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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

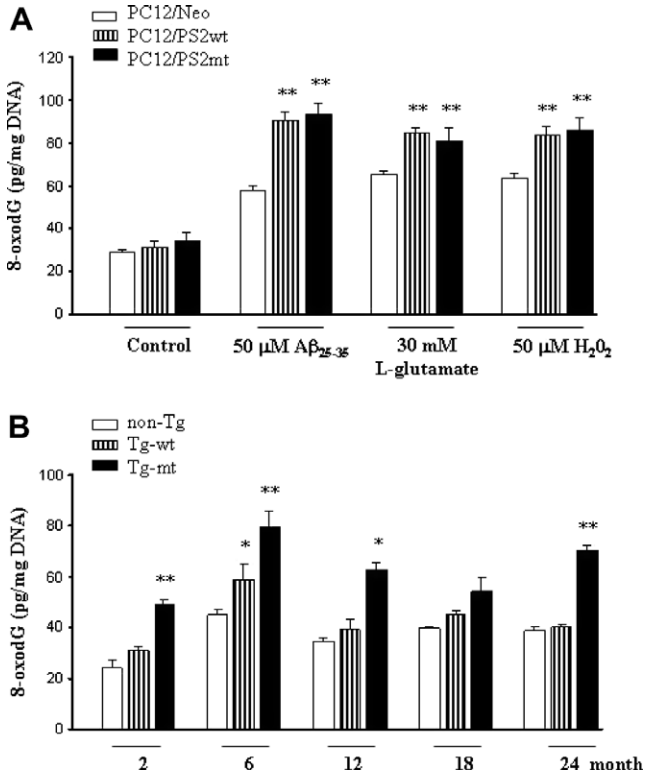

Fig. 1. The 8-oxodG levels were determined to the intact DNA isolated from the cells and brain tissue samples. (A) 8-oxodG levels of the PC12/Neo, PC12/PS2wt, PC12/PS2mt treated with 50 μM $A\beta_{25-35}$, 30 mM L-glutamate, 50 μM H_2O_2. The cells were cultured to reach 80–90% confluence and harvested 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