Neurosci Bull February 1, 2010, 26(1): 37-46. http://www.neurosci.cn
DOI: 10.1007/s12264-010-6122-3


·Original Article·

# Expression of the apoptosis-related proteins caspase-3 and NF-κB in the hippocampus of Tg2576 mice

Yan-Li NIU*, Wei-Juan ZHANG*, Ping WU, Bin LIU, Guo-Tao SUN, Dong-Ming YU, Jin-Bo DENG

*Institute of Neurobiology and Laboratory of Neurobiology, Nursing College of Henan University, Kaifeng 475004, China*



**Abstract: Objective** To investigate the relations between neuroapoptosis and the onset and development of Alzheimer's disease (AD), especially the role of NF-κB in the regulation of neuroapoptosis. **Methods** Caspase-3 and NF-κB (p50) expressions in the CA3 region of the hippocampus in APPswe Tg2576 transgenic mice were studied from postnatal day 0-180, using Nissl staining, immunohistochemistry and RT-PCR methods. **Results** Both neuronal apoptosis and NF-κB activity decreased gradually with the increase of age in wild type and Tg2576 mice. However, the number of caspase-3-positive or NF-κB-positive pyramidal cells in Tg2576 mice was greater than that in age-matched wild type mice, with significant differences after postnatal day 14 ($P < 0.01$ or $P < 0.05$). Linear regression analyses of caspase-3 and NF-κB expression demonstrated a correlation between neuroapoptosis and activity of NF-κB. **Conclusion** The process of neuroapoptosis is consistent with the onset and development of AD. Furthermore, the observed correlation between neuroapoptosis and NF-κB activity suggests a role of NF-κB in hippocampal neuroapoptosis.

**Keywords:** Alzheimer's disease; Tg2576 transgenic mice; caspase-3; hippocampus; apoptosis; NF-κB

## 1 Introduction

Alzheimer's disease (AD) is a devastating disease affecting the health of many people aged over 65 years. Symptoms of AD include memory loss, language deterioration, poor judgment, confusion and mood swings. The neuropathology of AD is characterized by amyloid plaques, neurofibrillary tangles and degeneration of neurons and synapses[1].

In the present study, neuroapoptosis was investigated in APPswe Tg2576 transgenic mice at different developmental stages using caspase-3 immunocytochemistry in an effort to further understand the development of neuroapoptosis in AD. In addition, activation of NF-κB in the hippocampus was analyzed. Understanding the role of NF-κB in neuroapoptosis will be beneficial to development of novel strategies for pharmacological intervention in many neurodegenerative diseases.

*These authors contributed equally to this work.
Corresponding author: Jin-Bo DENG
Tel: 0378-3880292; Fax: 0378-3880585
Email: jinbo_deng@henu.edu.cn
Article ID: 1673-7067(2010)01-0037-10
Received date: 2009-06-12; Accepted date: 2009-09-18

## 2 Materials and methods

**2.1 Animals** APPswe Tg2576 transgenic mice were used in the present study. Hemizygous males (transgenic mice, TM) encoding the Swedish FAD double mutation, HuAPP695SWE under the control of a hamster prion protein promoter[2], were crossed with wild type (WT) female mice on a hybrid C57BL/6-SJL background. All the mice were born and bred in the animal housing facilities at the Institute of Neurobiology at Henan University, under a 12-h light/dark cycle. Tail DNA was extracted from P10 animals (P = postnatal days; P0 = the first 24 h after birth) and genotyping was performed by PCR to detect hAPPswe positive animals using the following primers: 5'GTG GAT AAC CCC TCC CCC AGC CTA GAC CA

3' and 5' CTG ACC ACT CGA CCA GGT TCT GGG T 3'. Offspring genotypes were identified as WT (-/-, normal mice lacking a PCR band) and heterozygote (+/-, with a 466 bp PCR band). For the behaviour, Tg2576 mice appeared slower in reaction than WT mice did after postnatal 3 months. In addition, Tg2576 mice usually could not live for longer than 8 months. The survival rate at the age of 6 months for Tg2576 mice was 82% *vs* 95% for WT. At the age of one year, the survival rate of Tg2576 mice was less than 10% compared to 91% of WT mice. Behaviour and survival rates in Tg2576 mice were consistent with AD onset and progression. In order to find the progressive course of AD, several age points were selected to investigate neuroapoptosis and brain pathology of Tg2576 mice, including P0, P7, P14, P30, P90 and P180. Age-matched WT littermates served as control. For each age group, at least 8 mice were included, among which 5 were for histological analysis and 3 were for reverse transcription-polymerase chain reaction (RT-PCR). Totally 96 mice were used for pathological and RT-PCR analyses.

**2.2 HE staining and Nissl staining methods** Prior to sacrifice, animals were given a lethal overdose of sodium pentobarbital (80 mg/kg, i.p.) and then perfused transcardially with 4% paraformaldehyde in 0.1 mol/L phosphate buffer (PB; pH 7.2). The whole brain was removed using a fine spatula. After immersion in fresh fixative for 1-2 d at 4 ºC, brains were dehydrated in gradients of ethanol and embedded in paraffin. Coronal sections of hippocampus were cut at a 5-μm thickness, and HE staining and Nissl method were carried out.

**2.3 Caspase-3 / NF-κB immunohistochemistry and hematoxylin counterstaining** Hippocampal sections were prepared as described above (2.2). To eliminate the activity of endogenous peroxidases, sections were incubated with 3% $H_2O_2$ prepared in 0.1 mol/L PB for 15 min. After being washed, sections were incubated for 30 min in 0.5% normal goat serum to block non-specific binding. Then rabbit anti-activated caspase-3 (1:500; R&D) or rabbit anti-NF-κB, p50 antibody (1:200, Santa Cruz) was added and sections were incubated overnight at 4 ºC. After that, sections were incubated with HRP-conjugated goat anti-rabbit IgG (1:500, Upstate) for 3 h at room temperature. Finally, sections were incubated with the substrate diaminobenzidine (DAB) to detect HRP and allow visualization of the caspase-3- and NF-κB-positive hippocampal neurons. Following immunohistochemistry, some sections were counterstained with hematoxylin. In this way, positive cells were double-labeled with nuclear hematoxylin and cytoplasmic HRP, while normal neurons were negative for caspase 3 and NF-κB and stained only with hematoxylin in nuclei.

**2.4 Immunofluorescent double labeling** In order to determine whether cells were positive for both caspase-3 and NF-κB, immunofluorescent double labeling was employed. Sections were prepared as described above. After being rinsed for several times, sections were incubated with both polyclonal goat anti-caspase-3 (1:1 000, Santa Cruz) and polyclonal rabbit anti-p50 (1:200, Santa Cruz) antibodies overnight at 4 ºC. After that, the corresponding secondary antibodies goat anti-rabbit IgG (1:300, Invitrogen) and donkey anti-goat IgG (1:600, Invitrogen) were added and sections were incubated at room temperature for 3 h. After being washed for 3 times, sections were coverslipped with mounting medium and imaged with an epifluorescent microscope (BX61, Olympus). High quality sections were photographed with a confocal microscope (FV10, Olympus) using separate scans for the 568 and 488 nm laser lines.

**2.5 RT-PCR** Since RT-PCR is a sensitive method for detection of mRNA expression, caspase-3 mRNA expression was tested using this method. Hippocampal RNA was extracted using TRIzol reagent[3]. RNA was reverse transcribed into cDNA using a Reverse Transcription System Kit according to manufacturer's instructions (Promega Corporation, Promega). The resulting cDNA was used as a template for PCR amplification. The sequences of the primers for caspase-3 were 5' AGA TAC CGG TGG AGG CTG ACT 3' and 5' TCT TTC GTG AGC ATG GAC ACA 3'. β-Actin served as an internal control using the primers 5' GGG AAA TCG TGC GTG ACA T 3' and 5' TCA GGA GGA GCA ATG ATC TTG 3' (Beijing AuGCT Biotechnology Co., Ltd.). The DNA sequence was amplified for 30 cycles (denaturation at 94 ºC for 45 s, annealing at 58 ºC for 45 s and extension at 72 ºC for 45 s).

**2.6 The criteria for scoring caspase-3- and NF-κB-positive cells** Since active caspase-3 could be located in both cyto-

plasm and nucleus during apoptosis, any pyramidal cell displaying immunoreactivity (brown) was regarded as caspase-3-positive. Identification of NF-κB-positive cell is more complex since activation of NF-κB involves separation of the p50-p65 complex from IκB in the cytoplasm and transference to the nucleus[4]. Therefore, immunoreactive cells demonstrating brown staining in either nuclei only or in nuclei and cytoplasm were regarded as activated NF-κB-positive cells. Cells with or without brown staining in the cytoplasm were recognized as negative cells (Fig. 1A, B and C).

**2.7 Parameters and measurements** Due to the role of the hippocampus in learning and memory, and cognition, the pathological alterations that occur during AD are particularly visible in the hippocampus, predominantly in the CA3 region which is extremely sensitive to stimulation. Therefore, the CA3 area was targeted for measurement. Parameters were designed and measured in order to evaluate loss of neurons, neuroapoptosis and NF-κB activation. Although both neurons and neuroglia were stained by immunohistochemistry and hematoxylin, the two kinds of cells could be easily recognized according to their morphological features. The parameters used in the present study were as follows: (1) Density of pyramidal cells (DPC): DPC = number of pyramidal cells in the CA3 region/area of CA3; (2) Apoptotic index (AI): AI = number of apoptotic pyramidal cells (caspase-3-positive cells) in the CA3 region/total number of pyramidal cells (both apoptotic neurons and non-apoptotic neurons) in the CA3 region; (3) Density of caspase-3 neurons (DCN): DCN = number of caspase-3-positive pyramidal cells in the CA3 region/area of CA3; (4) Density of NF-κB-positive neurons (DκBN): DκBN = number of NF-κB-positive pyramidal cells in the CA3 region/area of CA3. All the parameters were measured in Tg2576 and wild type mice at the 6 developmental stages (P0, P7, P14, P30, P90, and P180). At least 5 animals were used in each group. For each animal, 5 sections were analyzed, and the mean values were calculated for all parameters and used for further statistical analysis.

**2.8 Data analysis** The developmental trend of neuronal loss and neuroapoptosis was compared between Tg2576 and WT mice. Data were presented as mean±SD and analyzed with *t* tests at each developmental stage. A correlative test between the density of caspase-3-positive neurons and density of NF-κB-positive neurons was carried out using linear regression analysis. $P < 0.05$ was considered as statistically significant.

## 3 Results

**3.1 General pathology and neuronal loss in Tg2576 mice** The hippocampus is highly laminated, in terms of its cell and fiber projections. HE staining and Nissl method demonstrated that the pyramidal layer of the Ammon's horn and the granule layer of the dentate gyrus interlocked to form a double "C" like shape. During the course of development, hippocampal morphology underwent a series of alterations. The developmental trend of the pyramidal layer in Tg2576 mice was similar to that of WT mice. Pyramidal cells matured and their number decreased with the increase of age (Table 1). This phenomenon of neuronal reduction resulted in a thinner pyramidal layer with less cells (Fig. 2A, B and C). In contrast, at P0, the pyramidal layer was thick, and pyramidal cells were densely packed. The pyramidal cells appeared young, with little cytoplasm and resembled lymphocytes. However, with the increase of age, pyramidal cells grew larger and developed a mature structure —— triangular in shape with abundant cytoplasm, large nuclei and clear nucleoli. The number of pyramidal cells was stable after P30 (Fig. 2A, B and C). Although the developmental trend was similar in both groups, differences did exist between Tg2576 and WT mice. There were obvious neuronal loss and neuroapoptosis in Tg2576 mice. The number of neurons in Tg2576 and WT mice throughout the developmental time course was shown in Table 1. In the Tg2576 mice, the density of pyramidal cells was smaller than that in WT mice after P7. In addition, there was obvious neuroapoptosis at P180, and irregular polarity of apoptotic cells, and the cells exhibited concentrated cytoplasm with visible apoptotic bodies in the pyramidal layer (Fig. 2D, E and F).

**3.2 Caspase-3-dependent neuroapoptosis**

**3.2.1 Activated caspase-3-positive pyramidal cells in the CA3 area** Caspase-3 is a pro-apoptotic cytoplasmic enzyme. Here, activated caspase-3 was used as a marker to label apoptotic cells. Apoptotic neurons were positive for DAB

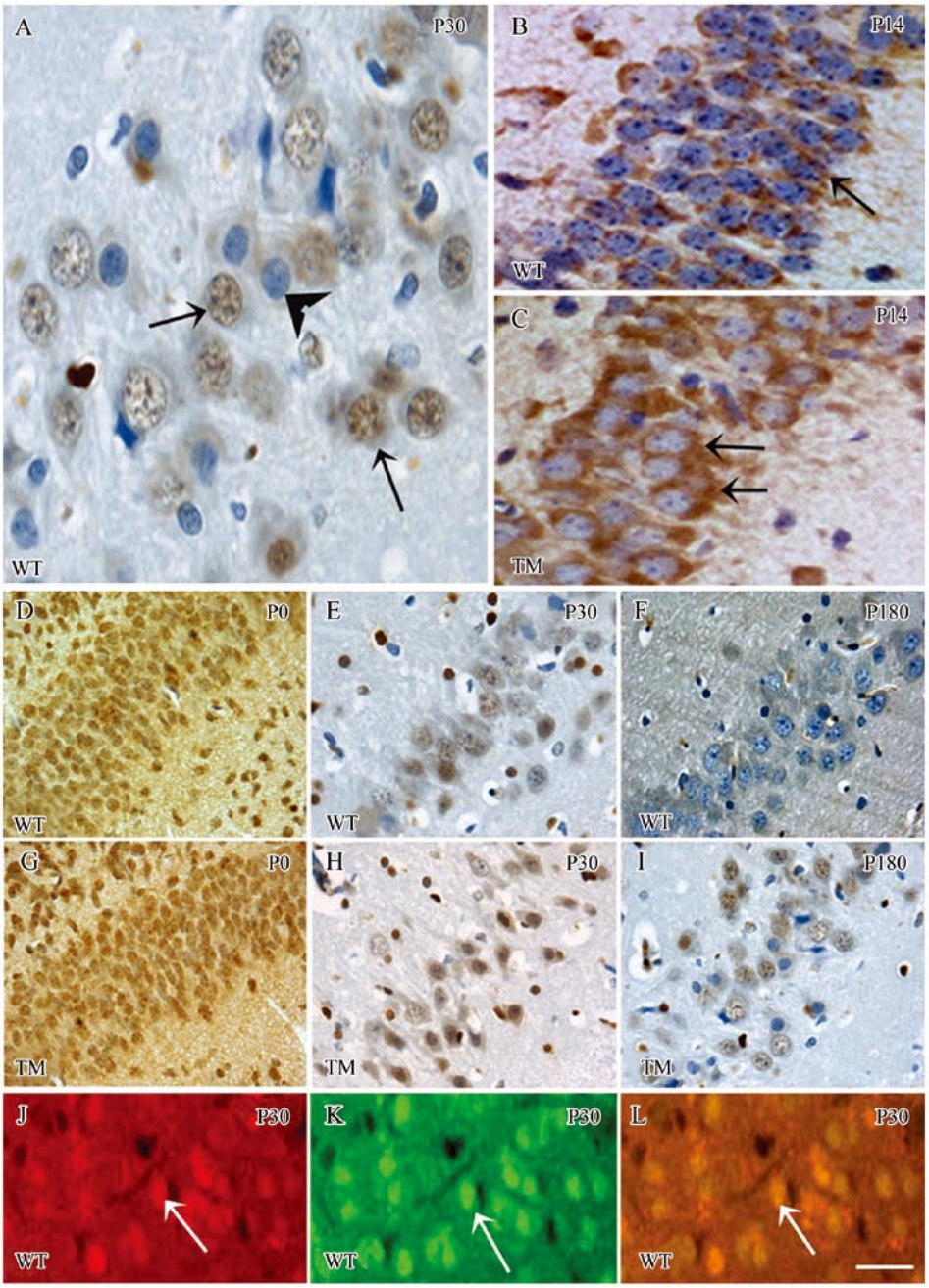


**Fig. 1** **Activation of caspase-3 and NF-κB in the CA3 region of the hippocampus, as detected by immunocytochemistry with hematoxylin counterstaining. A: A high magnification of NF-κB activation. Arrows pointed to NF-κB-activated cells displaying brown staining in nuclei or nuclei and cytoplasm. Cells lacking brown color in nuclei were regarded as NF-κB inactive cells (arrowheads). B and C: CA3 neuroapoptosis at P14 as determined by caspase-3 immunochemistry plus hematoxylin counterstaining. Caspase-3-positive cells displaying cellular brown staining were regarded as apoptotic cells (arrows), and cells with only hematoxylin in nuclei were regarded as healthy non-apoptotic cells. The neuroapoptosis in (C) Tg2576 mice was more severe than that in (B) WT mice. D-I: NF-κB activation in the CA3 region in Tg2576 (G-I) and WT mice (D-F) at P0, P30 and P180, as demonstrated by immunocytochemistry and hematoxylin counterstaining. NF-κB activation level in Tg2576 mice was higher than that in WT mice, particularly after P14. J-L: Co-expression of caspase-3 and NF-κB, as demonstrated by caspase-3 and NF-κB double immunolabeling. J and K demonstrated caspase-3 (red) and NF-κB (green) positive cells, respectively. L: A merge of J and K. Co-activation of caspase-3 and NF-κB was detected in some cells (arrows). Scale bar, A = 30 μm; B-I = 20 μm; J-L = 10 μm.**

staining which could reflect the level of activated caspase-3 in the cytoplasm, and were stained with hematoxylin marking the nucleus, while normal neurons were stained only with hematoxylin (Fig. 1B, C). During early development of the central nervous system (CNS), neuroapoptosis is often found in the proliferative zone and in young neurons, since numer-

**Table 1. Density of pyramidal cells in the CA3 area as determined by the Nissl method (mean±SD)**

| Postnatal day | *n* | TM mice (cells/mm$^2$) | WT mice (cells/mm$^2$) |
|---|---|---|---|
| P0 | 5 | 11646.4±1355.1 | 12308.7±1974.4 |
| P7 | 5 | 7067.0±932.4$^{**}$ | 8373.2±1300.7 |
| P14 | 5 | 5346.9±678.4$^{*}$ | 5548.4±879.9 |
| P30 | 5 | 4950.9±607.0 | 5431.7±670.9 |
| P90 | 5 | 4671.1±757.8$^{*}$ | 5346.9±678.4 |
| P180 | 5 | 3603.8±268.4$^{**}$ | 4515.8±652.1 |

$^{*}P < 0.05$, $^{**}P < 0.01$ *vs* WT mice.

**Table 2. Density of caspase-3-positive pyramidal cells in the CA3 area (mean±SD)**

| Postnatal day | *n* | TM mice (cells/mm$^2$) | WT mice (cells/mm$^2$) |
|---|---|---|---|
| P0 | 5 | 9077.3±821.6 | 8170.4±771.7 |
| P7 | 5 | 5545.8±996.0 | 4868.2±748.3 |
| P14 | 5 | 4588.1±958.5$^{**}$ | 1523.6±142.5 |
| P30 | 5 | 1426.7±260.6$^{*}$ | 715.99±296.3 |
| P90 | 5 | 3170.2±507.8$^{**}$ | 1828.1±717.0 |
| P180 | 5 | 1090.5±668.1$^{*}$ | 534.85±73.6 |

$^{*}P < 0.05$, $^{**}P < 0.01$ *vs* WT mice.

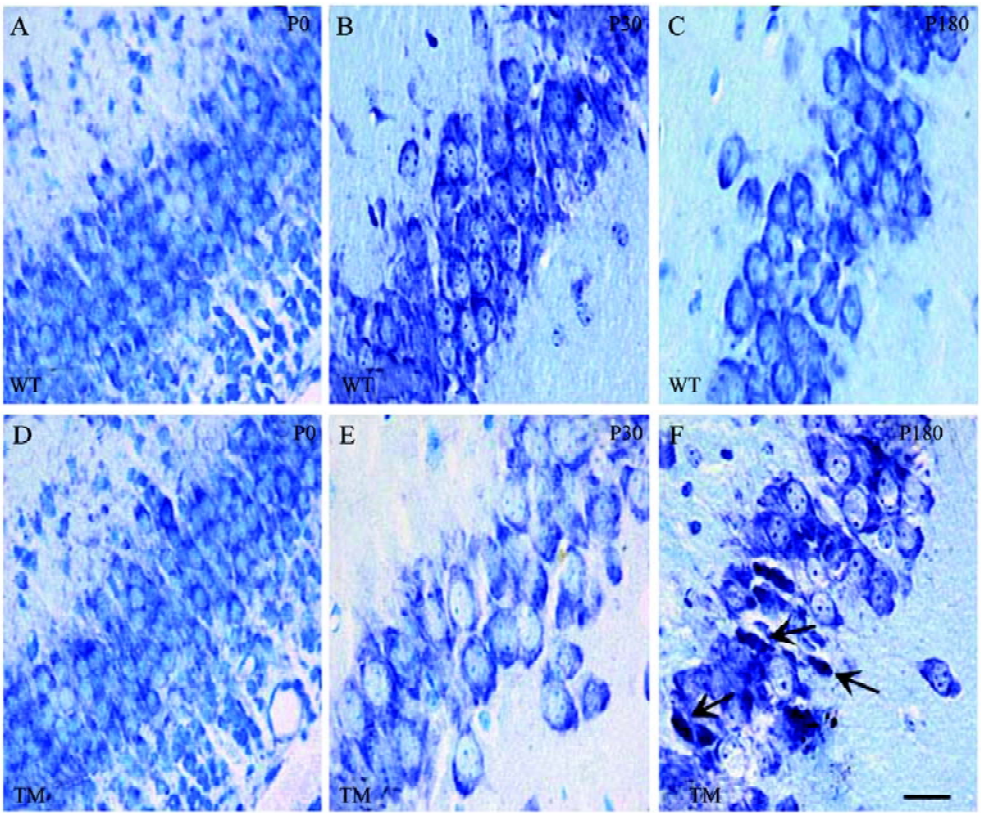


**Fig. 2 CA3 pyramidal cells in developing hippocampus with Nissl staining. A-C: CA3 pyramidal cells of WT mice at P0, P30 and P180, respectively. With the increase of age, the pyramidal layer showed a smaller density and pyramidal cells became more mature. Overall, the cell number decreased. For instance, at P0, pyramidal cells were young and resembled lymphocyte with a round shape and little cytoplasm. At P30 and P180, the pyramidal cells matured and the size increased. D-F: CA3 pyramidal cells of Tg2576 at P0, P30 and P180 respectively. Development of the pyramidal layer in Tg2576 and WT mice was similar, but neuronal loss and neuroapoptosis were more obvious in Tg2576 mice. In Tg2576 mice after P30, pyramidal cell polarity became irregular, and the cytoplasm became more concentrated. At P180, apoptotic bodies (arrows) could be observed in the pyramidal layer. Scale bar, 30 μm.**

ous non-functional cells are waiting to be eliminated through apoptosis. As development proceeds, neuroapoptosis decreases as mature synapses are established among neurons[5]. In the present study, developmental trend of neuroapoptosis in Tg2576 mice was similar to that observed in WT mice. Using immunocytochemistry and hematoxylin counterstaining, the apoptotic index of pyramidal cells in the CA3 area was calculated. The highest degree of neuroapoptosis (66.4% in WT and 68.0% in Tg2576 mice) was observed in pyramidal cells at P0. Neuroapoptosis gradually decreased with the increase of age (58.1% in WT and 59.1% in Tg2576 mice at P7, 27.5% in WT and 35.8% in Tg2576 mice at P14, 19.2% in WT and 25.1% in Tg2576 mice at P30, 13.4% in WT and 17.9% in TM at P90, 11.8% in WT and 15.3% in TM at P180). In addition, differences were observed between WT and Tg2576 mice (Fig. 1B, C). Neuroapoptosis in Tg2576 mice was more severe than that in age-matched WT mice (Fig. 1). Table 2 showed the density of caspase-3-positive pyramidal cells (cells/mm$^2$) in the CA3 area of WT and Tg2576 mice. Statistically significant differences appeared after P14 ($P > 0.05$ at P0 and P7, $P < 0.05$ from P14 - P180), indicating a close correlation between hippocampal neuroapoptosis and AD development.

**3.2.2 Caspase-3 mRNA expression in hippocampus** To further confirm the results of immunohistochemistry assay, semi-quantitative RT-PCR was carried out to measure expression of caspase-3 mRNA in Tg2576 and WT mice at different ages. β-Actin served as an internal control. To improve the accuracy of PCR results, each sample was measured in duplicate, and the average value was used for further analysis. At least 3 animals were included in each age group. As shown in Fig. 3, the results were consistent with that of the caspase-3 immunohistochemistry. Expression level of caspase-3 mRNA in Tg2576 mice was higher than that in WT mice after P14. However, before P14, there was no obvious difference between Tg2576 and WT mice. Table 3 listed the caspase-3/β-actin ratio measured by densitometry at different ages.

**3.3 NF-κB and caspase-3 activation**

**3.3.1 NF-κB activation in CA3 pyramidal cells** NF-κB-positive cells were observed in both Tg2576 and WT mice. Developmental trends were similar to that of caspase-3 activation. NF-κB was largely activated in young postmitotic neurons similar to the pattern of activated caspase-3. NF-κB-positive pyramidal cells appeared more frequently in young animals than in old ones, and NF-κB-positive cells appeared preferentially in the proliferative zone, such as the subventricle, rather than the cortical plate. Using NF-κB im-

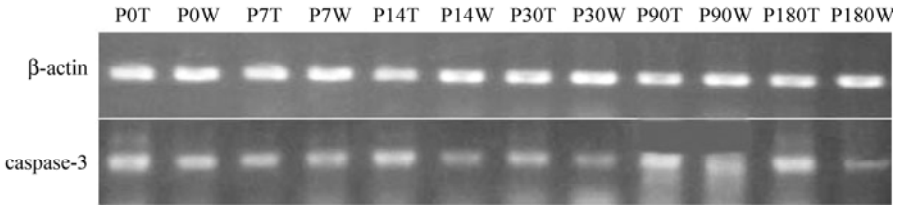


**Fig. 3 Caspase-3 expression measured by RT-PCR. W: wild type mice. T: transgenic mice. β-Actin was used as an internal control.**

**Table 3. Caspase-3/β-actin densitometric ratio (mean±SD)**

| Postnatal day | TM mice (%) | WT mice (%) |
|---|---|---|
| P0 | 69.0±13.5 | 54.9±34.3 |
| P7 | 60.5±21.8 | 52.8±36.2 |
| P14 | 55.9±35.6* | 45.1±22.6 |
| P30 | 55.0±24.2* | 39.2±11.6 |
| P90 | 48.8±23.1* | 31.7±18.5 |
| P180 | 42.9±19.1* | 28.6±17.2 |

*$P < 0.05$, *vs* WT mice.

**Table 4. Activation of NF-κB in CA3 pyramidal cells (mean±SD)**

| Postnatal day | *n* | TM mice (cells/mm$^2$) | WT mice (cells/mm$^2$) |
|---|---|---|---|
| P0 | 5 | 9064.2±3511.8 | 7802.5±1349.9 |
| P7 | 5 | 3357.5±706.5 | 2419.2±1319.2 |
| P14 | 5 | 2468.7±421.9** | 1687.1±528.0 |
| P30 | 5 | 3515.4±314.6** | 2547.3±361.7 |
| P90 | 5 | 3213.2±1251.2* | 2233.8±442.8 |
| P180 | 5 | 2012.1±390.9* | 1167.6±344.0 |

*$P < 0.05$, **$P < 0.01$ compared to WT mice.

munocytochemistry with hematoxylin counterstaining, NF-κB-positive cells were easily recognized. At P0, activation of NF-κB in pyramidal cells of the CA3 region was higher than that at all the other time points. With the increase of age, NF-κB activation gradually decreased (Table 4, Fig. 1D-I). Although the developmental trends of NF-κB activation in Tg2576 and WT mice were similar, NF-κB activation in Tg2576 mice was statistically greater than that in WT mice after P14 ($P > 0.05$ before P14, $P < 0.05$ from P14-P180, *t* test; Table 4, Fig. 1D-I).

**3.3.2 Correlation between caspase-3 activation and NF-κB activity** In order to examine the correlation between caspase-3 activation and NF-κB activity, linear regression and correlation tests were carried out. Table 2 and Table 4 listed the density of caspase-3-positive pyramidal cells and the density of NF-κB-positive pyramidal cells, respectively. Based on the data of caspase-3 *vs* NF-κB of Tg2576 and WT mice, the correlations between caspase-3 and NF-κB were made respectively. The results demonstrated a positive correlation between caspase-3 and NF-κB (r=0.825 and $P < 0.05$ in Tg2576 mice, r=0.885 and $P < 0.05$ in WT mice), which suggested a link between NF-κB and caspase-3 activation. In order to further examine the relationship between caspase-3 and NF-κB, co-immunofluorescent staining for caspase-3 and NF-κB immunofluorescence was carried out, and some cells co-positive for caspase-3 and NF-κB were demonstrated (Fig. 1 J-L).

## 4 Discussion

During normal development of CNS, apoptosis has been widely demonstrated to play a crucial role in sculpting and maintaining the architecture of the mature CNS. It is assumed that approximately 50% of the neurons produced during development die before CNS maturation, and nearly all neuronal classes are excessively produced during development. These oversized neuronal populations are then significantly eliminated primarily via apoptosis. Meanwhile, neuroapoptosis can be also found in neurodegenerative diseases, such as Parkinson's disease and AD. Neuroapoptosis plays an important role during CNS development. During the course of neuronal proliferation, differentiation and migration, numerous neurons migrate incorrectly and many non-functional synapses are formed with inappropriate neurons or at inappropriate development stages. Any ectopic neurons or neurons with non-functional synapses are eliminated during the normal development process through neuroapoptosis[6]. On the other hand, neuroapoptosis also plays an important role in clearing away sick cells in neurodegenerative disease[7]. Investigation in neuroapoptosis during CNS development may shed light on the pathogenesis of AD. The process of neuroapoptosis is highly controlled, in which many genes participate. NF-κB is considered as a candidate for active regulation of neuroapoptosis. Understanding the role of NF-κB in neuroapoptosis may be helpful for develop novel therapies for pharmacological intervention in AD.

**4.1 Pathological alterations in Tg2576 mice during development** Amyloid plaques and neurofibrillary tangles are the pathological hallmarks of AD. Amyloid plaques are dense insoluble deposits of Aβ protein and cellular material outside and around the neurons. Neurofibrillary tangles are insoluble twisted fibers built up inside the neuron[8]. Neurofibrillary tangle formation in the CA3 region of the hippocampus is an early event of AD pathogenesis[9]. As the disease progresses, more neurofibrillary tangles are formed with substantial neuronal loss in the hippocampus and cortex[10]. In AD, loss of synaptic connections and neurons occurs in the cerebral cortex, hippocampus and certain subcortical regions, which results in gross atrophy of the affected regions[11]. Surprisingly, some neuroregeneration can occur in tandem with chronic neuroapoptosis in an attempt to compensate for neuronal loss. Moreover, biochemical evidence demonstrates a loss of choline acetyltransferase and acetylcholine in the cerebral cortex of patients with AD[12].

In the current study using a combination of HE staining and Nissl method, hippocampal pathology and neuronal loss were investigated in Tg2576 mice. In general, the number of pyramidal cells in the CA3 region reduced gradually with age, but we observed that neuronal loss was more severe in Tg2576 mice compared with that in age-matched WT mice. At P180, apoptotic bodies were found among pyramidal cells, suggesting a link between amyloid plaques and neuronal loss in Tg2576 mice. These observations are consistent with the study of Schmitz *et al*.[13], in which Tg2576 mice express-

ing human mutant amyloid precursor protein APP751 (KM670/671NL and V717I) and human mutant presenilin-1 (PS-1 M146L) were investigated. Their data revealed a substantial age-related neuronal loss in the hippocampal pyramidal cell layer of APP/PS-1 double-transgenic mice. The apoptotic bodies found in the present study suggest the neuroapoptosis to be a main cause for neuronal loss, probably resuting from Aβ toxicity.

**4.2 Caspase-3-dependent apoptosis in Tg2576 and WT mice during development** It is well accepted that massive neuronal and glial death occurring in AD is caused by apoptosis. Using immunocytochemistry method, Su *et al*.[14] have demonstrated substantial neuroapoptosis in the hippocampus and entorhinal cortex of AD brain, consistent with the notion that apoptosis is the primary mechanism leading to neuronal death in AD. There are 2 types of apoptosis: caspase-dependent apoptosis and caspase-independent apoptosis (autophagy). The majority of cell death in neurodegenerative disease occurs through caspase-dependent apoptosis. Multiple extrinsic and intrinsic stimulations lead to cell death through different pathways culminating in proteolytic activation of caspases-3. Activated caspase-3 subsequently cleaves a specific set of protein substrates, such as procaspases themselves, resulting in mediation and amplification of the death signal and eventually cell death[15]. Since caspase-3 activation is the convergence of apoptotic pathways, the presence of active caspase-3 is often used to mark caspase-dependent apoptosis. We have previously used activated caspase-3 to detect neuroapoptosis occurring as a result of prenatal alcohol exposure with satisfactory results[16].

In the present study, neuroapoptosis in Tg2576 mice was studied at various developmental stages using caspase-3 immunohistochemistry. Development of neuroapoptosis in the 2 groups showed some similarities. In both Tg2576 and WT mice, neuroapoptosis declined gradually with age and the maximal apoptosis occurred at P0. However, there were significant differences in neuroapoptosis in the hippocampus between Tg2576 and WT mice after P14. In CA3 pyramidal cells, the rate of neuroapoptosis was faster in Tg2576 mice than in WT mice. RT-PCR results have demonstrated that caspase-3 mRNA expression showed a similar pattern to that of caspase-3 activity. Understanding the role of neuroapoptosis in Tg2576 mice has clinical significances. Prior to P14, no differences were found between the two groups, but after P14 differences appeared between WT and Tg2576 mice. The symptoms of AD usually appear in middle-aged patients and progress over time. Our data are consistent with the correlation between neuroapoptosis and AD development.

**4.3 Activation of NF-κB- and caspase-3-dependent apoptosis** NF-κB, a nuclear transcription factor, plays an important role in many physiological and pathological processes, including development, synaptic plasticity, immune regulation, inflammatory response, cancer and degenerative disease[17,18]. Given the role of NF-κB in inflammation, it is not surprising that NF-κB is found to be chronically active in many inflammatory diseases including inflammatory bowel disease, arthritis, sepsis and asthma. In eukaryotic cells, NF-κB also acts as a regulator for cell proliferation and survival. The role of NF-κB in apoptosis remains controversial. NF-κB has been demonstrated to promote cell proliferation and protect the cell from apoptosis[19]. The anti-apoptosis function of NF-κB most likely occurs through suppression of tumor necrosis factor α (TNF-α) signaling[20]. On the other hand, NF-κB is also suggested to up-regulate apoptosis[21], and may lead to an increase of caspase-3 activity and neuroapoptosis[22].

In the present study, the role of NF-κB in neuroapoptosis was investigated. In both Tg2576 and WT mice, the activities of NF-κB and caspase 3 were similar in CA3 pyramidal cells. The highest NF-κB activity was detected at P0, and gradually decreased with age. However, activation level of NF-κB was significantly higher in Tg2576 mice than that in WT mice after P14. The similarity in patterns of caspase-3 activation and NF-κB activity during development suggested a positive correlation between NF-κB activity and neuroapoptosis. To seek further evidence for a connection between NF-κB and caspase-3 activation, immunofluorescent double labeling was employed. The results demonstrated co-expression of caspase-3 and NF-κB in some cells, lending further credence to the notion of a connection between NF-κB activity and neuroapoptosis. The mechanism by which NF-κB acts to up-regulate neuroapoptosis has not been well understood.

Apoptosis-related proteins such as Bcl-2 and caspase-3 are suggested to be involved in the regulation of neuroapoptosis[23]. Since apoptosis is a common pathological event in AD, prevention of neuroapoptosis offers a novel therapeutic approach for treatment of the disease. Inhibition of NF-κB activity may suppress neuroapoptosis and ultimately slow the progression of AD.

In summary, a reduction in the number of hippocampal neuronal cells occurred during CNS development in WT mice, and a pathological neuronal loss occurred in Tg2576 mice. Neuroapoptosis in Tg2576 mice was more severe than that in WT mice after P14, which suggests that neuroapoptosis is correlated with AD development. A positive correlation was detected between NF-κB activity and caspase-3 activation, suggesting that NF-κB may lead to up-regulation of neuroapoptosis. Taken together, the present study suggests that inhibition of NF-κB may potentially provide a therapeutic target for AD treatment.

**Acknowledgments:** This work was supported by the National Natural Science Foundation of China (No. 30670688, 30771140), the Natural Science Foundation of Education Department of Henan Province (No. 2007180008), the Natural Science Key Program of Henan University (No. 2008YBGG048), and the International Cooperation Program of Science and Technique Department of Henan Province, China (No. 094300510044).

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# 凋亡相关基因 caspase-3 和 NF-κB 在阿尔茨海默病 Tg2576 转基因小鼠海马内的表达

牛艳丽，张维娟，吴萍，刘彬，孙国涛，于东明，李明善，邓锦波

河南大学护理学院神经生物学研究所，开封 475004

**摘要：目的** 探讨阿尔茨海默病(Alzheimer's disease，AD)的发生、发展与神经细胞凋亡之间的规律，尤其是NF-κB在神经细胞凋亡中的调节作用。**方法** 以0-180日龄的Tg2576转基因小鼠作为研究对象，正常野生型小鼠为对照，应用caspase-3和NF-κB免疫组织化学、免疫荧光双标、Nissl染色、RT-PCR等方法进行研究。**结果** 随着小鼠日龄的增长，对照组与模型组小鼠的海马CA3区锥体细胞中，凋亡细胞密度和NF-κB阳性细胞密度逐渐下降；出生14 d以后，模型组凋亡细胞密度和NF-κB阳性细胞密度均高于对照组($P < 0.05$)，且小鼠海马caspase-3 mRNA表达水平与caspase-3免疫组织化学结果基本吻合。模型组NF-κB和caspase-3的表达水平均高于对照组。**结论** 凋亡相关基因NF-κB和caspase-3在AD的发病机制中可能起重要作用，NF-κB与神经细胞凋亡有正相关关系。此外，caspase-3与NF-κB共同表达于同一细胞中，提示NF-κB的激活可能参与神经细胞凋亡的调节。

**关键词：**阿尔茨海默病；Tg2576转基因小鼠；caspase-3；海马；凋亡；NF-κB