Wild-type and mutated presenilins 2 trigger p53-dependent apoptosis and down-regulate presenilin 1 expression in HEK293 human cells and in murine neurons

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Presenilins 1 and 2 are two homologous proteins that, when mutated, account for most early onset Alzheimer's disease. Several lines of evidence suggest that, among various functions, presenilins could modulate cell apoptotic responses. Here we establish that the overexpression of presenilin 2 (PS2) and its mutated form Asn-141-Ile-PS2 alters the viability of human embryonic kidney (HEK)293 cells as established by combined trypan blue exclusion, sodium 3'-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate assay, and propidium iodide incorporation FACS analyses. The two parent proteins increase the acetyl-DEVD-al-sensitive caspase-3-like activity in both HEK293 cells and Telencephalon specific murine neurons, modulate Bax and bcl-2 expressions, and enhance cytochrome C translocation into the cytosol. We show that overexpression of both wild-type and mutated PS2 increases p53-like immunoreactivity and transcriptional activity. We also establish that wild-type- and mutated PS2-induced caspase activation is reduced by p53 antisense approach and by pifithrin- α , a chemical inhibitor of p53. Furthermore, mouse fibroblasts in which the PS2 gene has been knocked out exhibited strongly reduced p53-transcriptional activity. Finally, we establish that the overexpression of both wild-type and mutated PS2 is accompanied by a drastic reduction of endogenous presenilin 1 (PS1) expression. Interestingly, pifithrin- α diminished endogenous PS2 immunoreactivity, whereas the inhibitor increases PS1 expression. Altogether, our data demonstrate that wild-type and familial Alzheimer's disease-linked PS2 trigger apoptosis and down-regulate PS1 expression through p53-dependent mechanisms.

Alzheimer's disease is either sporadic or genetic in origin (1). In the latter case, the disease occurs early, is aggressive, and is very often associated with missense mutations located on two homologous proteins, named PS1 and PS2 (for reviews, see refs. 2–4). Although still debated, apoptosis appears, if not causative, at least associated with late stages of Alzheimer's disease neuropathology (5).

It is noteworthy that most presenilin 1 (PS1) and PS2 mutations trigger a selective phenotypic increase in A β 42 formation, and that the exacerbation of the production of this highly aggregable fragment is intimately linked with Alzheimer's pathology (6, 7). Interestingly, it was previously suggested that A β -related cell toxicity could be due to a modified apoptotic response in most cell types (8–10). Another clue of a direct link between presenilins and apoptotic response derived from the demonstration that presenilins could be targeted by caspases, the enzymatic effectors of programmed cell death.

More directly, it was shown that PS1 could decrease the susceptibility of neurons to apoptotic stimuli (11), whereas conversely, hippocampal neurons from knock-in mice expressing PS1 mutant exhibit enhanced vulnerability (12). Furthermore,

increased cell death was observed in antisense PS1-expressing NT2 cells that could be rescued by overexpression of the antiapoptotic protooncogene bcl-2 (13). Finally, it was reported that tumor suppressor p53 down-regulated PS1 expression (14), likely at the transcriptional level (15), and that this resulted in increased apoptosis (14).

Conversely, presenilin 2 (PS2) was reported to be proapoptotic (16), a phenotype potentiated by familial PS2 mutations (17, 18). Unlike PS1, the mechanisms by which PS2 displays its proapoptotic phenotype have been poorly investigated. Here we examine the influence of the overexpression of PS2 and its N141I-PS2 mutant on several cellular intermediates of the apoptotic cascade in human embryonic kidney (HEK)293 cells as well as in Telencephalon specific murine neurons (TSM 1). We demonstrate, to our knowledge for the first time, crosstalk between PS2 and p53 in both cell lines. Furthermore, we establish that PS2 expression could down-regulate endogenous PS1-like immunoreactivity, likely through a p53-dependent mechanism.

Materials and Methods

Cell Systems. HEK293 cells expressing wild-type (wt) PS2 or Asn-141-Ile-PS2 were obtained and cultured as previously reported (19). TSM1 neurons (20) were cultured as detailed previously (21) and stably transfected with 1 μ g of p53 cDNA subcloned in pcDNA3(–) antisense vector (provided by B. Vogelstein, Johns Hopkins University School of Medicine). Stable transfectants were screened for their endogenous p53-like immunoreactivity, as described below. Mice fibroblasts in which PS2-gene has been knocked out were previously described (22).

p53 Transcriptional Activity. The PG13-luciferase and p21^{waf-1}-luciferase p53 gene reporter constructs (provided by B. Vogelstein) have been described (23, 24). One microgram of PG13-luciferase or p21^{waf-1}-luciferase cDNA was cotransfected with 1 μ g of a β -galactosidase transfection vector (to normalize transfections efficiencies) in wt PS2-expressing HEK293 cells or PS2–/– mice fibroblasts. Forty-eight hours after transfection,

Abbreviations: wt, wild type; HEK, human embryonic kidney; XTT, sodium 3'-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate; PI, propidium iodide; FAD, familial early onset forms of Alzheimer's disease; β APP, β -amyloid precursor protein; PS2, presenilin 2; PS1, presenilin 1.

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luciferase and β -galactosidase activities were measured according to described procedures (23, 24).

Caspase Activity Measurements. HEK 293 cells [obtained as previously reported (19)] were cultured in six-well plates, as described (25), and then incubated for 24 h at 37°C in the presence or absence of 2 μ M staurosporine (Sigma). In some experiments, cells were preincubated with 100 μ M of the caspase inhibitor Ac-DEVD-al (Neosystem, Strasbourg, France) for 24 h before stimulation and induction of apoptosis by staurosporine. Some of the staurosporine-induced caspase responses were performed in the presence of 10 μ M of the p53 inhibitor pifithrin- α . Cells were then rinsed, gently scraped, pelleted by centrifugation, and assayed for their caspase-3-like activity, as extensively detailed (21).

Trypan Blue Exclusion. The viability of HEK293 cells was determined by the measurement of their capacity to exclude the vital dye trypan blue. In brief, HEK293 cells were cultured in 12-well plates, as described (25), and incubated for 24 h at 37°C in the presence or absence of 1 μ M staurosporine. After this incubation, cells were rinsed, gently scraped, pelleted by centrifugation, resuspended in 500 μ l of culture medium containing 0.1% trypan blue, loaded into a hemocytometer, and examined by light microscopy. Viable and nonviable (blue cells) cells were then counted, and the score obtained expressed dead cells in percent of total cells.

Sodium 3'-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene Sulfonic Acid Hydrate (XTT) Assay. HEK293 cells were grown in a 5% atmosphere in 96-well plates in a final volume of 100 μ l per well and incubated for 24 h at 37°C in the presence or absence of 2 μ M staurosporine. XTT metabolizing activity was determined as reported (21).

Cytochrome C Translocation Analysis. HEK293 cells were grown in a 5% atmosphere in six-well plates and incubated for 24 h at 37°C in the presence or absence of 2 μ M staurosporine. Cells were harvested, pelleted by centrifugation at 1,000 $\times g$ for 10 min at 4°C, lysed in 1 ml of hypotonic buffer [Hepes-NaOH 20 mM, pH 7.5/10 mM NaCl/1.5 mM MgCl₂/1 mM EDTA/1 mM DTT/1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride containing 250 mM sucrose] and homogenized with a Dounce homogenizer (100 strokes/300 rpm). Homogenates were then centrifuged at 750 $\times g$ for 10 min at 4°C for recovery of the nuclear fraction followed by centrifugation at 10,000 $\times g$ for 15 min to obtain the mitochondrial fraction. The mitochondrial pellets were resuspended in 25–50 μ l of the hypotonic buffer and the supernatants further centrifuged at 100,000 $\times g$ for 1 h at 4°C to obtain the cytosolic fractions. Both fractions (cytosolic and mitochondrial) were then submitted to Western blot analysis by means of Tris-Tricine gels as described (25). In brief, 25 μ g of proteins was separated on 16.5 Tris-Tricine gels, immobilized on nitrocellulose sheets, and probed with an anticytochrome C (rabbit polyclonal IgG, H104, Santa Cruz Biotechnology) antibody. Immunological complexes were revealed with an anti-rabbit peroxidase (Immunotech, Luminy, France) and electrochemiluminescence.

Flow Cytometry Analysis. HEK293 cells were grown in six-well plates and incubated for 24 h at 37°C in the presence or absence of 2 μ M staurosporine. Cells were harvested, pelleted by centrifugation at 1,000 $\times g$ for 10 min at 4°C, gently resuspended in 500 μ l of 0.1% sodium citrate buffer containing 50 μ g/ml of propidium iodide (PI), and incubated overnight under agitation. The PI fluorescence of individual nuclei was measured by using a FACScan flow cytometer (program CELLQUEST, Becton Dickinson). Red fluorescence due to PI staining of DNA was expressed on a logarithmic scale simultaneously to the forward scatter of the particles. Fifty thousand events were counted on the scatter gate. All measure-

ments were performed under identical conditions. This technique allows the discrimination of populations of apoptotic nuclei from debris and nonviable cells and also from diploid nuclei that show higher fluorescence staining. The number of apoptotic nuclei is expressed as a percentage of the total number of events.

Western Blot Analyses. Equal amounts of protein (50 μ g) were separated on 12 and 8% SDS/PAGE gels for the detection of PS1/PS2/p53/Bax/ β -tubulin and Bcl-2, respectively, and wet transferred to Hybond-C (Amersham Pharmacia Life Science) membranes. After transfer, membranes were blocked with nonfat milk and incubated overnight with the following primary antibodies: Ab444 and Ab333 [rabbit polyclonals anti-N-terminal fragment-PS1 (NTF-PS1) and anti-C-terminal fragment-PS2 (CTF-PS2) (donated by W. Araki and T. Tabira), anti-p53 (mouse monoclonal, Pab1801) and anti-Bcl-2 (mouse monoclonal IgG, clone C-2) were from Santa Cruz Biotechnology, and anti-Bax (rabbit polyclonal IgG from Upstate Biotechnology, Lake Placid, NY)]. Anti- β -tubulin was from Sigma. Immunological complexes were revealed either with an anti-rabbit peroxidase (Immunotech) or anti-mouse peroxidase (Amersham Pharmacia Life Science) antibodies, depending on the host used for obtaining the primary antibodies described above, followed by electrochemiluminescence (Amersham Pharmacia Biotech). All protein concentrations were determined by the BioRad procedure as described (26).

Statistical Analysis. Statistical analysis was performed with PRISM software (GraphPad Software, San Diego) by using the Newman Keuls multiple comparison test for one-way ANOVA.

Results

We previously set up and characterized stably transfected HEK293 cells overexpressing wt PS2 or its N141I-PS2 mutated counterpart (19). The two clones examined in the present study were chosen on the basis of their very similar expression of PS2-related holoproteins and C-terminal maturation fragment (Fig. 1A). Trypan blue exclusion experiments indicate clearly that both wt and mutated PS2 drastically lower cell viability either in basal and staurosporine-stimulated conditions when compared with mock-transfected cells (Fig. 1F). This was confirmed by XTT assay showing that, as expected, staurosporine affects mock-transfected cells viability, but that this effect is statistically significantly amplified in wt- and N141I-PS2-expressing cells (Fig. 1G). Nucleus incorporation of PI measured by FACS analysis indicated that mock-transfected cell nuclei (Fig. 1H Top) were poorly labeled in basal conditions (Fig. 1H Top Left), but that staurosporine increases PI incorporation (Fig. 1H Top Right). Interestingly, the wt (Fig. 1H Bottom) and mutated (Fig. 1H Middle) PS2-transfected cell lines display higher PI incorporation in basal conditions and show drastically exacerbated staurosporine-induced PI label. Altogether, these data indicate that wt and N141I-PS2 expressions lower HEK293 cell viability and exacerbate the responsiveness of these cells to staurosporine.

As staurosporine is an apoptotic effector classically linked to mitochondrial disturbance and later to caspase activation, we first examined the influence of PS2 expression on basal and staurosporine-stimulated caspase 3-like activity. Basal Ac-DEVD-al-sensitive caspase 3-like activity is higher in both PS2-expressing cells than in mock-transfected cells (Fig. 1B). Here again, staurosporine increases caspase activity in mock- and PS2-transfected cells but to a higher extent in the latter cell systems (Fig. 1B). This was corroborated by the observation that staurosporine-sensitive pro-caspase 3-like immunoreactivity appeared lower in PS2-expressing cells than in mock-transfected HEK293 cells (data not shown), in agreement with higher active caspase-3-like activity in the former cell systems.

Fig. 1. Apoptotic phenotypes of wt and N141I-PS2-expressing HEK293 cells. (A) Western blot analysis (see *Materials and Methods*) of PS2-like immunoreactivities in Mock-transfected HEK293 cells (Mock) and cells overexpressing wt (Wt) or N141I-(Mut) PS2. Arrows indicate either the migration position of PS2 holoprotein or its C-terminal catabolite (CPS2). Note that the smear of holoprotein immunoreactivity corresponds to the previously characterized polyubiquitinated forms of PS2 (19). (B) Basal and staurosporine-induced (STS, 2 μ M, 24 h) caspase 3-like activity (see *Materials and Methods*) in the indicated cell lines. Bars represent the Ac-DEVD-al-sensitive caspase 3-like activity and are the means $\pm$ SEM of six determinations carried out in duplicates. *, $P < 0.05$; **, $P < 0.01$ (versus staurosporine-treated Mock-transfected cells). Bax (C), Bcl-2 (D), and tubulin immunoreactivities (see *Materials and Methods*) in the indicated cell lines. Cytochrome C-like immunoreactivity (E) in cytosolic and mitochondrial fractions (see details in *Materials and Methods*). Bars represent the ratio of cytochrome C content in cytosol versus mitochondrial fractions. (F) Trypan blue exclusion measured (see *Materials and Methods*) on the indicated cell lines in the absence (black bars) or presence (white bars) of staurosporine. Trypan blue positive (nonviable) cells are expressed as percent of total cells. Bars are the means of six determinations $\pm$ SEM. **, $P < 0.01$; ***, $P < 0.001$ (versus Mock-transfected cells). (G) Cell viability measurements by XTT (see *Materials and Methods*). Bars represent residual cell viability after staurosporine treatment expressed as the percent (taken as 100) of cell viability in control conditions and are the means $\pm$ SEM of 10 determinations. **, $P < 0.05$; ***, $P < 0.01$. (H) Representative illustration of PI incorporation measured in the indicated cell lines in control and staurosporine-stimulated conditions (see F) by FACS analysis (similar data were obtained in four independent experiments). The number of apoptotic nuclei is expressed as a percentage of the total number of events (see *Materials and Methods* for details).

Fig. 2. p53-dependent PS2-induced caspase activation in HEK293 and TSM1 cells. (A, C) p53 and tubulin immunoreactivities (see *Materials and Methods*) in PS2-expressing HEK293 transfectants (A) or in TSM1 neurons (C) expressing empty vector (TSM1/Mock) or p53-antisense cDNA (TSM1/ASp53). (B) Effect of pifithrin- α . The indicated HEK293 transfectants were treated for 24 h with 2 μ M staurosporine in the absence (black bars, control) or presence (white bars) of 10 μ M pifithrin- α (PFT). Bars represent the means $\pm$ SEM of six determinations carried out in duplicate. (D) Staurosporine-stimulated (1 μ M, 2 h) caspase activity in TSM1/Mock and TSM1/ASp53 neurons transiently transfected with pcDNA3 empty or bearing wt or mutated PS2.

It is interesting to note that the extent of caspase activation is virtually identical in wt and mutated PS2-expressing cells (Figs. 1B and 2B). This could have been because of high expression levels of the proteins, leading to a “saturation” of the caspase machinery, thereby masking a putative difference that would have been linked to the pathogenic mutation. To examine such a possibility, we transiently transfected various amounts of cDNA (ranging from 0.1 to 3 μ g). At low cDNA doses, although PS2-like immunoreactivities were not different from that observed in mock-transfected cells, we did observe an increase of caspase activity, the extent of which was identical for wt and N141I-PS2-expressing cells (not shown). This indicates that similar caspase responses are triggered by both proteins, irrespective of their rate of expression, as we also observed in stably transfected cells.

To also rule out a possible cell-specificity problem in the paradigm examined, we transiently transfected TSM1 neurons with wt and mutated PS2cDNA. Our data indicate that both PS2-expressing cells clearly display a higher staurosporine-stimulated caspase 3-like activity than mock-transfected neurons (Fig. 2D, black bars), as was shown for HEK293-transfected cells (Fig. 1B).

The possibility that wt- and N141I-PS2-stimulated apoptotic response could also trigger modifications of the expressions of protooncogenes or could trigger cytochrome C translocation into the cytosol was examined. wt- and N141I-PS2-transfected cells display a higher expression of the apoptotic effector Bax (Fig. 1C) and lower Bcl-2-like immunoreactivity (Fig. 1D) when compared with mock-transfected cells. In apoptotic conditions, it has been hypothesized that Bax multimeric complexes are incorporated into the mitochondrial membrane, thereby creating pores by which cytochrome C could be released into the cytosol (27). Interestingly, an increased ratio of cytosolic versus mitochondrial cytochrome C-like immunoreactivity was observed in both PS2-transfected cell lines (Fig. 1E), reflecting an important translocation of this mitochondrial protein into the cytosol.

It was reported that Bax gene deficiency attenuates p53-dependent apoptosis (28, 29). This was in agreement with the observation that p53 activates Bax at the transcriptional level (30, 31). We therefore assessed whether the observed increase in Bax immunoreactivity observed in wt- and mutated PS2-expressing cells could be associated with an increased p53 expression. Indeed, Fig. 2A shows that p53-like immunoreactivity was drastically enhanced in cells expressing wt PS2 and to a lesser extent in those harboring mutated PS2.

Of most interest was the recent description of a chemical inhibitor of p53, namely pifithrin- α , which blocks p53 transcriptional activation and subsequent apoptosis (32). We took advantage of this pharmacological tool to delineate the contribution of p53 in the PS2-mediated apoptotic responses. Interestingly, pifithrin- α reduces the wt- and N141I-PS2-stimulated caspase activity in transfected cells by about 70 and 30%, respectively (Fig. 2B), and reverses the increase in Bax immunoreactivity (not shown). To further support a p53-mediated PS2-dependent phenotype, we took advantage of TSM1 neurons stably transfected with p53 antisense cDNA. As shown in Fig. 2C, transfected cells exhibit a lower p53-like immunoreactivity than mock-transfected neurons. Clearly, these cells also exhibit a reduced staurosporine-stimulated caspase response than mock-transfected cells (Fig. 2D).

We examined whether the PS2-mediated increase in the transcriptional activity of p53 correlated with the observed enhanced p53 immunoreactivity. By means of a p53 reporter gene construct (23), we demonstrated that PS2-expressing cells display drastically higher p53 transcriptional activity (Fig. 3A). This was corroborated by a concomitant increase (Fig. 3B) in the promoter activity of p21^{waf1}, a well characterized downstream effector gene of p53 (24).

To avoid the possibility that most of the observed effects were related to the fact that PS2 was overexpressed, we analyzed the influence of the deletion of endogenous PS2 in mice fibroblasts in which its gene had been knocked out. Our data clearly show that the p53-transcriptional activation was higher in parent fibroblasts than in fibroblasts devoid of PS2 (Fig. 3C). The transcriptional activity of p53 is fully blocked by pifithrin- α in both fibroblast cell lines (Fig. 3C).

It has been reported that p53 down-regulates PS1 expression (14). As wt- and mutated-PS2 increase p53 immunoreactivity, we examined whether the overexpression of PS2 could lead to a p53-dependent down-regulation of endogenous PS1-like immunoreactivity. Indeed, we showed that cells overexpressing wt and mutated PS2 strongly lower endogenous PS1 expression (Fig. 4A and B), revealing a crosstalk between PS2 and PS1 in human cells. Combined with the observed increase in p53 expression, our data suggest that PS2 could down-regulate PS1 expression likely through the increase of p53. If p53 displays an opposite effect on endogenous PS1 and PS2 expressions, one should expect to observe a different effect of pifithrin- α on the expression of both endogenous proteins. Indeed, we showed that pifithrin- α increases endogenous PS1 immunoreactivity as was expected but also reduces the endogenous expression of PS2 (Fig. 4C and D).

Discussion

Apoptosis is a normal process that occurs at various stages of cell life to maintain its homeostasis and controlled development. This programmed cell death involves a cascade of events which, when affected, may trigger major pathologies. Defect in apoptosis could lead to tumorigenicity and cancers whereas, conversely, neurodegenerative diseases often characterized by neuronal loss are usually associated with an exacerbated cell death.

The potentiation of apoptosis can be due to genetic factors. Familial early onset forms of Alzheimer's disease (FAD) are due to autosomal dominant mutations borne by three main proteins, namely the β -amyloid precursor protein (β APP) and PS1 and PS2

Fig. 3. p53 transcriptional activity in wt PS2-expressing HEK293 cells and in PS2^{-/-} fibroblasts. (A, C) p53 transcriptional activity measured with a p53 reporter gene construct (pG13-luciferase) in wt PS2-expressing HEK293 cells (A) or in PS2^{-/-} fibroblasts (C). (B) p53 transcriptional activity measured in wt PS2-HEK293 cells with a construct coding for the p21^{waf-1} promoter-luciferase as described in *Materials and Methods*. All data have been normalized for transfection efficiencies assessed by cotransfection experiments with a β -galactosidase expression vector.

(for review, see ref. 3). It is striking that all but one (25) of the mutations responsible for FAD are associated with increased amounts of β -amyloid peptides ($A\beta$), the β APP catabolites that mainly compose the senile plaques that invade the cortical and subcortical areas of affected patient brains. Although it is still under discussion, a network of biochemical evidences led to the suggestion that $A\beta$ could exacerbate the apoptotic responses of various cell

Fig. 4. Down-regulation of endogenous PS1 expression in wt- and mutated N141I-PS2-expressing HEK293 cells: effect of pifithrin- α . (A) Endogenous PS1-like and tubulin immunoreactivities (see *Materials and Methods*) in the indicated cell lines. (B) Densitometric analyses of the N-terminal PS1 fragment (NTF-PS1) are the means $\pm$ SEM of four determinations. Note that, as described (49), endogenous presenilin holoproteins are barely detectable as the protein is rapidly fully processed. (C) Effect of pifithrin- α on endogenous PS1- and PS2-related immunoreactivities. Cells (as in A) were treated for 24 h in the absence (-) or presence (+) of 10 μ M pifithrin- α (PFT), then endogenous PS1- and PS2-related immunoreactivities were monitored by Western blot with Ab444 and Ab333 (see *Materials and Methods*). Bars in D correspond to the densitometric analysis of the N-terminal PS1 (two left bars) and C-terminal PS2 (two right bars) fragments obtained in the absence (black bars) or presence (white bars) of pifithrin- α and are the means $\pm$ SEM of four determinations.

lines (8–10). That this apoptotic response could be modulated by β APP and presenilins was also supported by several reports indicating that both β APP (33–37) and presenilins (38–41) are cellular targets of caspases, a family of cysteine proteases playing a key role in the cascade of events leading to cell death (42, 43).

We show here that PS2 increases both basal and staurosporine-stimulated apoptotic response in HEK293 cells as well as in TSM1 murine neurons and that this phenotype was exacerbated by the N141I missense mutation responsible for FAD. First, PS2 and its mutated counterpart drastically lower cell viability as measured by trypan blue exclusion and XTT metabolism. PI nucleus incorporation and increased caspase 3-like activity indicated that the decrease of cell viability was likely due to enhanced caspase-mediated cell death. Accordingly, wt and mutated PS2 increase the expression of the proapoptotic oncogene Bax and reduce that of Bcl-2, the antiapoptotic effector. These data corroborate and complete previous works suggesting that PS2 was apparently required for apoptotic response in various cell lines and that this phenotype was potentiated by FAD mutations (16, 18, 44).

Our study clearly demonstrates that wt- and N141I-PS2-mediated caspase 3 activation is p53-dependent. The most straightforward arguments were that, (i) PS2 expression drastically enhanced both expression and transcriptional activity of p53; (ii) PS2 also enhanced the promoter activity of p21^{waf-1}, a p53 downstream effector gene; (iii) p53 antisense strongly reduced the PS2-induced caspase activation in neuronal cells; (iv) p53 transcriptional activity is markedly reduced in mice fibroblasts devoid of PS2; (v) finally, pifithrin- α , a chemical agent (that we confirmed to be a strong inhibitor of p53 transcriptional activity), antagonizes PS2-induced caspase-3 activation. The above network of evidence implicating p53, Bax, and caspase 3 activity was in good agreement with a study by Cregan *et al.*

demonstrating that p53-induced apoptosis was directly linked with Bax-dependent caspase-3 activation in neurons (45).

It should be noted that the effect of pifithrin- α on caspase activation (Fig. 2) and on the cleavage of poly(ADP ribose) polymerase (data not shown) was stronger on wt- than on N141I-PS2-expressing cells. This could be explained by the possibility that all of the wt PS2-mediated apoptotic response would be p53-dependent, whereas the phenotype elicited by N141I-PS2 would include a p53-independent component. This hypothesis is corroborated by the stronger enhancement of p53-like immunoreactivity triggered by wt PS2 than by its mutated counterpart. Whether this difference accounts for the pathogenic phenotype linked to the N141I mutation remains speculative.

It is noteworthy that previous studies on apoptotic responses triggered by β APP and PS1 (see above), and our present work on PS2, all led to the demonstration of a link between these distinct proteins and p53. Thus, wt β APP was reported to confer resistance to p53-induced apoptosis in rat neuroblastoma cells, whereas FAD-linked β APP did not (46). Roperch *et al.* also demonstrated that PS1, which is antiapoptotic or could confer resistance to apoptotic stimuli, was down-regulated during p53-induced apoptosis (14).

Our data indicate that PS2 overexpression was associated with increased p53 immunoreactivity. This crosstalk between p53 and PS2 was reinforced by the observation that pifithrin- α , which inhibits p53, lowers endogenous PS2-like immunoreactivity,

whereas conversely the inhibitor enhance endogenous PS1 expression. In line with this observation was the drastic lowering of endogenous PS1 expression when wt and mutated PS2 were overexpressed. Altogether, our study demonstrates, to our knowledge for the first time, a crosstalk between PS2 and p53 and suggests that a consequence of such a functional link is the down-regulation of PS1 expression. That PS2 and PS1 behave differently and sometimes in an opposite manner has been already suggested (for review, see ref. 4).

It should be noted that p53 expression has been reported to be higher in the temporal cortex of Alzheimer's disease brains (47). Also interesting was a recent study showing that pifithrin- α could protect neurons from cell death induced by amyloid β -peptide (48). Whether p53 could be seen as a therapeutic target of a strategy aimed at slowing down neuronal cell death in Alzheimer's disease still remains a matter of speculation.

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