salidroside attenuates hypoxia-induced abnormal processing of amyloid precursor protein by decreasing BACE1 expression in SH-SY5Y cells

Qing-Yun Li[a], Hong-Mei Wang[b], Zhi-Quan Wang[a], Jian-Fang Ma[b], Jian-Qing Ding[b,*], Sheng-Di Chen[a,b,**]

[a] Lab of Neurodegenerative Diseases and Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences(SIBS), Chinese Academy of Sciences(CAS), and Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[b] Department of Neurology and Institute of Neurology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

ARTICLE INFO

Article history:
Received 23 April 2010
Received in revised form 3 June 2010
Accepted 28 June 2010

Keywords:
Salidroside
Hypoxia
β-Site APP cleaving enzyme1
Amyloid-beta

ABSTRACT

Hypoxia which is mainly mediated by hypoxia-inducible factor 1 (HIF-1), can greatly contribute to the occurrence of Alzheimer's disease (AD) by increasing β-site APP cleaving enzyme (BACE1) gene expression, protein level and β-secretase activity, resulting in a significant generation of amyloid-beta (Aβ). Salidroside has been reported to have great neuroprotective effects. The aim of this study was to investigate the effects of salidroside on hypoxia-induced abnormal processing of the amyloid precursor protein (APP) in SH-SY5Y cells and its possible mechanism. Western blot analysis showed that 200 μM of salidroside pretreatment significantly decreased BACE1 protein level and promoted the secretion of sAPPα in hypoxic condition. Salidroside had no effect on the level of APP, ADAM10 and ADAM17. ELISA analysis revealed that salidroside was able to inhibit the increase of β-secretase activity and Aβ generation induced by hypoxia, with no effect on γ-secretase activity. Notably, under hypoxia condition, mRNA of *BACE1* and protein level of HIF-1α were decreased by salidroside pretreatment. These results demonstrated for the first time that salidroside was able to attenuate abnormal processing of amyloid precursor protein induced by hypoxia in SH-SY5Y cells, providing a new insight into prevention and treatment of Alzheimer's disease.

Alzheimer's disease (AD) is the most common type of dementia among older people. The aggregation and accumulation of extracellular Aβ in the brain are important pathologic features of AD [7]. Aβ is generated by sequential proteolysis of the amyloid precursor protein (APP) by β-secretase and then by γ-secretase [7,12]. BACE1 is a transmembrane aspartyl protease and is the principal β-secretase responsible for Aβ generation *in vivo* [1,4]. APP can also be cleaved by α-secretase (ADAM10, ADAM17) within the Aβ domain to generate soluble neurotrophic sAPPα [7,12].

Many studies have revealed that hypoxic/ischemic injury can greatly contribute to the occurrence of AD [13,15,17,26]. Hypoxia significantly increases *BACE1* mRNA and protein levels [10,19,20]. Recent studies have shown that *BACE1* promoter contains a physiological functional hypoxia response element (HRE), and hypoxia can facilitate AD pathogenesis by increasing HIF-1α binding to the HRE, thereby up-regulate *BACE1* gene expression, protein level and β-secretase activity, and eventually leading to a significantly generation of Aβ both *in vivo* and *in vitro* [8,16,24,25].

Salidroside(2-(4-hydroxyphenyl) ethyl β-D-glucopyranoside) is extracted from the root of *Rhodiola rosea* L., which has been used in traditional Tibetan medicine for a long history. Recently, salidroside has been found to have a protective effect against amyloid-beta induced cytotoxicity in PC12 cells [9], and against H_2O_2-induced cell apoptosis in SH-SY5Y human neuroblastoma cells, primary rat hippocampal neurons and PC12 cells [2,3,22]. Our previous studies revealed that salidroside conferred neuroprotection against MPTP-induced nigrostriatal dopaminergic damage in mice (unpublished data). Another study reported the protective effects of salidroside on hypoxia/hypoglycemia-induced injury in cultured neurons [23]. Taken together, these studies suggest that salidroside exerts great neuroprotective effects and potential pharmaceutical applications for neurodegenerative disorders. However, whether salidroside has protective effects against hypoxia facilitated Alzheimer's disease remains unknown. Therefore, the aim of present study was to test

Abbreviations: Aβ, amyloid-beta; AD, Alzheimer's disease; ADAM, a disintegrin and metalloproteinase; APP, amyloid precursor protein; BACE, β-site APP cleaving enzyme; sAPPα, soluble amyloid precursor protein α; HIF-1α, hypoxia-inducible factor 1α; HRE, hypoxia response element; PS, presenilin; PEN-2, presenilin enhancer 2; APH-1, anterior pharynx-defective 1.

* Corresponding author. Tel.: +86 21 6445 7249; fax: +86 21 6445 7249.
** Corresponding author at: Department of Neurology and Institute of Neurology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China. Tel.: +86 21 6445 7249; fax: +86 21 6445 7249.
E-mail addresses: jqding18@yahoo.com (J.-Q. Ding), chen_sd@medmail.com.cn (S.-D. Chen).

0304-3940/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2010.06.076

the effect of salidroside on hypoxia-induced abnormal processing of APP and the potential mechanism.

Human neuroblastoma SH-SY5Y cells stably transfected with human wild type APP695 cDNA (SH-SY5Y^{APP695}, kindly provided by Prof. Dai Zhang, Institute of Mental Health, Peiking University, Beijing) were cultured in DMEM/F12 (GIBCO, Carlsbad, CA) containing 1% non-essential amino acids, 1% glutamine, 300 μg/ml G418 (Sigma–Aldrich Inc.) and 10% heat-inactivated FBS (Hyclone, Rockford, IL) at 37 °C with 5% CO_2. Twelve to sixteen hours before drug treatment, the media was replaced with FBS-free DMEM/F12 media. Salidroside (Purity (HPLC) 99.74%. Shanghai Pharm Valley Corp., Shanghai, China) was dissolved in PBS and sterilized through a 0.2 μm filter prior to use. For drug and hypoxia treatment, SH-SY5Y^{APP695} cells were pretreated with indicated concentration of salidroside for 1 h then incubated in a 37 °C chamber containing 1% O_2 and 5% CO_2 as indicated time.

As for ELISA assay, the supernatants of SH-SY5Y^{APP695} cells were collected and the concentrations of secreted Aβ40 and Aβ42 were determined using commercial ELISA kits (Shanghai ExCell Biology, China), following manufacturer's protocols. β-Secretase activity and γ-secretase activity of cultured SH-SY5Y^{APP695} cells were detected using β-Secretase Activity Assay Kit (BioVision, CA, US) and γ-Secretase Activity Assay Kit (R&D Systems, Inc. Minneapolis, US), according to manufacturer's instructions.

LDH (lactate dehydrogenase) activity was determined using the Cytotoxicity Detection Kit (LDH) (Roche Applied Science, Mannheim, Germany), following manufacturer's protocols.

Western blot analysis: The culture media of SH-SY5Y^{APP695} cells were collected, concentrated with Centricon YM-30 filter units (Millipore, Bedford, MA) and frozen at −80 °C until further analysis. Treated cells were lysed in ice-cold RIPA (50 mM Tris–HCl, pH 7.4, 150 mM sodium chloride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate). Protein concentrations were determined using the Pierce BCA Protein Assay Kit (Thermo Scientific). Equal amounts of lysates were separated by SDS-PAGE, then transferred into PVDF membrane (Millipore). The membranes were blocked with 5% skim milk in 1× Tris-buffered saline containing 0.8‰ Tween-20 for 1 h. Then the membranes were incubated overnight at 4 °C with the following antibody: HIF-1α, Novus Biologicals, Littleton, US; BACE1, Alzheimer precursor protein A4 monoclonal antibody, 22C11, ADAM 10 and ADAM17, Millipore; Human β Amyloid (1-17), clone 6E10, Aph-1aL, β-actin and α-tubulin, Sigma–Aldrich Inc. The membranes were then incubated with goat anti-mouse or goat anti-rabbit horseradish (HRP) secondary antibody (Jakson ImmunoResearch Laboratories) at room temperature for 1 h. The signals were developed with Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA).

Quantitative real-time polymerase chain reaction (RT-PCR): Total cellular RNA was extracted by TRIzol reagent (Invitrogen). RT was performed with a cDNA synthesis kit according to manufacturer's instructions (Takara, Dalian, China). For quantitative SYBR Green (2×SYBR Green Real-time PCR Master Mix, TOYOBO, Osaka, Japan) real-time PCR, 50 ng of cDNA was used per reaction. The following specific oligonucleotide primers were used for BACE1 (sense, 5′-CCGGCGGGAGTGGTATTATG-3′, antisense, 5′-GCAAACGAAGGTTGGTGGT-3′), HIF-1α (sense, 5′-ACTAGCCGAGGAAGAACTATGAA-3′, antisense, 5′-TACCCACACTGAGGTTGGTTA-3′), with β-actin as an internal control (sense, 5′-CATGTACGTTGCTATCCAGGC-3′, antisense, 5′-CTCCTTAATGTCACGCACGAT-3′). Real-time RT-PCR was performed with ABI 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) and data were analyzed using ΔΔCT method.

All data in bar graphs were expressed as mean ± SEM ($n \geq 3$). Statistical analysis was performed by Student's *t*-test or ANOVA method using Prism software (GraphPad, San Diego, CA). A value of $p < 0.05$ was considered to be statistically significant.

We first evaluated whether hypoxia could increase the expression of BACE1 in human neuroblastoma SH-SY5Y^{APP695} cells. For this, we treated SH-SY5Y^{APP695} cells with hypoxic condition for 0, 3, 6, 12 or 24 h. As shown in Fig. 1A and B, hypoxia markedly increased the protein level of BACE1, with a significantly 2.6-fold increase at 12 h. Quantitative real-time PCR analysis showed 1.4-fold increased expression of *BACE1* mRNA (Fig. 1C). Concomitantly, the HIF-1α protein level elevated obviously at 6 h (Fig. 1A). We also observed a significant increase of total BACE1 activity (Fig. 1D) and significant higher level of secreted Aβ40 (Fig. 1E) and Aβ42

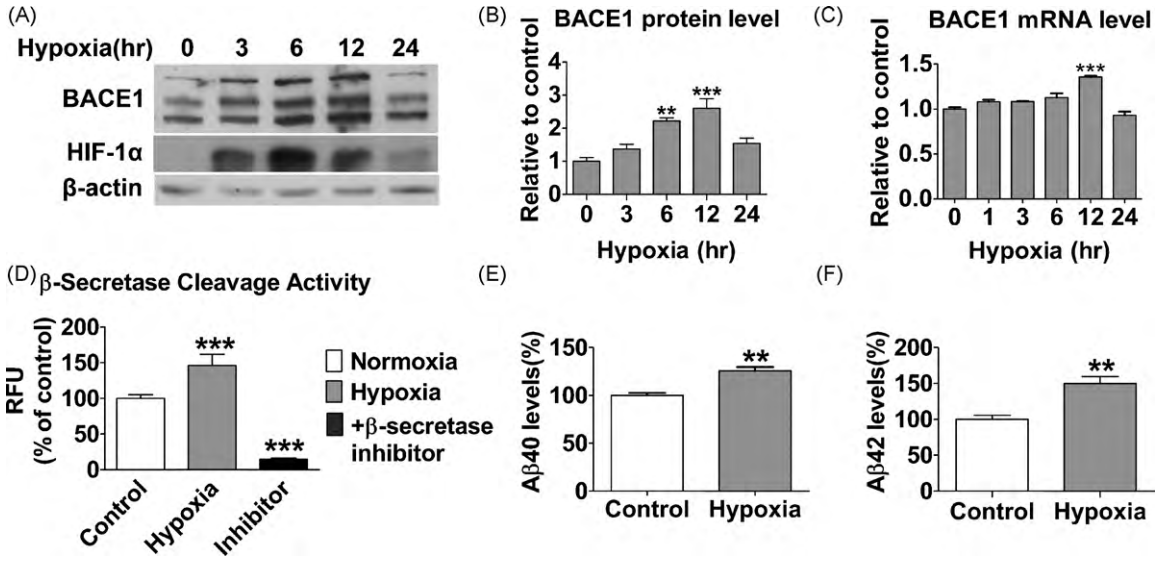

Fig. 1. Hypoxia increased BACE1 expression, β-secretase activity and Aβ generation in SH-SY5Y^{APP695} cells. SH-SY5Y^{APP695} cells were treated with 1% O_2 for indicated time. (A) BACE1 and HIF-1α were detected by Western blot with β-actin as an internal control. (B) Quantification of BACE1 protein levels. (C) *BACE1* mRNA level was detected by real-time quantitative RT-PCR. (D) Lysates of hypoxia-treated (1% O_2, 12 h) and untreated SH-SY5Y^{APP695} cells were assayed for β-secretase activity using β-Secretase Activity Assay Kit. β-secretase inhibitor was used as negative control. RFU, the relative fluorescence units per μg of protein sample. $^{**}p < 0.01$, $^{***}p < 0.001$ by ANOVA. (E and F) ELISA was performed to measure Aβ40(E) and Aβ42(F) in conditioned media from hypoxia-treated (1% O_2, 12 h) and untreated SH-SY5Y^{APP695} cells. $^{**}p < 0.01$ by Student's *t*-test.

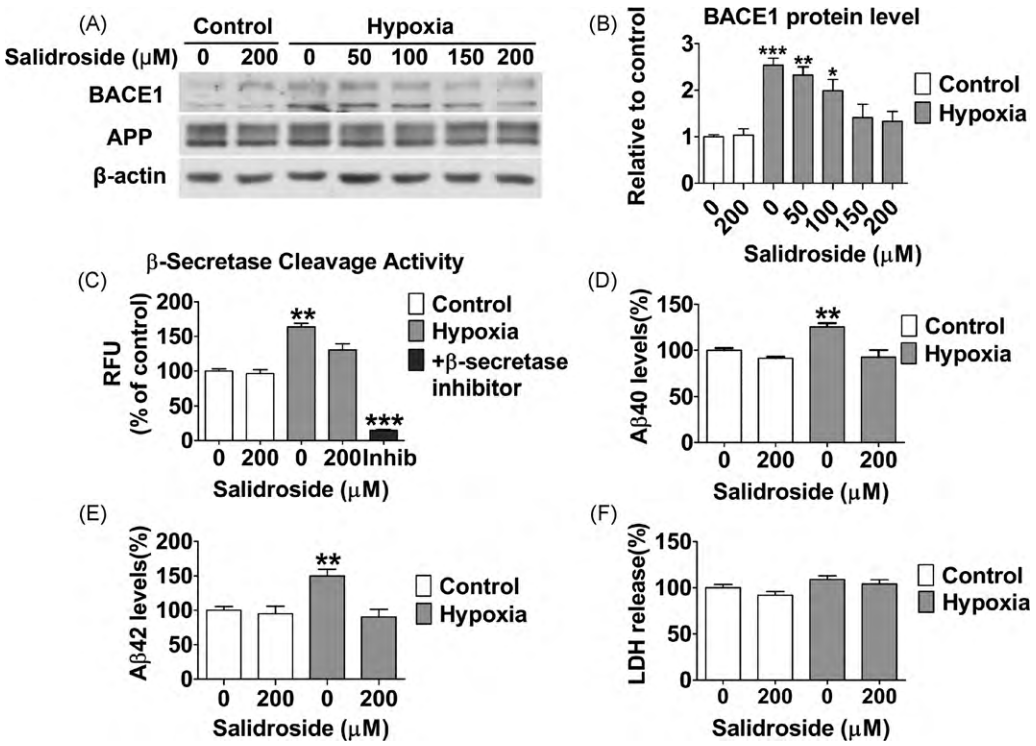

Fig. 2. Salidroside inhibited hypoxia-induced increase of BACE1 protein level, β-secretase activity and Aβ generation in SH-SY5Y^{APP695} cells. After pretreatment for 1 h in the presence or absence of indicated concentration of salidroside, SH-SY5Y^{APP695} cells were treated with normoxic or 1% O_2 for 12 h. (A) BACE1 and APP protein were detected by Western blot with β-actin as a loading control. (B) Densitometric analysis of BACE1 protein levels. (C) Lysates of SH-SY5Y^{APP695} cells were applied to ELISA assay for β-secretase activity using β-Secretase Activity Assay Kit. RFU, the relative fluorescence units per μg of protein sample. β-Secretase inhibitor was used as negative control. (D and E) Conditioned cell culture media were collected, ELISA was performed to measure Aβ40(D) and Aβ42(E). (F) LDH was assayed using the Cytotoxicity Detection Kit. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ by ANOVA.

(Fig. 1F). These observations strongly indicated that hypoxia could increase BACE1 expression, β-secretase activity and Aβ level in SH-SY5Y^{APP695} cells.

To investigate the effect of salidroside on BACE1, SH-SY5Y^{APP695} cells were preincubated with salidroside (0, 50, 100, 150 or 200 μM) for 1 h, then incubated in hypoxic condition for 12 h. As shown in Fig. 2A and B, 100–200 μM of salidroside pretreatment significantly decreased BACE1 protein level in a dose-dependent manner. In the presence of 150 or 200 μM of salidroside, there were only 1.412- or 1.332-fold BACE1 protein as compared with only hypoxia-treated cells (2.635-fold).

We further tested the effect of salidroside on β-secretase activity in hypoxia condition by ELISA analysis. As shown in Fig. 2C, 200 μM of salidroside pretreatment significantly attenuated hypoxia-induced up-regulation of β-secretase activity. Based on the results of BACE1 protein level and β-secretase activity, we expected that salidroside might inhibit the generation of Aβ. Therefore, we examined extracellular Aβ level in cultured medium from SH-SY5Y^{APP695} cells by ELISA analysis. The results indicated that 200 μM of salidroside preincubation significantly reduced hypoxia-induced excessively release of Aβ40 (Fig. 2D) and Aβ42 (Fig. 2E) to normal level. Further, we did not find obviously change in APP protein level either by salidroside or by hypoxia treatment (Fig. 2A), this implied that the reduced Aβ generation was not the result of decreased APP, but may due to down-regulation of BACE1 by salidroside.

LDH analysis showed that 200 μM of salidroside preincubation had no significant effect on LDH release into the culture medium under normoxia or hypoxia condition (Fig. 2F). This suggested that the decreased BACE1 protein level, β-secretase activity and Aβ generation after salidroside pretreatment were not induced by toxicity of salidroside to cells.

To investigate the effect of salidroside on α-secretase and sAPPα, we performed Western blot analysis to detect sAPPα release in the supernatants of SH-SY5Y^{APP695} cells using the 6E10 antibody, which recognized amino acid resides 1-17 of the human amyloid-beta. Compared with normoxia, hypoxia had no significant effect on the level of sAPPα (Fig. 3A and B). Notably, 200 μM of salidroside pretreatment significantly increased sAPPα level (about 2-fold) either in hypoxia-treated or in untreated SH-SY5Y^{APP695} cells (Fig. 3A and B). Consistent with some studies [18,25], hypoxic treatment did not significantly influence the levels of ADAM10 and ADAM17 (Fig. 3A). However, 200 μM of salidroside pretreatment failed to alter the expression of APP, ADAM10 and ADAM17 in hypoxic condition (Fig. 2A and Fig. 3A). It was thus implied that the promotion of sAPPα secretion was not the result of an increase in cellular APP, ADAM10 and ADAM17 expression, but was possibly due to decrease in β-secretase activity and other pharmacological properties.

As documented [10,18], hypoxia dramatically enhanced APH-1α protein expression. Here, we also observed a significantly increase (1.6-fold) in the APH-1α protein level by 12 h of hypoxic treatment and 200 μM of salidroside pretreatment reduced APH-1α levels to 1.446-fold (Fig. 3C and D). Hypoxia treatment had no effect on the levels of PEN-2, PS1 and Nicastrin, and their protein levels remained unchanged by 200 μM of salidroside pretreatment (data not shown). We next investigated the effect of salidroside on γ-secretase activity. As can be seen in Fig. 3E, 12 h of hypoxic treatment with or without 200 μM of salidroside preincubation failed to significantly affect γ-secretase activity in SH-SY5Y^{APP695} cells. This could be attributed to the unchanged other three components of γ-secretase (PEN-2, PS1 and Nicastrin) and their coordinate regulation. This indicated that only the increase of APH-1α was insufficient to alter the activity of γ-secretase complex.

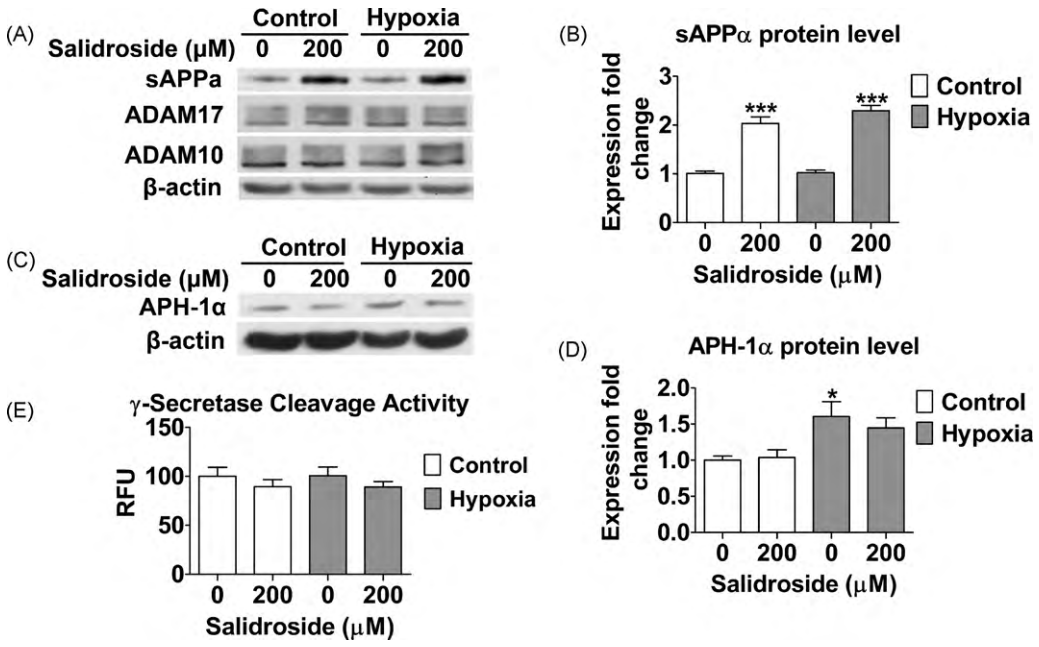

Fig. 3. Effects of salidroside on sAPPα release and γ-secretase activity. After pretreatment for 1 h in the presence or absence of 200 μM of salidroside, SH-SY5Y^{APP695} cells were treated with normoxic or 1% O_2 for 12 h. (A) sAPPα level in the supernatants of SH-SY5Y^{APP695} cells was detected. The cell lysates were analyzed and immunoblotted with antibodies against ADAM10, ADAM17, with β-actin as an internal control. (B) Quantification of sAPPα protein level. (C) APH-1α was detected by Western blot with β-actin as an internal control. (D) Quantification of APH-1α protein level. (E) Lysates of SH-SY5Y^{APP695} cells were applied to ELISA assay for γ-secretase activity using γ-Secretase Activity Assay Kit. RFU, the relative fluorescence units per μg of protein sample. $^{***}p < 0.001$, $^{*}p < 0.05$ by ANOVA.

To understand the potential mechanism underlying the reduced expression of BACE1 protein after salidroside pretreatment, *BACE1* mRNA was measured in salidroside and/or hypoxia-treated SH-SY5Y^{APP695} cells by real-time quantitative RT-PCR. Under hypoxic condition, endogenous *BACE1* mRNA levels showed significantly 1.4-fold increase compared with normoxia, and 200 μM of salidroside pretreatment could reduce *BACE1* mRNA to 1.051-fold (Fig. 4A). These results suggested that salidroside could inhibit hypoxia-induced up-regulation of *BACE1* transcription. Since hypoxia-induced *BACE1* mRNA and protein up-expression was principally mediated by activation of HIF-1α [8,16,25], we continued to analyze whether HIF-1α protein level was decreased by salidroside pretreatment under hypoxic condition. As shown in Fig. 4B and C, 200 μM of salidroside pretreatment significantly reduced HIF-1α protein level (0.55 ± 0.06229) compared with only hypoxia-treated SH-SY5Y^{APP695} cells, with no influence on *HIF-1α* mRNA level (Fig. 4D). These results indicated that salidroside attenuated hypoxia-induced increase of BACE1 possibly through down-regulating HIF-1α protein level.

Our study validated the findings that hypoxia could increase BACE1 expression, β-secretase activity and Aβ generation [10,16,19,25] in human neuroblastoma SH-SY5Y cells. Here we

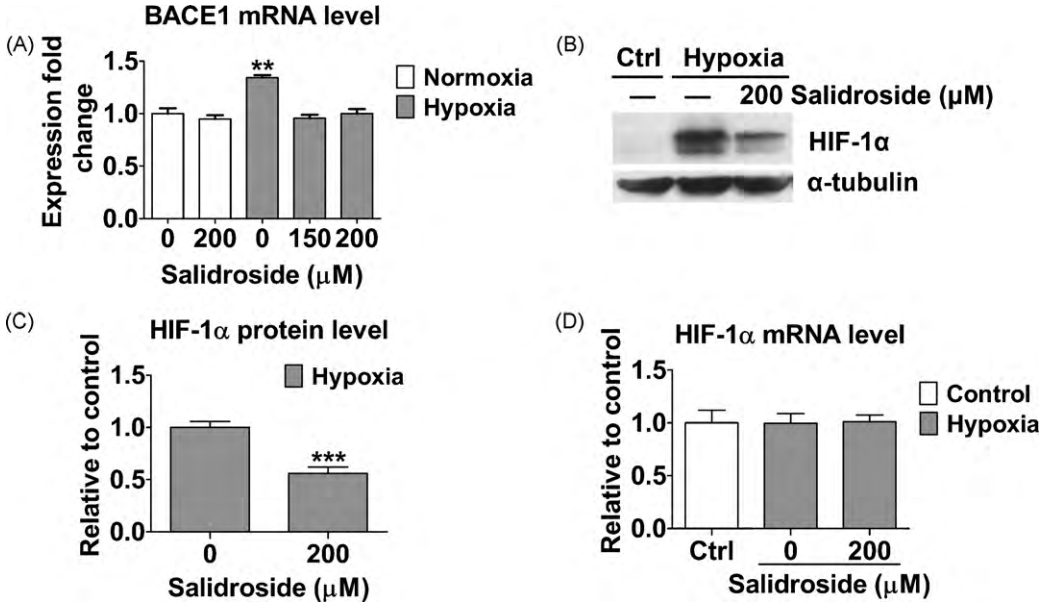

Fig. 4. Effects of salidroside on mRNA level of *BACE1* and protein expression of HIF-1α in hypoxia-treated SH-SY5Y^{APP695} cells. SH-SY5Y^{APP695} cells were treated with or without 150 or 200 μM of salidroside for 1 h then incubated in normoxic or 1% O_2 for 12 h. (A) *BACE1* mRNA level was detected by real-time quantitative RT-PCR. $^{**}p < 0.01$ by ANOVA. (B) HIF-1α was detected by Western blot with α-tubulin as an internal control. (C) Quantification of HIF-1α protein levels. $^{***}p < 0.001$ by Student's *t*-test. (D) *HIF-1α* mRNA level was detected by real-time quantitative RT-PCR.

found that salidroside pretreatment markedly attenuated hypoxia-induced increase in BACE1 protein levels and β-secretase activity. We also examined extracellular Aβ level by ELISA analysis and detected that 200 μM of salidroside preincubation significantly reduced hypoxia-induced excessively release of Aβ. LDH analysis confirmed that pretreatment with salidroside had no neurotoxicity to SH-SY5Y^{APP695} cells. Thus, the reduced BACE1 protein level, β-secretase activity and Aβ generation by salidroside pretreatment were not induced by neurotoxicity of salidroside to SH-SY5Y^{APP695} cells. We also found that 200 μM of salidroside pretreatment significantly promoted sAPPα secretion. However, hypoxia and salidroside had no significant effects on the levels of ADAM10, ADAM17, APP and γ-secretase activities, this indicated that the elevated release of sAPPα was due to reduction in BACE1 protein level, β-secretase activity and other pharmacological properties of salidroside, not appear to be mediated by the promotion of APP, ADAM10 and ADAM17 expression.

Multiple studies have shown significantly elevated BACE1 protein level and activity in sporadic AD patients, suggesting that abnormal increased BACE1 may constitute a determining factor in the pathogenesis of sporadic AD [5,6,21]. Some studies demonstrated that BACE1 inhibition dramatically reduced cerebral Aβ levels, and BACE1 deficiency rescued memory deficits in AD mouse model without profound side effects [1,11,14]. These findings indicate that BACE1 is a prime therapeutic target for prevention and therapy of AD. Several studies have revealed that *BACE1* gene promoter contains a physiological functional HIF-1α binding element, and HIF-1α can modulate *BACE1* mRNA and protein level by binding to the *BACE1* promoter [8,16,24,25]. Overexpression of HIF-1α with *HIF-1α* cDNA increased *BACE1* mRNA and protein level, while down-regulation of HIF-1α by siRNA reduced the level of BACE1 in both hypoxia-treated and untreated cells [8,16,25]. Furthermore, in hippocampus and cortex of HIF-1α conditional knock-out mice, BACE1 protein level was significantly reduced [25]. Thus, we tested whether the inhibition of BACE1 by salidroside under hypoxia condition was mediated by regulation of HIF-1α. As we observed, salidroside significantly reduced HIF-1α protein and *BACE1* mRNA level. This indicated that salidroside may inhibit binding of HIF-1α to *BACE1* promoter by reducing HIF-1α protein level, thereby down-regulated *BACE1* mRNA and protein level, modulating process of APP.

In conclusion, this work showed for the first time that salidroside could inhibit hypoxia-induced increase of BACE1 expression, β-secretase activity, Aβ generation, and promote the secretion of sAPPα. These findings suggest a new potential application value of salidroside in the treatment of AD.

Acknowledgments

We thank Dr. Guoqiang Chen (Department of Pathophysiology, Key Laboratory of Cell Differentiation and Apoptosis of the Chinese Ministry of Education, Shanghai Jiao Tong University School of Medicine) for kindly providing hypoxia incubator. This work was supported by grants from State Key Basic Research Program (2006CB500706, 2010CB945200), Shanghai Key Project of Basic Science Research (07DJ14005, 09DZ1950400), Shanghai Key Discipline Program (S30202) and Program for Outstanding Medical Academic Leader (LJ 06003).

References

[1] H. Cai, Y. Wang, D. McCarthy, H. Wen, D.R. Borchelt, D.L. Price, P.C. Wong, BACE1 is the major beta-secretase for generation of Abeta peptides by neurons, Nature Neuroscience 4 (2001) 233–234.

[2] L. Cai, H. Wang, Q. Li, Y. Qian, W. Yao, Salidroside inhibits H_2O_2-induced apoptosis in PC12 cells by preventing cytochrome c release and inactivating of caspase cascade, Acta Biochimica et Biophysica Sinica 40 (2008) 796–802.

[3] X. Chen, Q. Zhang, Q. Cheng, F. Ding, Protective effect of salidroside against H_2O_2-induced cell apoptosis in primary culture of rat hippocampal neurons, Molecular and Cellular Biochemistry 332 (2009) 85–93.

[4] S.L. Cole, R. Vassar, BACE1 structure and function in health and Alzheimer's disease, Current Alzheimer Research 5 (2008) 100–120.

[5] H. Fukumoto, B.S. Cheung, B.T. Hyman, M.C. Irizarry, Beta-secretase protein and activity are increased in the neocortex in Alzheimer disease, Archives of Neurology 59 (2002) 1381–1389.

[6] H. Fukumoto, D.L. Rosene, M.B. Moss, S. Raju, B.T. Hyman, M.C. Irizarry, Beta-secretase activity increases with aging in human, monkey, and mouse brain, The American Journal of Pathology 164 (2004) 719–725.

[7] S Gandy, The role of cerebral amyloid beta accumulation in common forms of Alzheimer disease, The Journal of Clinical Investigation 115 (2005) 1121–1129.

[8] M. Guglielmotto, M. Aragno, R. Autelli, L. Giliberto, E. Novo, S. Colombatto, O. Danni, M. Parola, M.A. Smith, G. Perry, E. Tamagno, M. Tabaton, The up-regulation of BACE1 mediated by hypoxia and ischemic injury: role of oxidative stress and HIF1alpha, Journal of Neurochemistry 108 (2009) 1045–1056.

[9] S.I. Jang, H.O. Pae, B.M. Choi, G.S. Oh, S. Jeong, H.J. Lee, H.Y. Kim, K.J. Kang, Y.G. Yun, Y.C. Kim, H.T. Chung, Salidroside from Rhodiola sachalinensis protects neuronal PC12 cells against cytotoxicity induced by amyloid-beta, Immunopharmacology and Immunotoxicology 25 (2003) 295–304.

[10] L. Li, X. Zhang, D. Yang, G. Luo, S. Chen, W. Le, Hypoxia increases Abeta generation by altering beta- and gamma-cleavage of APP, Neurobiology of Aging 30 (2009) 1091–1098.

[11] Y. Luo, B. Bolon, M.A. Damore, D. Fitzpatrick, H. Liu, J. Zhang, Q. Yan, R. Vassar, M. Citron, BACE1 (beta-secretase) knockout mice do not acquire compensatory gene expression changes or develop neural lesions over time, Neurobiology of Disease 14 (2003) 81–88.

[12] M.P. Mattson, Pathways towards and away from Alzheimer's disease, Nature 430 (2004) 631–639.

[13] N.N. Nalivaevaa, L. Fisk, E.G. Kochkina, S.A. Plesneva, I.A. Zhuravin, E. Babusikova, D. Dobrota, A.J. Turner, Effect of hypoxia/ischemia and hypoxic preconditioning/reperfusion on expression of some amyloid-degrading enzymes, Annals of the New York Academy of Sciences 1035 (2004) 21–33.

[14] M. Ohno, E.A. Sametsky, L.H. Younkin, H. Oakley, S.G. Younkin, M. Citron, R. Vassar, J.F. Disterhoft, BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease, Neuron 41 (2004) 27–33.

[15] J.A. Schneider, R.S. Wilson, E.J. Cochran, J.L. Bienias, S.E. Arnold, D.A. Evans, D.A. Bennett, Relation of cerebral infarctions to dementia and cognitive function in older persons, Neurology 60 (2003) 1082–1088.

[16] X. Sun, G. He, H. Qing, W. Zhou, F. Dobie, F. Cai, M. Staufenbiel, L.E. Huang, W. Song, Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression, Proceedings of the National Academy of Sciences of the United States of America 103 (2006) 18727–18732.

[17] E. Tamagno, M. Guglielmotto, M. Aragno, R. Borghi, R. Autelli, L. Giliberto, G. Muraca, O. Danni, X. Zhu, M.A. Smith, G. Perry, D.G. Jo, M.P. Mattson, M. Tabaton, Oxidative stress activates a positive feedback between the gamma- and beta-secretase cleavages of the beta-amyloid precursor protein, Journal of Neurochemistry 104 (2008) 683–695.

[18] R. Wang, Y.W. Zhang, X. Zhang, R. Liu, X. Zhang, S. Hong, K. Xia, J. Xia, Z. Zhang, H. Xu, Transcriptional regulation of APH-1A and increased gamma-secretase cleavage of APP and Notch by HIF-1 and hypoxia, FASEB J. 20 (2006) 1275–1277.

[19] Y. Wen, O. Onyewuchi, S. Yang, R. Liu, J.W. Simpkins, Increased beta-secretase activity and expression in rats following transient cerebral ischemia, Brain Research 1009 (2004) 1–8.

[20] S. Xue, L. Jia, J. Jia, Hypoxia and reoxygenation increased BACE1 mRNA and protein levels in human neuroblastoma SH-SY5Y cells, Neuroscience Letters 405 (2006) 231–235.

[21] L.B. Yang, K. Lindholm, R. Yan, M. Citron, W. Xia, X.L. Yang, T. Beach, L. Sue, P. Wong, D. Price, R. Li, Y. Shen, Elevated beta-secretase expression and enzymatic activity detected in sporadic Alzheimer disease, Nature Medicine 9 (2003) 3–4.

[22] L. Zhang, H. Yu, Y. Sun, X. Lin, B. Chen, C. Tan, G. Cao, Z. Wang, Protective effects of salidroside on hydrogen peroxide-induced apoptosis in SH-SY5Y human neuroblastoma cells, European Journal of Pharmacology 564 (2007) 18–25.

[23] W.S. Zhang, L.Q. Zhu, F.L. Niu, R.C. Deng, C.X. Ma, Protective effects of salidroside on injury induced by hypoxia/hypoglycemia in cultured neurons, Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China Journal of Chinese Materia Medica 29 (2004) 459–462.

[24] X. Zhang, W. Le, Pathological role of hypoxia in Alzheimer's disease, Experimental Neurology 223 (2004) 299–303.

[25] X. Zhang, K. Zhou, R. Wang, J. Cui, S.A. Lipton, F.F. Liao, H. Xu, Y.W. Zhang, Hypoxia-inducible factor 1alpha (HIF-1alpha)-mediated hypoxia increases BACE1 expression and beta-amyloid generation, The Journal of Biological Chemistry 282 (2007) 10873–10880.

[26] C. Zhiyou, Y. Yong, S. Shanquan, Z. Jun, H. Liangguo, Y. Ling, L. Jieying, Upregulation of BACE1 and beta-amyloid protein mediated by chronic cerebral hypoperfusion contributes to cognitive impairment and pathogenesis of Alzheimer's disease, Neurochemical Research 34 (2009) 1226–1235.