Alzheimer's Disease and Apoptosis

Young-Hoon Kim, M.D., Ph.D.,* Hye-Sun Kim, Ph.D., Cheol-Hyoung Park,**
Sung-Jin Jeong,** Young-Kyung Kim,* Sun-Hee Kim,*
Sang-Kyeng Lee, M.D.,* Yoo-Hun Suh, M.D., Ph.D.,** Sung Su Kim, Ph.D.*†**

ABSTRACT

Apoptosis is a form of cell death in which the cells shrink and exhibit nuclear chromatin condensation and DNA fragmentation, and yet maintain membrane integrity. Many lines of evidence have shown that brain neurons are vulnerable to degeneration by apoptosis. Also it has been suggested that apoptosis is one of the mechanism contributing neuronal loss in Alzheimer's disease(AD), since the conditions in the disease(Aβ peptide, oxidative stress, low energy metabolism) are the inducers that activate apoptosis. Indeed some neurons in vulnerable regions of the AD brain show DNA damage, chromatin condensation, and apoptic bodies. Consistently, mutations in AD causative genes(Amyloid precursor protein, Presenilin-1 and Presenilin-2) increase Aβ peptide$_{1-42}$(Aβ$_{1-42}$) and sensitize neuronal cell to apoposis. However, several lines of evidence have shown that the location of neuronal loss and Aβ peptide deposition is not correlated in AD brain and transgenic mice brain over-expressing Aβ$_{1-42}$. Taken together, these data may indicated that Aβ peptide(and other causative factors of AD) can interact with other cellular insults or risk factors to exacerbate pathological mechansim of AD through apoptosis. Thus, this review discusses possible role and mechanism of apoptosis in AD.

KEY WORDS : Apoptosis · Alzheimer's disease · β-Amyloid protein · Amyloid precursor protein · Presenilin 1 · Presenilin 2.

Introduction

Apoptosis(programmed cell death), a genetically controlled cell deletion process, plays an important role in the regulation of cellular and tissue homeostasis. Furthermore, apoptosis is essential for normal development and disregulation of the process can lead a spectrum of defects ranging from embryonic lethality, to tissue specific perturbation of postnatal development, to a high susceptibility to cancer(Cohen 1993). Also, apoptosis is a common feature of the nervous system occurring physiologically during development and pathologically in several disease. Therapeutics for modulating apoptosis can provide a new possibility for the treatment of diseases including cancer, viral pathogenesis, neuronal degeneration, lymphoproliferation, inflammation, and immuno-deficiency and so on(Arends and wyllie 1991). Therefore there is a need to understand and identify both positive and negative regulators of apoptosis. Cell death can be occurred either by necrosis or apoptosis. Necrosis is usually considered to result from physical injury and is not genetically controlled, in contrast apoptosis is deliberate and genetically controlled cellular response to specific developmental and enviromental stimuli(Oppenheim 1991). The symptoms of apoptosis and necrosis are generally different. Necrosis is indicated by loss of plasma membrane integrity and cytoplasmic organelle destruction, while apoptosis is characterized by cytoplasmic membrane blebbing, chromatin condensation and nuclear DNA fragmentation. One major distinction between apoptosis and necrosis *in vivo* is that complete elimination of the apoptotic cell prevents an inflammatory response, while necrosis does not. Apoptosis can be thought of as a neat and tidy process to kill cells with minimal damage to surrounding cells or tissue (Wyllie et al. 1980). While the pathways for apoptosis and necrosis may be distinct, there is likely to be overlap and cross-

*Institute of Neuroscience and Department of Psychiatry, Medical College, Inje Univerisity, Pusan, Korea
**Department of Pharmacology, College of Medicine, Seoul National University, Seoul, Korea
†Corresponding author
Sung Su Kim, Ph.D. Institute of Neuroscience, Inje University, College of Medicine, 633-165 Kaekum-Dong, Jin-Ku, Pusan 614-735, Korea
Tel : 051-890-6749, Fax : 051-892-4463
Internet ID : sungsu@cau.ac. kr

talk between the pathway *in vivo*. An event that produces necrosis may trigger apoptosis in surrounding tissues as a result of the accumulation of cellular debris and inflammation. Likewise, it may be possible that induction of apoptosis could indirectly produce necrosis in some circumstances.

There are many ways to measure apoptosis：probably none alone are sufficient criterion but several in combination are usually definitive. Loss of cell viability（failure to either exclude vital dye or uptake MTT）, DNA fragmentation（assayed by agarose gel electrophoresis）, in situ tunnel（terminal transferase labeling）, cell and nuclear morphology（microscopy to visualize chromatin condensation, DNA organization, and cytoplasmic integrity）, cysteine protease activation（PARP or lamin cleavage *in vivo* or *in vitro*, and inhibition by cysteine protease inhibitors）and inhibition by Bcl-2 are some of means for measuring apoptosis（Lazebnik et al. 1995；Newmeyer et al. 1994）.

Regulators of apoptosis

The knowledge of the components of the apoptotic regulatory machinary, which include Bcl-2 family members（White 1996）and caspase continue to develop（Yuan et al. 1993）. Signal transduction pathways that regulate apoptosis and control the function of these family members have begun to be identified. The Bcl-2 related proteins either suppress or promote aptosis by interacting with and fuctionally antagonizing each other. These interactions either prevent caspase activation in the case of apoptosis inhibiton, or promote caspase activation in the case of apoptosis induction.

Sevaral proto-oncogenes are known to play an active part in the regulation of apoptosis. Interestingly several of them（p53, c-myc, c-fos）are trascription factors（Freeman et al. 1993）. The findings that c-fos plays a role in the apoptotic death of sympathetic neurons undergoing NGF withdrawal support the idea that apoptosis is an active process involving the synthesis or repression of specific genes（Estus et al 1994）. Still the role of reactive oxygen species（ROS）in apoptosis is not well determined. Although known to cause cell damage, one new hypothesis is that ROS may be part of a signaling system for cell death（Greenlund et al. 1995）. It is likely that several signaling pathways are able to initiate or influence the apoptotic cascade.

Is Apoptosis Involved in Alzheimer's Disease?

Alzheimer's disease（AD）, the most common dementia of the elderly, results from a significant loss of neuronal cells in brain regions important in memory and cognition. Approximately 10% of AD can be classified as early onset（<60 years）familial Alzheimer's disease（FAD）. FAD causing mutations were so far found in three genes, the APP gene on chromosome 21, the PS1 gene on chromosome 14 and PS2 gene on chromosome 1. Mutations in all three gene cause an increase of the amount of Aβ terminating after amino acid 42（Aβ_{1-42}）. Aβ is now widely believed to play an important role in AD. However APP mutations account for 5－10% of all FAD case（Van Broeckhoven 1995）. The mutations in PS1 gene contribute to about 70% of FAD. To date, 45 different PS1 mutations have been described and two FAD mutations have been identified in its close homologue PS2（Hardy 1997；Tanzi et al. 1996）. Therefore, the cell biology and pathobiology of PS proteins is of great interest for the understanding of the molecular mechanisms causing AD.

1. The Aβ toxicity

One of the most significant feature of AD is a subsequent loss of neuronal cell in the hippocampus and cerebral cortex, regions that are known to be involved in memory and cognition. This cell loss is accompanied by the neuropathological hallmarks of AD, extracellular deposit of β amyloid（Aβ-plaque）and intracellular neurofibrillary tangles（NTFs）. Several lines of evidence have led to the hypothesis that the Aβ peptide, the main component of the Aβ plaque, is directly responsible for the cell loss observed in AD. Aβ is a 39－43 amino acid peptide derived from the much longer amyloid precursor protein（APP）. Genetic studies of familial AD（FAD）identified several different mutations in or around the Aβ sequence of APP that cause AD（Chartier-Harlin et al. 1991；Citron at al. 1992；Goate et al. 1991）.

Cell death induced by Aβ has been examined *in vitro* culture system by several groups. Forloni et al.（1993）treated primary cultures of fetal rat hippocampal neurons with Aβ 25－35 on alternative days for 10 day. Significant cell death was observed at 100 uM accompanied by apoptotic fluorescent nuclear staining and oligoneucleosomal ladder. Also Loo et al.（1993）showed similar, more detail results. The primary mouse cortical or hippocampal cultures, treated continuously with Aβ, were assayed after 24 and 48 hr of exposure for viability, mitochondrial function（MTT assay）, and lactate dehydrogenase（LDH）release. At 24 hr after Aβ treatment, there was a significant decrease in cell viability. Scanning and transmission electron microscopy of the cells revealed plasma membrane blebbing and nuclear chromatin condensation with intact organ-

ellar structures. A biochemical analysis of the nuclear DNA showed the protypical oligonucleosomal ladder, which increased in intensity in a time dependent manner. These studies suggest the Aβ induce an apoptotic form of cell death in cultured neurons. However, not all studies have reported that Aβ induced an apoptotic response. Exposure of PC12 cells, both native and NGF-treated, to Aβ resulted in significant LDH release and reduction of mitochondrial activity. DNA isolated from Aβ treated cells appeared randomly degraded (Behl et al. 1994). This disagreement over the type of cell death in these systems might be owing to differences in culture conditions. Cotman et al. (1994) have reported that Aβ induces changes in intracellular Ca^{2+} levels only under certain conditions. When Aβ treated neurons in media containing 5 mM glucose were imaged for Ca^{2+}, there was a progressive increase in intracellular Ca^{2+}, levels in individual cells. Electron microscopy of the cell revealed signs of necrosis within as little as 3 hours. Interestingly when the level of glucose in the imaging media was increased to 25 mM, there was no significant increase in intracellular Ca^{2+} levels. These results suggest that type of cell death induced by a specific external insults may depend on the energy state of the cell. Several reports have established that Aβ increases the sensitivity of primary cortical neurons to changes in the extracellular milieu (Copani et al. 1991；Koh et al 1990). Apparently, the enhanced vulnerability results from disregulation of intracellular Ca^{2+} homeostasis (Mattson et al. 1992). A final explanation for the different results among culture systems may necessitate more defines data on which internal and external conditions influence cell death pathways.

2. Cell death in AD brains

Although *in vitro* studies indicate that Aβ is capable of inducing neuronal apoptosis, until recently there was little evidence that apoptosis was actually accurring in AD. However, during the past year, some groups have investigated if apoptosis occurs in AD brain. Lassmann et al. (1995) used the TUNNEL technique to identify dying cells in situ in AD brain sections. The study utilized 18 neuropathologically conformed AD cases and 15 age matched nondemented controls. Statistical analysis indicated there was no correlation between the number of TUNNEL labeled cells and either age of the individuals or the postmortem interval. The relative number of labeled cells in the temporal lobe was increased up to 50-fold in AD brain. Using double-labeling techniques, the majority of TUNNEL-positive cells appeared to be glial. However, one third of labeled cells were identified as neurons. Although there was no correlation bet-ween the number of apoptotic cells and density of Aβ plaques, there was a six fold elevation in the number of labeled cells located within areas of amyloid staining. There was also a weak correlation between TUNNEL-positive cell and NFTs. At the light microscopy level, the majority of labeled cells did not appear to show the typical unclear morphology of apoptotic cells. This implicated that the manner of cell death in AD may be the type of cell death of necrotic or an alternative forms of apoptotic cell death. Clarification of the type of cell death may require electron microscopy to examine the intracellular and nuclear morphological changes in greater detail. Using smaller sample size (nine AD, and three age-matched controls) Su et al. (1994) performed similar study. Although the authors showed no quantitative data, they did report a qualitative increase in labeled cells in AD cases. Interestingly the majority of labeled cells were neurons as identified by double labeling with AT8, and antibody to paired helical filrament (PHF). A third report from Smale et al. (1995) also examined the hippocampal formation using the TUNNEL technique. The greatest density of positive cell was found in the stratum lacunosum-moleculare of CA1 and CA3 and outer two thirds of the dentate molecular layer. Both neurons and astrocytes were labeled. However the majority of cells were identified as glial. No corelation was found between of labeled cells and Aβ plaques or NFTs. The umber of TUNNEL staining cells was significantly increased in some, but not all AD patients.

Despite some difference, there is a general consensus among the three papers. All three group report that levels of cell death were significantly elevated in AD. Finally, both glial cells as well as neurons labeled by the TUNNEL technique. Clearly, the first two observations would be expected if apoptosis were occurring in AD. The observation that glical cells are also dying in AD brain may be a normal response to increased glial cell proliferation. On the other hand, if there is an actual reduction in the number of glial support cells this may icrease the vulnerability of neurons that are dependent in these cells to environmental or physiological insults. Alternatively, it has been shown that significant loss of glial cells may iduce apoptosis in surrounding neurons (Xiong and Montell 1995).

Participation of PS1 and 2 in apoptosis：Most autosomal dominant inherited forms of early onset Alzheimer's disease (AD) are caused by mutations in the PS1 and PS2 gene on chromosome 14 and 1, respectively. PS1 and 2 are integral membrane proteins with six to nine membrane-spanning domains and are expressed in neurons throughout the brain wherein these are localized mainly in endoplasmic reticulum (ER). The mec-

hanism or mechanisms whereby PS1 and 2 mutations promote neuron degeneration in AD are unknown.

However, several lines of evidence, showing apoptotic function of PS1 and 2, has been reported recently. Initially Vito et al. showed that expression of C-terminal portin of mouse PS2 (also called ALG-3) rescued the T cell hybridoma undergoing Fas-induced apoptosis (Vito et al. 1996a). Subsequently Vito and his colleagues suggested that the PS2 gene is required for some forms of cell death in diverse cell types by showing that overxpression of PS2 in ALG-3 transfected 3DO cells reconstitutes sensitivity to receptor-induced cell death. The result implied that the artificial PS2 polypeptide functions as a dominant negative mutant of PS2 (Vito et al. 1996b). Other groups also showed the apoptotic function of PS2 using PC12 cell either transiently or stably trasfected with PS2 gene. Deng et al. (1996) showed that stable overexpression of PS2 in PC12 conferred increased sensitivity to the apoptotic stimuli stausporine and hydrogen peroxide. Similarly, transient overexpression of PS2 in nerve growth factor (NGF)-differentiated PC12 cell enhanced apoptosis induced by trophic factor withdrawal, while transfection with antisense PS2 construct rescued cells form apoptosis (Wolozin et al. 1996). Compared to PS2, little is known the function of PS1 concerning with apoptosis. However, it is likely that PS1 also related with apoptosis. Guo et al. (1997) showed that expression of the human PS1 L286V mutation in PC12 cells increases their susceptibility to apoptosis induced by trophic factor withdrawal and Aβ. Increases in oxidative stress and intracellular calcium levels induced by the apoptotic stimuli were exacerbated greatly in cells expressing the PS1 mutation, as compared with control cell lines and lines overxpressing wild-type PS1. The antiapoptotic gene product Bcl-2 prevented apoptosis after NGF withdrawal from differentiated PC12 cells expressing mutant PS1. Elevations of $[Ca^{2+}]$ in response to thapsigargin, an inhibitor of the ER Ca^{2+}-ATPase, were increased in cells expressing mutant PS1, and this adverse effect was abolished in cells expressing Bcl-2. Antioxidants and blockers of calcium influx and release from ER protected cells against the adverse consequences of the PS1 mutation. By perturbing cellular calcium regulation and promoting oxidative stress, PS1 mutations may sensitize neurons to apoptotic death in AD. Further work will be required to identify if PSs affect directly on ER calcium signaling alteration which possibly alter APP processing, also.

The relationship between caspase and PSs has been revealed (Kim et al. 1997). The study indentified that PSs are cleaved by caspase 3 and caspase-induced cleavage of PS2 is increased when PS2 is mutated. The hypothesis that caspase-induced cleavage of PSs plays a role in apoptosis or is only a consequence of activation of apoptotic pathways remains to be determined.

Conclusions

Many inducers of apoptosis correspond to conditions present in the AD brain, so apoptosis is highly possible pathogenic pathway in the AD. Consistently, AD brain shows many neurons with DNA damage and some of these neuron display apoptotic morphology. Aβ accumulated in AD brain can cause neuron to die by an apoptotic pathway, also. Furthermore, the presenilin genes might be involved in the regulation of apoptosis. Howeber there is discrepancy between the location of Aβ plaque and neuronal death in AD brain. So, it is still remains to be studied what is the true apoptotic fuction of Aβ *in vivo*. Similarly, it is still largely unknown how the presenilins involve in apoptotic pathway *in vivo*, so far. As the mechanisms that induce and mediate apoptosis in AD brain are elucidated, the patholgy of AD wil also be revealed. In principle, the understanding about the mechanism of neuronal cell death in AD might provide target for therapeutic strategies. Together with more accurate predictors of risk of AD, it may be possible to prevent or at least delay the onset of AD.

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