Biochemical and Biophysical Research Communications 386 (2009) 175–180

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Constitutive JAK2/STAT1 activation regulates endogenous BACE1 expression in neurons

Hyun Jin Cho, Seok Min Jin, Sung Min Son, Yong Woo Kim, Ji Yeon Hwang, Hyun Seok Hong, Inhee Mook-Jung *

Department of Biochemistry and Biomedical Sciences, Seoul National University College of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 2 June 2009
Available online 7 June 2009

Keywords:
AD
Amyloid-β
BACE1
JAK2/STAT1
SOCS
IFN-γ
Neuron
Astrocyte

ABSTRACT

The protease BACE1 (β-site APP-cleaving enzyme 1) is essential for the generation of amyloid beta (Aβ) from amyloid precursor protein (APP). Although BACE1 is expressed primarily in neurons, which are a principal source of Aβ in the brain, the mechanism that underlies basal expression of BACE1 in neurons has not been studied thoroughly. In the present study, we found that endogenous BACE1 expression was mediated by constitutive JAK2/STAT1 activation in neurons. Inhibition of the JAK2/STAT1 signaling pathway, using AG490 (a JAK2 inhibitor), a dominant-negative form of STAT1, and SOCS1 and SOCS3 overexpression, reduced levels of BACE1 promoter activity, expression of endogenous BACE1, and generation of Aβ. These results were recapitulated in the SH-SY5Y neuronal cell line, primary cultured neurons, and mouse brains. Therefore, we propose that constitutive JAK2/STAT1 activation mediates endogenous BACE1 expression in neurons and that inhibition of JAK2/STAT1 signaling abrogates basal levels of BACE1 expression and Aβ generation.

Introduction

Deposition of extracellular senile plaques in the brain is one of major pathological hallmarks of Alzheimer disease (AD) [1]. BACE1 (β-site APP-cleaving enzyme 1) is a transmembrane aspartyl protease that cleaves amyloid precursor protein (APP), generating amyloid beta (Aβ), a principal component of senile plaques [2,3]. Aβ is derived from the sequential proteolysis of APP by BACE1 and γ-secretase [4,5]. Inhibition of BACE1 or γ-secretase activity has long been considered to be an attractive target for AD prevention and therapy [6,7]. Obstruction of γ-secretase activity, however, results in severe side effects, because γ-secretase acts on several essential substrates, such as Notch, cadherin, and low-density lipoprotein receptor-related protein (LRP) [8–11]. Nevertheless, abrogation of BACE1 expression in BACE1-knockout mice eliminates Aβ production without inducing any remarkable abnormalities [12], although some aberrations recently have been reported [13]. Moreover, the partial reduction of BACE1 hampers amyloid plaque formation dramatically in AD mouse models [14]. In our previous study, BACE1 expression was induced in IFN-γ-stimulated astrocytes via direct interaction between activated signal transducer and activator of transcription 1 (STAT1) and the BACE1 promoter. In the present study, we investigated the underlying mechanisms of BACE1 expression for nonresponsiveness to IFN-γ treatment in neurons. To this end, we examined the potential involvement of JAK2/STAT1 signaling using AG490, as well as suppressor of cytokine signaling (SOCS) 1/3 expression and BACE1 promoter activity in primary neuron cultures, neuroblastoma cell lines, and mouse brains. Although IFN-γ had no effect on neuronal BACE1 levels *in vivo*, STAT1 appeared to be involved in endogenous neuronal BACE1 expression. Blockage of STAT1 activation by overexpression of dominant-negative STAT1 reduced BACE1 expression levels in neuroblastoma cells. Also, endogenous BACE1 levels were decreased by overexpression of SOCS, an endogenous negative regulator of JAK2/STAT1 signaling [15] demonstrating that downregulation of JAK2/STAT1 signaling suppresses BACE1 expression and Aβ generation in neurons. These results suggest that JAK2/STAT1 signaling is constitutively active in neurons and regulates endogenous neuronal BACE1 expression.

Materials and methods

Cell culture and drug treatment. Human SH-SY5Y neuroblastoma cells and U373MG astrocytoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM; HyClone, Irvine, CA) with 10% fetal bovine serum (FBS; HyClone) and a 1% penicillin/streptomycin mixture in a 5% CO_2 incubator at 37 °C. To differentiate SH-SY5Y

* Corresponding author. Address: Department of Biochemistry and Biomedical Sciences, Seoul National University College of Medicine, 28 Yungun-dong, Jongro-gu, Seoul 110-799, Republic of Korea. Fax: +82 2 744 4534.
E-mail address: inhee@snu.ac.kr (I. Mook-Jung).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.06.006

cells, 10 μM all-trans-retinoic-acid (Tocris Cookson, Bristol, U.K.) was fed to the cells in 1% serum media for 5 days in poly-D-lysine-coated plates (Sigma, Saint Louis, MO). Cells were treated with 100 ng/ml recombinant human IFN-γ (R&D System, Minneapolis, MN) or AG490, a JAK2 inhibitor (Tocris), in serum-free DMEM. For pure cultures of mouse neurons, cerebral cortices of embryonic day 16 (E16) C57BL/6 mouse brains were isolated in ice-cold Hanks Balanced Salt Solution (HBSS, Life Technologies, Grand Island, NY). The cortices were digested with papain (Sigma) in dissociation medium. The dissociated cells were plated on poly-D-lysine-coated 12-well culture plates at 1×10^6 cells/well. Neurons were maintained in Neurobasal medium with 1% B-27 (Gibco BRL, USA). On Day 6, cells were treated with AG490 for 24 h.

Stereotaxic injection and immunohistochemistry. AG490 was injected into 4-month-old B6/SJL mouse brains by stereotaxic injection. Briefly, a mouse (average weight, 30 g) was anesthetized with sodium pentobarbital (20 mg/kg, i.p.) and then positioned on a Kopf stereotaxic apparatus (Model 900, David Kopf Instruments, Tujunga, CA). The stereotaxic coordinates for the cortex were; anterior–posterior [AP], -2.2 mm; medial–lateral [ML], 0.5 mm; dorsal–ventral [DV], 1.2 mm. The injection was performed using a microinjection autopump and a 10-μl Hamilton microsyringe filled with AG490 (10 μg) at a rate of 0.15 μl/min. Mice were sacrificed at 3 days after injection. All animal procedures were approved by the Seoul National University Animal Care and Use Committee. The brain sections (40-μm thick) were immunostained using human BACE1 (1:500) and NeuN (1:500) and observed under a fluorescence microscope (Olympus DP50, Japan). Photographs were taken from randomly selected cortex regions.

Constructs and BACE1 promoter activity assay. Two constructs were used to assess BACE1 promoter activity: uBACE-1Ka, −1 to −994 bp; and uBACE-2K, +50 to −2100 bp of the human BACE1 gene, as previously reported [16]. Luciferase assay was performed using a dual luciferase kit (Promega, Madison, WI). Dominant-negative STAT1 (STAT1Y701F was provided by Dr. YY Kong, Seoul National University, Korea) was used to block endogenous STAT1; for overexpression of SOCS1 and SOCS3 proteins, the respective cDNA constructs (SOCS1; pEF-FLAG-I/m4A2, SOCS3; pEF-FLAG-I/mSOCS-3 were provided by Dr. MH Song, Chungnam University, Daejeon, Korea) were used, and the amount of DNA was normalized with an appropriate mock vector. Each construct was transfected into cells using the Lipofectamine and Plus reagents (Invitrogen, Carlsbad, CA).

In vitro β-secretase activity assay. To measure BACE1 enzymatic activity, cells were lysed with 20 mM MES, 0.5% Triton X-100, and 0.15 M NaCl (pH 6.0). The prepared proteins were incubated with a synthetic peptide substrate, MCA-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys(DNP)-Arg-Arg-NH2 (Bachem, Bubendorf, Switzerland) in 0.15 M Na-acetate (pH 5.2) for 1 h at 37 °C, and inhibitor peptides (Bachem) were preincubated with cell lysates for 30 min at 37 °C, as previously reported [16]. Signals (ex 320 nm, em 405 nm) were detected by a fluorescence luminometer (LS-55, Perkin Elmer, Norwalk, CT).

Antibodies. BACE1 was detected using anti-BACE1 polyclonal antibodies (Calbiochem, San Diego, CA, USA) and an anti-actin monoclonal antibody (Sigma) were used for immunoblotting. For the immunostaining, Anti-BACE AB-1 polyclonal antibody (Oncogene, Darmstadt, Germany), which recognizes amino acids 44–59, and anti-NeuN monoclonal antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) were used.

Electrophoretic mobility shift assay (EMSA). A double-stranded probe was generated by annealing two biotin-labeled oligonucleotides to the STAT1 recognition site (−1857 to −1865 bp) within the human BACE1 gene promoter, as previously reported [17]. Nuclear extracts were incubated with biotin-labeled BACE1-STAT1 probes at room temperature for 30 min. For competition experiments, a 100-fold molar excess of cold BACE1-STAT1 probes were preincubated with the nuclear extracts. Signals were detected using the Light Super Shifted Module Kit (Pierce) according to the manufacturer's instructions.

Sandwich ELISA for measuring mouse Aβ40 levels. Aβ40 levels in conditioned media (CM) and RIPA extracts of mouse primary neuronal cultures were analyzed using sandwich Aβ ELISA kit for mouse (Biosource International, Camarillo, CA) [18].

Statistical analyses. The differences between the groups were examined for their statistical significance with a one-way analysis of variance (ANOVA) or Student's *t*-test using Prism4 (GraphPad software Inc., La Jolla, CA) software program. All the data are expressed as a means ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Results

Constitutive BACE1 expression in SH-SY5Y was not affect by IFN-γ

We have found that IFN-γ stimulates astrocytic BACE1 expression *in vivo* as well as in an astrocytoma cell line [17,19]. To examine the direct effect of IFN-γ in neurons, SH-SY5Y cells were stimulated by 100 ng/ml IFN-γ for 24 h. BACE1 expression in SH-SY5Y cells remained constant with IFN-γ treatment, while BACE1 protein was robustly upregulated by IFN-γ in U373MG cells, a human astrocytoma cell line (Fig. 1A). To test whether IFN-γ had any effect on BACE1 enzymatic activity in neuronal cells, an *in vitro* peptide cleavage assay was performed. In SH-SY5Y cells, when endogenous BACE1 catalytic activity was detected in the absence of IFN-γ stimulation, the level was unchanged by IFN-γ treatment (Fig. 1B). U373MG cells had no endogenous BACE1 activity in the absence of IFN-γ. Unlike in SH-SY5Y cells, BACE1 activity was induced and increased dose-dependently with IFN-γ treatment (Fig. 1B). These results suggest that IFN-γ does not regulate the levels of BACE1 protein or enzymatic activity in neurons.

Constitutive STAT1 binding to the BACE1 promoter in SH-SY5Y cells

In our previous study, the uBACE-2K construct, which contains a STAT1-binding site, showed IFN-γ-enhanced luciferase activity in U373MG cells [17]. Here, the regulation of BACE1 transcription by IFN-γ in neuronal cells was assessed by BACE1 promoter activity assay in SH-SY5Y cells. When IFN-γ was applied to cells that were transfected with uBACE-2K or uBACE-1Ka, no increase in BACE1 promoter activity was observed (Fig. 1C), suggesting that IFN-γ has no effects on BACE1 at the transcriptional level in SH-SY5Y cells. However we found that phosphorylated STAT1 and nuclear translocated STAT1 were detected in unstimulated SH-SY5Y cells (data not shown). We hypothesized that BACE1 promoter is constitutively activated by STAT1 in SY5Y cell. To test our hypothesis, nuclear extracts from both U373MG and SY5Y cells were incubated with biotin-labeled STAT1 probes for BACE1 promoter, and then EMSA was performed to measure the level of STAT1. A band that represented the protein–DNA complex appeared in the nuclear extracts of IFN-γ-treated U373MG cells (left panel, Fig. 1D). Interestingly, protein–DNA complex appeared in PBS-treated SH-SY5Y cells, the interaction of which was unchanged despite treatment with IFN-γ (right panel, Fig. 1D). These data indicate that STAT1 is highly existed in the nucleus and binds to the BACE1 promoter on non-stimulated SH-SY5Y cells.

Inhibition of STAT1 attenuates endogenous BACE1 expression in SH-SY5Y cells

To determine whether basal expression of BACE1 was due to constitutive binding of STAT1 to the BACE1 promoter in SH-SY5Y cells, we inhibited STAT1 activity. Treatment of SH-SY5Y cells with

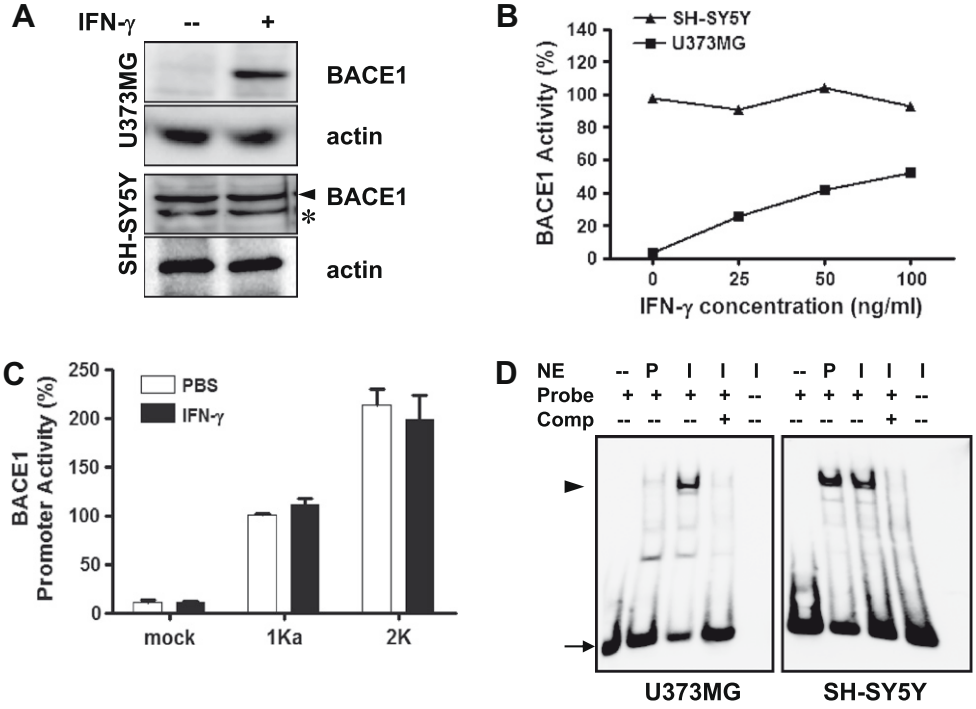

Fig. 1. Constitutive BACE1 expression in SH-SY5Y was not affected by IFN-γ. (A) U373MG and differentiated SH-SY5Y cells were stimulated with 100 ng/ml IFN-γ in serum-free media for 24 h. Representative Western blot analysis of cell lysates using BACE1 and endogenous actin antibody are shown. Arrow head; BACE1, asterisk; non-specific band. (B) *In vitro* peptide cleavage assay for BACE1 activity. (C) BACE1 promoter activity on SH-SY5Y was measured using pGL3-Basic that contained the BACE1 upstream region: 1Ka (uBACE-1Ka) and 2K (uBACE-2K). (D) The cells were incubated with PBS (P) or IFN-γ (I) for 30 min, and then the nuclear extracts were obtained. Gel shift assay was performed using biotin-labeled probes against the STAT1 binding sequences. NE, nuclear extracts; Probe, biotin-labeled probes; Comp, competitor; arrowheads, STAT1/DNA complex; arrow, free probes.

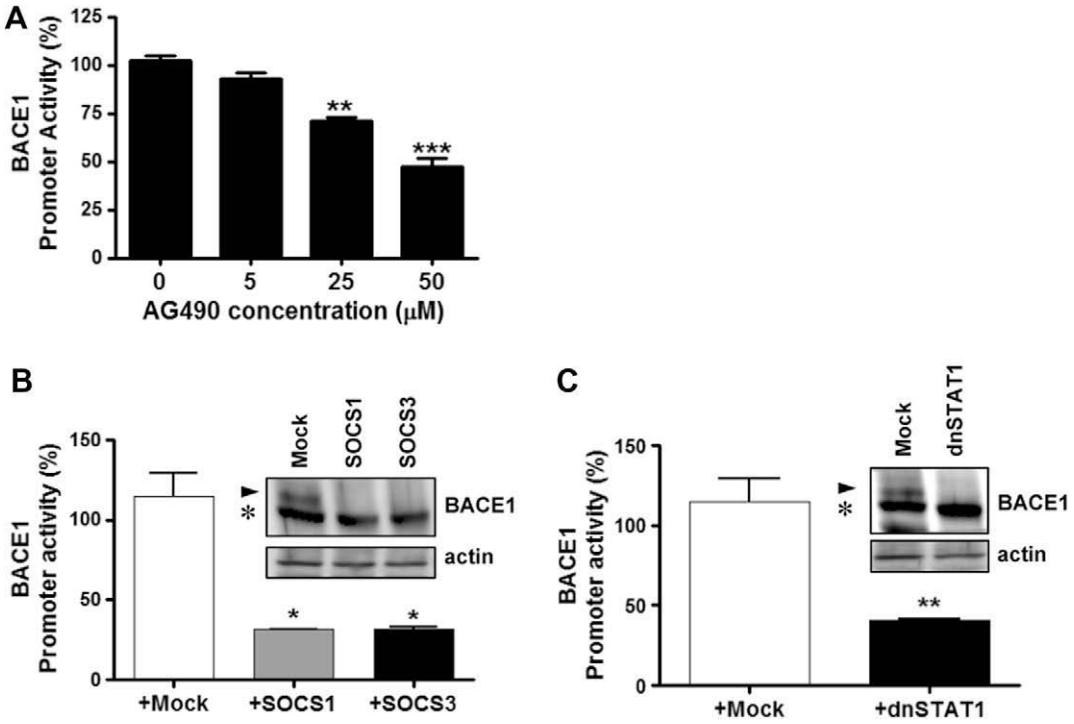

Fig. 2. Inhibition of STAT1 attenuates endogenous BACE1 expression in SH-SY5Y cells. (A) BACE1 promoter activity was measured from uBACE-2K transfected SH-SY5Y cells that were incubated with various doses of AG490 (0, 5, 25, and 50 μM) for 24 h. The activity was compared and presented as % of the 0 μM AG490 treatment. (B) uBACE-2K with SOCS1- or SOCS3-encoding constructs and (C) uBACE-2K with pcDNA3-dnSTAT1 (Y701F) constructs were co-transfected into SH-SY5Y cells for 24 h, BACE1 promoter activity was measured and presented as % of the mock vector. Representative Western blot analysis of cell lysates using BACE1 and actin antibody are shown (inset). Arrowhead, BACE1; asterisk, non-specific band; Mock, empty vector.

AG490, a JAK2/STAT1 inhibitor, reduced BACE1 promoter activity in a dose-dependent manner (Fig. 2A). Because SOCS1 and SOCS3 block JAK2 activation [20,21], cDNA constructs that encoded the SOCS genes were transfected into SH-SY5Y cells. The expression of both SOCS1 and SOCS3 diminished endogenous BACE1 promoter activity substantially ($p < 0.05$ Fig. 2B). In addition, BACE1 protein levels were reduced when SOCS1 and SOCS3 were overexpressed in SH-SY5Y cells (Fig. 2B, inset). When dominant-negative STAT1 (dnSTAT1) was overexpressed in SH-SY5Y cells, BACE1 promoter activity decreased (Fig. 2C). Moreover, BACE1 protein levels dwindled when dnSTAT1 was transfected into SH-SY5Y cells (Fig. 2C inset). Taken together, abrogation of STAT1 function attenuates endogenous BACE1 expression in SH-SY5Y cells, indicating that constitutive BACE1 expression is tightly associated with STAT1 activity in neurons.

AG490 attenuates BACE1 expression and Aβ generation in primary cultured neurons and mouse brains

To examine the involvement of JAK2/STAT1 signaling in neuronal BACE1 expression, we treated primary cultured neurons from E16 mouse brains with AG490. Phosphorylated JAK2 and STAT1 were detected in untreated cells, indicating that JAK2/STAT1 signaling was constitutively active in primary neurons. AG490 reduced phospho-JAK2 and STAT1 levels dose-dependently (Fig. 3A). Under the same conditions, BACE1 protein levels also were down regulated (Fig. 3A), suggesting that AG490 reduces endogenous BACE1 levels by interrupting constitutive JAK2/STAT1 signaling in neurons. Because neuronal BACE1 protein levels were decreased by AG490, the extent of Aβ40 generation was measured. AG490 treatment for 24 h lowered secreted and intracellular Aβ40

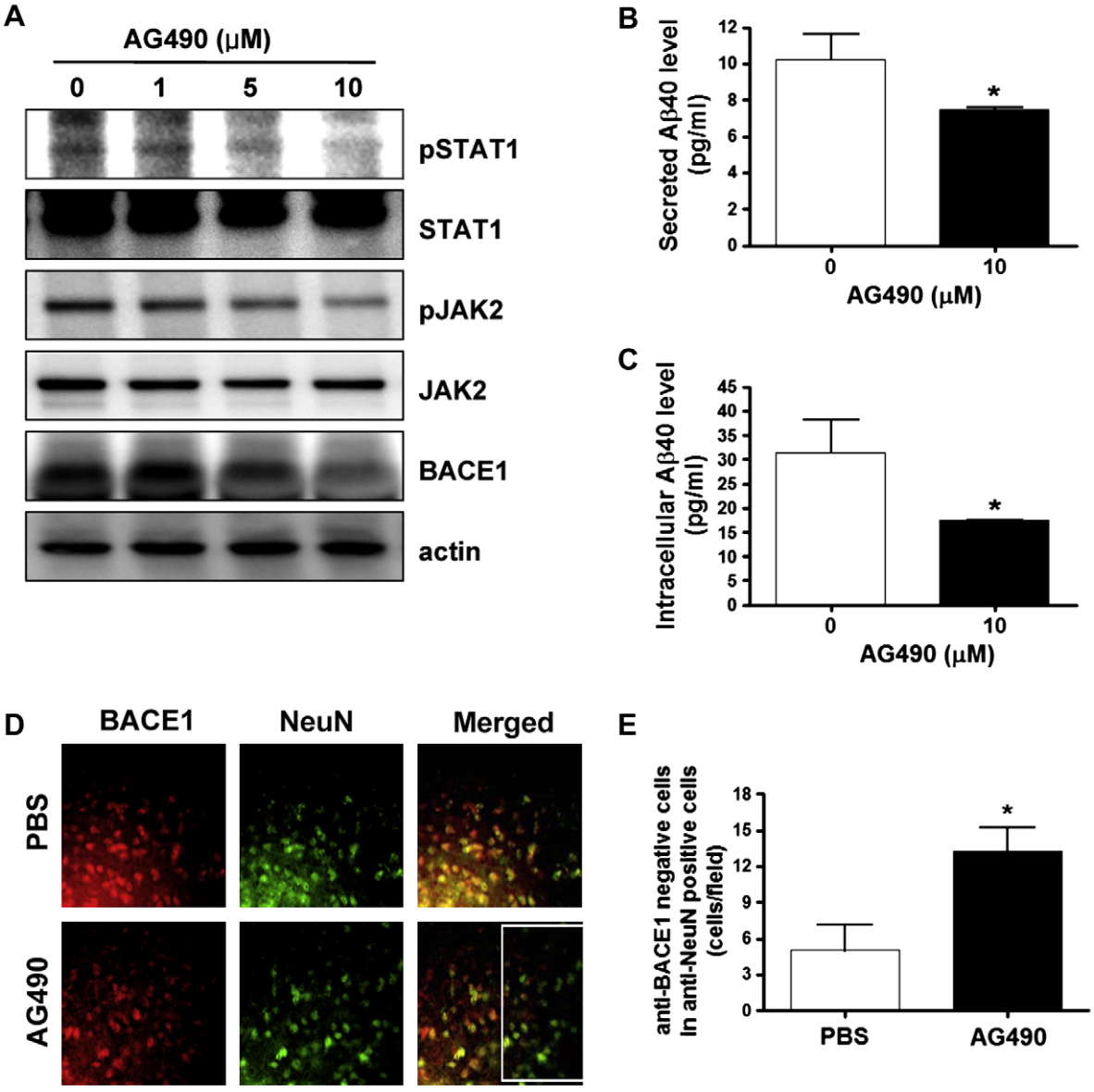

Fig. 3. AG490 attenuates BACE1 expression and Aβ generation in primary cultured neurons and mouse brains. (A) Various doses of AG490 (0, 1, 5, and 10 μM) were administrated to mouse primary cultured neurons for 24 h. Total lysates were analyzed by Western blotting to observe the levels of pJAK2/JAK2, pSTAT1/STAT1, and BACE1. (B and C) Secreted and intracellular Aβ40 level were measured from mouse neuron cultures described in *A*. (D) AG490 treated group decreased BACE1 staining (red) in NeuN positive cells (green) compared to PBS treated group. Box in merged figure represented lower BACE1 expression in neurons. (E) The anti-BACE1 negative cells among anti-NeuN positive cells were counted from PBS ($n = 3$) and AG490 ($n = 5$) injected mice brain sections. White bar; PBS-injected mice, black bar; AG490-injected mice. $^{*}p < 0.05$, *t*-test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

levels (Fig. 3B and C, respectively). These results indicate that inhibition of constitutively active JAK2/STAT1 signaling attenuates endogenous BACE1 levels as well as Aβ generation in neurons. To ensure the effects of JAK2/STAT1 on neuronal BACE1 levels *in vivo*, AG490 was injected into mouse brain. The mouse brain sections were immunostained using anti-BACE1 and anti-NeuN antibodies (Fig. 3D). Since AG490 treatment reduced BACE1 expression in neurons, the number of anti-BACE1 negative cells with anti-NeuN positive cells was significantly increased compared to PBS treated groups (Fig. 3E). It is implying that BACE1 expression in neurons is attenuated by AG490 injection into mouse brains.

Discussion

Neurons are a primary source of Aβ in the brain, because BACE1, a rate-limiting secretase of Aβ generation, is expressed in neurons but not astrocytes [22–25]. Cho et al. [17] reported that IFN-γ, a proinflammatory cytokine, modulates astrocytic BACE1 gene expression at the transcription level by activating STAT1. JAK2 and ERK1/2 phosphorylate Tyr701, and Ser727 of STAT1, respectively, following the direct interaction of activated STAT1 with specific binding sites in the human BACE1 promoter region. Further, astrocytic BACE1 expression was observed in mouse brains that were injected with IFN-γ. Neuronal BACE1 levels, however, did not change on administration of IFN-γ.

To investigate the regulation mechanism of BACE expression in neuron, we used human SH-SY5Y neuroblastoma, U373MG astrocytoma cells, and mouse primary cultured neurons. Our results reveal that SH-SY5Y cells, in contrast to U373MG, did not show significant changes on the enzymatic function, transcriptional activity, or protein levels of BACE1 by IFN-γ treatment. However, we found that SH-SY5Y cells have abundant STAT1 in nucleus and higher promoter activity than U373MG cell. We observed that differentiated as well as undifferentiated SH-SY5Y cells harbored constitutively phosphorylated JAK2 and STAT1, and pSTAT1 proteins were translocated into the nucleus in the absence of IFN-γ (data not shown). In addition, we found phosphorylated JAK2 and STAT1 on mouse primary cultured neuron. These results suggest that BACE1 is constitutively expressed in neurons and neuroblastoma cell line. Also, it is due to endogenous STAT1 binding to the BACE1 promoter, regardless of the presence of IFN-γ.

Inhibition experiments that blocked constitutive JAK2/STAT1 activation using AG490, SOCS1 or 3 expression, or dnSTAT1 expression in primary cultured neurons as well as in SH-SY5Y cells demonstrated that basal levels of BACE1 transcription were reduced in neurons. Furthermore, stereotaxic injection of AG490 into mouse brains downregulated neuronal BACE1 levels, and AG490 treatment impeded generation of Aβ as well as of BACE1 proteins in primary cultured neurons by blocking constitutive JAK2/STAT1 activation. JAK2 is activated by cytokines and numerous growth factors [26]. Binding of JAK2 to its receptor causes receptor oligomerization and autophosphorylation of JAK2 [27]. Although little is known about the molecular mechanisms that underlie constitutive activation of the JAK2 pathway, several studies have reported that constitutive JAK2 activation has cytokine- and growth factor-independent effects in several cell types [28,29]. For example, constitutive JAK2/STAT5 activation is accompanied by cell proliferation in the factor-independent Dami/HEL and Meg-01 leukemic cell lines, the nuclear extracts of which express constitutively active STAT5 in the absence of cytokine stimulation [29]. In addition, several groups have reported on cytokine receptor-independent JAK2 activation [30–32]. Ferrand et al. [32] showed that G protein-coupled receptors (GPCRs) are associated with activation of the JAK2 signaling pathway. Like cytokine receptors, GPCRs activate JAK kinases and STAT family members. Future studies are required to identify the underlying mechanism of constitutive activation of the JAK2/STAT1 pathway in neurons. Inhibition of BACE1 is considered to be an attractive target for AD therapy, although complete elimination of BACE1 has been shown to elicit several side effects in mice, such as reduction of myelination and cognitive deficiencies [13,33,34]. Because JAK2/STAT1 signal transduction is associated with BACE1 expression in both neurons and astrocytes, it may be a more effective target in preventing BACE1 and Aβ generation in the brain.

Acknowledgments

This work was supported by grants from the 21C Frontier Functional Proteomics Project (FPR08K1301-02210), the AARC program project (R11-2002-097-08001), and WCU-Neurocytomics program project for I.M.J.

References

[1] M. Citron, Strategies for disease modification in Alzheimer's disease, Nat. Rev. Neurosci. 5 (2004) 677–685.
[2] C. Haass, M.G. Schlossmacher, A.Y. Hung, C. Vigo-Pelfrey, A. Mellon, B.L. Ostaszewski, I. Lieberburg, E.H. Koo, D. Schenk, D.B. Teplow, et al., Amyloid beta-peptide is produced by cultured cells during normal metabolism, Nature 359 (1992) 322–325.
[3] S.S. Sisodia, D.L. Price, Role of the beta-amyloid protein in Alzheimer's disease, FASEB J. 9 (1995) 366–370.
[4] C. Haass, D.J. Selkoe, Cellular processing of beta-amyloid precursor protein and the genesis of amyloid beta-peptide, Cell 75 (1993) 1039–1042.
[5] R. Vassar, M. Citron, Abeta-generating enzymes: recent advances in beta- and gamma-secretase research, Neuron 27 (2000) 419–422.
[6] M. Citron, Emerging Alzheimer's disease therapies: inhibition of beta-secretase, Neurobiol. Aging 23 (2002) 1017–1022.
[7] A.K. Ghosh, L. Hong, J. Tang, Beta-secretase as a therapeutic target for inhibitor drugs, Curr. Med. Chem. 9 (2002) 1135–1144.
[8] J. Shen, R.T. Bronson, D.F. Chen, W. Xia, D.J. Selkoe, S. Tonegawa, Skeletal and CNS defects in Presenilin-1-deficient mice, Cell 89 (1997) 629–639.
[9] S. Lammich, M. Okochi, M. Takeda, C. Kaether, A. Capell, A.K. Zimmer, D. Edbauer, J. Walter, H. Steiner, C. Haass, Presenilin-dependent intramembrane proteolysis of CD44 leads to the liberation of its intracellular domain and the secretion of an Abeta-like peptide, J. Biol. Chem. 277 (2002) 44754–44759.
[10] A. Geling, H. Steiner, M. Willem, L. Bally-Cuif, C. Haass, A gamma-secretase inhibitor blocks Notch signaling in vivo and causes a severe neurogenic phenotype in zebrafish, EMBO Rep. 3 (2002) 688–694.
[11] P. Marambaud, P.H. Wen, A. Dutt, J. Shioi, A. Takashima, R. Siman, N.K. Robakis, A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations, Cell 114 (2003) 635–645.
[12] Y. Luo, B. Bolon, S. Kahn, B.D. Bennett, S. Babu-Khan, P. Denis, W. Fan, H. Kha, J. Zhang, Y. Gong, L. Martin, J.C. Louis, Q. Yan, W.G. Richards, M. Citron, R. Vassar, Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation, Nat. Neurosci. 4 (2001) 231–232.
[13] M. Willem, A.N. Garratt, B. Novak, M. Citron, S. Kaufmann, A. Rittger, B. DeStrooper, P. Saftig, C. Birchmeier, C. Haass, Control of peripheral nerve myelination by the beta-secretase BACE1, Science 314 (2006) 664–666.
[14] L. McConlogue, M. Buttini, J.P. Anderson, E.F. Brigham, K.S. Chen, S.B. Freedman, D. Games, K. Johnson-Wood, M. Lee, M. Zeller, W. Liu, R. Motter, S. Sinha, Partial reduction of BACE1 has dramatic effects on Alzheimer plaque and synaptic pathology in APP Transgenic Mice, J. Biol. Chem. 282 (2007) 26326–26334.
[15] B.T. Kile, W.S. Alexander, The suppressors of cytokine signalling (SOCS), Cell. Mol. Life Sci. 58 (2001) 1627–1635.
[16] H.J. Cho, S.M. Jin, H.D. Youn, K. Huh, I. Mook-Jung, Disrupted intracellular calcium regulates BACE1 gene expression via nuclear factor of activated T cells 1 (NFAT 1) signaling, Aging Cell 7 (2008) 137–147.
[17] H.J. Cho, S.K. Kim, S.M. Jin, E.M. Hwang, Y.S. Kim, K. Huh, I. Mook-Jung, IFN-gamma-induced BACE1 expression is mediated by activation of JAK2 and ERK1/2 signaling pathways and direct binding of STAT1 to BACE1 promoter in astrocytes, Glia 55 (2007) 253–262.
[18] S.M. Jin, H.J. Cho, M.W. Jung, I. Mook-Jung, DNA damage-inducing agent-elicited gamma-secretase activity is dependent on Bax/Bcl-2 pathway but not on caspase cascades, Cell Death Differ. 14 (2007) 189–192.
[19] H.S. Hong, E.M. Hwang, H.J. Sim, H.J. Cho, J.H. Boo, S.S. Oh, S.U. Kim, I. Mook-Jung, Interferon gamma stimulates beta-secretase expression and sAPPbeta production in astrocytes, Biochem. Biophys. Res. Commun. 307 (2003) 922–927.
[20] T.A. Endo, M. Masuhara, M. Yokouchi, R. Suzuki, H. Sakamoto, K. Mitsui, A. Matsumoto, S. Tanimura, M. Ohtsubo, H. Misawa, T. Miyazaki, N. Leonor, T. Taniguchi, T. Fujita, Y. Kanakura, S. Komiya, A. Yoshimura, A new protein

containing an SH2 domain that inhibits JAK kinases, Nature 387 (1997) 921–924.
[21] S.E. Nicholson, T.A. Willson, A. Farley, R. Starr, J.G. Zhang, M. Baca, W.S. Alexander, D. Metcalf, D.J. Hilton, N.A. Nicola, Mutational analyses of the SOCS proteins suggest a dual domain requirement but distinct mechanisms for inhibition of LIF and IL-6 signal transduction, EMBO J. 18 (1999) 375–385.
[22] S. Sinha, J.P. Anderson, R. Barbour, G.S. Basi, R. Caccavello, D. Davis, M. Doan, H.F. Dovey, N. Frigon, J. Hong, K. Jacobson-Croak, N. Jewett, P. Keim, J. Knops, I. Lieberburg, M. Power, H. Tan, G. Tatsuno, J. Tung, D. Schenk, P. Seubert, S.M. Suomensaari, S. Wang, D. Walker, J. Zhao, L. McConlogue, V. John, Purification and cloning of amyloid precursor protein beta-secretase from human brain, Nature 402 (1999) 537–540.
[23] R. Yan, M.J. Bienkowski, M.E. Shuck, H. Miao, M.C. Tory, A.M. Pauley, J.R. Brashier, N.C. Stratman, W.R. Mathews, A.E. Buhl, D.B. Carter, A.G. Tomasselli, L.A. Parodi, R.L. Heinrikson, M.E. Gurney, Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity, Nature 402 (1999) 533–537.
[24] M. Bigl, J. Apelt, E.A. Luschekina, C. Lange-Dohna, S. Rossner, R. Schliebs, Expression of beta-secretase mRNA in transgenic Tg2576 mouse brain with Alzheimer plaque pathology, Neurosci. Lett. 292 (2000) 107–110.
[25] 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, Nat. Neurosci. 4 (2001) 233–234.
[26] J.N. Ihle, B.A. Witthuhn, F.W. Quelle, K. Yamamoto, W.E. Thierfelder, B. Kreider, O. Silvennoinen, Signaling by the cytokine receptor superfamily: JAKs and STATs, Trends Biochem. Sci. 19 (1994) 222–227.
[27] J.N. Ihle, The Janus protein tyrosine kinases in hematopoietic cytokine signaling, Semin. Immunol. 7 (1995) 247–254.
[28] C.B. Liu, T. Itoh, K. Arai, S. Watanabe, Constitutive activation of JAK2 confers murine interleukin-3-independent survival and proliferation of BA/F3 cells, J. Biol. Chem. 274 (1999) 6342–6349.
[29] R.Y. Liu, C. Fan, R. Garcia, R. Jove, K.S. Zuckerman, Constitutive activation of the JAK2/STAT5 signal transduction pathway correlates with growth factor independence of megakaryocytic leukemic cell lines, Blood 93 (1999) 2369–2379.
[30] B. Rodriguez-Linares, S.P. Watson, Phosphorylation of JAK2 in thrombin-stimulated human platelets, FEBS Lett. 352 (1994) 335–338.
[31] M.B. Marrero, B. Schieffer, W.G. Paxton, L. Heerdt, B.C. Berk, P. Delafontaine, K.E. Bernstein, Direct stimulation of Jak/STAT pathway by the angiotensin II AT1 receptor, Nature 375 (1995) 247–250.
[32] A. Ferrand, A. Kowalski-Chauvel, C. Bertrand, C. Escrieut, A. Mathieu, G. Portolan, L. Pradayrol, D. Fourmy, M. Dufresne, C. Seva, A novel mechanism for JAK2 activation by a G protein-coupled receptor, the CCK2R: implication of this signaling pathway in pancreatic tumor models, J. Biol. Chem. 280 (2005) 10710–10715.
[33] F.M. Laird, H. Cai, A.V. Savonenko, M.H. Farah, K. He, T. Melnikova, H. Wen, H.C. Chiang, G. Xu, V.E. Koliatsos, D.R. Borchelt, D.L. Price, H.K. Lee, P.C. Wong, BACE1, a major determinant of selective vulnerability of the brain to amyloid-beta amyloidogenesis, is essential for cognitive, emotional, and synaptic functions, J. Neurosci. 25 (2005) 11693–11709.
[34] X. Hu, C.W. Hicks, W. He, P. Wong, W.B. Macklin, B.D. Trapp, R. Yan, Bace1 modulates myelination in the central and peripheral nervous system, Nat. Neurosci. 9 (2006) 1520–1525.