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Contribution of presenilin/γ-secretase to calsenilin-mediated apoptosis

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Abstract

Mutant presenilins cause early-onset of familial Alzheimer's disease and render cells vulnerable to apoptosis. Calsenilin/DREAM/KChIP3 is a multifunctional calcium-binding protein that interacts with presenilin and mediates calcium-mediated apoptosis. In the present study, we report that the calsenilin-mediated apoptosis is regulated by presenilin. The expression of calsenilin was highly up-regulated in neuronal cells undergoing Aβ42-triggered cell death. The incidence of calsenilin-mediated apoptosis was diminished in *presenilin-1*$^{-/-}$ mouse embryonic fibroblast cells or neuronal cells stably expressing a loss-of-function presenilin-1 mutant. On the contrary, an array of familial Alzheimer's disease-associated presenilin mutants (gain-of-function) increased calsenilin-induced cell death. Moreover, γ-secretase inhibitors, including compound E and DAPT, decreased the calsenilin-induced cell death. These results suggest that the pro-apoptotic activity of calsenilin coordinates with presenilin/γ-secretase activity to play a crucial role in the neuronal death of Alzheimer's disease.

Keywords: Calsenilin; Presenilin; γ-Secretase; Cell death; Alzheimer's disease; Aβ

Alzheimer's disease (AD) is the most common cause of senile dementia in humans and is characterized by the deposition of amyloid-β peptide (Aβ) in the extracellular compartments, the formation of neurofibrillary tangles in the neuronal cell body, and massive neuronal death (reviewed in [1]). Although mechanism for neuronal death in the brain affected by AD remains unknown, results from several model systems indicate that Aβ itself seems to induce neurodegenerative characteristics of apoptosis [2,3]. In addition, neurons undergoing apoptosis were reported to generate elevated levels of cytotoxic Aβ [4,5] though apoptosis may be far down the cascade event driven by Aβ. Mutations in the genes encoding presenilins and β-amyloid precursor protein (APP), which are known to cause early-onset familial Alzheimer's disease (FAD), can increase the production of Aβ and neuronal susceptibility to apoptosis (reviewed in [6]). In addition, extensive evidence suggests the involvement of presenilins in programmed cell death or apoptosis. Over-expression of presenilin 1 (PS1) and presenilin 2 (PS2) in the transfected cell lines increased their susceptibility to apoptosis [7–10], while FAD-associated PS1 and PS2 mutants are constitutively pro-apoptotic [10–12]. Especially, the C-terminus of presenilin has been suggested to play an important role in apoptosis and Aβ42 production. Interestingly, ALG-3, which codes for the calsenilin/DREAM/KChIP3-binding region spanning the cytoplasmic C-terminal 103 amino acids of PS2, rescues T-cell hydridoma and PC12 cells from apoptosis probably by antagonizing the pro-apoptotic function of PS2 [9,10,13]. Moreover, modifications of amino acids at the C-terminus of PS abrogated the ability of presenilin mutations to overproduce Aβ [14].

Calsenilin/DREAM/KChIP3 (hereafter referred to as calsenilin) was identified as a Ca^{2+}-binding protein that binds to the last 103 amino acids of PS1 and PS2 [15] that are identical to ALG3. Other groups reported that calsenilin also binds to the A-type voltage-gated potassium channel [16] and serves as a transcription factor known as downstream regulatory element antagonist

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modulator [17]. In addition, our previous findings suggest that calsenilin is also pro-apoptotic [18]. The aim of the present study was to characterize functional interaction between calsenilin and FAD-associated presenilin/γ-secretase activity in neuronal apoptosis. Here we demonstrate that calsenilin-mediated cell death is functionally associated with presenilin/γ-secretase activity as examined with PS1-deficient cells, loss or gain of function mutant of presenilin, and selective γ-secretase inhibitors.

Materials and methods

Cell cultures and preparation of Aβ peptide. B103 rat neuroblastoma cells, SK-N-BE2(c) human neuroblastoma cells, and mouse embryonic fibroblasts (MEFs) from wild-type or PS1-null mice [19] were grown in DMEM (Gibco-BRL) supplemented with 10% heat-inactivated fetal bovine serum. $PS1^{-/-}$ or $PS1^{+/+}$ MEFs at the passage between four and seven were used in the experiments. SK-N-BE2(c) cells stably expressing wild-type PS1 or PS2, PS1-M146V, PS2-N141I, or empty plasmid (pcDNA3); B103 cells expressing wild-type PS1, PS1ΔE9, PS1-D385A or empty plasmid (pBabe); and B103 cells expressing both PS1 and APP were established by selecting with G418 or puromycine for 4 weeks. Commercially available Aβ42 was purchased from Sigma and dissolved in phosphate-buffered saline (PBS, 500 μM). The stock solutions of Aβ were incubated for 1 week at 4 °C and then divided into small aliquots for storage at −70 °C.

Immunocytochemistry. For immunocytochemical analysis, B103 cells were seeded on poly-L-lysinated glass cover slips. After experimental treatments, B103 cells were fixed with 4% paraformaldehyde for 10 min at room temperature, washed in PBS, and permeablized with 0.1% Triton X-100 in PBS for 5 min. Cells were then pre-coated with blocking buffer (4% BSA in PBS) and incubated overnight with affinity purified anti-calsenilin antibody or pre-immune serum at 4 °C. After washing, the cells were incubated with anti-rabbit TRITC-conjugated secondary antibody (Santa Crutz Biotechnology, Inc.) for 1 h. Slides were mounted and analyzed using a fluorescence microscope (Leica DMLB).

Assessing cell viability. Cell viability was determined with trypan blue exclusion assay or by staining with Hoechst 33258 (0.1 μg/ml, bis-benzimide; Sigma). For the transfected cells, cell viability was determined based on the morphology of GFP-positive cells under a fluorescence microscope (Leica DMRBE).

Inhibition of γ-secretase activity. γ-Secretase activity was inhibited using DAPT (Calbiochem) and compound E [20,21]. γ-Secretase inhibitors were diluted from stock solutions in dimethyl sulfoxide to the concentrations described.

Data analysis. In all experiments, means ± SE is reported. Statistical comparisons among groups were determined using one-way analysis of variance (ANOVA); where indicated, individual comparisons were performed using Student's *t* test.

Results and discussion

The accumulation of Aβ in the brains of AD patients has been implicated as a cause of the neuronal loss that occurs in AD. Previously, we have shown that the pro-apoptotic activity of calsenilin mediated calcium-signal-triggered cell death [18]. We also observed that calsenilin was highly up-regulated in the brains of AD patients and AD transgenic mice (manuscript in preparation). However, the apoptotic signaling pathway mediated by calsenilin has not been fully elucidated. To determine the role of calsenilin in the neuronal apoptosis of AD, we examined the effect of Aβ on the expression of pro-apoptotic calsenilin with immunocytochemical analysis (Fig. 1). The results demonstrated that the expression of calsenilin increased in B103 neuronal cells incubated with Aβ (Fig. 1, middle panel) and undergoing apop-

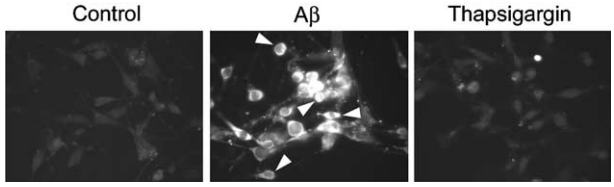

Fig. 1. Induction of calsenilin expression in B103 neuronal cells exposed to Aβ42. B103 cells were left untreated (Control) or treated with Aβ42 (10 μM) and thapsigargin (0.5 μM) for 36 h, and then immunostained with anti-calsenilin antibody. Staining was performed at least three times and representative images were shown. Arrowheads indicate apoptotic cells with the increased immunoreactivity of calsenilin (middle panel).

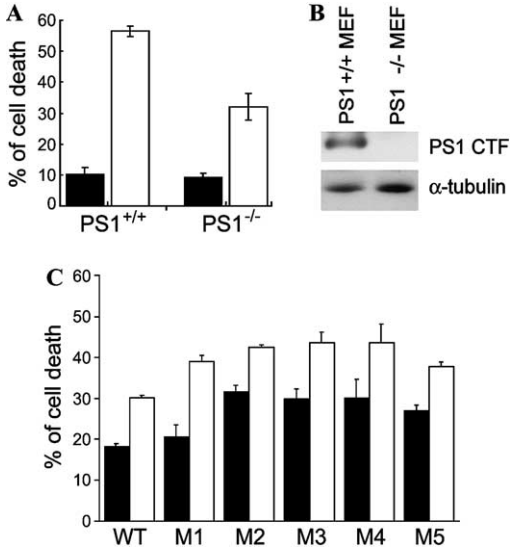

Fig. 2. Effects of presenilin 1 deficiency on the calsenilin-induced apoptosis. (A) $PS1^{-/-}$ MEF cells exhibit decreased susceptibility to calsenilin-induced cell death. $PS1^{-/-}$ and $PS1^{+/+}$ MEF cells were transiently co-transfected with 3 μg of either empty vector (black bars) or pcDNA3.1-calsenilin (white bars) and 0.3 μg of pEGFP. After 36 h, biochemical and morphological changes of GFP-positive cells were examined under the fluorescence microscope. Values depict means ± SD ($n = 5$). Expression of PS1 and α-tubulin in $PS1^{-/-}$ and $PS1^{+/+}$ MEF cells. (B) The PS1 C-terminal fragment (CTF) was detected with an anti-PS1 loop antibody. (C) Augmentation of calsenilin-mediated cell death by transient reconstitution with a wild-type of PS1 (WT) or a variety of FAD-causing presenilin mutants: PS1-L286V (M1), PS1-L235P (M2), PS1-A246E (M3), PS1-L250S (M4), and PS1-M146V (M5). $PS1^{-/-}$ MEFs were transiently co-transfected with one of diverse forms of PS1 alone (black bars) or together with pEGFP-calsenilin (white bars).

tosis with morphological changes, rounded and shrunken (Fig. 1, arrow heads). However, calsenilin expression did not change in B103 cells undergoing apoptosis by treatment with thapsigargin (Fig. 1, right panel), an endoplasmic reticulum Ca^{2+}-ATPase inhibitor, and by staurosporine, a kinase inhibitor, and etoposide, a DNA damaging agent (data not shown). These results show that pro-apoptotic calsenilin is selectively induced during Aβ toxicity of AD model.

Because calsenilin is known to bind to the presenilins, we examined pro-apoptotic activity of calsenilin in mouse embryonic fibroblast (MEF) cells from $PS1^{+/+}$ and $PS1^{-/-}$ mice. Although transfection of $PS1^{-/-}$ MEF cells with calsenilin induced cell death (32%), the effect was much less marked than in $PS1^{+/+}$ cells (56%) (Fig. 2A). As expected, we observed no expression of the PS1 C-terminal fragments in $PS1^{-/-}$ MEF cells (Fig. 2B). These results indicate that PS1 is required for calsenilin-induced cell death. Point mutation in the PS1 is the chief cause of FAD and has been shown to increase neuronal vulnerability to apoptotic cell death. Therefore, we next examined the effects of calsenilin expression on death of cells expressing a variety of FAD-causing PS mutants (PS1-L286V, -L235P, -A246E, -L250S, and -M146V). When $PS1^{-/-}$ MEF cells transiently over-expressing PS1 wild-type or each of PS1 mutants were transfected with calsenilin, the calsenilin-induced cell death was potentiated without apparent selectivity in the fibroblasts (Fig. 2C).

To further characterize the functional relation between calsenilin and FAD-causing PS mutants in neuronal cells, we constructed various human and rat neuroblastoma cell lines stably expressing wild-type, one of the FAD-causing mutants (PS1, PS2, PS1-M146V, PS1ΔE9, and PS2-N141I), or loss-of-function PS1-D385A mutant (Fig. 3). We consistently observed that the calsenilin-induced cell death was potentiated by stable expression of PS1 and PS2 in SK-N-BE2(c) human neuronal cells. Interestingly, the stable expression of a FAD-causing mutant (e.g., PS1-M146V and PS2-N141I) further and significantly increased the incidence of cell death (Fig. 3A). The importance of PS1 activity was confirmed by comparing the effects of PS1ΔE9 (gain of function mutant), a functional FAD-associated PS1 variant that lacks exon 9 and does not undergo conventional endoproteolysis, and PS1-D385A (loss-of-function mutant) in which the aspartate (Asp385) essential for endoproteolysis and γ-secretase activity is replaced with an alanine. As expected, expression of PS1-D385A did not affect the incidence of calsenilin-induced cell death, while PS1ΔE9 significantly increased it (Figs. 3B and C). These results are indicative of

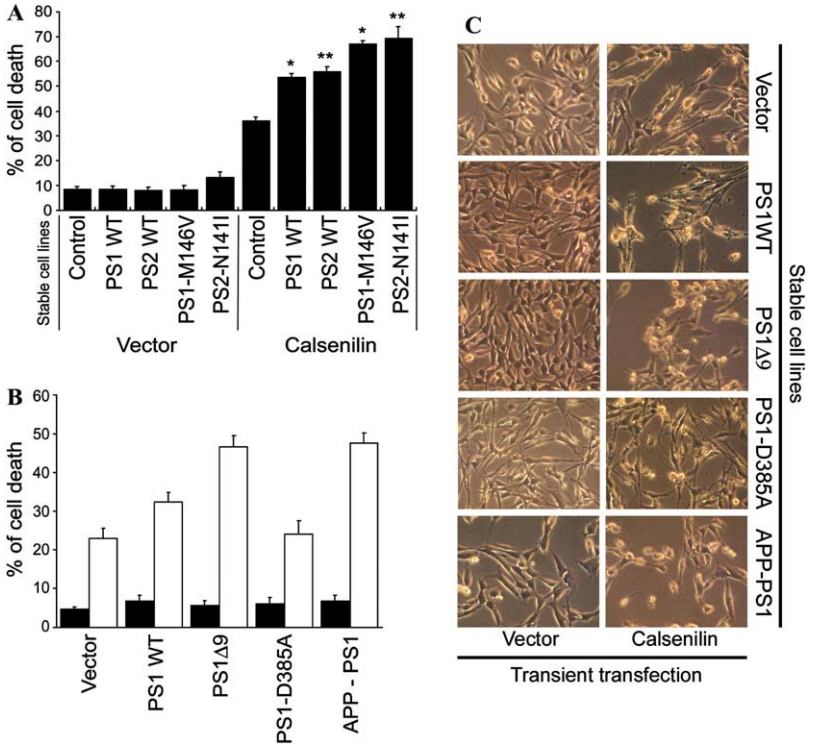

Fig. 3. Potentiation of calsenilin-induced death by FAD mutants of presenilin. Calsenilin was transfected into SK-N-BE2(c) human (A) or B103 rat (B and C) neuronal cells stably expressing FAD-associated mutants (gain-of-function; PS1ΔE9, PS1-M146V, PS2-N141I, and loss-of-function; PS1-D385A) or wild-type of presenilin. White and black bars indicate calsenilin and empty vector transfection, respectively (B). The incidence of apoptotic cell death was determined by evaluation of Hoechst 33258-stained condensed nuclei and cell morphology. Values are means ± SD ($n = 5$) (*$p < 0.01$; **$p < 0.01$).

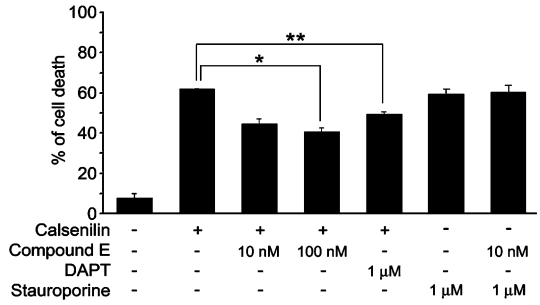

Fig. 4. Inhibition of calsenilin-induced cell death by γ-secretase inhibitors. HeLa cells were transfected with calsenilin and incubated with DAPT or compound E. After 36 h, death rates (means ± SD, $n = 3$) of the GFP-positive cells were determined based on the biochemical and morphological changes under the fluorescence microscope. Statistically significant differences compared with control (transfection with calsenilin) are indicated with asterisks (*$p < 0.005$; **$p < 0.01$).

functional coordination between calsenilin and presenilin/γ-secretase.

Evidence suggests that presenilins are either the catalytic subunit or a necessary co-factor of a high molecular weight multi-subunit aspartyl protease complex that exhibits γ-secretase activity [22,23]. Directed mutagenesis of either of two transmembrane-situated aspartates that are conserved in all presenilins similarly interferes with γ-secretase activity [24]. Furthermore, well-characterized γ-secretase inhibitors that mimic the transition state of an aspartyl protease mechanism directly bind to presenilins to inhibit enzymatic activity of presenilin [25]. By using such γ-secretase inhibitors, we further examined the role of PS/γ-secretase in the calsenilin-mediated cell death. Indeed, treatment of the calsenilin-transfected cells with selective γ-secretase inhibitors, compound E or DAPT, significantly decreased the calsenilin-induced cell death compared with controls but did not affect staurosporine-triggered apoptosis (Fig. 4), showing the first evidence for the inhibitory effects of γ-secretase inhibitors on apoptosis and indicating that the PS-associated γ-secretase activity is involved in the calsenilin-mediated cell death. DAPT and compound E are selective γ-secretase inhibitors that directly block the γ-secretase-mediated production of Aβ. Moreover, compound E is known to directly bind to presenilin [21]. We also found that these inhibitors reduced other types of cell death triggered by increase of intracellular calcium concentration (data not shown) in which calsenilin was previously implicated to play a role [18]. Still, partial suppression of calsenilin-mediated cell death by γ-secretase inhibitors may imply a presenilin-independent function of calsenilin such as gene regulation.

While numerous presenilin-binding proteins have been identified as a part of the characterization of the normal and pathological functions of presenilin, the extent to which these binding proteins require presenilin for activity and the effect of presenilin mutation on their activity are still incompletely understood. Our findings demonstrate that γ-secretase activity and FAD-linked mutants of presenilins sensitize calsenilin-mediated apoptosis, though the molecular architecture of the protein complexes containing presenilin mutant and calsenilin remains to be solved. In summary, the study presented here suggests that the increased expression of pro-apoptotic calsenilin in neuronal cells coordinates with presenilin/γ-secretase in the neuronal loss of AD.

Acknowledgments

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References

[1] D.L. Price, S.S. Sisodia, Mutant genes in familial Alzheimer's disease and transgenic models, Anuu. Rev. Neurosci. 21 (1998) 479–505.
[2] D.T. Loo, A. Copani, C.J. Pike, E.R. Whittemore, A. Walencewicz, C.W. Cotman, Apoptosis is induced by β-amyloid in cultured central nervous system neurons, Proc. Natl. Acad. Sci. USA 90 (1993) 7951–7955.
[3] S. Estus, H.M. Tucker, C. van Rooyen, S. Wright, E.F. Brigham, M. Wogulis, R.E. Rydel, Aggregated amyloid-β protein induces cortical neuronal apoptosis and concomitant apoptotic pattern of gene induction, J. Neurosci. 17 (1997) 7736–7745.
[4] A. LeBlanc, Increased production of 4 kDa amyloid β peptide in serum deprived human primary neuron cultures: possible involvement of apoptosis, J. Neurosci. 15 (1995) 7837–7846.
[5] F.G. Gervais, D. Xu, G.S. Robertson, J.P. Vaillancourt, Y. Zhu, J. Huang, A. LeBlanc, D. Smith, M. Rigby, M.S. Shearman, E.F. Clarke, H. Zheng, L.H. Van Der Ploeg, S.C. Ruffolo, N.A. Thornberry, S. Xanthoudakis, R.J. Zamboni, S. Roy, D.W. Nicholson, Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-*rbeta* precursor protein and amyloidogenic A β peptide formation, Cell 97 (1999) 395–406.
[6] J. Yuan, N.A. Yankner, Apoptosis in the nervous system, Nature 407 (2000) 802–809.
[7] G. Deng, C.J. Pike, C.W. Cotman, Alzheimer-associated presenilin-2 confers increased sensitivity to apoptosis in PC12 cells, FEBS Lett. 397 (1996) 50–54.
[8] Q. Guo, K. Furukawa, B.L. Sopher, D.G. Pham, N. Robinson, G.M. Martin, M.P. Mattson, Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid β-peptide, NeuroReport 8 (1996) 379–383.
[9] P. Vito, E. Lacana, L. D'Adamio, Interfering with apoptosis: Ca^{2+}-binding protein ALG-2 and Alzheimer's disease gene ALG-3, Science 271 (1996) 521–525.
[10] B. Wolozin, K. Iwasaki, P. Vito, J.K. Ganjei, E. Lacana, T. Sunderland, B. Zhao, J.W. Kusiak, W. Wasco, L. D'Adamio, Participation of presenilin 2 in apoptosis: enhanced basal activity conferred by an Alzheimer mutation, Science 274 (1996) 1710–1713.
[11] Q. Guo, B.L. Sopher, K. Furukawa, D.G. Pham, N. Robinson, G.M. Martin, M.P. Mattson, Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor

withdrawal and amyloid β-peptide: involvement of calcium and oxyradicals, J. Neurosci. 17 (1997) 4212–4222.
[12] S. Janicki, M.J. Monteiro, Increased apoptosis arising from increased expression of the Alzheimer's disease-associated presenilin-2 mutation (N141I), J. Cell Biol. 139 (1997) 485–495.
[13] P. Vito, B. Wolozin, J.K. Ganjei, I. Katsunori, E. Lacan, L. D'Adamio, Requirement of the familial Alzheimer's disease gene PS2 for apoptosis. Opposing effect of ALG-3, J. Biol. Chem. 271 (1996) 31025–31028.
[14] T. Tomita, R. Takikawa, A. Koyama, Y. Morohashi, N. Takisugi, T.C. Saido, K. Maruyama, T. Iwatsubo, C terminus of presenilin is required for overproduction of Aβ42 through stabilization and endoproteolysis of presenilin, J. Neurosci. 19 (1999) 10627–10634.
[15] J.D. Buxbaum, E. Choi, Y. Luo, C. Lilliehook, A.C. Crowley, D.E. Merriam, W. Wasco, Calsenilin: a calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment, Nat. Med. 4 (1998) 1177–1181.
[16] W. Frank An, M.R. Bowlby, M. Betty, J. Cao, H.P. Ling, G. Mendoza, J.W. Hinson, K.I. Mattsson, B.W. Strassle, J.S. Trimmer, K.J. Rhodes, Modulation of A-type potassium channels by a family of calcium sensors, Nature 403 (2000) 553–556.
[17] A.M. Carrión, W.A. Link, F. Ledo, B. Mellström, J.R. Naranjo, DREAM is a Ca^{2+}-regulated transcriptional repressor, Nature 398 (1999) 80–84.
[18] D.G. Jo, M.J. Kim, Y.H. Choi, I.K. Kim, Y.H. Song, H.N. Woo, C.W. Chung, Y.K. Jung, Pro-apoptotic function of calsenilin/DREAM/KChIP3, FASEB J. 15 (2001) 589–591 (first published on January 19, 2001: 10.1096/fj.00-0541fje).
[19] B. De Strooper, W. Annaert, P. Cupers, P. Saftig, K. Craessaerts, J.S. Mumm, E.H. Schroeter, V. Schrijvers, M.S. Wolfe, W.J. Ray, A. Goate, R. Kopan, A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain, Nature 398 (1999) 518–522.
[20] H.F. Dovey, V. John, J.P. Anderson, L.Z. Chen, P. de Saint Andrieu, L.Y. Fang, S.B. Freedman, B. Folmer, E. Goldbach, E.J. Holsztynska, K.L. Hu, K.L. Johnson-Wood, S.L. Kennedy, D. Kholodenko, J.E. Knops, L.H. Latimer, M. Lee, Z. Liao, I.M. Lieberburg, R.N. Motter, L.C. Mutter, J. Nietz, K.P. Quinn, K.L. Sacchi, P.A. Seubert, G.M. Shopp, E.D. Thorsett, J.S. Tung, J. Wu, S. Yang, C.T. Yin, D.B. Schenk, P.C. May, L.D. Altstiel, M.H. Bender, L.N. Boggs, T.C. Britton, J.C. Clemens, D.L. Czilli, D.K. Dieckman-McGinty, J.J. Droste, K.S. Fuson, B.D. Gitter, P.A. Hyslop, E.M. Johnstone, W.Y. Li, S.P. Little, T.E. Mabry, F.D. Miller, J.E. Audia, Functional γ-secretase inhibitors reduce β-amyloid peptide levels in brain, J. Neurochem. 76 (2001) 173–181.
[21] D. Seiffert, J.D. Bradley, C.M. Rominger, D.H. Rominger, F. Yang, J.E. Meredith, Q. Wang Jr., A.H. Roach, L.A. Thompson, S.M. Spitz, J.N. Higaki, S.R. Prakash, A.P. Combs, R.A. Copeland, S.P. Arneric, P.R. Hartig, D.W. Robertson, B. Cordell, A.M. Stern, R.E. Olson, R. Zaczek, Presenilin-1 and -2 are molecular targets for γ-secretase inhibitors, J. Biol. Chem. 275 (2000) 34086–34091.
[22] Y.M. Li, M.T. Lai, M. Xu, Q. Huang, J. DiMuzio-Mower, M.K. Sardana, X.P. Shi, K.C. Yin, J.A. Shafer, S.J. Gardell, Presenilin 1 is linked with γ-secretase activity in the detergent solubilized state, Proc. Natl. Acad. Sci. USA 97 (2000) 6138–6143.
[23] W.P. Esler, W.T. Kimberly, B.L. Ostaszewski, W. Ye, T.S. Diehl, D.J. Selkoe, M.S. Wolfe, Activity-dependent isolation of presenilin–γ-secretase complex reveals nicarstin and a γ substrate, Proc. Natl. Acad. Sci. USA 99 (2002) 2720–2725.
[24] M.S. Wolfe, W. Xia, B.L. Ostaszewski, T.S. Diehl, W.T. Kimberly, D.J. Selkoe, Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity, Nature 398 (1999) 513–517.
[25] Y.M. Li, M. Xu, M.T. Lai, Q. Huang, J. Castro, J. DiMuzio-Mower, T. Harrison, C. Lellis, A. Nadin, J. Neduvelil, R. Register, M. Sardana, M. Shearman, A. Smith, X.P. Shi, K.C. Yin, J. Shafer, S.J. Gardell, Photoactivated γ-secretase inhibitors directed to the active site covalently label presenilin 1, Nature 405 (2000) 689–694.