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Mutant presenilin 2 increased oxidative stress and p53 expression in neuronal cells

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Abstract

The learning and memory impairment of presenilin 2 transgenic mice was mentioned previously. In this study, exposing the presenilin 2 transfected PC12 cells to the 50 μM $A\beta_{25-35}$, 30 mM L-glutamate and 50 μM H_2O_2 resulted in significant increase 8-oxodG and p53 levels of the cells expressing the mutant gene. The increase was also found in the mutant presenilin 2 transgenic mice brains age-dependently in comparison to that in the wild-type presenilin 2-transgenic mice and non-transgenic ones. These findings indicated that mutant presenilin 2 clearly increases oxidative stress and p53 expression, which could be implicated in promoting mutant presenilin 2-induced neurodegeneration in Alzheimer's disease, and the influence of mutant presenilin 2 in Alzheimer's disease may be brain regional and age related effects.

Keywords: Neuronal cell; Presenilin 2; 8-oxodG; p53; Alzheimer's disease

The effects of presenilin 1 mutation to the neurodegeneration of Alzheimer's disease (AD) have been thoroughly investigated [1,2]. The participation of presenilin 2 (PS2) in AD has also been considered [3,4]. The learning and memory impairment of PS2 transgenic mice, particularly in cases of mutant PS2 transgenic ones [5] strongly suggested the involvement of PS2 gene in the neurodegeneration of AD. The PS2 mutation N141I was also found to cause the enhancement of beta amyloid fragment 42 (Aβ42) production [5]. The beta amyloid fragment 25–35 ($A\beta_{25-35}$) treatment on PC12 cells induced to increase the apoptotic cell death [6,7]. Moreover, a PS2 fragment, which consists of the 166 amino terminal aminoacid, was found to be sufficient for inhibition of DNA synthesis and induction of apoptosis [8]. Overexpression of the wild-type or mutant-type (N141I) of PS2 in neuronal cells renders significant increase of basal cell death [3,4,9–11]. It was suggested a connection between oxidative stress and apoptosis in neurodegeneration disease including AD [1,12]. Emerging data indicate that oxidative damage is an early event in neurodegeneration in AD [13,14]. Inamura and the coworkers strongly suggested that p53 expression and neuronal cell death induced by DNA damaging agents [15]. In many types of post-mitotic neurons, p53 may mediate apoptosis induced by a range of insults including DNA damage and oxidative stress [16]. Furthermore, a large number of neurons with fragmented DNA in post-mortem AD brains was found [17] suggesting the further study involving in DNA damage in AD. AD being

Abbreviations: PS2, presenilin 2; wt, wild type; mt, mutant type; AD, Alzheimer's disease; Neo, empty vector; Tg, transgenic mice; $A\beta_{25-35}$, beta amyloid fragment 25–35; 8-oxodG, 8-hydroxy-2′-deoxyguanosine base.

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classified according to its age of onset [18] suggested the investigation at different ages of PS2 transgenic mice.

This study, therefore, investigate the oxidative DNA base 8-hydroxy-2′-deoxyguanosine (8-oxodG) induction and p53 expression in both in vitro and in vivo AD models to further understand the role of presenilin 2 and relationship between this gene mutation and the neuronal cell death in AD.

Materials and methods

Gene construction and stable transfection of mutant or wild-type PS2 into PC12 cells. The resulting construct of pNSE-PS2mt carries the 1.8 kb promoter fragment fused to a PS2mt fusion gene and the transfection into PC12 cells was carried out as described previously [5,19]. The stable transfected clones were selected by Western blotting for the present study based on their similar increased expression of PS2-like relative holoproteins (54 kDa) and N- and C-terminal maturation fragment (data not shown).

Mutant and wild-type PS2 transgenic mice. Transgenic mice expressing wild-type PS2 (Tg-wt) and mutant PS2 (Tg-mt), which was described previously [5], were used in this study.

Cell culture and treatment. Cells were seeded and cultured in poly D-lysine coated plastic dishes as described previously [19]. $A\beta_{25-35}$, which is the most toxic peptide fragment derived from amyloid precursor protein and retains full biological activity as compared to the naturally occurring full length Aβ protein; L-glutamate and H_2O_2 were used in the 6 h treatment.

8-oxodG assay. Among many oxidative DNA bases, 8-oxodG is high mutagenicity and sensitivity of its measurement [20]. Isolated DNA from the cells was completely digested with the enzymes: DNase I, Nuclease P1 and Alkaline phosphatase and analyzed the 8-oxodG formation with ELISA assay kit (Japan Institute for the control of Aging, Shizuoka, Japan).

RT-PCR. RNA was extracted using RNeasy mini kit according to the supplier's instruction. The primers are as follows: p53 of 560 bp, 5′-CTC TGT CAT CTT CCG TCC CTT C-3′ (forward), 5′-AGG ACA GGC ACA AAC ACG AAC-3′ (reverse) or of 261 bp, 5′-CGC TGC TCC GAA CCT CAT C-3′ (forward), 5′-CCG TCC CAG AAG GTT GCC A-3′ (reverse) and β-actin of 203 bp, 5′-CGA TAA GGA GAA GAT TTG GCA CC-3′ (forward), 5′-TAC GAC CAG AGG CAT ACA GGG AC-3′ (reverse). The RT-PCR process was carried out for 30 min at 50 °C, denature 2 min at 94 °C, annealing 30 s or 1 min at the primer's melting temperature, elongate 1 min 20 s or 2 min at 72 °C and prolonged elongation 3 min to 10 min at 72 °C.

Western blotting. Cells were lysed for Western blotting analysis as previously described [6,21]. Rabbit polyclonal antibodies against p53 (1:500 dilution) (Santa Cruz, CA, USA Santa Cruz Biotechnology Inc.) were used in this study. The relative density of the protein bands was quantified by densitometry using Electrophoresis Documentation and Analysis System 120 (Eastman Kodak Com., Rochester, NY).

Statistical analysis. Data were analyzed using one way analysis of variance followed by Tukey's test as a post hoc test. Differences were considered significantly at $p < 0.05$ (marked with *) and $p < 0.001$ (marked with **).

Results

Increase the 8-oxodG formation of PC12/PS2mt under apoptotic stimulating conditions

Based on the previous investigations of PC12 cells viability under stimulation of L-glutamate, H_2O_2, and $A\beta_{25-35}$ with various doses [6,7], we chose the L-glutamate

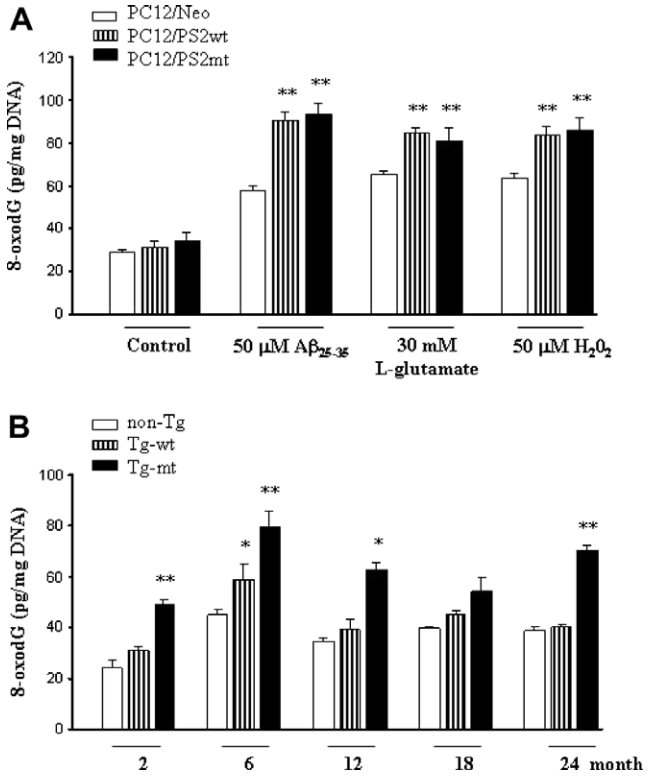

Fig. 1. The 8-oxodG levels were determined to the intact DNA isolated from the cells and brain tissue samples. (A) 8-oxodG levels of the PC12/Neo, PC12/PS2wt, PC12/PS2mt treated with 50 μM $A\beta_{25-35}$, 30 mM L-glutamate, 50 μM H_2O_2. The cells were cultured to reach 80–90% confluence and harvested for DNA extract after treatment for 6 h, parallel with untreated cells (control). (B) 8-oxodG levels from DNA of the mice brain tissues: non-Tg, Tg-wt, and Tg-mt at all ages of 2, 6, 12, 18, and 24 month. The data are means ± SD (bars) values of determinations of at least at least three animals ($n \geqslant 3$). Significant differences versus control and non-Tg samples were assessed by one way analysis of variance followed by Tukey's post hoc test (*$p < 0.05$, **$p < 0.001$).

of the 30 mM, H_2O_2 of the 50 μM and $A\beta_{25-35}$ of the 50 μM concentrations for this study. The 8-oxodG levels increased in all the treated samples compared to the corresponding untreated cells (Fig. 1A). Under the treatment conditions, the increment of 8-oxodG inductions in PC12 cells expressing PS2wt (PC12/PS2wt) and PC12 cells expressing PS2mt (PC12/PS2mt) (about three times higher than in untreated cells) were clearly higher than that in PC12 cells carrying control vector (PC12/Neo) (about 1.5–2 times higher than in untreated cells) (Fig. 1A).

Increase the 8-oxodG levels of the PS2 transgenic mice brain tissues

The 8-oxodG levels in all the Tg-wt and Tg-mt brains were clearly higher than that in the non-Tg samples. In which, the 8-oxodG levels of Tg-mt brains were always higher than that of Tg-wt brains (Fig. 1B). Interestingly, the enhancement of the 8-oxodG levels in Tg-mt brains is approximately 1.8-fold higher than that in non-Tg brains

and this ratio relatively consistent at all the considered ages (except the ratio of 1.3-fold increase in the case of 18-month old-mice), whereas these enhancements in Tg-wt brains were decreased age-dependently in comparison to non-Tg. Compared with the corresponding non-Tg brains, the 8-oxodG inductions in the Tg-wt brains of 2-months or 6-months old (young mice) were about 1.3-fold increase, but this is down to 1.2-fold at 12 months and 1.1-fold at 18-months old (old mice), particularly not higher at 24-months old (Fig. 1B).

Increase p53 mRNA levels of PC12/PS2mt under apoptotic stimulating conditions

Study the expression of p53 in time course treatment (from 2 to 24 h) indicated that the p53 levels were expressed highest at 6 h treatment (data not shown) and there were some other findings supported this result [22]. Therefore, we harvested the cells after 6 h treatment to detect p53 mRNA differences. The high levels of p53 mRNA were found in all of the treated cells in comparison to the corresponding control cells (Fig. 2). The level of p53 mRNA in the control samples of the PC12/PS2mt cells was higher than in that of the two other cell types. Under the 50 μM $A\beta_{25-35}$ treatment conditions, the increments of p53 mRNA of PC12/PS2mt and PC12/PS2wt were clearly higher than that of PC12/Neo, in which the enhancement of the PC12/PS2mt was about two times higher than that of the PC12/PS2wt in comparison to the control samples (Fig. 2).

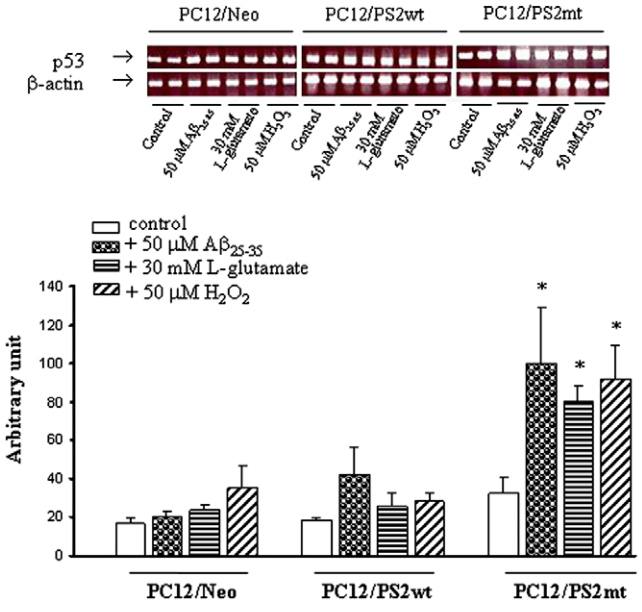

Fig. 2. p53 mRNA expression of three cell types PC12/Neo, PC12/PS2wt, PC12/PS2mt treated with 50 μM $A\beta_{25-35}$, 30 mM L-glutamate, 50 μM H_2O_2. Each lane was applied with the same volume of corresponding RT-PCR product of each sample. The cells were cultured to reach 80–90% confluence and harvested for RNA extract after treatment for 6 h, parallel with untreated cells (control). The data are means ± SD (bars) values of determinations of three independent experiments with duplicate ($n = 6$). Differences versus control samples were considered significant at $p < 0.05$ (*) followed by Tukey's post hoc test. The result was expressed as arbitrary unit, which is the ratio of the average of the p53 densities to the corresponding β-actin gene and amplified percentagewise.

Increase the p53 mRNA levels of the PS2 transgenic mice brain tissues

The p53 mRNA levels of the Tg-mt brain were higher than that of the Tg-wt and non-Tg samples and clearer in the cortical brains than in the hippocampal ones (Fig. 3). These results were very clearly different in the cortical samples among Tg-mt, Tg-wt and non-Tg and seemed to increase age-dependently, as well as much clearer in the old mice brains than in the young mice (Fig. 3A).

Increase p53 protein levels of PC12/PS2mt cells under apoptotic stimulating conditions

The increase of p53 protein levels in the 50-μM $A\beta_{25-35}$ and 30 mM L-glutamate treated cells was also detected (Fig. 4). The p53 protein expressed significantly higher in PC12/PS2wt and PC12/PS2mt than in PC12/Neo samples, in which p53 protein levels were always higher in PC12/PS2mt than in PC12/PS2wt (Fig. 4).

Increase p53 protein levels in the PS2 transgenic mice brain tissues

In in-vivo study results, p53 protein levels were found to increase age-dependently in all samples, even in non-Tg brain tissues (Fig. 5A and B). In which, the p53 protein levels in Tg-wt and Tg-mt brains were higher than that in non-Tg samples.

Discussion

The learning and memory impairment of PS2 transgenic mice, correlated with the increase in expression of PS2 wild-type (PS2wt) and PS2 mutant type (PS2mt) in the cortex and hippocampus of the transgenic mice were described previously [5]. The levels of PS2-like protein and of the C and N terminal PS2 fragments were find greater in all the transfected PC12 cells than in the PC12 [23]. The apoptotic cell death was observed to occur in dose- and time-dependent manner after 24–72 h stimuli treatments with 50 μM $A\beta_{25-35}$, 30 mM L-glutamate or 50 μM H_2O_2. In this study, we further investigate how the mutant PS2 influences 8-oxodG formation and p53 expression in both in vitro and in vivo AD models.

The key role for oxidative stress in the neurotoxic Aβ action was considered with the evidence that Aβ can also induce apoptosis in cultured neurons [24] and it can be prevented by antioxidants [1]. The increase of 8-oxodG levels in PC12/PS2wt and PC12/PS2mt indicated that PS2 more or less influences the 8-oxodG inductions. Despite no significant difference of 8-oxodG levels between the PC12/

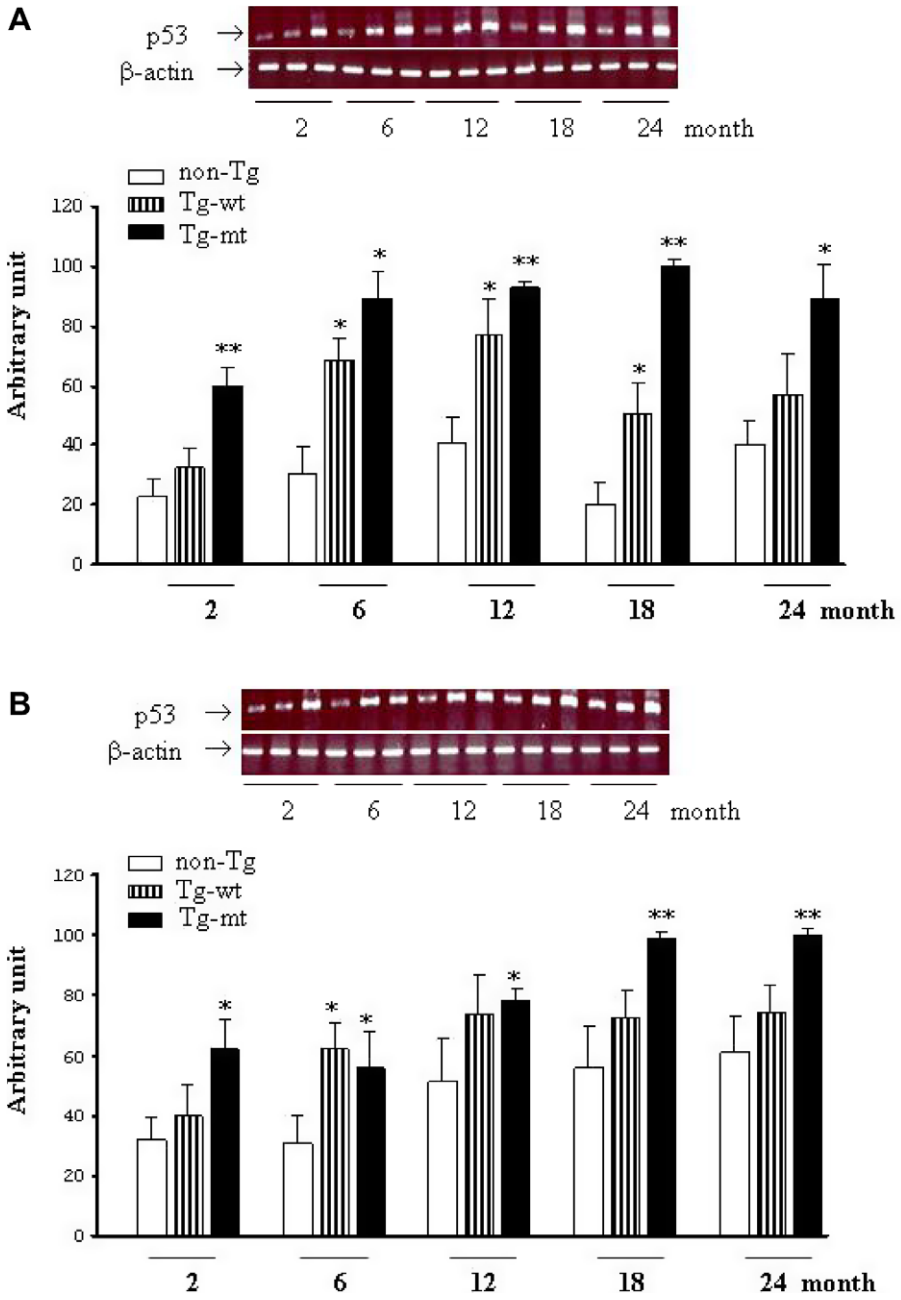

Fig. 3. p53 mRNA expression of mice brain tissues. Each lane was applied with the same volume of corresponding RT-PCR product of each sample. The brain of non-transgenic mice, wild-type PS2 transgenic mice and mutant PS2 transgenic mice at all ages of 2, 6, 12, 18, and 24 month were separated into cortex (A) and hippocampus (B) for RNA extract distinctly. The data are means ± SD (bars) values of at least three animals ($n \geqslant 3$). Significant differences versus corresponding non-Tg samples were assessed by one way analysis of variance followed by Tukey's post hoc test ($^{*}p < 0.05$, $^{**}p < 0.001$). The result was expressed as arbitrary unit, which is the ratio of the average of the p53 densities to the corresponding β-actin gene and amplified percentagewise.

PS2wt and PC12/PS2mt cells, there were remarkable increments of 8-oxodG levels in Tg-mt brains in comparison to that in non-Tg and Tg-wt samples. These results indicated the high susceptibility of mutant PS2 transgenic mice brains to 8-oxodG formations. Moreover, the DNA damage was found to be feature in AD brain [25]. Together with this evidence, the finding suggested that the enhancement of 8-oxodG formation in neuronal cells expressing PS2mt increases risk of DNA damage, which could lead to the cell death in AD brain. Further supports for the involvement of oxidative stress in the parthogenesis of AD also come from the studies that antioxidants such as vitamin E and estrogen can protect neurons against Aβ toxicity [1]. Our findings also suggested that overexpression of PS2wt may render a significant susceptibility of neurons to degeneration like as effect of PS2mt and this effect was

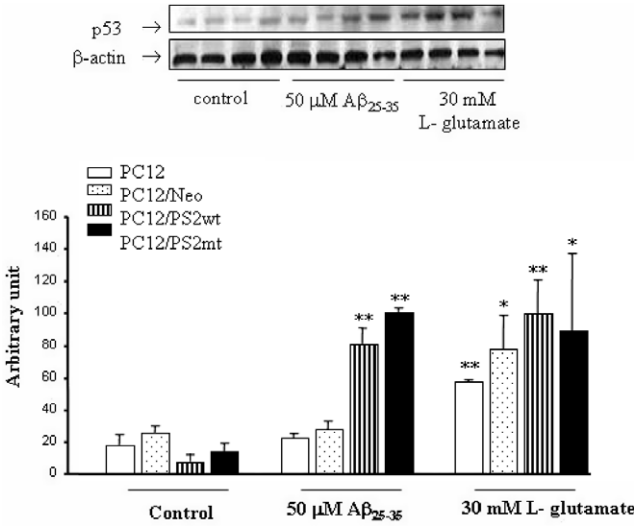

Fig. 4. p53 protein levels of four cell types PC12, PC12/Neo, PC12/PS2wt, and PC12/PS2mt treated with 50 μM $A\beta_{25-35}$, 30 mM L-glutamate. Each lane was applied with the same volume of corresponding total protein of each sample. The data are means ± SD (bars) values of determinations of two independent experiments with duplicate ($n = 4$). Significant differences versus control samples were assessed by one way analysis of variance followed by Tukey's post hoc test ($^{*}p < 0.05$, $^{**}p < 0.001$). The result was amplified percentagewise based on the ratio of the average of the p53 densities to the corresponding β-actin and was expressed as arbitrary unit.

clearer at the young mice brains than at the old mice brains. Support for this study, another report indicating the clear decrease in neuronal survival after two days PS2 post-infection demonstrated that the PS2 overexpression enhances apoptosis of cultured neurons under basal conditions [10]. These may help to understand why the Tg-wt mice exhibit increase responses in comparison to non-Tg animals.

Correlated with these results, the great expression of p53 in PC12/PS2mt, particularly in the treated cells, was found demonstrating that the mutation of PS2 alters the p53 expression toward increase of sensitivity to apoptosis. Although it could be influenced by some other mediated factors during protein process, it was a tendency towards age-dependent increase in p53 in the neuronal cells carrying PS2mt. Moreover, the similar results were also found in the in vivo study. The interesting finding of the age-dependent increase of p53 expression in neuronal cells expressing PS2mt indicated the noteworthy contribution of PS2mt to the neurodegeneration during aging. On the other hand, the great increments of p53 mRNA in Tg-mt cortex in comparison to that in hippocampus suggested that cortical neurons may be more sensitive to PS2mt-induced apoptosis than hippocampal neurons. We also found the developmental atrophy of cortex in transgenic mice brain expressing mutant PS2 in another our study (data not published yet). Moreover, the finding of high atrophy rate in entorhinal cortex in comparison to hippocampus in AD brains [26] agrees with the deduction of the tight involvement of

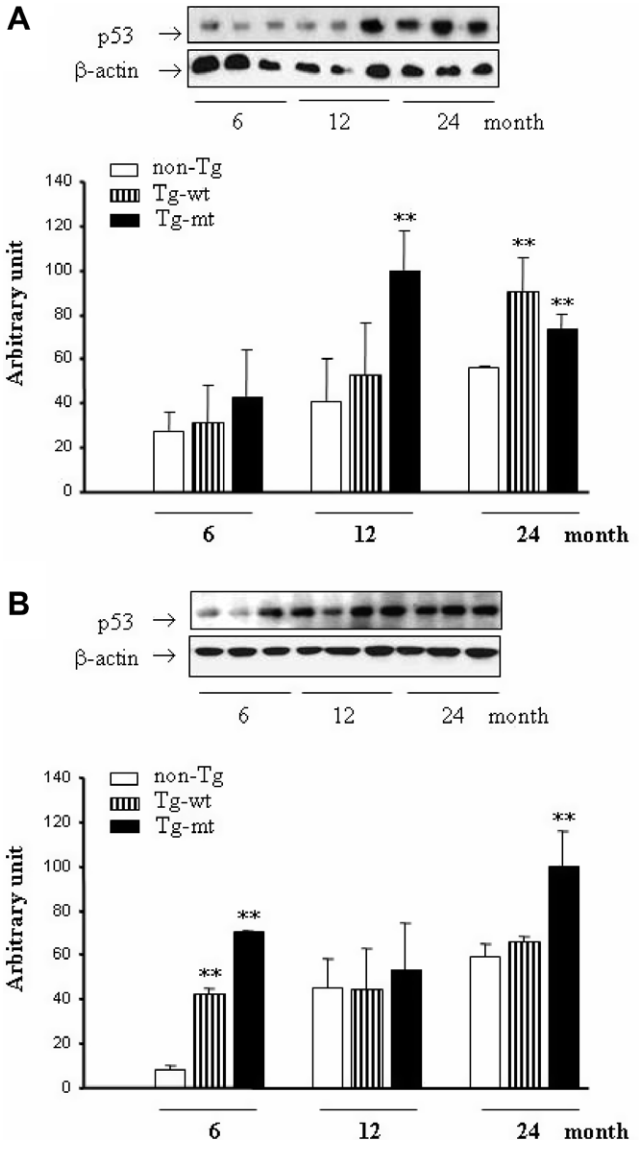

Fig. 5. p53 protein levels of mice brain tissues. Each lane was applied with the same volume of corresponding total protein of each sample. The brain of non-Tg, Tg-wt, Tg-mt at all ages of 6, 12, and 24 month were separated into cortex (A) and hippocampus (B) for protein extract distinctly. The data are means ± SD (bars) values of determinations of at least three animals ($n \geqslant 3$). Differences versus corresponding non-Tg samples were considered significant at $p < 0.001$ (**) followed by Tukey's post hoc test. The result was amplified percentagewise based on the ratio of the average of the p53 densities to the corresponding β-actin and was expressed as arbitrary unit.

cortex in AD. Additionally, the induction of p53 mRNA following exitotoxic or ischemic injury in the central nervous system is found to temporally and regionally relate to DNA damage and apoptosis [27]. This evidence may also support the finding of the current study. The finding leads us to the hypothesis that PS2mt may aggravate the oxidative stress-induced impairment membrane ion-motive ATPases and glucose transporters, which were mentioned elsewhere [28–31], thereby increasing susceptibility of the cells to excitotoxicity. However, other possible mechanism

should not be excluded. For instant, PS2mt decrease transcription activities of NF-κB, AP-1, SP-1 [19], which may be influenced by DNA damage and may be involved in inhibition of protein synthesis [32] including Bcl-2 [10,11] and regulatory proteins for p53. This idea is being further studied.

In conclusion, this study demonstrated that mutant PS2 clearly increases oxidative stress and p53 expression, which could be implicated in promoting mutant presenilin 2-induced neurodegeneration in AD, and the influence of mutant PS2 in AD may be brain regional and age related effects.

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