Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 7547–7552, June 1999
Neurobiology

Wild-type but not Alzheimer-mutant amyloid precursor protein confers resistance against p53-mediated apoptosis

XIAO XU*†, DASENG YANG*, TONY WYSS-CORAY*†, JIM YAN*, LI GAN*†‡, YI SUN§, AND LENNART MUCKE*†‡¶

*Gladstone Institute of Neurological Disease, †Department of Neurology, and ‡Neuroscience Program, University of California, San Francisco, CA 94141-9100; and §Department of Molecular Biology, Parke–Davis Pharmaceutical Research, Division of Warner–Lambert Company, Ann Arbor, MI 48105

Edited by Eugene Roberts, Beckman Research Institute of the City of Hope, Duarte, CA, and approved April 8, 1999 (received for review December 2, 1998)

ABSTRACT **Amyloid precursor proteins (APPs) are expressed in multiple organs and cell types in diverse species. Their conservation across species and high abundance in brain and the association of various APP missense mutations with autosomal dominant forms of familial Alzheimer's disease (FAD) suggest important roles for APP in the central nervous system. However, the basic functions of APP in the central nervous system remain largely unknown. To assess potential effects of APP on neuronal death and survival, we transfected APP-deficient rat neuroblastoma cells (B103) with DNA constructs encoding wild-type or FAD-mutant human APP. Wild-type, but not FAD-mutant, APP effectively protected cells against apoptosis induced by ultraviolet irradiation, staurosporine, or p53. Wild-type APP also strongly inhibited p53 DNA-binding activity and p53-mediated gene transactivation, whereas FAD-mutant APP did not. We conclude that APP protects neuronal cells against apoptosis by controlling p53 activation at the post-translational level. Disruption of this function by mutations or alterations in APP processing could enhance neuronal vulnerability to secondary insults and contribute to neuronal degeneration.**

Amyloid precursor proteins (APPs) are type I integral membrane proteins that resemble glycosylated membrane receptors (1). In species as diverse as *Drosophila* and humans, APP or closely related homologues are expressed in multiple organs and cell types (2–4). Different APP isoforms are derived from a single gene by alternative splicing (5, 6). The most prevalent forms of APP are 695- to 770-amino acid glycoproteins with a large extracellular region, a single hydrophobic transmembrane domain, and a short cytoplasmic segment (1). APP can be processed proteolytically into various fragments. Cleavage of APP by presumed α-secretase(s) results in the secretion of a 100-kDa N-terminal ectodomain (α-s-APP) (7). An alternative processing pathway, involving presumed β- and γ-secretases, gives rise to a shorter ectodomain (β-s-APP) and to the 39- to 43-amino acid Aβ peptide, which appears to play a central role in Alzheimer's disease (AD) (8, 9). Various APP mutations have been linked to autosomal dominant forms of familial AD (FAD), and many studies have shown that diverse FAD mutations increase production of the Aβ42 peptide (refs. 10–12, and refs. 8 and 13 for review). However, few studies have examined whether FAD mutations impair or counteract specific APP functions (14, 15). In this study we compared the ability of wild-type and FAD-mutant APPs to protect neuronal cells against apoptosis.

Apoptosis, a sequence of regulated cellular events culminating in cell death, is a fundamental biological process that fulfills many important functions in the developing and adult organism (16, 17). However, the aberrant induction of apoptosis can have dire consequences, particularly in largely irreplaceable cells, such as neurons. Activation of proapoptotic pathways appears to contribute to AD and other important neurological diseases (16, 18, 19). Because inhibitors of apoptosis can prevent cell death even in the continued presence of the apoptosis-inducing trigger, these pathways are attractive targets for therapeutic intervention (20).

The p53 protein is a stress-activated transcription factor that functions as a critical molecular switch between life and death (21, 22). Expression of wild-type p53 in neurons increases after brain injury; loss of this factor prevents, whereas overexpression induces, neuronal apoptosis (23–28). Therefore, molecules that block the proapoptotic activation of p53 could regulate cell survival and may be particularly important for protecting neurons against apoptosis-inducing pathogens.

Here we demonstrate that wild-type human APP effectively prevents neuronal apoptosis, and we provide evidence that it does so by inhibiting p53 activation at the posttranslational level. In addition, we show that FAD mutations interfere with this function.

MATERIALS AND METHODS

Cells and Treatment. B103 cells (29) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies) containing 10% fetal bovine serum and 5% horse serum at 37°C in a 5% CO_2/95% air atmosphere. After transfection in the undifferentiated state, cells were washed twice with serum-free DMEM and cultured in Neurobasal medium containing N-2 supplement (Life Technologies) for differentiation. After 48 h, neurites and cell bodies of differentiated cells were immunolabeled with antibodies against the neuronal marker microtubule-associated protein 2 (data not shown). Differentiated B103 cells were exposed to UV-C (UV) (20 J/m^2) or staurosporine (ST) (Sigma; diluted in differentiation medium to a final concentration of 5 μM) or infected with the recombinant adenoviruses p53-rAd or LacZ-rAd [multiplicity of infection (moi) = 5]. DNA fragmentation increased significantly after infection with p53-rAd (see *Results*) with further increases seen at higher infectious doses (data not shown).

Human APP (hAPP) Constructs and Transfections. cDNAs encoding wild-type or FAD-mutant hAPP695 (hAPP695wt or hAPP695mut, respectively) were isolated by *Hin*dIII digestion from NSE-hAPP constructs described previously (30) and subcloned into a cytomegalovirus (CMV) promoter/

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PNAS is available online at www.pnas.org.

This paper was submitted directly (Track II) to the *Proceedings* office. Abbreviations: APP, amyloid precursor protein; α-s-APP, α-secretase-cleaved APP; hAPP, human APP; AD, Alzheimer's disease; FAD, familial AD; ST, staurosporine; moi, multiplicity of infection; TUNEL, terminal deoxynucleotidyltransferase-mediated UTP end labeling; EMSA, electrophoretic mobility-shift assay.

¶To whom reprint requests should be addressed at: Gladstone Institute of Neurological Disease, P.O. Box 419100, San Francisco, CA 94141-9100. e-mail: Lmucke@gladstone.ucsf.edu.

enhancer–driven expression vector (31). The resulting constructs were transfected transiently into B103 cells by using Lipofectamine (Life Technologies). Cotransfection with pGreen Lantern (Life Technologies) was used in parallel experiments to monitor transfection efficiencies. Select findings in transiently transfected B103 cells were confirmed in B103 cells stably transfected with the above hAPP cDNA constructs, with a previously characterized hAPP minigene construct (6, 32) encoding the FAD-associated V642F substitution (hAPP695 numbering) (33), or with a similar hAPP minigene encoding wild-type hAPP. Cell lines stably expressing these constructs were selected by cotransfection with pSV2neo (CLONTECH) and incubation in G418-containing medium, essentially as described (34). Expression levels of hAPP in stably transfected B103 lines were ascertained by Western blot analysis.

Apoptosis Assessments. Three assays were used to assess apoptosis. (*i*) Nuclear fragmentation was detected by *in situ* labeling with the terminal deoxynucleotidyltransferase-mediated UTP end labeling (TUNEL) assay kit (Boehringer Biotech). Briefly, cells in 8-well chamberslides (Fisher) were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and incubated with fluorescently labeled dUTP and terminal deoxynucleotidyltransferase at 37°C for 1 h. After three rinses with PBS, cells (≈500 per well) were inspected with an epifluorescence microscope to determine the percentage of apoptotic cells. (*ii*) Genomic DNA was isolated from cell cultures and separated by electrophoresis on 3% agarose gels. DNA laddering typical of apoptosis was visualized by ethidium bromide staining and UV. (*iii*) DNA fragments released into the cytoplasm during apoptosis were measured with the ELISA apoptosis kit (Boehringer). Briefly, 1×10^4 B103 cells were seeded into each well of a 96-well plate and incubated overnight. The medium was then replaced with differentiation medium containing BrdUrd, and cells were incubated for 48 h. DNA fragmentation induced by subsequent exposure to UV, ST, or p53-rAd was quantitated in cell lysates with a peroxidase-conjugated anti-BrdUrd antibody.

Immunostaining and Microscopy. Cells were fixed in methanol at −20°C for 5 min and then with acetone at 4°C for 2 min and washed in PBS. Nonspecific immunostaining was blocked by incubation for 30 min at 37°C in PBS with 3% normal rabbit serum. hAPP expression was assessed 72 h after transient transfection with hAPP695 cDNA constructs and 48 h after exposure to differentiation medium by incubating cells with the monoclonal antibody (mAb) 22C11 (Boehringer; final concentration 0.5 μg/ml) at 4°C overnight. For p53 immunostaining, cells were incubated with mAb p53Ab1 (Oncogene; final concentration 0.5 μg/ml) at 4°C overnight. After three washes with PBS, cells were incubated with fluorescein isothiocyanate–labeled rabbit anti-mouse IgG for 1 h at room temperature. After three washes with PBS, immunolabeled cells were examined by laser scanning confocal microscopy (MRC 1024, Bio-Rad).

Western Blot Analysis. B103 cell lysates (lysis buffer: 20 mM Tris·HCl, pH 7.8/150 mM NaCl/0.5% SDS/0.5% NP40/1 μM DTT/1 μM EDTA/1 μM PMSF) or cell culture media concentrated with a PD-11 column (Pharmacia Biotech/Amersham Life Science) were subjected to SDS/polyacrylamide gel electrophoresis. Gels were blotted onto Immobilon membranes (Millipore). Immobilized proteins were then detected with mAbs against p53 (p53Ab1; Oncogene, 0.1 μg/ml final concentration), hAPP (8E5; Athena Neurosciences, 1:2000 in PBS), or APP (22C11; Boehringer, 0.1 μg/ml final concentration). Target proteins were revealed with the ECL (enhanced chemiluminescence) system (Amersham).

Electrophoretic Mobility-Shift Assay (EMSA). Nuclear proteins were extracted from cell cultures essentially as described previously (35). p53 nuclear binding activities were determined by EMSA using the p53 consensus binding site sequence (5′-AGACATGCCTAGACATGCCT-3′) as a probe. Protein–DNA binding reaction mixtures optimized for this probe contained 5 μg of total nuclear protein, 0.5 ng of the radioactive probe, 200 ng of the nonspecific competitor poly[d(I-C)], and 1 μg of p53Ab1, which stabilizes the p53 DNA-binding complexes. Mixtures were incubated for 30 min at room temperature in 40 μl of 10 mM Tris·HCl, pH 7.5/75 mM NaCl/10 mM EDTA/7% (vol/vol) glycerol/1 mM DTT. Free and protein-bound DNA probes were then resolved by polyacrylamide gel electrophoresis. Gels were dried and autoradiographed. For EMSA competition assays, the p53 DNA-binding activities were assessed in the presence of increasing molar excess of nonradioactive probe (see above). As a control, we used a nonradioactive probe (5′-AGA**G**AT**C**CCTA-GA**G**AT**C**CCT-3′) carrying point mutations (in boldface) that diminish its specific DNA-binding activity.

Luciferase Assay. A p53-responsive ($p53^+$luc) or a p53-unresponsive ($p53^-$luc) luciferase construct (36) was transiently cotransfected with hAPP695wt or hAPP695mut into B103 cells. After UV, ST treatment, or infection with p53-rAd, protein extracts were prepared from cells, and luciferase activity was measured with the luciferase assay system (Promega) and a luminometer.

RESULTS AND DISCUSSION

To assess the antiapoptotic capacity of hAPP, we transfected hAPP constructs into rat neuroblastoma B103 cells (29). Lacking expression of endogenous APP and APP-like proteins, this cell line allows comparison of wild-type and mutant hAPP without interference by rodent APP. Apart from their deficiency in endogenous APP, B103 cells share many typical neuronal properties with other commonly used APP-expressing neuronal cell lines, including outgrowth of neurites upon differentiation, synthesis of neurotransmitters, possession of neurotransmitter receptors, and electrical excitability of surface membranes (37).

Expression of Wild-Type Versus FAD-Mutant hAPP695. B103 cells were transfected with a wild-type hAPP695 cDNA construct (hAPP695wt) or a mutant hAPP695 cDNA construct (hAPP695mut) containing a valine-to-isoleucine substitution at amino acid 642 (V642I). This mutation (corresponding to V717I with respect to hAPP770 sequence), the first hAPP mutation implicated by genetic linkage analysis in the pathogenesis of early-onset FAD (38), has been identified in at least 16 different families (8). Similar expression levels of hAPP were detected by Western blot analysis in cell lysates and medium from B103 cultures transfected with hAPP695wt or hAPP695mut (Fig. 1*a*). Wild-type and mutant hAPP695 also showed a similar intracellular distribution in transfected B103 cells; APP immunoreactivity was detectable in soma and neurites but not in the nucleus (Fig. 1*b*), closely resembling the distribution of endogenous APP observed in untransfected primary neurons (39).

High-level expression of FAD-mutant hAPP695 has been reported to cause apoptosis in other cell lines (40). In contrast, hAPP695mut-transfected B103 cells showed no increase in apoptosis compared with mock-transfected and hAPP695wt-transfected B103 cultures (Fig. 1 *c–e*). That expression of V642I-mutant hAPP695 *per se* did not induce apoptosis of differentiated B103 cells in the absence of other apoptosis-inducing challenges is consistent with the fact that humans carrying FAD mutations live normally for decades before developing overt neurological disease (8, 41, 42).

Wild-Type, but Not FAD-Mutant, hAPP Inhibits Apoptosis. To compare the effect of wild-type and mutant hAPP695 on the susceptibility of B103 cells to apoptosis, we first challenged B103 cells with two well-established methods of apoptosis induction: UV and ST. The extent of apoptosis in differentiated B103 cells was measured by DNA laddering, TUNEL

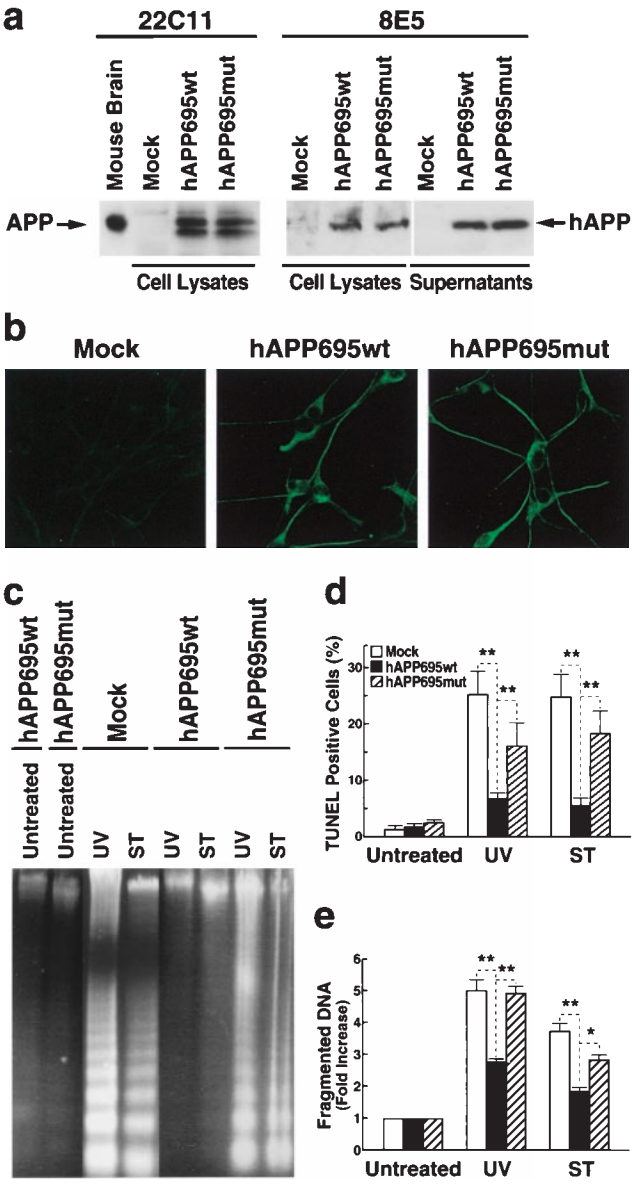

FIG. 1. Wild-type, but not FAD-mutant, hAPP695 protects APP-deficient B103 cells against apoptosis induced by UV or ST. (*a*) Western blot analysis of hAPP expression by B103 cells transfected with hAPP695wt, hAPP695mut, or plasmid containing the CMV promoter but no cDNA insert (Mock). Positive control: nontransgenic mouse brain homogenate. APP was detected with 22C11 (crossreacts with human and rodent APP) or 8E5 (recognizes human but not rodent APP). (*b*) Similar intracellular distribution of hAPP695wt and hAPP695mut after transfection of B103 (confocal images of 22C11 immunostaining; ×100). (*c–e*) Expression of hAPP695wt, but not hAPP695mut, significantly reduced DNA laddering on agarose gels (*c*), the proportion of condensed or fragmented nuclei labeled by TUNEL (*d*), and DNA fragmentation assessed by ELISA (*e*) after UV or ST treatment. Columns and error bars represent means and standard deviations of data obtained in three separate experiments. The ELISA results represent average fold increases over baseline levels in untreated cultures (arbitrarily defined as 1.0). *, $P < 0.05$; **, $P < 0.01$ by Tukey–Kramer posthoc test. Differences between mock-transfected and hAPP695mut-transfected cells were not statistically significant.

assay, and ELISA DNA fragmentation analysis. Six hours after UV or ST treatment, mock-transfected B103 cells showed significantly increased DNA fragmentation and nuclear segmentation (Fig. 1 *c–e*) consistent with apoptosis. Expression of hAPP695wt, but not of hAPP695mut, in transfected cells substantially inhibited UV- or ST-induced apoptosis (Fig. 1 *c–e*). The differential antiapoptotic capacity of wild-type and mutant hAPP695 was quantitated independently by counting TUNEL-positive nuclei and by measuring levels of fragmented DNA by ELISA. Both approaches yielded comparable results: expression of hAPP695wt decreased the number of cells showing fragmented or condensed TUNEL-positive nuclei by approximately 60% (Fig. 1*d*) and reduced the level of fragmented DNA by 50% (Fig. 1*e*). In contrast, expression of hAPP695mut did not significantly decrease either measure of apoptosis, although an inhibitory tendency was seen compared with mock-transfected B103 cells (Fig. 1 *d* and *e*). Thus, wild-type hAPP695 protects B103 cells against apoptosis induced by UV or ST, whereas V642I-mutant hAPP695 does not.

Limited Antiapoptotic Capacity of Secreted hAPP695. As indicated by Western blot analysis (Fig. 1*a*) and previous studies (29), B103 cells synthesize and secrete hAPP effectively despite their lack of endogenous APP. One might speculate that the antiapoptotic effect of wild-type hAPP is mediated by the large secreted N-terminal ectodomain of hAPP that results from cleavage of APP at the α-secretase site (α-s-APP) and protects neurons against excitotoxicity (30, 43). However, the V642I substitution, which significantly reduced the antiapoptotic capacity of hAPP, does not affect the production and secretion of α-s-APP (Fig. 1*a*) (44). Furthermore, untransfected B103 cells treated with conditioned medium from hAPP695wt-transfected B103 cells or with recombinant hAPP695 (rhAPP695) corresponding to α-s-hAPP (43) were significantly less protected against ST-induced apoptosis than hAPP695wt-transfected B103 cells and were not at all protected against apoptosis induced by UV (data not shown). Although minor contributions from α-s-hAPP cannot be excluded, these results suggest that the antiapoptotic effects in hAPP695wt-transfected cultures may depend primarily on the activity of full-length, membrane-anchored hAPP. Our observations in transfected B103 cells could explain why some populations of primary neurons from APP-deficient mice had diminished viability in cell culture that could not be improved by coculturing with APP-secreting primary astrocytes from wild-type mice (45).

Wild-Type, but Not FAD-Mutant, hAPP Inhibits p53 Activation and p53-Dependent Transcription. What mechanisms mediate the antiapoptotic function of hAPP? To address this question, we examined whether hAPP695 inhibits critical proapoptotic pathways. We focused on the tumor suppressor protein p53, a key transcription factor that targets genes involved in cell-cycle control and apoptosis (23, 46). In neuron-like cells, latent p53 is mainly in the cytoplasm (47). Although the precise mechanisms that convert latent into active p53 have not yet been defined, it is clear that activated p53 translocates into the nucleus, binds to DNA, and up-modulates the expression of diverse target genes (23, 48, 49). In B103 cells, UV or ST prominently increased p53 immunoreactivity in the nucleus, indicating nuclear translocation of p53 (Fig. 2*a*). Consistent with this nuclear translocation of p53, UV or ST also strongly increased specific nuclear p53 DNA-binding activity (Fig. 2*b*) and p53-mediated transactivation of a p53-responsive indicator gene (Fig. 2*c*). As in many other cell types (50, 51), activation of endogenous p53 by UV or ST in B103 cells was associated with apoptosis, as evidenced by TUNEL, DNA laddering, and cell loss (not shown).

Next, we compared p53 DNA-binding activity and p53-mediated gene transactivation in mock-, hAPP695wt-, and hAPP695mut-transfected B103 cells. Expression of hAPP695wt, but not of hAPP695mut, effectively inhibited p53 DNA-binding activity (Fig. 2*d*) and p53-mediated transactivation (Fig. 2*e*) after UV or ST treatment. This differential effect of wild-type and mutant hAPP695 on p53 activation is consistent with their differential effect on apoptosis (Fig. 1 *c–e*).

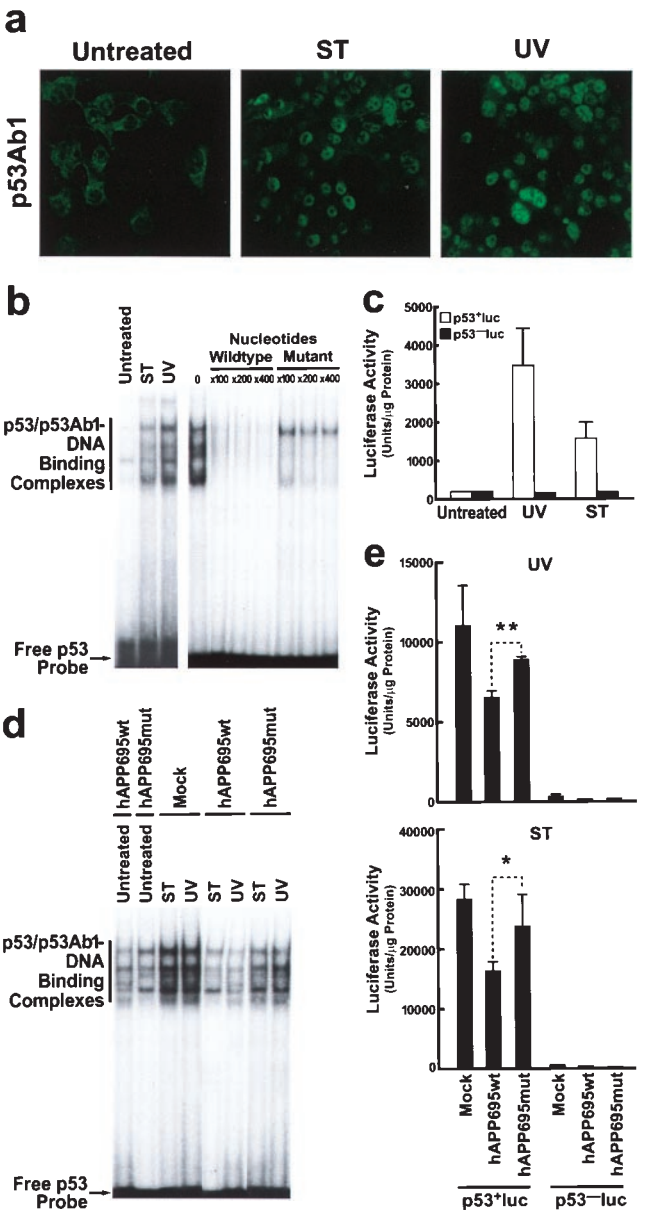

FIG. 2. Expression of wild-type hAPP695 in B103 cells inhibits p53 activation induced by UV or ST. (*a*) UV and ST induce nuclear translocation of p53 (confocal images of p53Ab1 immunostaining; ×100). (*b*) UV and ST increase p53 DNA-binding activity. Nuclear extracts were analyzed by EMSA with a ^{32}P-labeled p53 probe. The specificity of the p53/p53Ab1 DNA binding was confirmed in nuclear extracts from UV-treated untransfected B103 cells (*Right*) by competition assay using wild-type or mutant p53 oligonucleotides (36). (*c*) UV and ST increase luciferase activity in B103 cells transfected with a p53-responsive (p53$^+$luc) or a p53-unresponsive (p53$^-$luc) luciferase indicator construct. (*d* and *e*) Differential effects of wild-type and FAD-mutant hAPP695 on p53 DNA-binding activity and p53-responsive transactivation. (*d*) EMSA showing reduced levels of p53/p53Ab1 DNA-binding complexes in hAPP695wt-transfected B103 cells compared with mock- and hAPP695mut-transfected B103 cells. (*e*) Mock-, hAPP695wt-, and hAPP695mut-transfected B103 cells were cotransfected with p53$^+$luc or p53$^-$luc and treated with UV or ST. Luciferase activities in lysates of treated and untreated cells were assayed. Each column represents the mean of measurements obtained in six wells. Error bars indicate SD. *, $P < 0.05$; **, $P < 0.01$ by Tukey–Kramer posthoc test. Similar results were obtained in two additional experiments.

Differential Effects of Wild-Type Versus FAD-Mutant hAPP on Apoptosis and Indicator Gene Transactivation Induced Specifically by p53. Because UV and ST can also cause p53-independent apoptosis (52, 53), APP-mediated antiapoptotic effects and APP-dependent inhibition of p53 activation might be parallel events. To determine whether hAPP695 protects against p53-mediated apoptosis, we used a p53-encoding recombinant adenovirus (p53-rAd) that elicits apoptosis in primary neuronal cultures (24, 25). After differentiation, B103 cells were infected with an equal dose of p53-rAd or a control recombinant adenovirus encoding β-galactosidase (LacZ-rAd). p53-rAd infection increased p53 expression to similar levels in hAPP695wt-transfected and hAPP695mut-transfected B103 cells (Fig. 3*a*). hAPP695wt strongly protected B103 cells against p53-induced apoptosis, whereas hAPP695mut did not (Fig. 3*b*). These results clearly pinpoint p53 as a prime target for antiapoptotic hAPP functions; they do not exclude potential additional effects of hAPP on other transcription factors.

Wild-type and FAD-mutant forms of hAPP695 were also compared for their ability to inhibit p53-mediated gene transactivation. B103 cells cotransfected with p53$^+$luc and either hAPP695wt or hAPP695mut were infected with p53-rAd or LacZ-rAd. Consistent with the DNA fragmentation analysis (Fig. 3*b*), expression of hAPP695wt, but not of hAPP695mut,

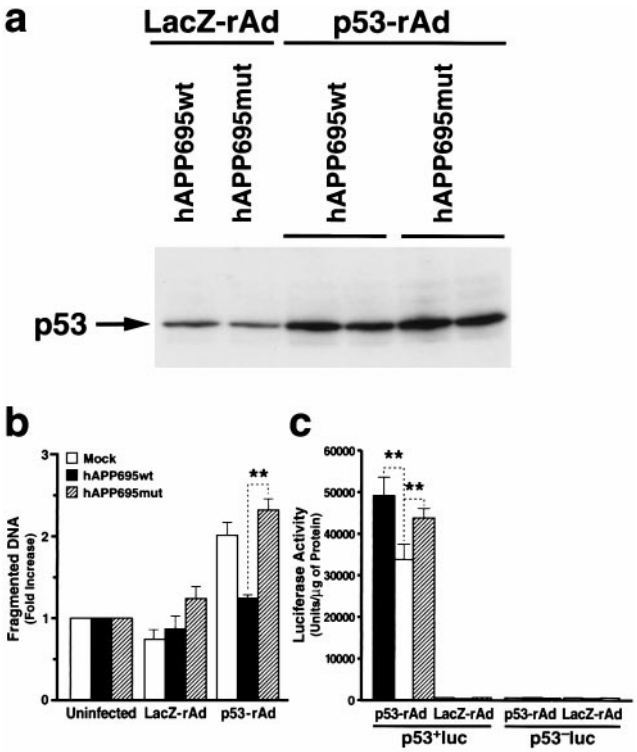

FIG. 3. Differential capacity of wild-type and FAD-mutant hAPP695 to inhibit apoptosis induced by p53. (*a*) p53 protein levels were measured by Western blotting with p53Ab1 in hAPP695wt- or hAPP695mut-transfected B103 cells infected with LacZ-rAd or p53-rAd (moi = 5). Similar levels of p53 protein expression were detected in B103 cells transfected with wild-type or FAD-mutant hAPP695. (*b*) Decreased capacity of FAD-mutant hAPP695 to inhibit p53-induced apoptosis. The extent of apoptosis was assessed by DNA fragmentation ELISA. Data represent average fold increases over baseline levels in uninfected cultures (arbitrarily defined as 1.0). Twelve culture wells were analyzed per condition in three independent experiments. (*c*) Decreased capacity of FAD-mutant hAPP695 to inhibit p53-dependent transactivation. Mock-, hAPP695wt, or hAPP695mut-transfected B103 cells were cotransfected with a p53-responsive (p53$^+$luc) or p53-unresponsive (p53$^-$luc) luciferase indicator construct. Luciferase activities in cell lysates were measured after infection with LacZ-rAd or p53-rAd. Each column represents the mean of measurements obtained in six wells. Similar results were obtained in two additional experiments. Error bars indicate SD. **, $P < 0.01$ by Tukey–Kramer posthoc test.

significantly inhibited p53-mediated transcriptional transactivation of the p53^{+}luc reporter gene (Fig. 3*c*).

Although overall expression levels of wild-type and FAD-mutant forms of hAPP695 were comparable in the above experiments, transient transfections do not allow reliable control of hAPP levels in individual cells. Therefore, we established two expression-matched sets of B103 cell lines stably transfected to express wild-type or mutant hAPP695 at high or low levels (Fig. 4*a*). At both levels of expression, only wild-type hAPP695 protected B103 cells against p53-mediated apoptosis (Fig. 4*b*). That wild-type and mutant hAPP695 differed in their antiapoptotic capacity over a range of APP expression levels indicates that this difference is robust and did not result from minor variations in APP expression that may occur in transiently transfected cultures. Another FAD-linked mutation, V642F (33), also significantly interfered with hAPP's ability to prevent p53-mediated apoptosis and gene transactivation when wild-type and V642F-mutant forms of hAPP were expressed at comparable levels in stably transfected B103 cell lines (not shown).

At what level, then, does APP regulate p53 activation? Both UV and ST activated p53 in B103 cells without altering overall p53 protein levels, and similar overall p53 protein levels were found in B103 cells expressing hAPP695wt or hAPP695mut (not shown), despite the differential effects these hAPPs had on p53 activation. These results strongly suggest that hAPP regulates p53 activity at the posttranslational level. However,

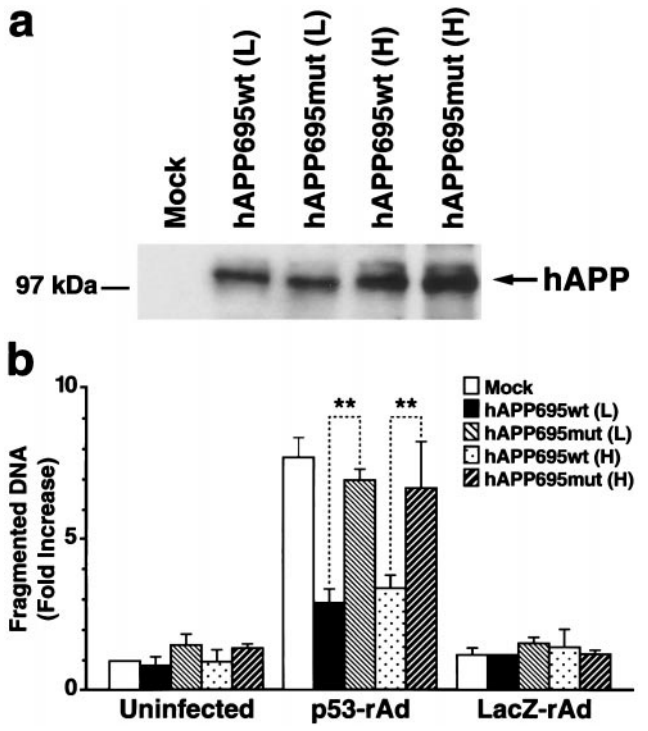

FIG. 4. Differential antiapoptotic capacity of wild-type and mutant hAPP695 revealed in stably transfected B103 cells. B103 cells were stably transfected with hAPP695wt or hAPP695 mut, and two pairs of B103 lines were selected by Western blot analysis with 8E5. (*a*) Representative Western blots showing high (H) or low (L) levels of hAPP expression (arrow) in stably transfected B103 lines (cell lysates). A B103 line stably transfected with the neomycin-resistance plasmid alone (Mock) was used as a negative control. (*b*) These stably transfected B103 lines were infected (moi = 5) with p53-rAd or LacZ-rAd, and apoptosis was assessed by DNA fragmentation ELISA. Data represent average fold increases over baseline levels in uninfected neomycin-resistant (Mock) B103 cells (arbitrarily defined as 1.0). Four culture wells were analyzed per condition in two independent experiments. Error bars indicate SD. **, $P < 0.01$ by Tukey–Kramer posthoc test.

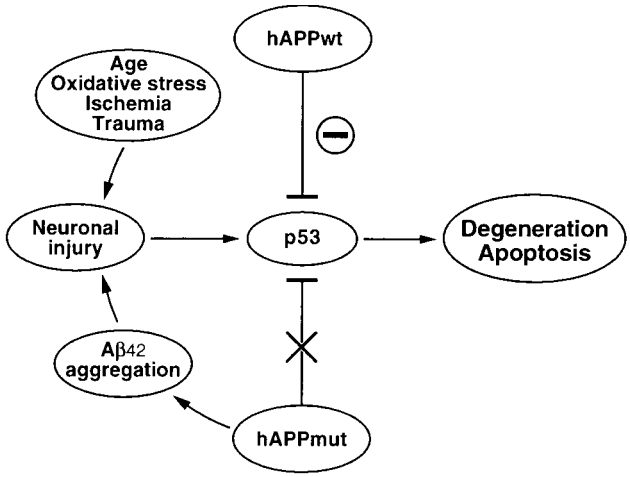

FIG. 5. Hypothetical model of hAPP effects in AD. In aging brain, neurons are exposed to increasing oxidative stress and other injurious factors that could trigger the p53 pathway and cause neuronal degeneration. Wild-type hAPP helps prevent p53 activation, whereas FAD-mutant hAPP or hAPP variants resulting from "molecular misreading" in sporadic AD (56, 57) may be deficient in or counteract this function. Increased production of Aβ42 in FAD (8, 54) and unknown factors in sporadic AD promote Aβ aggregation, resulting in a vicious pathogenetic cycle that could sensitize neurons to other insults and promote neurodegeneration. For clarity, the diagram focuses on hAPP and p53; additional pathways are likely to contribute to AD pathogenesis.

hAPP did not prevent nuclear translocation of p53 in B103 cells treated with UV or ST, and coimmunoprecipitation experiments have so far failed to reveal evidence for a direct interaction between hAPP and p53 (not shown). Further studies are necessary to determine whether APP-mediated signaling alters the phosphorylation, acetylation, or redox state of p53, all of which could potentially affect p53 activity (49).

Increasing evidence suggests that activation of p53 induces apoptosis in postmitotic neurons (24, 25) and that this process may contribute to AD and other neurodegenerative diseases of the adult central nervous system (23). Our study indicates that APP can protect neurons against apoptosis by blocking p53 activation. There are at least two potential mechanisms by which APP mutations could interfere with this function (Fig. 5). First, FAD mutations, including those assessed in the current study, increase the production of Aβ42 (8, 13, 54). In the absence of apoptosis-inducing treatments, we found no evidence for increased apoptosis or p53 activity in hAPP695 mut-transfected versus hAPP695wt-transfected B103 cells. However, it is possible that increased production of Aβ42 somehow primed the p53 pathway for subsequent activation and thereby counteracted antiapoptotic APP functions when hAPP695mut-transfected cells were challenged with UV, ST, or p53 overexpression. Second, because gain- and loss-of-function effects are not mutually exclusive, it is also possible that FAD mutations directly impair the antiapoptotic capacity of APP. Interestingly, down-regulation of presenilin 1 by antisense treatment has recently been shown to enhance p53-mediated apoptosis (55). Thus, the p53 pathway may be an important functional convergence point for different proteins affected by genetic alterations in AD.

We thank M. Masliah for the B103 cell line, X. Fan for p53-rAd, M. Mattson for recombinant hAPP695, B. Conklin, R. Messing, and M. Sofroniew for reviewing the manuscript, J. Carroll and S. Gonzales for help with graphics, G. Howard and S. Ordway for editorial assistance, and G. Costa for manuscript preparation. This research was supported by the U.S. Public Health Service (Grant AG11385 to L.M.) and the Alzheimer's Disease Program of the State of California (Grant

98-15725 to X.X.). L.G. was supported, in part, by Neuroscience Postdoctoral Training Grant NS07067.

1. Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. & Müller-Hill, B. (1987) *Nature (London)* **325,** 733–736.
2. Torroja, L., Luo, L. Q. & White, K. (1996) *J. Neurosci.* **16,** 4638–4650.
3. Neve, R. L., Finch, E. A. & Dawes, L. R. (1988) *Neuron* **1,** 669–677.
4. Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A. P., St. George-Hyslop, P., Van Keuren, M. L., Patterson, D., Pagan, S., Kurnit, D. M. & Neve, R. L. (1987) *Science* **235,** 880–884.
5. Sandbrink, R., Masters, C. L. & Beyreuther, K. (1994) *J. Biol. Chem.* **269,** 1510–1517.
6. Rockenstein, E. M., McConlogue, L., Tan, H., Gordon, M., Power, M., Masliah, E. & Mucke, L. (1995) *J. Biol. Chem.* **270,** 28257–28267.
7. Checler, F. (1995) *J. Neurochem.* **65,** 1431–1444.
8. Lendon, C. L., Ashall, F. & Goate, A. M. (1997) *J. Am. Med. Assoc.* **277,** 825–831.
9. Selkoe, D. J. (1994) *J. Neuropathol. Exp. Neurol.* **53,** 438–447.
10. Cai, X.-D., Golde, T. E. & Younkin, S. G. (1993) *Science* **259,** 514–516.
11. Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A. Y., Seubert, P., Vigo-Pelfrey, C., Lieberburg, I. & Selkoe, D. J. (1992) *Nature (London)* **360,** 672–674.
12. Suzuki, N., Cheung, T. T., Cai, X.-D., Odaka, A., Otvos, L., Jr., Eckman, C., Golde, T. E. & Younkin, S. G. (1994) *Science* **264,** 1336–1340.
13. Younkin, S. G. (1995) *Ann. Neurol.* **37,** 287–288.
14. Furukawa, K., Sopher, B. L., Rydel, R. E., Begley, J. G., Pham, D. G., Martin, G. M., Fox, M. & Mattson, M. P. (1996) *J. Neurochem.* **67,** 1882–1896.
15. Li, H. L., Roch, J. M., Sundsmo, M., Otero, D., Sisodia, S., Thomas, R. & Saitoh, T. (1997) *J. Neurobiol.* **32,** 469–480.
16. Thompson, C. B. (1995) *Science* **267,** 1456–1462.
17. White, E. (1996) *Genes Dev.* **10,** 1–15.
18. Cotman, C. W. & Su, J. H. (1996) *Brain Pathol.* **6,** 493–506.
19. Shi, B., De Girolami, U., He, J., Wang, S., Lorenzo, A., Busciglio, J. & Gabuzda, D. (1996) *J. Clin. Invest.* **98,** 1979–1990.
20. Kroemer, G. (1997) *Nat. Med.* **3,** 614–620.
21. Wyllie, A. (1997) *Nature (London)* **389,** 237–238.
22. Béroud, C. & Soussi, T. (1998) *Nucleic Acids Res.* **26,** 200–204.
23. Hughes, P. E., Alexi, T. & Schreiber, S. S. (1997) *NeuroReport* **8**, v–xii.
24. Slack, R. S., Belliveau, D. J., Rosenberg, M., Atwal, J., Lochmüller, H., Aloyz, R., Haghighi, A., Lach, B., Seth, P., Cooper, E. & Miller, F. D. (1996) *J. Cell Biol.* **135,** 1085–1096.
25. Xiang, H., Hochman, D. W., Saya, H., Fujiwara, T., Schwartzkroin, P. A. & Morrison, R. S. (1996) *J. Neurosci.* **16,** 6753–6765.
26. Wood, K. A. & Youle, R. J. (1995) *J. Neurosci.* **15,** 5851–5857.
27. Jordán, J., Galindo, M. F., Prehn, J. H. M., Weichselbaum, R. R., Beckett, M., Ghadge, G. D., Roos, R. P., Leiden, J. M. & Miller, R. J. (1997) *J. Neurosci.* **17,** 1397–1405.
28. Sakhi, S., Bruce, A., Sun, N., Tocco, G., Baudry, M. & Schreiber, S. S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7525–7529.
29. Schubert, D. & Behl, C. (1993) *Brain Res.* **629,** 275–282.
30. Masliah, E., Westland, C. E., Rockenstein, E. M., Abraham, C. R., Mallory, M., Veinberg, I., Sheldon, E. & Mucke, L. (1997) *Neuroscience* **78,** 135–146.
31. Dai, Y., Schwarz, E. M., Gu, D., Zhang, W. W., Sarvetnick, N. & Verma, I. M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 1401–1405.
32. Games, D., Adams, D., Alessandrini, R., Barbour, R., Berthelette, P., Blackwell, C., Carr, T., Clemens, J., Donaldson, T., Gillespie, F., *et al*. (1995) *Nature (London)* **373,** 523–527.
33. Murrell, J., Farlow, M., Ghetti, B. & Benson, M. D. (1991) *Science* **254,** 97–99.
34. Wyss-Coray, T., Masliah, E., Toggas, S. M., Rockenstein, E. M., Brooker, M. J., Lee, H. S. & Mucke, L. (1996) *J. Clin. Invest.* **97,** 789–798.
35. Xu, X., Brown, D. A., Kitajima, I., Bilakovics, J., Fey, J. W. & Nerenberg, M. I. (1994) *Mol. Cell. Biol.* **14,** 5371–5383.
36. Sun, Y., Bian, J., Wang, Y. & Jacobs, C. (1997) *Oncogene* **14,** 385–393.
37. Schubert, D., Heinemann, S., Carlisle, W., Tarikas, H., Kimes, B., Patrick, J., Steinbach, J. H., Culp, W. & Brandt, B. L. (1974) *Nature (London)* **249,** 224–227.
38. Goate, A., Chartier-Harlin, M.-C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., *et al*. (1991) *Nature (London)* **349,** 704–706.
39. Yamazaki, T., Selkoe, D. J. & Koo, E. H. (1995) *J. Cell Biol.* **129,** 431–442.
40. Yamatsuji, T., Okamoto, T., Takeda, S., Murayama, Y., Tanaka, N. & Nishimoto, I. (1996) *EMBO J.* **15,** 498–509.
41. Mullan, M., Tsuji, S., Miki, T., Katsuya, T., Naruse, S., Kaneko, K., Shimizu, T., Kojima, T., Nakano, I., Ogihara, T., *et al*. (1993) *Neurobiol. Aging* **14,** 407–419.
42. Mullan, M., Bennett, C., Figueredo, C., Hughes, D., Mant, R., Owen, M., Warren, A., McInnis, M., Marshall, A., Lantos, P., *et al*. (1995) *Am. J. Med. Genet.* **60,** 44–52.
43. Mattson, M. P., Cheng, B., Culwell, A. R., Esch, F. S., Lieberburg, I. & Rydel, R. E. (1993) *Neuron* **10,** 243–254.
44. Sisodia, S. S., Koo, E. H., Beyreuther, K., Unterbeck, A. & Price, D. L. (1990) *Science* **248,** 492–495.
45. Perez, R. G., Zheng, H., Van der Ploeg, L. H. T. & Koo, E. H. (1997) *J. Neurosci.* **17,** 9407–9414.
46. Agarwal, M. L., Taylor, W. R., Chernov, M. V., Chernova, O. B. & Stark, G. R. (1998) *J. Biol. Chem.* **273,** 1–4.
47. Eizenberg, O., Faber-Elman, A., Gottlieb, E., Oren, M., Rotter, V. & Schwartz, M. (1996) *Mol. Cell. Biol.* **16,** 5178–5185.
48. Shaulsky, G., Goldfinger, N., Tosky, M. S., Levine, A. J. & Rotter, V. (1991) *Oncogene* **6,** 2055–2065.
49. Giaccia, A. J. & Kastan, M. B. (1998) *Genes Dev.* **12,** 2973–2983.
50. Lu, X. & Lane, D. P. (1993) *Cell* **75,** 765–778.
51. Jiang, M. C., Yang-Yen, H. F., Lin, J. K. & Yen, J. J. (1996) *Oncogene* **13,** 609–616.
52. Xu, C., Meikrantz, W., Schlegel, R. & Sager, R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7829–7833.
53. Kawai, H., Kitamura, Y., Nikaido, O., Tatsuka, M., Hama-Inaba, H., Muto, M., Ohyama, H. & Suzuki, F. (1998) *Rad. Res.* **149,** 41–51.
54. Selkoe, D. J. (1996) *J. Biol. Chem.* **271,** 18295–18298.
55. Roperch, J., Alvaro, V., Prieur, S., Tuynder, M., Nemani, M., Lethrosone, F., Piouffre, L., Gendron, M. C., Israeli, D., Dausset, J., *et al*. (1998) *Nat. Med.* **4,** 835–838.
56. van Leeuwen, F. W., de Kleijn, D. V. P., van den Hurk, J. J., Neubauer, A., Sonnemans, M. A. F., Sluijs, J. A., Köycü, S., Ramdjielal, R. D. J., Salehi, A., Martens, G. J. M., *et al*. (1998) *Science* **279,** 242–247.
57. van Leeuwen, F. W., Burbach, J. P. H. & Hol, E. M. (1998) *Trends Neurosci.* **21,** 331–335.