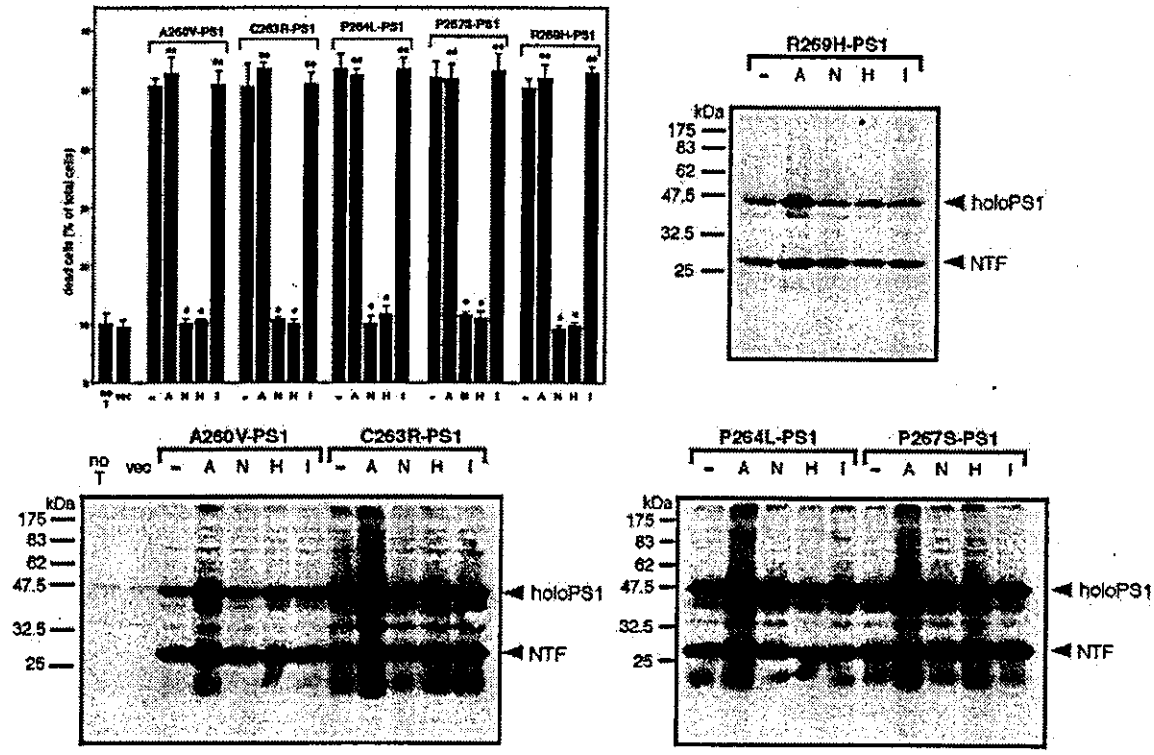

Fig. 5. A260V-, C263R-, P264L-, P267S-, R269H-PS1. F11 cells were transfected with an empty vector (vec), A260V-, C263R-, P264L-, P267S-, or R269H-PS1 cDNA and cultured in the presence of N2 supplement with or without 300 μM APO (A), 1 mM L-NMMA (N), 10 μM HN (H), or 10 nM IGF-I (I). Seventy-two hours after the onset of transfection, cell mortality was measured by Trypan blue exclusion assay (a). *Significant vs. corresponding controls without inhibitors. **Not significant vs. corresponding controls without inhibitors. Expression of the transfected PS1 mutants was examined by immunoblotting with anti-PS1 antibody (b–d). The upper and lower regions of the two major bands correspond to the holoproteins and the N-terminal fragments of mutant PS1s. no T, no transfection cases; holoPS1, holoprotein of mutant PS1; NTF, NTF of mutant PS1.

L250S-PS1 induce cell death by triggering the same pathway as M146L-PS1 triggers.

A260V, C263R, P264L, P267S, and R269H. As shown in Figure 5, cell deaths induced by A260V-, C263R-, P264L-, P267S-, and R269H-PS1 were inhibited by 1 mM L-NMMA, but resistant to 300 μM APO. Expression of A260V-, C263R-, P264L-, P267S-, or R269H-PS1 was not inhibited by 1 mM L-NMMA and 300 μM APO (Fig. 5). As compared to the internal control actin, the expression levels of these PS1 mutants were thought to be equivalent, even when APO or other inhibitors were treated (data not shown). These results suggest that A260V-, C263R-, P264L-, P267S-, and R269H-PS1 induce cell death by triggering the same pathway as that triggered by M146L-PS1.

E280A, E280G, E318G, G384A, and L392V. As shown in Figure 6, cell deaths induced by E280A-PS1 and E280G-PS1were inhibited by 1 mM L-NMMA, but resistant to 300 μM APO. In contrast, cell deaths induced by E318G-, G384A-, and L392V-PS1 were inhibited by 300 μM APO, but resistant to 1 mM L-NMMA. Expression of E280A-, E280G-, E318G-, G384A-, or L392V-PS1 was not inhibited by 1 mM L-NMMA and 300 μM APO (Fig. 6). As compared to the internal control actin, the expression levels of these PS1 mutants were thought to be equivalent, even when treated with APO or other inhibitors (data not shown). These results suggest that E280A-PS1 and E280G-PS1 induce cell death by triggering the same pathway as M146L-PS1 triggers, whereas E318G-, G384A-, and L392V-PS1 induce cell death by triggering a pathway different from the pathway that M146L-PS1 triggers and same as that triggered by C410Y-PS1.

Sensitivities of FAD-Linked Mutant PS1-Induced Cell Death to HN and IGF-I in F11 Neurohybrid Cells

We next examined whether cell death by each FAD-linked mutant of PS1 was sensitive to HN or IGF-I. We transfected F11 cells with various PS1 mutant cDNAs and cultured cells with HN or IGF-I in the presence of N2 supplement.

V82L, V96P, Y115H, E120K, M139T/V/I, I143T/F, M146V, H163R/Y, I213T, A231T, L250S,

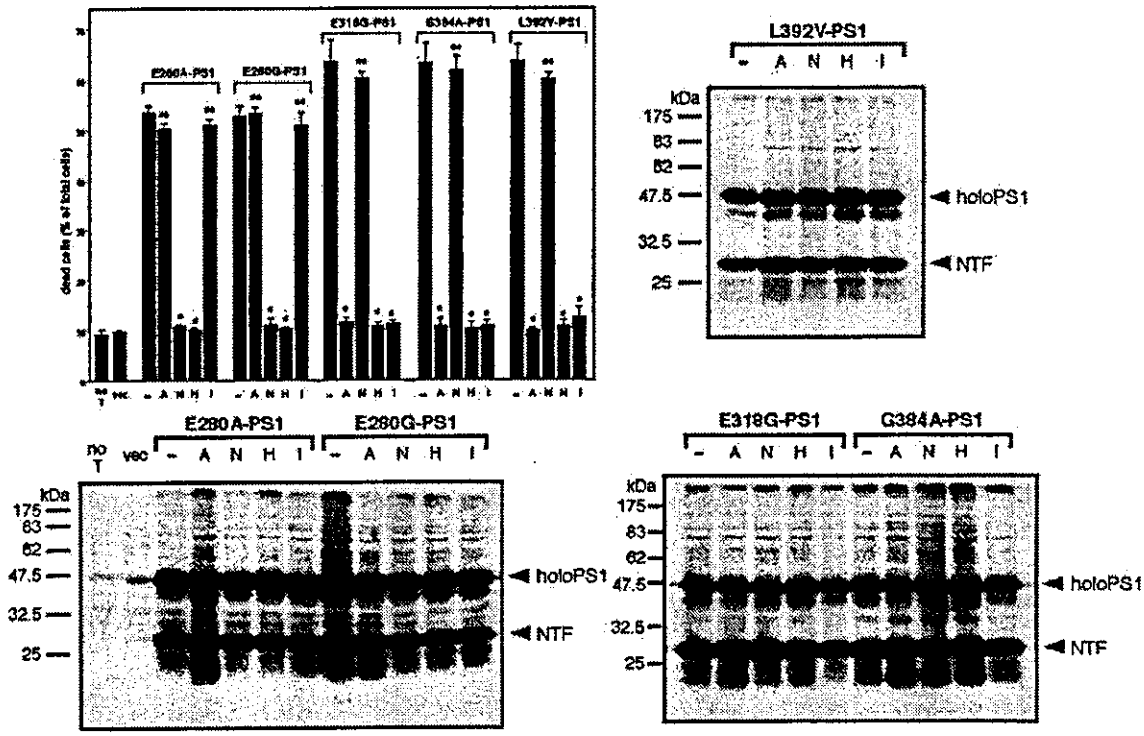

Fig. 6. E280A/G-, E318G-, G384A-, L392V-PS1. F11 cells were transfected with an empty vector (vec), E280A-, E280G-, E318G-, G384A-, or L392V-PS1 cDNA and cultured in the presence of N2 supplement with or without 300 μM APO (A), 1 mM L-NMMA (N), 10 μM HN (H), or 10 nM IGF-I (I). Seventy-two hours after the onset of transfection, cell mortality was measured by Trypan blue exclusion assay (a). *Significant vs. corresponding controls without inhibitors. **Not significant vs. corresponding controls without inhibitors. Expression of the transfected PS1 mutants was examined by immunoblotting with anti-PS1 antibody (b–d). The upper and lower regions of the two major bands correspond to the holoproteins and the N-terminal fragments of mutant PS1s. no T, no transfection cases; holoPS1, holoprotein of mutant PS1; NTF, NTF of mutant PS1.

A260V, C263R, P264L, P267S, R269H, E280A, and E280G. As shown in Figure 2–6, cell death induced by V82L, V96P, Y115H, E120K, M139T/V/I, I143T/F, M146V, H163R/Y, I213T, A231T, L250S, A260V, C263R, P264L, P267S, R269H, E280A, and E280G were inhibited by 10 μM HN, but resistant to 10 nM IGF-I. Expression of V82L, V96P, Y115H, E120K, M139T/V/I, I143T/F, M146V, H163R/Y, I213T, A231T, L250S, A260V, C263R, P264L, P267S, R269H, E280A, or E280G-PS1 was not inhibited by 10 μM HN and 10 nM IGF-I (Fig. 2–6). These data confirm that these NTF-mutants of PS1 induce cell death by triggering the same pathway as M146L-PS1 triggers, which is sensitive to HN, but resistant to IGF-I.

E318G, G384A, and L392V. As shown in Figure 6, cell deaths induced by E318G-, G384A-, and L392V-PS1 were inhibited by 10 μM HN and 10 nM IGF-I. Expression of E318G-, G384A-, or L392V-PS1 was not inhibited by 10 μM HN and 10 nM IGF-I (Fig. 6). These data confirm that these CTF-mutants of PS1 induce cell death by triggering a pathway different from that triggered by M146L-PS1.

Sensitivities of Mutant PS1-Induced Cell Death to L-NMMA and APO in NSC-34 Cells

We investigated further whether FAD-linked mutants of PS1 induce cell death in NSC-34 cells in the same sensitivity profile as in F11 cells. NSC-34 cells are a well-established immortalized motoneuron hybrid cell line. As shown in Figure 7A and B, each of V96P-PS1, M146L-PS1, I213T-PS1, A246E-PS1, G384A-PS1, L392V-PS1, and C410Y-PS1 induced cell death in NSC-34 cells. V96P-PS1, M146L-PS1, I213T-PS1, A246E-PS1 are the NTF-mutant PS1, and G384A-PS1, L392V-PS1, and C410Y-PS1 are the CTF-mutant PS1. Cell death induced by NTF-mutant PS1 was inhibited by 1 mM GEE and 1 mM L-NMMA, but not 300 μM APO (Fig. 7A,B). In contrast, cell death induced by CTF-mutant PS1 was inhibited by 1 mM GEE and 300 μM APO, but not 1 mM L-NMMA (Fig. 7A,B). As was the case in F11 neurohybrid cells (Hashimoto et al., 2000b), the data of Trypan blue exclusion assay were precisely reciprocal with the data of WST-8 assay (Fig. 7C), indicating that Trypan blue exclusion assay is a reliable method to assess cell viability.

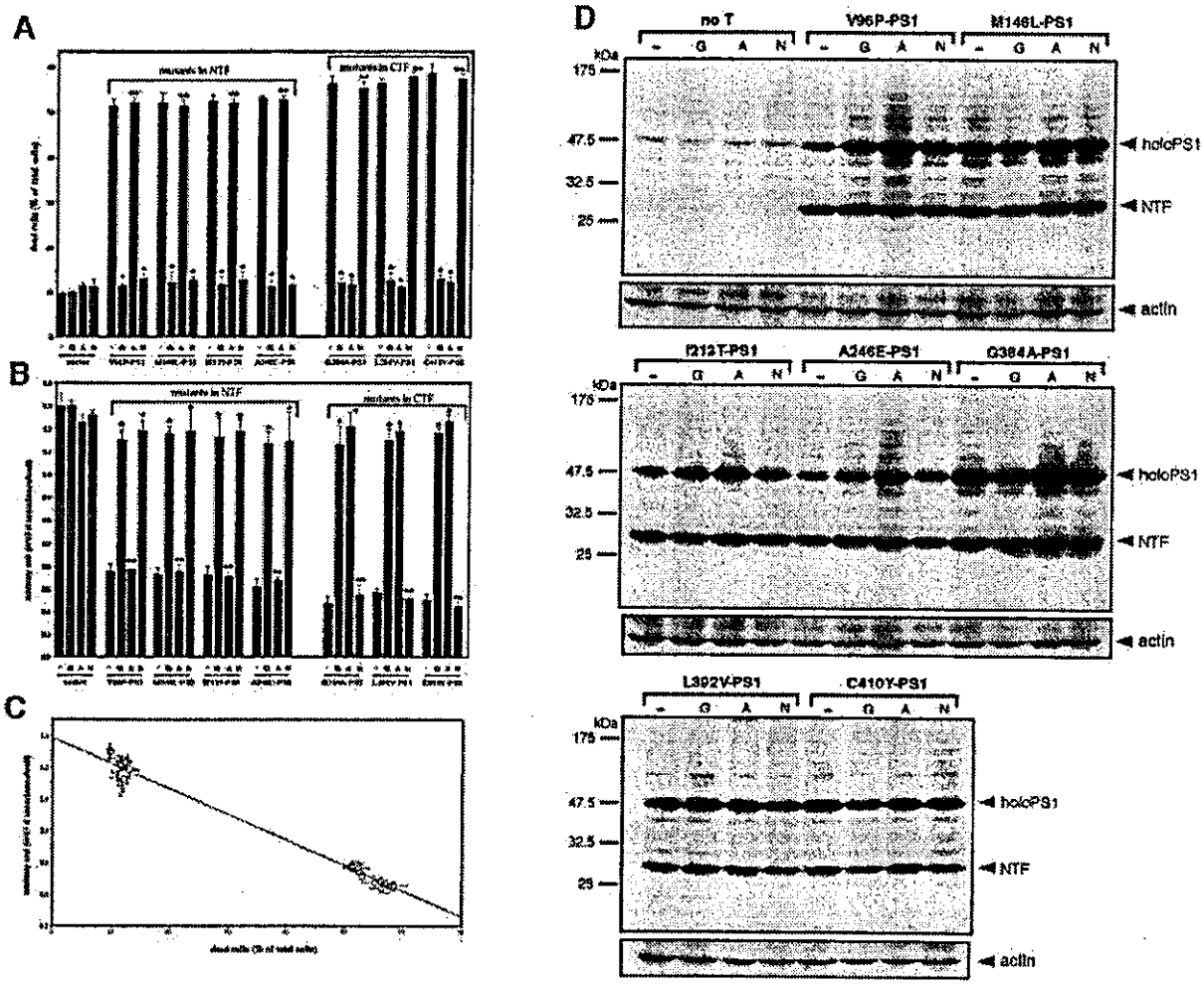

Fig. 7. Inhibitor profiles of cell death by M146L-PS1 and C410Y-PS1 in NSC-34 cells. **A, B:** NSC-34 cells were transfected with empty vector (vec), V96P-PS1, M146L-PS1, I213T-PS1, A246E-PS1, G384A-PS1, L392V-PS1, or C410Y-PS1 cDNA and cultured in the presence of N2 supplement with or without 1 mM GEE (G), 1 mM L-NMMA (N), or 300 μM APO (A). Cell mortality was measured by Trypan blue exclusion assay 72 hr after the onset of transfection (A) and WST-8 cell viability assay (B). *Significant vs. corresponding controls without inhibitors; **not significant vs. corresponding controls without inhibitors. **C:** Plot of relationship between data from the Trypan blue exclusion and WST-8 cell viability assays (r^2 = 0.989). **D:** Expression of the transfected PS1 mutants was examined by immunoblotting with anti-PS1 antibody. The upper and lower arrowheads indicate the holoproteins and the N-terminal fragments of mutant PS1s. "no T" denotes no transfection cases.

As compared to the internal control actin amount, expression of the NTF-mutants and the CTF-mutants of PS1 was inhibited little by 1 mM L-NMMA and 300 μM APO (Fig. 7D). These data not only indicate that FAD-linked mutants of PS1 also induce cell death in neuronal cells other than F11 neurohybrids, but also confirm that in neuronal cells other than F11 cells, FAD-linked NTF-mutants of PS1 induce L-NMMA-sensitive/APO-resistant cell death and FAD-linked CTF-mutants of PS1 induce L-NMMA-resistant/APO-sensitive cell death.

DISCUSSION

We have herein shown that FAD-linked NTF-mutants and CTF-mutants of PS1 induce neuronal cell death, but through different toxic mechanisms. Figure 8 summarizes the results. We examined the 27 FAD-linked PS1 mutants (V82L-, V96P-, Y115H-, E120K-, M139T/V/I-, I143T/F-, M146L/V-, H163R/Y-, I213T-, A231T-, L250S-, A260V-, C263R-, P264L-, P267S-, R269H-, E280A/G-, E318G-, G384A-, L392V-, C410Y-PS1) and used two neuronal cell lines to conclude this finding. Neuronal cell death by FAD-linked NTF-

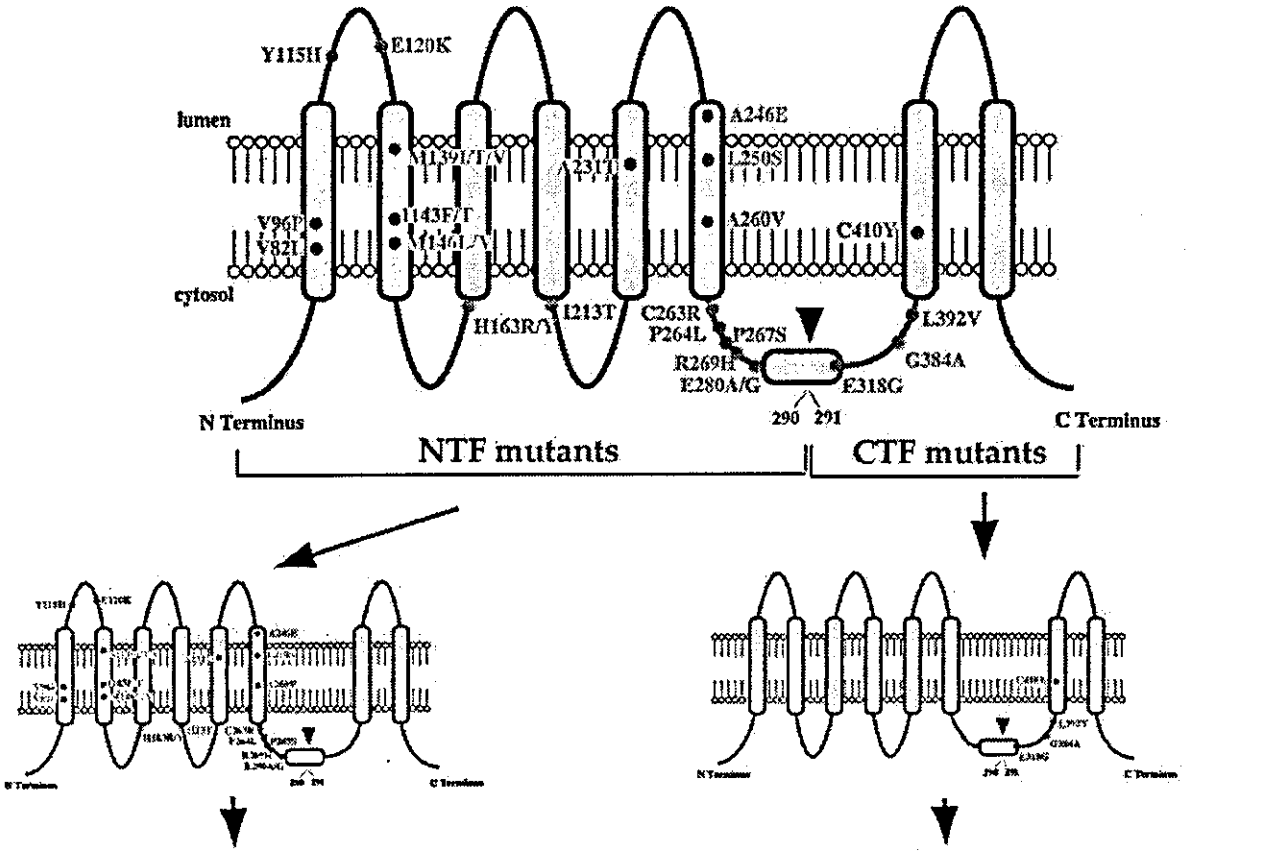

Fig. 8. Schematic illustration of the examined mutants of PS1 and their neurotoxic mechanisms. This study examined the neurotoxic mechanisms of the indicated PS1 mutants. The PS1 mutants whose mutations are located in the NTF cause NOS inhibitor-sensitive neurotoxicity, which is HN-sensitive, but IGF-I-resistant. The PS1 mutants whose mutations are located in the CTF cause NADPH oxidase inhibitor-sensitive neurotoxicity, which is HN-sensitive and IGF-I-sensitive. The position 290 is the C-terminal end of the NTF and the position 291 is the first amino acid residue of the CTF. See the text for detail.

mutants of PS1 was sensitive to L-NMMA, but resistant to APO. Neuronal cell death by FAD-linked CTF-mutants of PS1 was sensitive to APO, but resistant to L-NMMA. These results indicate that FAD-linked NTF-mutants of PS1 would induce cell death by triggering the NOS-mediated toxic pathway and that FAD-linked CTF-mutants of PS1 induce cell death by triggering the NADPH oxidase-mediated toxic pathway. Further details of the relationship between expression and neurotoxicity of PS1 mutants requires investigation.

We also examined the effects of HN and IGF-I on neurotoxicity by various PS1 mutants. HN is a newly identified rescue factor that inhibits cell death induced by FAD-linked mutants of AβPP, PS1, and PS2 (Hashimoto et al., 2001a,b,c). In the F11 system, 10 μM HN suppresses M146L-PS1-induced cell death to the basal level (Hashimoto et al., 2001a). HN can also suppress the NADPH oxidase-mediated cell death induced by N141I-PS2 (Hashimoto et al., 2001a, 2002a). Therefore, it is likely that HN also suppresses cell death induced by PS1 mutants in addition to M146L-PS1. In fact, HN inhibited neurotoxicity by H163R-PS1, A246E-PS1, L286V-PS1, and C410Y-PS1, in addition to M146L-PS1 (Hashimoto et al., 2001c).

In contrast, IGF-I cannot inhibit M146L-PS1-induced cell death under the same condition as HN inhibits M146L-PS1-induced cell death (Hashimoto et al., 2001a). Yet in the F11 system, IGF-I can inhibit V642I-AβPP-induced cell death (Niikura et al., 2001), which is mediated by NADPH oxidase (Hashimoto et al., 2002a). Therefore, it is possible that IGF-I suppresses NADPH oxidase-mediated cell death induced by the CTF-mutant

PS1, but may not suppress the NOS-mediated cell death induced by NTF-mutant PS1.

We thus examined whether cell death by each FAD-linked mutant of PS1 was sensitive to HN or IGF-I. The results indicated that the neurotoxic pathway induced by the NTF-mutant PS1 was inhibited by HN, but not IGF-I, and that the neurotoxic pathway induced by the CTF-mutant PS1 was inhibited by both HN and IGF-I. These data are consistent with our earlier studies that HN, but not IGF-I, inhibits NOS-mediated cell death by M146L-PS1, one of the FAD-linked NTF-mutants of PS1 (Hashimoto et al., 2001c, 2002b), and that both HN and IGF-I inhibit NADPH oxidase-mediated cell death (Niikura et al., 2001; Hashimoto et al., 2001a, 2002a, 2003). The present data indicate not only that the neurotoxic pathways are different between NTF- and CTF-mutant PS1, but also suggest that the rescue mechanism of HN is different from that of IGF-I. In this regard, it should be noted that the rescue function of IGF-I is inhibited by genistein (tyrosine kinase inhibitor) and wortmanin (PI-3 kinase inhibitor), but that the rescue function of HN is sensitive to genistein, but resistant to wortmanin (Hashimoto et al., 2001a). It should be noted that it is controversial genetically whether E318G-PS1 causes FAD (Aldudo et al., 1998; Mattila et al., 1998). E318G-PS1 expression, however, is associated with increased production of Aβ42 (Murayama et al., 1999) and augmented levels of neuronal cell death (this study), indicating that E318G-PS1 has the same biological potential as other FAD-linked mutants of PS1.

These data, taken collectively, indicate that FAD-linked mutants of PS1 whose mutations are located in the NTF induce L-NMMA-sensitive/APO-resistant cell death by triggering the same pathway as M146L-PS1 triggers, and that FAD-linked mutants of PS1 whose mutations are located in the CTF induce L-NMMA-resistant/APO-sensitive cell death by triggering a pathway different from the pathway triggered by M146L-PS1. At present, it remains unknown how NTF-mutants and CTF-mutants of PS1 induce cell death via different mechanisms. Only a few clues include that N141I-PS2 triggers the NADPH oxidase-mediated toxic mechanism under the same condition as M146L-PS1 triggers the NOS-mediated toxic mechanism (Hashimoto et al., 2002a,b). NADPH oxidase-mediated cell death by N141I-PS2 is mediated by a trimeric G protein, Go (Hashimoto et al., 2002a), and NOS-mediated cell death by M146L-PS1 is mediated by a novel target of pertussis toxin (PTX) not Go, Gi1, Gi2, or Gi3 (Hashimoto et al., 2002b). Therefore, a simple interpretation of the present study is that the CTF of PS1 interacts with Go, which is activated by the CTF-mutants, and that the NTF of PS1 interacts with the novel PTX target, which is activated by the NTF-mutants. This notion is partly consistent with the report showing that PS1 interacts with Go at the CTF of PS1 (Smine et al., 1998) as well as the experimental results that cell deaths by M146L-PS1, A246E-PS1, and C410Y-PS1 were sensitive to PTX (Hashimoto et al., 2002b for M146L-PS1; not shown for A246E-PS1 and C410Y-PS1). To determine whether the NTF of PS1 interacts with the novel target of PTX, it is necessary to identify the novel PTX target.

In summary, this is the first report showing that FAD-linked NTF-mutants and CTF-mutants of PS1 induce neuronal cell death through different toxic mechanisms. Although the mechanisms of cell death by some of FAD-linked mutants of PS1 have been studied individually, no study thus far has comprehensively investigated cell death mechanisms by various FAD-linked mutants of PS1. This study provides an important clue to understanding the entire array of neurotoxic signals generated by FAD mutants of PS1 and will provide a novel insight into the pathologic and probably physiologic functions of PS1.

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Planel E, Miyasaka T, Launey T, Chui D-H, Tanemura K, Sato S, Murayama O, Ishiguro K, Tatebayashi Y, Takashima A	Alterations in glucose metabolism induce hypothermia leading to tau hyperphosphorylation: mechanism and implications for Alzheimer's disease	J Neurosci	24	2401-2411	2004
Hashimoto Y, Tsukamoto E, Niikura T, Yamagishi Y, Ishizaka M, Aiso S, Takashima A, Nishimoto I	Amino- and carboxyl-terminal mutants of presenilin 1 cause neuronal cell death through distinct toxic mechanisms: Study of 27 different presenilin 1 mutants	J Neurosci Res	75 (3)	417-428	2004

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Novel function of PS2V: change in conformation of tau proteins☆

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Abstract

Neurofibrillary tangles (NFTs) are associated with many neurodegenerative disorders, such as Alzheimer's disease (AD). The major components of NFTs are hyper-phosphorylated tau proteins. The alternatively spliced form of the presenilin-2 (*PS2*) gene (*PS2V*) has been observed in sporadic AD brains. However, it is not known whether there is a relationship between tau aggregation/hyper-phosphorylation and PS2V expression. In this manuscript, we make the first report of PS2V alterations in the conformation of the tau protein (unknown form of tau) in the human neuroblastoma cell line.

Keywords: Presenilin-2; Splice variant; Tau; Oxidative stress; Conformation change; Neurodegenerative disease

Neurofibrillary tangles (NFTs) are found in many neurodegenerative disorders, such as Alzheimer's disease (AD) and frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) caused by mutations in the *tau* gene. The major components of NFTs are hyper-phosphorylated tau proteins. Alzheimer's disease is a neurodegenerative disorder that is pathologically characterized by severe neuron loss, glial proliferation, extracellular deposition of senile plaques composed of β-amyloid, and deposition of intracellular neurofibrillary tangles [1–5]. Recently, we discovered that an alternative splice variant that lacks exon 5 of the *presenilin-2* (*PS2*) gene (PS2V) is significantly expressed in some brains of AD patients compared with those of controls [6]. This PS2V encodes aberrant proteins, which form intracellular inclusion bodies (PS2V bodies; [7]), and was observed in pyramidal cells of the cerebral cortex and the hippocampus of sporadic AD brains [8]. Further, PS2V-expressing cell lines became fragile in response to various endoplasmic reticulum (ER) stresses [6,8]. The expression of PS2V observed in sporadic AD brains mimics hypoxia-exposed human neuroblastoma SK-N-SH cells [6,8,9]. The PS2V was induced by the action of the high mobility group protein A1a (HMGA1a) that directly binds to specific sequences on the PS2 pre-mRNA in SK-N-SH cells under hypoxia stimulation [9]. However, the interaction between PS2V and tau proteins was previously unknown.

Materials and methods

Cell culture and plasmid transfection. Cell culture and plasmid transfection were performed as previously described [6,8]. Transfection

☆ *Abbreviations:* AD, Alzheimer's disease; FTDP-17, frontotemporal dementia with Parkinsonism linked to chromosome 17; HMGA1a, high mobility group protein A1a; NFT, Neurofibrillary tangle; PS2, presenilin-2; PS2V, alternative splice form of PS2 gene that lacks exon 5.

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of expression plasmids was carried out using LipofectAmine (Gibco-BRL). The full-length cDNAs of PS2 and PS2V were amplified by RT-PCR with attached *Eco*RI and *Xho*I sites and subcloned into the pcDNA3(+) vector (Invitrogen) between corresponding restriction enzyme sites.

Immunoblotting assays. Immunoblotting was performed as previously described [10,11] with minor modifications. In brief, the protein contents of each fraction were determined and aliquots of these proteins were mixed at a ratio of 4:1 with 10 mM Tris–HCl buffer (pH 6.8), containing 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.01% bromophenol blue, and 5% mercaptoethanol. This mixture was then boiled at 100 °C for 10 min. Each aliquot was then electrophoresed on a polyacrylamide gel containing 0.1% SDS at a constant current of 15 mA/plate for 2 h at room temperature. The gel was then blotted onto a polyvinylidene fluoride (PVDF) membrane that was previously activated with 100% methanol. The membrane was blocked with 5% skimmed milk dissolved in PBS containing 0.05% Tween 20, and reacted with an anti-tau2 antibody diluted in the latter buffer that contained 1% skimmed milk. The second antibody used was an anti-mouse immunoglobulin G (IgG) conjugated with horseradish peroxidase (HRP). Proteins reactive with the antibody were detected with the aid of ECLTM detection reagents, followed by exposure to X-ray films for different periods to obtain films that were adequate for detection. The anti-tau2 mouse monoclonal antibody was purchased (Sigma). Protein content was measured using a Bio-Rad Protein Assay Kit (Bio-Rad, CA, USA).

Results and discussion

PS2V changes phosphorylation and conformation of tau proteins

Tau proteins are part of a family of six alternative splice forms, with their molecular weights ranging from 45 to 65 kDa on SDS–PAGE (reviewed in [12]). Further, the phosphorylated forms of the tau proteins are electrophoresed at molecular weights from 55 to 74 kDa

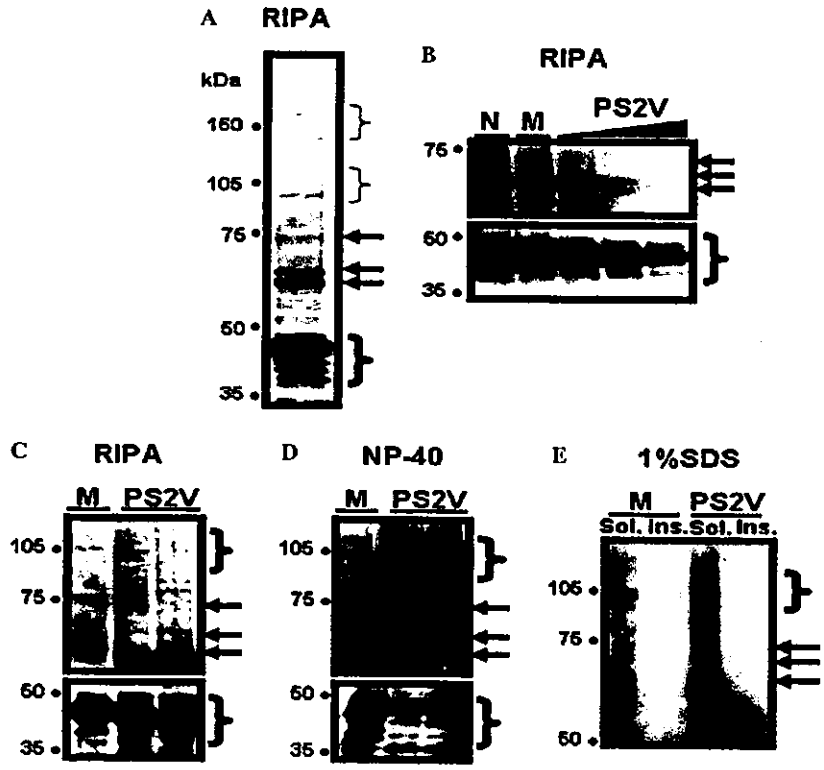

Fig. 1. Effects of PS2V on tau protein expression, phosphorylation, and aggregation in human neuroblastoma SK-N-SH cells. (A) Anti-tau2 antibody-positive tau proteins in normal SK-N-SH cells. Cell lysates were homogenized in RIPA buffer containing 50 mM Tris, 150 mM NaCl, 1% NP-40, 5 mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM sodium fluoride (NaF), 10 mM sodium β-glycerophosphate (NaGP), 10 mM sodium pyrophosphate (NaPP), 1 mM sodium orthovanadate (NaOV), and 1 μg/ml (*p*-amidinophenyl)methanesulfonyl fluoride (RIPA fractions). The fractions were separated with SDS–PAGE, followed by an immunoblotting assay using an anti-tau2 antibody. (B) Effects of PS2V on tau protein expression and/or phosphorylation in SK-N-SH cells. RIPA fractions were prepared from normal or PS2V-expressing cells, followed by SDS–PAGE and an immunoblotting assay using an anti-tau2 antibody. (C and D) Comparison of sampling buffer on tau protein conformation in PS2V-expressing cells. RIPA (C)- or NP-40 (D)- fractions were separated by SDS–PAGE, followed by an immunoblotting assay using an anti-tau2 antibody. NP-40 fractions were prepared by the method of Schreiber et al. [13] with minor modifications [14–16]. In brief, each cultured cell was homogenized in 50 volumes of PBS containing 10 mM KCl, 1 mM EDTA, 1 mM EGTA, 5 mM dithiothreitol (DTT), 10 mM NaF, 10 mM NaGP, 10 mM NaPP, 1 mM NaOV, and 1 μg/ml of (*p*-amidinophenyl)methanesulfonyl fluoride. This was followed by the addition of 10% Nonidet P-40 to make a final concentration of 0.6%. (E) The β-sheet structure of the tau proteins in 1% SDS-insoluble fractions. One percent SDS-insoluble fractions were prepared by the method of Tanemura et al. [17]. The fractions were separated with SDS–PAGE, followed by an immunoblotting assay using an anti-tau2 antibody.

(reviewed in [12]). First, we detected anti-tau2 antibody-positive tau proteins, which include all forms of tau, in human neuroblastoma SK-N-SH (Fig. 1A). Then, we examined the effects of PS2V on the expression, phosphorylation, and aggregation of tau proteins in SK-N-SH cells (Fig. 1B). No marked changes of the unphosphorylated tau proteins were observed in the PS2V-expressing SK-N-SH cells compared with those in the mock-expressing cells (Fig. 1B, lower panel). Surprisingly, PS2V was more potent than the mock-expressing cells at decreasing the intensity of bands at the molecular weight position of the phosphorylated tau proteins that were immunoreactive with the anti-tau2 antibody (Fig. 1B, upper panel). Thereafter, we changed the conditions for dissolving from RIPA to NP-40 (Figs. 1C and D). Then, both phosphorylated and unphosphorylated tau proteins were dramatically decreased by PS2V in the NP-40-lysates (Fig. 1D). The high mobility-shifted immunoreactivities against the anti-tau2 antibody by PS2V appeared in these lysates (Fig. 1D). However, the shifted tau protein smears (possibly the aggregate form) by PS2V were not detected in RIPA-treated cell lysates (Fig. 1C). Furthermore, PS2V-induced mobility-shifted tau proteins were not observed in 1% SDS insoluble fractions, which include the β-sheet form of the tau protein (Fig. 1E). In general, tau proteins are considered to become easy to condense as the phosphorylation state gets worse. However, although the phosphorylation object of tau proteins had decreased, condensation objects of tau proteins increased in number. It is reasonable to suppose that this is a result of being made to condense by PS2V proteins rather than by phosphorylation objects of tau proteins that decreased in number and disappeared from the original molecular weight position on the SDS–PAGE. These results suggest that PS2V may make tau proteins change their conformation. The mobility-shifted tau protein smears were the weak form against the RIPA buffer and may be the intermediate form between the normal form and the β-sheet form of the tau proteins. However, it is still unclear whether the β-sheet structures of the tau proteins are not permanently formed by PS2V or rather that they are the intermediate form of β-sheet constitution. This is the case because the sporadic development of symptoms of neurodegenerative disorders and aging occurs and progresses over an extended period of time.

ER stress increases the action of PS2V

The PS2V-expressing SK-N-SH cells become fragile in response to ER# stresses [6]. Therefore, we next examined the effects of tunicamycin (Tm) stimulation on tau phosphorylation and/or a conformational change in the PS2V-expressing cells (Fig. 2). Intriguingly, it was demonstrated that the high mobility-shifted tau protein smears produced by PS2V were more potently shifted by

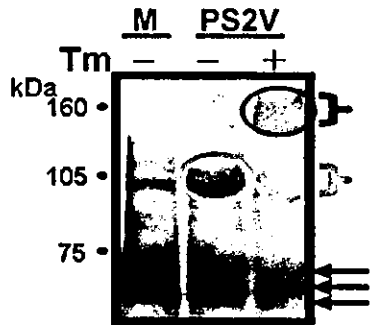

Fig. 2. Effects of tunicamycin (TM) on the conformational change of the tau protein by PS2V in SK-N-SH cells. PS2V-expressing cells were exposed to 0.2 μg/ml TM for 5 h, followed by preparation of NP-40 fractions. The fractions were separated by SDS–PAGE following an immunoblotting assay using an anti-tau2 antibody.

the Tm stimulation than those of the controls (Fig. 2). It is likely that PS2V does not become the core of a condensation object of tau proteins directly. Rather, it is probable that it becomes easy to condense tau proteins as a result of many proteins with faulty protein increase in number and is easy to condense in a cell because PS2V keeps unfolded protein response (UPR) of ER from increasing in number.

At the very least, these results suggest that the cell toxicity of the PS2V-ER stress system is a risk factor for tau abnormalities in oxidative stress-concerned neurodegenerative disorders, including in sporadic cases of AD.

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