REVIEW —Current Perspective—

Apoptotic Cell Death in Neurons and Glial Cells: Implications for Alzheimer's Disease

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ABSTRACT—It is now generally accepted that massive neuronal death due to oxidative stress is a common characteristic of brains in neurodegenerative diseases. Recently, numerous apoptosis-regulating factors and multiple pathways have been identified, and apoptotic cell death has been implicated in neuronal loss in Alzheimer's disease. Although glial cells are more resistant to oxidative stress than neurons, extensive oxidative stress seems to cause apoptotic cell death in glial cells. In fact, signs of apoptosis are observed in both neurons and glial cells in the brains of patients with Alzheimer's disease. This review summarizes current findings regarding apoptotic processes and discusses the possible involvement of apoptosis-regulating factors in the pathology of Alzheimer's disease.

Keywords: Alzheimer's disease, Neuron, Glial cell, Apoptotic cell death

1. Introduction

An increased level of reactive oxygen species (ROS) in cells, referred to as oxidative stress, is cytotoxic, and this has been postulated to be the cause of neurodegenerative diseases such as Alzheimer's disease, which is characterized by the progressive deterioration of cognition and memory in association with widespread senile plaques including β -amyloid (A β) (1). With respect to A β -induced neurotoxicity, which involves the production of hydrogen peroxide (H₂O₂), A β reportedly causes apoptotic neuronal death accompanied by DNA fragmentation, both in vitro and in vivo (2). In the brains of patients with Alzheimer's disease, DNA-end labeling, a technique for visualizing DNA fragmentation, suggests that at least some neurons die by apoptosis (2, 3). Previously, cell loss in Alzheimer's disease brain was believed to be confined to neurons, with gliosis being secondary to neuronal loss and degeneration. However, recent studies have suggested that DNA end-labeled glial cells in Alzheimer's disease brain were oligodendrocytes, microglia and astrocytes (2, 3). Degeneration of microglia in senile plaques either may be related to the cytotoxic effects of A β or may reflect immune activation and self-destruction by the release of toxic substances. Glial cells produce several neurotrophic factors, and direct communication has been observed be-

tween glial cells and neurons. It is possible that the degeneration of glial cells also underlies neuronal degeneration. However, the mechanism of cell death in neurons and glial cells is still unclear.

Recently, numerous apoptosis-regulating factors have been identified, e.g., Bcl-2 family (4), apoptotic protease activating factors (Apaf) (5), caspase family (6), caspase-activated DNase (CAD) (7), p53 (8), c-Jun N-terminal kinase (JNK) (9), nuclear factor- κ B (NF- κ B) (10), tissue-type plasminogen activator (tPA) and plasminogen (11), etc. Mitochondria and ROS also regulate apoptotic cell death (12). In addition, these factors cross-talk with one another, and this interaction also participates in apoptotic cell death (Fig. 1).

2. Different susceptibility of neurons and glial cells to oxidative stress and p53

Neurons have a tenfold higher rate of oxidative metabolism than glial cells (13). On the other hand, glial cells contain more vitamin E and glutathione (GSH), more of the enzymes involved in GSH metabolism, and more superoxide dismutase than neurons. In addition, heme oxygenase-1 (HO-1) is easily induced by oxidative stress, predominantly in glial cells (14, 15). HO-1 catalyzes the production of biliverdin and its metabolite

Fig. 1. Schematic representation of multiple pathways of apoptotic cell death. $\rightarrow$: activation, $\dashv$: inhibition, A β : β -amyloid, AGE: advanced glycation end products, Apaf-1: apoptotic protease activating factor-1, CAD: caspase-activated DNase, COX: cyclooxygenases, Cyt-c: cytochrome c, dATP: 2'-deoxy-ATP, ECM: extracellular matrix, GSH: glutathione, HO-1: heme oxygenase-1, IAP: inhibitor of apoptosis protein, ICAD: inhibitor of CAD, I κ B: inhibitory protein κ B, JNK: c-Jun N-terminal kinase, NF- κ B: nuclear factor- κ B, NOS: nitric oxide synthases, tPA: tissue-type plasminogen activator.

bilirubin, which are also more potent antioxidants. Therefore, neurons seem to be more vulnerable than glial cells. However, extensive oxidative stress seems to cause apoptosis in glial cells (16).

The susceptibility to kainate (KA)-induced neurotoxicity varies among mouse strains (17). This suggests that the vulnerability of neurons may be influenced by genetic background. A transcription factor p53 protein has a critical regulatory function at the G₁-cell cycle checkpoint and in apoptosis caused by exposure to DNA-damaging substances (8). It has been reported that p53 protein induces the upregulation of pro-apoptotic Bax and the downregulation of anti-apoptotic Bcl-2, and that neurons in the hippocampal CA1 and CA4 of p53-deficient mouse, which has a mixed genetic background of the 129/Sv and C57BL/6 strains, are invulnerable to KA-induced neuronal death (17). However, our recent observations suggest that nitric oxide (NO)-induced p53 expression did not change protein levels of Bax and Bcl-2 in human neuroblastoma SH-SY5Y cells (18) and that H₂O₂-induced p53 expression caused an increase in Bak, p21^{WAF1/CIP1} and GADD45, but did not change Bcl-2 or Bax in human glioblastoma A172 cells (16). In the CBA and C57BL/6 strains, which are both invulnerable to KA-induced neurotoxicity, the p53-null mutation inhibit-

ed H₂O₂-induced glial apoptosis (16) but did not affect neuronal apoptosis. In addition, a more recent paper presents the interesting speculation that p53 protein results in apoptosis through a three-step process: i) the transcriptional induction of redox-related genes, ii) the formation of ROS, and iii) the oxidative degradation of mitochondrial components, culminating in cell death (19).

In Alzheimer's disease brain, p53 protein is expressed predominantly in glial cells (20). Similarly, protein levels of cyclin-dependent kinase-4 and its inhibitor p16 are also increased in neurons in Alzheimer's disease (21). However, the re-entry of neurons into the cell cycle may be deleterious, and may underlie neurodegenerative abnormalities, since almost all neurons in adult and senescent brains, which are terminally differentiated cells, are believed to remain permanently in the G₀ phase. These observations suggest that the vulnerability of neurons and glial cells may be influenced by endogenous antioxidant production, genetic background and the cell-cycle phase. In addition, p53 protein may play a role in glial apoptosis rather than in neuronal apoptosis, and glial apoptosis may also underlie the neurodegeneration in Alzheimer's disease brain.

3. Alteration of Bcl-2 family, caspases and transcription factors in Alzheimer's disease brain

We further examined the protein levels of the Bcl-2 and caspase families in the particulate and cytosolic fractions of the temporal cortex in Alzheimer's disease and control brains (22) (Table 1). In the particulate fraction, the levels of both anti-apoptotic Bcl-2 and Bcl-x and those of pro-apoptotic Bak and Bad were increased in Alzheimer's disease brain. Although pro-apoptotic Bax was abundantly found in the cytosolic fraction, there was no difference between Alzheimer's disease and the control. These results suggest that Bak and Bad (rather than Bax) may contribute to neuronal and glial apoptosis in Alzheimer's disease. On the other hand, upregulation of Bcl-2 and Bcl-x proteins may represent a compensatory response in Alzheimer's disease to protect remaining neurons from apoptosis. We consider that the balance of relative pro-apoptotic (Bax, Bak and Bad) and anti-apoptotic (Bcl-2 and Bcl-x) proteins may be critical to the survival of individual neurons. Therefore, neurons with increased Bak and Bad will die. Furthermore, neurons

with normal or low Bcl-2 and Bcl-x expression will die, while neurons with high levels of these proteins are prevented from entering the apoptotic pathway. Further assessment of a single cell, as by immunocytochemistry, will be necessary to determine neuronal or glial changes in these proteins in Alzheimer's disease brain.

Caspase-3 is a key enzyme in apoptosis (6, 7) and neuronal selection during embryonic brain development (23). It is known that 32-kDa caspase-3 is activated through cleavage to 12-kDa and 20/17-kDa fragments by apoptotic signals, and poly (ADP-ribose) polymerase (PARP) is subsequently cleaved to 85-kDa fragments. However, caspase-3, PARP and these fragments were either only slightly detectable or undetectable in Alzheimer's disease and control brains, compared to human neuroblastoma SH-SY5Y cells that were treated with or without apoptotic stimuli such as H_2O_2 and 1-methyl-4-phenylpyridinium (MPP^+) (Fig. 2). In the immune system, PARP cleavage and apoptosis occur even in *caspase-3*-deficient mouse, suggesting that other caspase-3-like proteases such as caspase-2 and -7 may exhibit a compensatory

Table 1. Summary of immunochemical detection of Bcl-2 family, caspases, transcription factors and enzymes in the temporal cortex of patients with Alzheimer's disease

Proteins	M.W. ^{a)} (kDa)	Alzheimer's disease brain	
		Particulate	Cytosol
<i>Bcl-2 family</i>			
Bcl-2	27	++ (1.8*) ^{b)}	—
Bcl-x	29/30	++ (1.9*)	+ (1.0)
Bax	21	—	+ (0.8)
Bak	30	++ (3.2*)	+ (1.0)
Bad	23	++ (2.5*)	+ (1.4)
<i>Caspase family</i>			
caspase-2	48	—	++ (1.1)
caspase-3	32	±	±
<i>Transcription factors</i>			
NF-κB p65	65	—	+ (1.7*)
STAT1	91	+ (2.1*)	+ (1.9)
p53	53	—	+ (2.1*)
PARP	116	—	—
<i>Enzymes^{c)}</i>			
iNOS	130	n.d.	— or ±
COX-2	70	+ (1.8*)	—

^{a)} M.W., molecular weight. Detectable (+), faintly detectable (±) or undetectable (—) by densitometrical analysis of each immunoblot (Refs. 20, 22, 25). ^{b)} The number in parentheses is the ratio of the protein levels in Alzheimer's disease vs control. *, significant change. ^{c)} Unpublished observations. n.d., not determined.

Fig. 2. Caspase-3 and poly (ADP-ribose) polymerase (PARP) in human neuroblastoma SH-SY5Y cells and Alzheimer's disease brain. SH-SY5Y cells were treated for 3 days with vehicle (lane 1), 0.1 mM H_2O_2 (lane 2) or 1 mM 1-methyl-4-phenylpyridinium (MPP^+) (lane 3). Cytosolic (lanes 4 and 6) and particulate fractions (lanes 5 and 7) from control (lanes 4 and 5) and Alzheimer's disease brains (lanes 6 and 7). Samples (10 μ g protein/lane) were subjected to immunoblotting with antibodies against pro-enzyme/active fragments of caspase-3 (Pharmingen, A) and the caspase-3 cleavage site of human PARP (Upstate Biotechnology, B). In SH-SY5Y cells, apoptotic stimuli (H_2O_2 and MPP^+) induced decreases in protein levels in 32-kDa caspase-3 and 116-kDa PARP, and then these fragments cleaved into 20/17-kDa active forms of caspase-3 and a 85-kDa PARP fragment. In the control and Alzheimer's disease brains, 32-kDa caspase-3 was slightly detectable, while 116-kDa PARP and these fragments were undetectable.

response (23). In fact, there is more caspase-2 than caspase-3 in Alzheimer's disease and control brains, but there was no difference between Alzheimer's disease and the control (22) (Table 1). In comparison with neuronal apoptosis in the embryo, neurons in the brains of patients with Alzheimer's disease are terminally differentiated. Therefore, caspases or pathways involving molecules other than caspase-3 may underlie neuronal apoptosis in Alzheimer's disease brain.

Transcription factors such as NF- κ B and c-Jun are known to play harmful and helpful roles in neuronal viability (10, 24). c-Jun phosphorylated by JNK (Ser73 and Ser63), rather than non-phosphorylated c-Jun, may participate in neuronal apoptosis (9). In Alzheimer's disease brain, the expressions of NF- κ B and c-Jun were significantly enhanced (24, 25), suggesting these transcription factors also may participate in neuronal apoptosis. In addition, activation of NF- κ B and signal transducer and activator of transcription-1 (STAT1) in glial cells may underlie the inflammatory events in Alzheimer's disease brain (25, 26) and the induction of both inducible NO synthase (iNOS) and cyclooxygenase (COX-2) in glial cells. The inflammatory events in the brain are also believed to participate in neurodegeneration in Alzheimer's disease (26).

4. Other possibilities involving NOS, COX and degradation of extracellular matrix (ECM) in neurodegeneration

NO synthases such as nNOS and iNOS catalyze to produce massive amounts of NO and its oxidant peroxy-nitrite, which also participate in neuronal degeneration in Alzheimer's disease brain (27). Although nNOS is constitutively expressed in neurons of human brains, iNOS induction by cytokines and lipopolysaccharide is less potent in humans than in rats and mice. In our previous study, 130-kDa iNOS was faintly detectable in the cytosolic fraction from a patient with Alzheimer's disease, but was undetectable in 5 other patients and 6 controls (25). However, since iNOS immunoreactivity in astrocytes is associated with A β plaques (28), iNOS protein may be expressed in the particulate fraction from Alzheimer's disease brain.

ROS is formed during the enzymatic reaction of COX to produce prostaglandins from arachidonic acid. In Alzheimer's disease brain, it has been reported that both the mRNA expression and protein level of COX-2 are increased (29). We found a similar result that the levels of both COX-1 and COX-2 proteins in AD brain are significantly higher than those in the control (unpublished observation). In addition, the long-term administration of a non-steroidal anti-inflammatory drug (NSAID), indomethacin, to patients with Alzheimer's disease appears to slow the course of the disease (30). Therefore, COX-2-

mediated neurodegeneration is believed to participate in the pathology of Alzheimer's disease. In contrast, COX-2 also underlies the inhibition of apoptosis in mouse macrophages. Thus, COX-2 has both helpful and harmful effects. A recent interesting paper suggests that COX-2 induces an increase in adhesion to an ECM protein such as laminin (31). Laminin is constitutively expressed in hippocampal pyramidal layers in rats and mice (11) and its ECM protein is degraded by the extracellular tPA-plasminogen system (11). Thus, COX-2 may be involved in maintaining the interaction between neurons and ECM in pyramidal layers under normal conditions. In the pathological state, however, COX-2 may participate in neurodegeneration.

Thus, numerous apoptosis-regulating factors and multiple pathways have been identified by *in vitro* assay. However, it is unknown what kinds of pathways predominantly underlie the apoptotic process *in vivo*. In contrast to the immune system, since almost all of the neurons in the human senescent brain are terminally differentiated, an analysis of neuronal apoptosis *in vivo* is more difficult. Further detailed studies on apoptotic processes in Alzheimer's disease brain are necessary before definite conclusions can be drawn. Elucidating these mechanisms in detail will aid in the development of useful therapeutics or treatments with which to combat Alzheimer's disease.

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