FEBS Letters 583 (2009) 1403–1408

FEBS Letters

journal homepage: www.FEBSLetters.org

An alternative spliced mouse presenilin-2 mRNA encodes a novel γ-secretase inhibitor

Yoshihiro Suzuki [a,b,1], Kazunori Ohta [a,1], Masanori Itoh [a,1], Yukari Sakoh-Sumitomo [a,1], Teruhiko Mitsuda [a], Masashi Ueda [a], Yoshika Hayakawa-Yano [a], Shimo Li [a], Yoko Hida [a], Takashi Inuzuka [b], Yong-Keun Jung [c], Toshiyuki Nakagawa [a,*]

[a] Department of Neurobiology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
[b] Department of Neurology and Geriatrics, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan
[c] School of Biological Sciences, Seoul National University, Shillim-dong, Gwanak-gu, Seoul 151-742, South Korea

ARTICLE INFO

Article history:
Received 12 February 2009
Revised 2 April 2009
Accepted 3 April 2009
Available online 17 April 2009

Edited by Jesus Avila

Keywords:
Presenilin
Alternative splicing
γ-Secretase
Amyloid-β
Nicastrin
Alzheimer's disease

ABSTRACT

The γ-secretase, composed of presenilin-1 (PS1) or presenilin-2 (PS2), nicastrin (NCT), anterior pharynx-defective phenotype 1 (APH-1), and PEN-2, is critical for the development of Alzheimer's disease (AD). PSs are autoproteolytically cleaved, producing an N-terminal fragment (NTF) and a hydrophilic loop domain-containing C-terminal fragment. However, the role of the loop domain in the γ-secretase complex assembly remains unknown. Here, we report a novel PS2 isoform generated by alternative splicing, named PS2β, which is composed of an NTF with a hydrophilic loop domain. PS2β disturbed the interaction between NCT and APH-1, resulting in the inhibition of amyloid-β production. We concluded that PS2β may inhibit γ-secretase activity by affecting the γ-secretase complex assembly.

Structured summary:
MINT-7025654: *APH1* (uniprotkb:Q96BI3) *physically interacts* (MI:0218) with *PEN2* (uniprotkb:Q9NZ42), *PS2 beta* (uniprotkb:Q61144-2) and *PS1* (uniprotkb:P49769) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-7025631: *APH1* (uniprotkb:Q96BI3) *physically interacts* (MI:0218) with *NCT* (uniprotkb:Q92542), *PEN2* (uniprotkb:Q9NZ42) and *PS1* (uniprotkb:P49769) by *anti tag coimmunoprecipitation* (MI:0007)

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment and amyloid-β protein (Aβ) deposition in the brain. Aβ is generated from the amyloid precursor protein (APP) by two distinct enzymes, β-secretase (or BACE) and γ-secretase. γ-Secretase is an intramembrane aspartyl protease composed of presenilin-1 (PS1) or presenilin-2 (PS2), nicastrin (NCT), presenilin enhancer 2 (PEN-2), and anterior pharynx defective-1 (APH-1) [1]. Presenilins are endoproteolytically processed at the hydrophilic loop domain near transmembrane domain 6 into two fragments that associate as a heterodimer: an N-terminal fragment (NTF) and a loop domain containing C-terminal fragment (CTF). NCT is glycosylated during γ-secretase complex assembly, which occurs during transport from the endoplasmic reticulum (ER) to the Golgi apparatus [2–4]. All four components (PS1 or PS2, NCT, PEN-2, APH-1) are required for γ-secretase activity and NCT maturation [5–7]. The current model for γ-secretase complex assembly progresses in three steps: initial assembly of NCT with APH-1 [8], presenilin binding to the NCT/APH-1 dimer, and PEN-2 incorporation into the PS/NCT/APH-1 trimer. The final step allows endoproteolysis of PS and formation of the complete γ-secretase complex [6], which includes one of each component [9]. Missense mutations in PS1 or PS2 increase the ratio of $A\beta_{1-42}$ to $A\beta_{1-40}$, leading to formation of Aβ aggregates, thereby leading to AD. By analysis of PS1 and PS2 knockout mice, Chen et al. [10] observed the differential effect of PS1 and PS2 on NCT maturation. However, a role of PS2 in the γ-secretase complex assembly remains obscure. In this study, we cloned a novel splicing isoform of PS2, PS2β containing a hydrophilic loop domain, but missing the C-terminal transmembrane domain. The interaction between NCT and APH-1 was inhibited in the presence of PS2β,

Abbreviations: PS, presenilin; PEN-2, presenilin enhancer 2; APH-1, anterior pharynx-defective phenotype 1; NCT, nicastrin; CHAPS, 3-cholamidopropyl dimethylammonio propanesulfonic acid; mRFP, monomeric red fluorescence protein

* Corresponding author. Fax: +81 58 230 6484.
E-mail address: tnakagaw@gifu-u.ac.jp (T. Nakagawa).

[1] These authors contributed equally to this work.

0014-5793/$36.00 © 2009 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2009.04.014

leading to a disturbance in NCT maturation and a decrease in γ-secretase activity.

2. Materials and methods

2.1. Antibodies

Anti-PS2β antibody was generated in rats by serial immunization with the synthetic peptide CESKGA conjugated to KLH (Biogate Co., Ltd., Japan). Anti-FLAG antibody was purchased from Sigma (Saint Louis, MO).

2.2. DNA constructs

Mouse presenilins (PS1, PS2, PS2β) and human PEN-2, APH-1, and NCT cDNAs were tagged with HA (PS1, PS2, PS2β, and PEN-2), FLAG (APH-1), Myc (PS1), V5 (NCT), Xpress (PS2β), and monomeric red fluorescence protein (mRFP) (PS1, PS2β). FLAG, Myc, and V5 are tagged at C-terminal and the others are tagged at N-terminal. Human mannosidase II cDNA was inserted into pEGFP (Clontech, Mountain View, CA) as the Golgi apparatus marker [11]. The transmembrane domain (100–134 amino acid residues) of cb5 was fused to GFP and mRFP to generate the ER markers, ER-GFP and ER-mRFP, respectively.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR) and real-time PCR

RT-PCR was performed according to a previously described procedure [12]. PCR was carried out for 30 cycles with an annealing temperature of 65 °C. The PCR primer pairs were as follows: mouse PS2, 5′-AGGAGCTGGAGGAGGAGGAG-3′ (PS2W-1), 5′-ATGTAGAGCTGGTGGGAGGC-3′ (PS2W-2); mouse PS2β, 5′-AGGAGGAGGAAAGTAAGGGG-3′ (PS2BETA-2); mouse β-actin, 5′-GTTTGAGACCTTC-

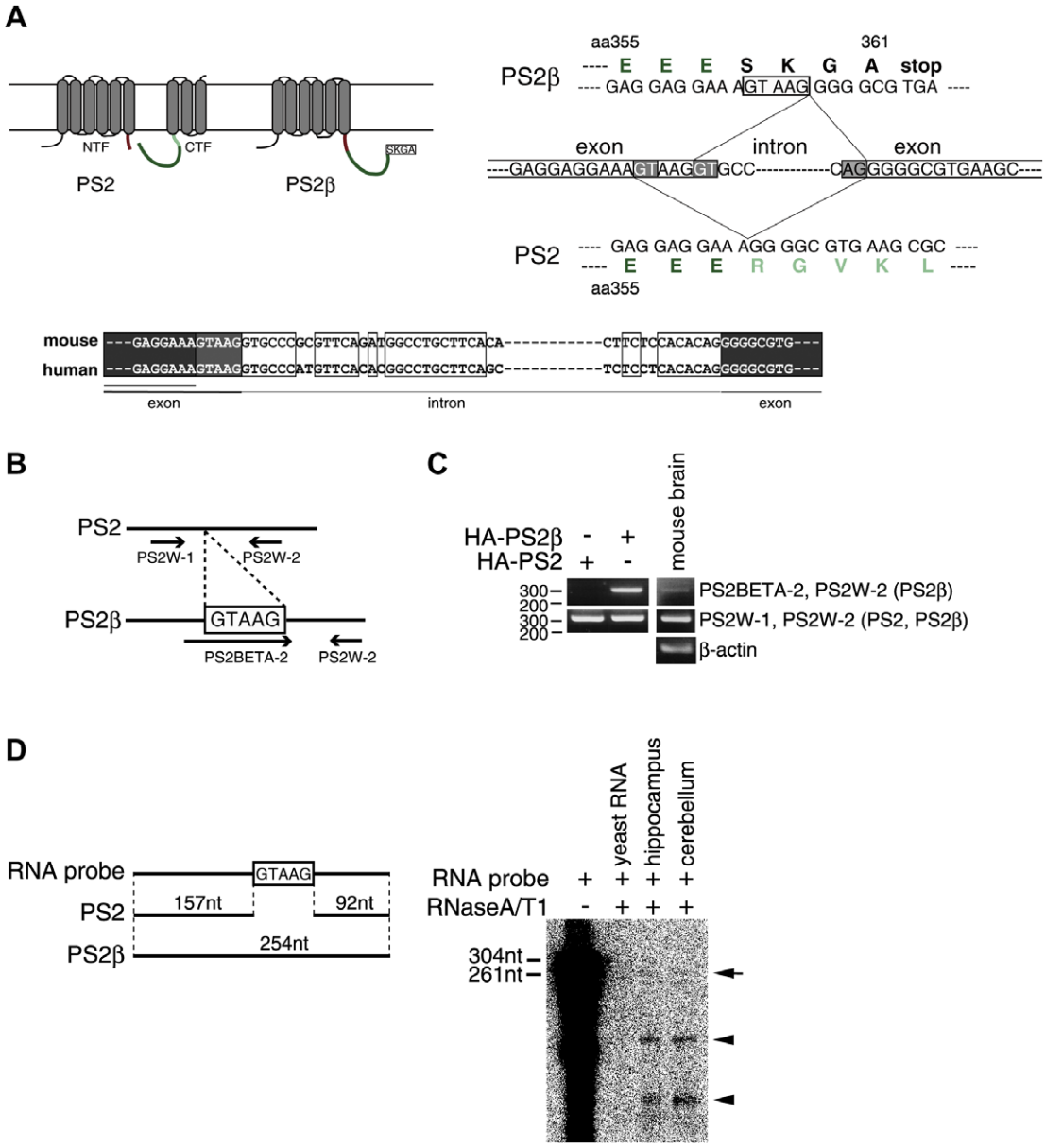

Fig. 1. Expression of an alternatively spliced isoform of mouse PS2. (A) Predicted structure of the PS2β (left). Alternative splicing of PS2 (right). PS2 gene (middle); PS2β cDNA (top); PS2 cDNA (bottom). Alignment between the mouse and human PS2 (NG_007381) genes (bottom). (B) Position of PCR primer. (C) PCR using plasmid as template (left) and RT-PCR (right). (D) RNase protection assay. The level of PS2β mRNA is about 12% of that of PS2 mRNA in hippocampus and cerebellum of adult mouse brain. RNA probe and protected fragment (left). arrow: PS2β; arrowhead: PS2.

AACACC-3′, 5′-GTGGTGGTGAAGCTGTAG-3′. Real-time PCR was performed using SYBR Green by Thermal Cycler Dice (Takara, Japan) according to the manufacturer's protocol. The PCR primer pairs have been described previously [13].

2.4. PS2β/SC100 transformant and ELISA

Xpress-tagged PS2β and pTK-Hyg (Clontech) were co-transfected into HEK293 cells stably expressing SC100 [13], selected with 600 μg/ml of hygromycin, by calcium-phosphate and DNA precipitation. ELISA was performed as described previously [13] using Aβ ELISA Kits (Wako, Japan). All measurements were performed in three independent experiments.

2.5. Immunoprecipitation

HEK293 cells were lysed in lysis buffer [50 mM Tris–HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.1% 3-cholamidopropyl dimethylammonio propanesulfonic acid (CHAPS) (Dojindo, Japan)] supplemented with protease inhibitor (Roche Applied Science, Mannheim, Germany) and centrifuged at 12 000×*g* for 30 min. After the supernatant was precleared with protein A, immunoprecipitation was performed using an ANTI-FLAG M2 affinity gel (Sigma) according to the manufacturer's protocol.

2.6. Western blot

Subcellular fractionation was performed as described previously [12]. Cells and tissues were lysed in RIPA buffer (50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.1% Na deoxycholate, 0.1% SDS) or TTS buffer (50 mM Tris–HCl pH 8.0, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 5 mM EDTA) containing protease inhibitors. Equal amounts of cell or tissue lysates were separated on SDS–PAGE and transferred to PVDF membranes (Immobilon P, Millipore, Billerica, MA).

2.7. Microscopic analysis

Cells were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 24 h after transfection and mounted on Perma Fluor™ (Thermo Shandon, Pittsburgh, PA). Samples were examined with confocal microscopy (LSM 510, Carl Zeiss, Oberkochen, Germany).

2.8. RNase protection assay

RNase protection assay was performed according to a previously described procedure [14]. Briefly, 0.6 μg of mRNA was hybridized with antisense RNA probe and then samples were digested by RNase A/T1 using RPA III™ Ribonuclease Protection Assay Kit (Ambion, Austin, TX). Antisense RNA probe was synthesized using the MAXIscript In Vitro Transcription Kit (Ambion) with [α-^{32}P]UTP (800 Ci/mmol, MP Biomedicals Inc., Solon, OH). The reactions were assessed on a 5% polyacrylamide/8M urea gel. Signal was calculated using Image J, corrected by number of UTP.

3. Results

3.1. Molecular cloning and expression of PS2β

To understand the role of PS2 in γ-secretase complex formation, we amplified the full-length mouse PS2 cDNA from mRNA of the mouse eye by RT-PCR. By coincidence, we cloned a novel isoform of PS2, whose molecular weight was smaller than the predicted size. It had an extra five nucleotides (GTAAG), leading to the translation of an additional four out-of-frame residues (SKGA) at the loop domain (Fig. 1A). By cloning the intron at the insertion point, we found that the exon was extended. Interestingly, the mouse and human splicing site sequences were highly conserved, especially at the boundary of the 5′ end of the intron (5′ splicing site). This splicing isoform, named PS2β, was composed of NTF and a hydrophilic loop domain, but it lacked the seventh transmembrane domain and downstream C-terminal regions. Under highly stringent conditions, the PS2β transcript was detected by RT-PCR (Fig. 1C) and RNase protection assay (Fig. 1D) from mouse brain mRNA, and the PS2β protein was enriched in the microsomal fraction detected by anti-ESKGA antibody, which specifically reacted with the PS2β protein (Fig. 2A and B). The PS2β loop domain-cleaved product was not observed in lysates from HEK293 cells stably expressing PS2β as observed with the active site mutated PS1 in the seventh transmembrane domain (data not shown). We performed confocal microscopy analysis using ER and Golgi apparatus markers to

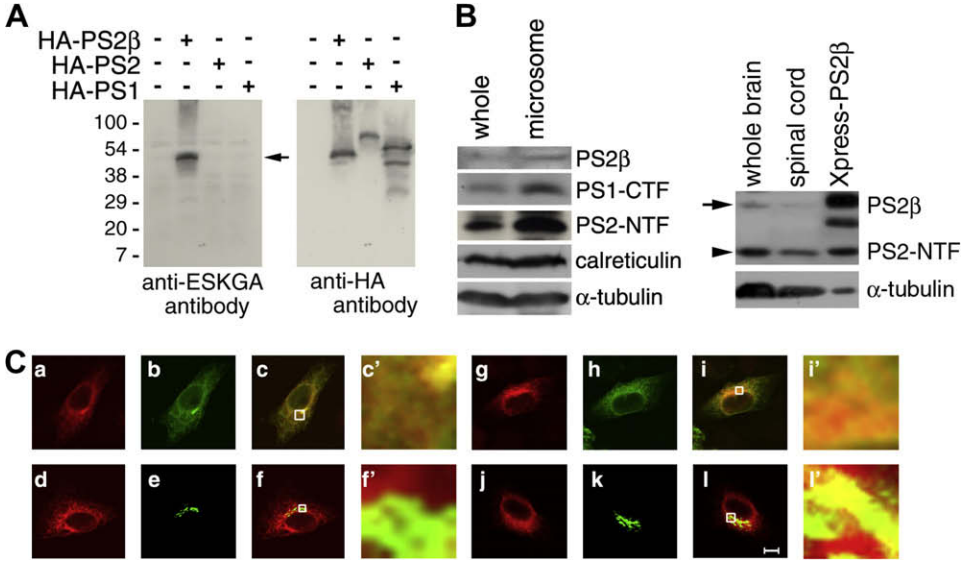

Fig. 2. Expression of PS2β protein. (A) Specificity of anti-ESKGA antibody. (B) Endogenous PS2β protein in the microsomal fraction of mouse brain (left). PS2β was detected by anti-PS2NTF antibody (right). Arrow: PS2β; arrowhead: PS2. (C) Localization of PS2β. (a, d) monomeric red fluorescence protein (mRFP)–PS2β; (g, j) mRFP–PS1; (b, h) ER–GFP; (e, k) mannosidase II-GFP; (c, f, i, l) merge; (c′, f′, i′, l′) higher magnification of the region marked by the square in c, f, i, l, respectively. Scale bars represent 5 μm.

examine the subcellular localization of PS2β (Fig. 2C). PS1 localized at the Golgi as well as ER (Fig. 2Cg–l), whereas PS2β mainly localized at the ER (Fig. 2Ca–f). Thus, we conclude that PS2β, which is composed of NTF and a hydrophilic loop domain, is expressed in the mouse brain and mainly localized at the ER.

3.2. PS2β inhibits the interaction between NCT and APH-1, leading to disturbance of NCT maturation

To examine the role of PS2β in NCT maturation, we determined whether PS2β was integrated into the γ-secretase complex. After transfection of PS2β with four γ-secretase components (NCT-V5, PS1-Myc, HA-PEN-2, APH-1-FLAG), an immunoprecipitation assay with the anti-FLAG antibody was carried out using lysis buffer containing 0.1% CHAPS (Fig. 3A). We found that the association between APH-1 and NCT was significantly inhibited in the presence of PS2β. The interaction of PS1 or PEN-2 with APH-1 was not disturbed. Interestingly, the NTF of PS2 (PS2N, Met^{1}-Lys^{306}) did not disturb the interaction between NCT and APH-1 (Fig. S1). To investigate whether γ-secretase complex was affected by the presence of PS2β, blue native polyacrylamide gel electrophoresis (BN-PAGE) was performed after transfection of all four γ-secretase components (NCT, PS1, PEN-2, APH-1) into parental HEK293 cells and HEK293 cells stably expressing PS2β. The anti-NCT antibody recognized a 440 kDa protein complex, which was observed in the other three components (PS1, PEN-2, and APH-1) by western blot (Fig. S2). The signal for the endogenous as well as exogenously expressed NCT band was drastically reduced, indicating that the incorporation of NCT into the γ-secretase complex was disturbed in the presence of PS2β (Fig. 3B). Next, we examined whether transportation of NCT was affected. A Western blot analysis revealed that brefeldin A, which disturbs Golgi function, decreased the upper (possibly glycosylated mature) NCT (Fig. 3C). The maturation of endogenous NCT was consistently inhibited in HEK293

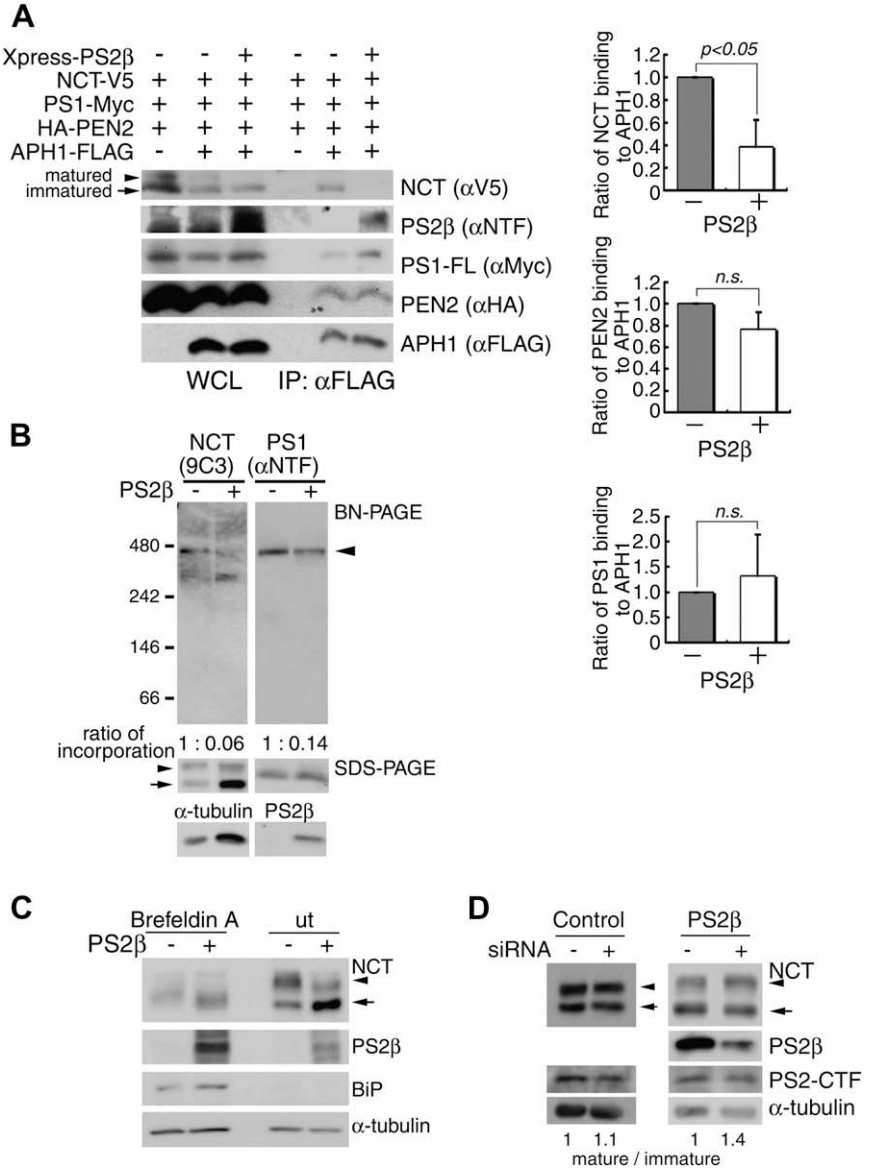

Fig. 3. PS2β disturbed γ-secretase complex assembly. (A) Immunoprecipitation of γ-secretase components with anti-Flag antibody (APH-1) after co-transfection into HEK293 cells. Data represent mean ± S.E.M in three independent experiments corrected by the APH-1 protein (right). (B) PS2β inhibited the incorporation of NCT into the γ-secretase complex in HEK293 cells stably expressing PS2β (arrowhead). top, BN-PAGE; middle and bottom, SDS–PAGE. (C) 10 μg/ml of brefeldin A treatment. (D) Matured NCT increased in HEK293 cells stably expressing PS2β after transfection of PS2 siRNA. arrowhead, matured form of endogenous NCT; arrow, immature NCT. Control: parental HEK293 cells; PS2β: HEK293 cells stably expressing PS2β.

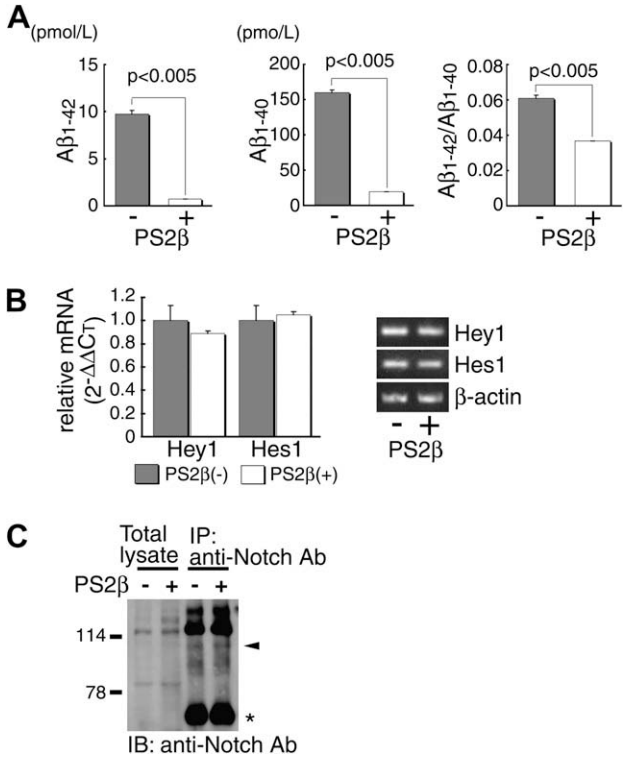

Fig. 4. PS2β disturbed γ-secretase activity. (A) Secreted $Aβ_{1-42}$ and $Aβ_{1-40}$ were measured by ELISA. (B) Real-time PCR of downstream target genes of notch. (C) NICD was similarly detected by western blotting after immunoprecipitation with anti-Notch antibody in both parental HEK293 cells and HEK293 cells stably expressing PS2β (arrowhead) PS2β(−), HEK293 cells; PS2β(+), HEK293 cells stably expressing PS2β. ∗: heavy chain.

cells stably expressing PS2β, which was rescued by PS2β knockdown (Fig. 3D). Thus, these findings suggest that the loop domain may be critical for γ-secretase complex assembly.

3.3. PS2β inhibits γ-secretase activity

To determine the role of PS2β in relation to γ-secretase activity, we first analyzed Aβ secretion by ELISA using HEK293 cells stably expressing PS2β, which also constitutively expressed the C-terminal region of APP SC100. $Aβ_{1-42}$ and $Aβ_{1-40}$ secretion was significantly inhibited in the presence of PS2β (Fig. 4A). In HEK293 cells, the expression of the downstream notch target genes hes1 and hey1 was regulated by γ-secretase activity [13]. In contrast, in HEK293 cells stably expressing PS2β, real-time PCR showed that hes1 and hey1 expression was not inhibited (Fig. 4B). Moreover, immunoprecipitation with anti-Notch antibody indicated that NICD in HEK293 cells stably expressing PS2β was detected similarly to that in HEK293 cells (Fig. 4C). These data suggest that PS2β mainly affect A production, but not notch signaling.

4. Discussion

In this study, we showed that an alternative splicing isoform of mouse PS2, composed of NTF and a hydrophilic loop domain, disturbs the interaction between NCT and APH-1 leading to inhibition of γ-secretase activity.

PS2β is produced by extension of the mouse PS2 gene exon 9 (NM_011183.2), which causes a frameshift mutation that creates a stop codon in the following exon and encodes for translation of four additional out-of-frame residues (SKGA). Sequences around the splicing sites were conserved between mouse and human (intron 14, NG_007381), suggesting that the extended exon may not be specific to mouse PS2 (Fig. 1A). A PS2β fragment containing GTAAG was detected in the mouse brain even under highly stringent PCR conditions, although its expression level was low (Fig. 1C and D). The PS2β protein was enriched in the microsomal fraction of mouse brain (Fig. 2B). Consistent with a previous study that endoproteolysis in the PS1 mutation of active sites (D385A in TM7) does not occur [15], endoproteolytic cleavage of PS2β was not observed by Western blot. Thus, PS2β existed in cells without endoproteolytic processing at the conventional site of the loop domain.

PS1 CTF, especially the C-terminal residues, interacts with NCT [16] through its transmembrane domain [17], which may be the trimeric assembly intermediate (PS/NCT/APH-1) during γ-secretase complex assembly [8]. In our study, PS2β interfered with the binding of NCT to APH-1 and with NCT incorporation into the γ-secretase complex even in the presence of PS1. However, the NTF of PS2 (PS2N) did not affect binding of NCT to APH-1 (Fig. S1). PS2β also inhibited NCT maturation (Fig. 3C), which was not due to disturbance of transportation, because wild-type CFTR protein was detected at the plasma membrane in the presence of PS2β (Fig. S3). Following treatment with brefeldin A, an inhibitor of intracellular protein transport at the ER–Golgi junction and in trans-Golgi, the upper band of NCT (arrowhead in Fig. 3C) disappeared, but the size of the lower band did not change, suggesting that transportation of NCT was specifically prevented at the ER–Golgi junction by PS2β which is mainly localized at the ER. The PS loop domain is dispensable for PS localization and γ-secretase activity [18,19]. Consistently, NTF of PS2 (PS2 N) interacted with APH-1 and did not affect the γ-secretase complex formation (Fig. S1). In contrast, PS2β, which is PS2N fused with the C-terminal loop domain, prevented the interaction of NCT to APH-1. Since APH-1 stabilize the PS holoprotein in the γ-secretase complex and endoproteolytic processing of PS is occurred after PEN-2 is joined to the complex[6], the holoprotein of PS may be critical for complex formation although the cleaved loop domain does not disturb the complex after all four components of γ-secretase are joined to the complex. These results indicate that a hydrophilic loop domain, which is fused to the C-terminal and not the NTFs of PS, may be crucial for completion of γ-secretase complex assembly. Consistently, $Aβ_{1-42}$ secretion and the ratio of $Aβ_{1-42}/Aβ_{1-40}$ were significantly decreased in the presence of PS2β. However, we could not detect the difference of Notch signaling in HEK293 cells stably expressing PS2β, using two different methods, NICD detected by immunoprecipitation and expression of Notch target genes by real-time PCR. This result suggests the differential effect of PS2β on Aβ generation and Notch signaling. We did not determine the precise mechanism, but one possibility is that the γ-secretase complex formation is not completely destroyed by PS2β (Fig. 3B). The other possibility is that PS2β may regulate γ-secretase activity as TMP21, which differentially regulates γ-secretase activity [20]. However, it requires further experiments to address the precise mechanism. Based on these data, we conclude that PS2β prevented formation of γ-secretase complex assembly at the ER, resulting in inhibition of γ-secretase activity.

Acknowledgements

We are grateful to Drs. T. Iwatsubo, T. Tomita (University of Tokyo, SC100) and RY. Tsien (University of California, San Diego, mRFP), W. Annaert, B. De Strooper (K.U. Leuven, Belgium, anti-NCT antibodies) for providing plasmids and antibodies. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan (to TN).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2009.04.014.

References

[1] Selkoe, D.J. and Wolfe, M.S. (2007) Presenilin: running with scissors in the membrane. Cell 131, 215–221.

[2] Yang, D.S. et al. (2002) Mature glycosylation and trafficking of nicastrin modulate its binding to presenilins. J. Biol. Chem. 277, 28135–28142.

[3] Kimberly, W.T., LaVoie, M.J., Ostaszewski, B.L., Ye, W., Wolfe, M.S. and Selkoe, D.J. (2002) Complex N-linked glycosylated nicastrin associates with active gamma-secretase and undergoes tight cellular regulation. J. Biol. Chem. 277, 35113–35117.

[4] Yu, G. et al. (2000) Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing. Nature 407, 48–54.

[5] Edbauer, D., Winkler, E., Regula, J.T., Pesold, B., Steiner, H. and Haass, C. (2003) Reconstitution of gamma-secretase activity. Nat. Cell Biol. 5, 486–488.

[6] Takasugi, N., Tomita, T., Hayashi, I., Tsuruoka, M., Niimura, M., Takahashi, Y., Thinakaran, G. and Iwatsubo, T. (2003) The role of presenilin cofactors in the gamma-secretase complex. Nature 422, 438–441.

[7] Kimberly, W.T., LaVoie, M.J., Ostaszewski, B.L., Ye, W., Wolfe, M.S. and Selkoe, D.J. (2003) Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2. Proc. Natl. Acad. Sci. USA 100, 6382–6387.

[8] Shirotani, K., Edbauer, D., Prokop, S., Haass, C. and Steiner, H. (2004) Identification of distinct gamma-secretase complexes with different APH-1 variants. J. Biol. Chem. 279, 41340–41345.

[9] Sato, T. et al. (2007) Active gamma-secretase complexes contain only one of each component. J. Biol. Chem. 282, 33985–33993.

[10] Chen, F. et al. (2003) Presenilin 1 and presenilin 2 have differential effects on the stability and maturation of nicastrin in Mammalian brain. J. Biol. Chem. 278, 19974–19979.

[11] Velasco, A., Hendricks, L., Moremen, K.W., Tulsiani, D.R., Touster, O. and Farquhar, M.G. (1993) Cell type-dependent variations in the subcellular distribution of alpha-mannosidase I and II. J. Cell Biol. 122, 39–51.

[12] Hayakawa, Y., Itoh, M., Yamada, A., Mitsuda, T. and Nakagawa, T. (2007) Expression and localization of Cayman ataxia-related protein, Caytaxin, is regulated in a developmental- and spatial-dependent manner. Brain Res. 1129, 100–109.

[13] Mitsuda, T., Hayakawa, Y., Itoh, M., Ohta, K. and Nakagawa, T. (2007) ATF4 regulates gamma-secretase activity during amino acid imbalance. Biochem. Biophys. Res. Commun. 352, 722–727.

[14] Nakagawa, T., Okano, H., Furuichi, T., Aruga, J. and Mikoshiba, K. (1991) The subtypes of the mouse inositol 1,4,5-trisphosphate receptor are expressed in a tissue-specific and developmentally specific manner. Proc. Natl. Acad. Sci. USA 88, 6244–6248.

[15] Wolfe, M.S., Xia, W., Ostaszewski, B.L., Diehl, T.S., Kimberly, W.T. and Selkoe, D.J. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 398, 513–517.

[16] Bergman, A., Laudon, H., Winblad, B., Lundkvist, J. and Naslund, J. (2004) The extreme C terminus of presenilin 1 is essential for gamma-secretase complex assembly and activity. J. Biol. Chem. 279, 45564–45572.

[17] Kaether, C., Capell, A., Edbauer, D., Winkler, E., Novak, B., Steiner, H. and Haass, C. (2004) The presenilin C-terminus is required for ER-retention, nicastrin-binding and gamma-secretase activity. Embo J. 23, 4738–4748.

[18] Kaether, C., Lammich, S., Edbauer, D., Ertl, M., Rietdorf, J., Capell, A., Steiner, H. and Haass, C. (2002) Presenilin-1 affects trafficking and processing of betaAPP and is targeted in a complex with nicastrin to the plasma membrane. J. Cell Biol. 158, 551–561.

[19] Saura, C.A. et al. (2000) The nonconserved hydrophilic loop domain of presenilin (PS) is not required for PS endoproteolysis or enhanced abeta 42 production mediated by familial early onset Alzheimer's disease-linked PS variants. J. Biol. Chem. 275, 17136–17142.

[20] Chen, F. et al. (2006) TMP21 is a presenilin complex component that modulates gamma-secretase but not epsilon-secretase activity. Nature 440, 1208–1212.