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APOPTOSIS IN ACUTE AND CHRONIC NEUROLOGICAL DISORDERS

Oleksandr Ekshyyan and Tak Yee Aw

Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport, Louisiana

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1. ABSTRACT

Programmed cell death or apoptosis is a physiologically important process in neurogenesis wherein ~50% of the neurons apoptose during maturation of the nervous system. However, premature apoptosis and/or aberrations in apoptosis control contribute to the pathogenesis of a variety of neurological disorders including acute brain injury such as trauma, spinal cord injury, ischemic stroke and ischemia/reperfusion as well as chronic disease states such as Alzheimer's, Parkinson's, Huntington's, amyotrophic lateral sclerosis, spinal muscular atrophy, and diabetic neuropathy. The current review will focus on two major topics, namely, the general concepts of our current understanding of the apoptosis death machinery, its mediators and regulation, and the relationship between aberrant apoptosis and genesis of neurodegenerative disorders. This knowledge of apoptosis mechanisms will underpin the basis for development of novel therapeutic strategies and treatment modalities that are directed at control of the neuronal apoptotic death program.

2. INTRODUCTION

Apoptosis is an orchestrated form of physiological cellular suicide common in a variety of biological processes, such as embryogenesis, synaptogenesis, immune response, and normal tissue and organ involution. Apoptosis is best defined by distinct morphological changes characterized by chromatin condensation, cytoplasmic and nuclear compartment shrinkage, vesicular apoptotic body formation and DNA degradation into oligonucleosome-length fragments. Apoptosis of targeted cells within a tissue is mediated by activation of cell signaling that results from engagement of the apoptotic stimuli and cell surface death receptors or from direct disruption of the mitochondria and the subsequent activation of executioner caspases. This organized apoptotic death program is distinguished from necrotic cell death which occurs in random and involves the loss of plasma membrane integrity, increased cell swelling and lysis. In recent years, the recognition that aberrant apoptosis may contribute to the pathogenesis of neurodegeneration has spurred apoptosis research in this field. The current chapter will focus on the specific concepts and aspects salient to understanding the control of cellular apoptosis that are relevant to acute and chronic neurological disorders.

3. THE APOPTOSIS DEATH MACHINERY

3.1. Signaling via death receptors and mitochondria

Apoptosis commitment at cell surfaces occurs through death receptor clustering. Death receptors are unique cell surface sensors that belong to the tumor necrosis factor (TNF) gene superfamily of which Fas (also called CD95) and TNF receptor 1 (TNFR1) are the two best known. These receptors are defined by cysteine-rich extracellular domains and homologous cytoplasmic death domains, and are activated by ligands of structurally related molecules of the TNF gene superfamily (1). Activation steps in the apoptotic cascade involve formation of the death inducing signaling complex (DISC), recruitment of adaptor proteins, and proteolytic cleavage and activation of caspases-8 and-3 (1-3). The interaction of TNF or Fas Ligand with their respective receptors and the associated adaptor proteins, the TNFR-associated or Fas-associated

death domains (TRADD and FADD) independently activates the mitogen activated protein kinase (MAPK) and the NFkappaB pathways (4). The TNF-alpha/TNFR1 and the Fas/Fas Ligand are the two best-described death receptor pathways in the immune system, and mutations of the CD95 or CD95 Ligand death receptor system in mice and humans lead to fatal autoimmune syndromes that result from an inability to eliminate self-reactive lymphocytes by apoptosis (3). The role of death receptor activation in neuronal apoptosis associated with neurological disorders is unclear.

Mitochondrial signaling typically mediates cell apoptosis induced by oxidative stress (5). The central paradigm in this process is the release of cytochrome *c* which couples with the apoptotic protease activating factor-1 (Apaf-1) and procaspase-9 to form the apoptosome complex during activation of caspase-3 (6). Mitochondrial cytochrome *c* release occurs by opening of a specific channel, the permeability transition pore in the mitochondrial membrane. Current evidence favors loss of the mitochondrial inner transmembrane potential (Delta psi) and transition pore opening in apoptosis onset (5). Members of the BcL-2 family are the gatekeepers of channel-mediated mitochondrial loss of cytochrome *c* (6), and the balance between the expression of pro-apoptotic members (*e.g.*, the BH3 and Bax subfamilies) and anti-apoptotic members (BcL-2 and BcL-xL subfamilies) controls the permeability transition pore function and mitochondrial cytochrone *c* release (5). Other contributing mitochondrial soluble factors include apoptosis inducing factor, pro-apoptotic Smac/DIABLO protein, procaspases, and specific inhibitor apoptosis proteins, IAPs (3, 5). Reactive oxygen species (ROS) have been implicated as modulators of mitochondrial apoptotic signaling. Consistent with this contention, a disturbance of the electron transport chain which promotes ROS production has been shown to be intricately linked to the loss of mitochondrial cytochrome *c* (7). The combination of the mitochondrial ROS signaling mechanism as well as the pathway of cytochrome *c*-caspase-3 activation would together, guarantee cell death. In cerebral ischemia/reperfusion, an ROS mechanism of apoptosis signaling is expected to contribute to post-ischemic neuronal cell death.

3.2. Caspases: Apoptosis initiation and execution

Caspases are cysteine proteases where enzyme catalysis is dependent on four amino acids N terminal to the cleavage site at the aspartate residues (8). Caspase members that are associated with cell apoptosis belong to the groups II and III classes that include caspases 3, 8, and 9 with caspase-8 and 9 serving as the respective initiators in the death receptor and mitochondrial pathways (8). Caspase activation is a tightly regulated process which is achieved through autocatalysis through interactions with adaptor proteins. Activation has been shown to be modulated by specific decoy proteins; pro-caspase-8 activation is prevented by the FADD-like ICE inhibitory proteins which share sequence homology with procaspase-8 but lack the catalytic domain while pro-caspase-9 activation is modulated by the apoptosis repressor or inhibitor proteins (8). Caspase-3 is the best characterized downstream effector caspase with forty known substrates. Among these are the inhibitor of caspase-activated deoxyribonuclease, the BcL-2 family of proteins, cytoskeletal proteins like gelsolin, focal adhesion kinase and p21-activated kinase, and proteins involved in DNA repair, mRNA splicing and DNA replication (3, 8). Because of their defined function, cysteine proteases are a popular target protein class for strategic development of inhibitors and mimics in various diseases including apoptosis associated inflammatory disorders and stroke.

3.3. Mediators of cellular apoptosis

Oxidants are well known triggers of apoptosis of which hydrogen peroxide (H_2O_2) is best studied (9). Physiologically important H_2O_2 producers include mitochondrial and extra-mitochondrial sources, such as cytoplasmic cytochrome P-450 and membrane bound NADPH oxidase. Popular paradigm supports H_2O_2 as a mediator of mitochondrial membrane potential collapse that leads to the release of cytochrome *c* and the activation of caspase-9 (7, 9) although existing evidence also implicate H_2O_2 in apoptosis initiation in the Fas/Fas Ligand signaling pathway. Support for this latter mechanism comes from the findings that chemotherapeutic drugs induce intracellular ROS production and Fas upregulation (10) and that ROS directly upregulate the Fas Ligand (11). There is evidence that low concentrations of H_2O_2 can directly activate caspases while prolonged H_2O_2 exposure inactivates caspases through oxidation of critical sulfhydryl moieties (12). A role for cellular redox in apoptosis is supported by the findings that glutathione (GSH) decreases were associated with enhanced cellular apoptosis (13) and GSH increases were associated with expression of BcL2 (14). More recently, we documented that it is the change in cellular GSH-to-GSSG ratio rather than changes in GSH *per se* that specifically mediated cell apoptosis and that this redox shift preceded caspase-3 activation (15). Furthermore, changes in GSSG-to-GSH ratio rather than GSH alone uniquely activate the nuclear transcription factor, kappa B (NFkappaB) (16). These results underscore an important mechanistic distinction between apoptosis regulation by GSH or by the GSH-to-GSSG (or redox) status. Among the components of the apoptotic machinery, the mitochondrial permeability transition pore and caspases are susceptible targets for redox control. Disulfide crosslinking in the transition pore alters the gatekeeping function of BcL-2 (17), while oxidation or thiol alkylation of cysteine active sites in caspases results in loss of enzyme activity (9). An anti-apoptotic role for the thioredoxin/thioredoxin reductase system in cellular apoptosis has been described. The findings that expression of redox-inactive thioredoxin promoted apoptosis and overexpression of thioredoxin protected cells from apoptotic death (18) are consistent with this notion. The extent to which redox contributes to neuronal apoptosis that is associated with acute cerebral ischemic insults or neurodegenerative disorders *in vivo* is not known. In recent studies, a role of cellular redox in oxidant-mediated apoptosis has been defined in

the mitotic competent undifferentiated PC-12 cell model (19 - 21).

While TNF-alpha is best known for its role in inflammation, it has been shown to induce apoptosis in non-immune tissues via the death domain of its cell surface receptor, TNF-R1. In the pathogenesis of neurological diseases, TNFα has been implicated in infectious and immunological conditions such as multiple sclerosis, bacterial meningitis, and AIDS, as well as non-infectious, acute cerebral insults like ischemic stroke (22). In addition, TNF-alpha has been shown to participate in chronic neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, and cerebral degeneration (22). A duality for nitric oxide (NO) in cellular apoptosis has been described. NO can promote or inhibit cellular apoptosis depending on the cell type, the concentration of NO and the nature of the apoptotic stimuli. The generally accepted view is that NO is anti-apoptotic as evidenced by studies showing that inhibition of endogenous NO synthase enhanced apoptosis (23), and activation of inducible nitric oxide synthase inhibits cell apoptosis (24). NO acts on cysteine caspases wherein S-nitrosylation of the cysteine thiol results in the formation of the signaling species, R-S-NO (9). This NO-mediated nitrosylation and denitrosylation in caspase-3 activation (25) is akin to protein phosphorylation/dephosphorylation in kinase activation. The redox control of the transcription factors, NFkappaB and activator protein-1 (AP-1) may also be mediated by S-nitrosylation.

NFkappaB is a well studied nuclear transcription factor in cellular apoptosis. Support for an anti-apoptotic function comes from studies showing that mice deficient in the NFkappaB gene die embryonically from extensive cellular apoptosis (26). The findings that NFkappaB activation was protective against TNF-induced apoptosis while NFκB inhibition sensitized TNF-induced death (4) and that inhibition of NFkappaB activation promoted H_2O_2 and superoxide-induced apoptosis (27) are consistent with an anti-apoptotic role. The paradigm that NFkappaB can mediate aspects of apoptosis is less well documented. AP-1 has been reported to be a pathophysiological regulator of apoptosis in the endothelium (28).

4. APOPTOSIS AND NEURODEGENERATIVE DISORDERS

4.1. Apoptosis involvement in neurodegeneration

During normal development of the central nervous system, ~50% of neurons are eliminated by apoptosis. Naturally occurring neuronal death is characterized by increased intracellular caspase activation and defined apoptotic morphology. Impairment in apoptosis regulation that results in premature cell death during ontogenesis can lead to pathological consequences and development of neurodegenerative disorders. Current evidence supports a role for apoptosis in a variety of neurological disorders.

4.1.1. Alzheimer's disease

Alzheimer's disease (AD) is a neurological condition characterized by progressive loss of memory and intellectual function. The pathological hallmarks of AD include extracellular amyloid plaques and intraneuronal fibrillar structures such as neurofibrillary tangles, neuropil threads, and dystrophic neuritis invading amyloid plaques. A role for apoptosis in AD is supported by clinical as well as experimental evidence. Elevated incidence of apoptosis and coincidence of DNA fragmentation with increased c-Jun protein (29), expression of early proapoptotic gene (30), decreased expression of BcL-2 concomitant with increased Bax (31), and the presence of activated caspase 3 within autophagic granules (32) in the brain of AD patients are consistent with enhanced apoptotic activity. Beta-amyloid involvement in neuronal apoptosis is well studied in cultured neurons wherein beta-amyloid causes membrane blebbing, nuclear chromatin condensation, and caspase activation (33 - 36). Mechanistically, beta-amyloid induces neuronal apoptosis by an oxidative mechanism. Treatment of neuronally differentiated neuroblastoma cells with ß-amyloid peptides induced an early and simultaneous production of H_2O_2 and 4-hydroxynonenal (HNE), followed by activation of JNK and p38 MAP kinases and nuclear changes characteristic of apoptosis (37). Cell apoptosis and disease progression were prevented by antioxidants such as alpha-tocopherol and N-acetylcysteine, and elevated lipid peroxidation (38) and decreased glutathione transferase activity (39) have been detected in AD brain or cerebrospinal fluid, consistent with an involvement for oxidative stress in AD pathogenesis. Whether oxidative stress is causal or coincidental in neuronal apoptosis in AD development remains to be defined.

The role for presenilin (PS1) in neuronal apoptosis in AD pathology has been demonstrated in cell and mutant mouse models. Mutant PS1 proteins were shown to enhance neuronal susceptibility to various insults such as trophic-factor withdrawal, beta-amyloid or glutamate exposure, and energy deprivation (40). An involvement of PS1 in apoptosis under oxidative stress and mitochondrial dysfunction in the AD brain was evidenced by the blockade of mutant PS1-mediated apoptotic induction by agents that prevent oxidative stress and intracellular calcium elevation (41). Apart from parenchymal damage, cerebrovascular lesions such as cerebral endothelial microvascular degeneration and cerebral amyloid angiopathy have been documented in AD cases, indicating an involvement of cellular elements of the blood brain barrier (42). Notably, barrier dysfunction is most evident in AD patients who exhibit peripheral vascular defects associated with hypertension, diabetes or cardiovascular disease. The mediators of brain endothelial apoptosis and dysfunction are unknown, and it is unclear whether cerebrovascular lesions are causal in AD pathogenesis.

4.1.2. Parkinson's disease

Parkinson's (PD) is a progressive neurodegenerative disease characterized by a loss of dopaminergic neurons in the substantia nigra pars compacta. Familiar PD (~5%) is linked to mutations in alpha-synuclein, parkin, and ubiquitin C-terminal hydrolase L1. A pathogenic role for apoptosis in PD is supported by

findings of activated caspase-3, -8 and –9 and higher activities of caspases-1 and -3 in dopaminergic substantia nigra (SN) neurons in PD brains (43, 44). The neurotoxin (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydro-pyridine, MPTP) mouse model is currently the most widely used experimental animal model of PD wherein drug administration increases apoptotic neurons in SN. The findings that BcL-2 overexpression (45) and Bax deficiency (46) attenuates MPTP neurotoxicity are consistent with involvement of an apoptotic pathway. Furthermore, MPTP neurotoxicity was diminished in transgenic mice deficient in the proapoptotic p53 gene (47). An oxidative mechanism for neuronal apoptosis has been suggested to involve ROS generation, inhibition of mitochondrial complex I activity, and caspase-3 activation (43, 48).

A role for alpha-synuclein in neuronal apoptosis is supported by studies showing that alpha-synuclein deficiency in mice prevents MPTP-induced neurodegeneration (49). Overexpression of mutant alpha-synuclein promoted apoptosis (50) in association with elevated 8-hydroxyguanine, protein carbonyls, lipid peroxidation and 3-nitrotyrosine, consistent with oxidative stress (51). The findings that mutant alpha-synuclein overexpression enhanced cell susceptibility induced by serum deprivation, H_2O_2, MPP^+, lactacystin or staurosporine are consistent with the notion that alpha-synuclein mutation promoted vulnerability of dopaminergic SN neurons in PD to toxic agents (52). The formation of alpha-synuclein-immunopositive inclusion-like structures within the mitochondria and increased free radical production suggests a role for alpha-synuclein in mediating mitochondrial alterations and cellular oxidative stress (53). The selective vulnerability of dopaminergic neurons in parkinsonian SN is believed to be mediated by generation of quinone species, superoxide radicals and H_2O_2 (54). Consistent with a state of oxidative stress in PD brain, tissue GSH and the GSH-to-GSSG ratio were significantly decreased while lipid, protein and DNA oxidations and iron contents were elevated in the SN region (51, 55, 56).

4.1.3. Huntington's disease

Huntington's disease (HD) is an autosomal dominant disorder characterized by selective neuronal loss in the striatum and the cerebral cortex. A pathogenic role of apoptosis in HD is supported by findings of activated caspases and mitochondrial cytochrome *c* loss in brain tissues of HD patients and experimental HD models (57 - 59). The slowing of disease progression in transgenic mouse models of HD with caspase inhibitors (58, 60) further support an involvement of apoptosis in disease pathogenesis. HD is caused by a mutation within the huntingtin gene through expansion of CAG repeats (61). While wild-type huntingtin protects against apoptosis by inhihiting procaspase-9 processing (62), mutant huntingtin induces death by activating caspases (59, 63). The proapoptotic function of mutant huntingtin is attributed to its caspase-generated toxic N-terminal fragments containing the expanded polyglutamine tract. Thus, during HD progression, cell survival is attenuated by the continual caspase cleavage of wild-type huntingtin and the formation of toxic fragments (64). Aberrant interaction of mutant huntingtin with huntingtin interacting protein (HIP-1) also contributes to HD pathogenesis. In this regard, overexpression of native huntingtin to sequester HIP-1 reduces its cytotoxicity (65).

4.1.4. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by selective and progressive degeneration of motor neurons. About 20% of familial ALS are linked to mutations of the Cu/Zn superoxide dismutase (SOD1) gene (66). Notably, the neuronal degeneration associated with SOD1 mutation is attributed to a gain of new toxicity rather than a reduced SOD1 activity (67). Current evidence supports a pathogenic role for apoptosis in ALS. For instance, the expression of apoptotic markers, such as, LeY antigen and fractin as well as the prostate apoptosis response-4 (Par-4) protein were detected in the spinal cord of ALS patients (68) and transgenic SOD1 mice (69). Accordingly, antisense treatment with complementary Par-4 oligonucleotide attenuated mitochondrial dysfunction and the accompanying oxidative stress-induced apoptosis (70). In addition, ALS is associated with decreased BcL-2 and increased Bax expression (71), and BcL-2 overexpression results in delayed neurodegeneration and motor neuron death (72).

4.1.5. Spinal muscular atrophy

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by progressive atrophy of the voluntary muscles of the limb and trunk consequent to degeneration and loss of spinal motor neurons. SMA is caused by mutations in the survival motor neuron (SMN)1 gene (73) wherein disease severity correlates with the homozygous deletions of the telomeric neuronal apoptosis inhibitory protein (NAIP) gene. NAIP has been shown to inhibit caspase-3 and caspase–7 (74), implicating its role in apoptosis. Full-length SMN displays antiapoptotic properties and inhibits apoptotic cell death triggered by withdrawal of trophic support that is related to the suppression of mitochondrial activation of caspases. The anti-apoptotic effect of full-length SMN and the proapoptotic effect of mutant SMN have been documented *in vitro* and *in vivo* (75). The interaction of the exon 6 region of SMN with the BH4 domain of Bcl-2 proteins confers a synergistic anti-apoptotic effect against Bax- or Fas-mediated apoptosis ((76). Accordingly, SMN with a missense mutation at exon 6 or lacking exon 7 failed to synergize the anti-apoptotic activity of BcL-2 (76). The C-terminal region of the SMN protein is pivotal for survival. SMN mutants resistant to caspase cleavage exhibit enhanced survival while cleavage of the C-terminal of SMN (which include exon 6 and exon 7) by caspases induces a proapoptotic phenotype via generation of a proapoptotic fragment (75). Interestingly, SMN also binds the proapoptotic tumor suppressor protein p53, and point mutations of full-length SMN or deletion of the SMN1 gene increases p53 proapoptotic activity (77). Thus, pathological changes in specific exons in SMN alter its

interactions with BcL-2 and p53 that results in ~~anti- or~~ pro-apoptotic phenotypes.

4.1.6. Diabetic neuropathy and retinopathy

Diabetic neuropathy and retinopathy are important complications of diabetes mellitus. While a pathogenic role of apoptosis in disease process is poorly understood, patients with type 2 diabetes do exhibit elevated activities of caspase-1, -3, -4, and -6 in the retinas (78), consistent with enhanced apoptotic activity. Moreover, hyperglycemia has been shown to induce apoptosis in neuronal cells that is characterized by activated caspase-3, reduced BcL-2 and mitochondrial cytochrome *c* loss (79). An oxidative mechanism is implicated in that hyperglycemia promotes mitochondrial superoxide production and the formation of advanced glycation end products (AGEs). Apoptosis induction by AGEs has been described in cultured cortical neurons (80); however, the extent to which AGEs induces vascular and neuronal apoptosis *in vivo* remains to be defined.

4.2. Apoptosis in cerebral stroke and ischemia/reperfusion

Although current dogma views ischemia/reperfusion (I/R) as an inflammatory process, growing evidence implicate apoptotic death in I/R-induced microvascular and parenchymal cell death and organ failure (81). Excessive apoptosis has been linked to nerve cell loss in stroke. That apoptosis mediates neuronal death in cerebral I/R is supported by the findings of elevated caspase-3 (82) and protection by caspase inhibitors (83). Other evidence implicates oxidative stress as a trigger of I/R-induced neuronal apoptosis (84), and the involvement of MAPKs and NFkappaB in apoptotic signaling (85). The possibility that endothelial apoptosis can mediate aspects of post ischemic cerebrovascular pathology suggests that the pathophysiology of I/R-induced cerebral dysfunction is likely to involve apoptosis of both neuronal and microvascular endothelial cells. Evidence of apoptotic cells and activated caspase-3 has been described in various models of traumatic brain injury (86 - 88). Accordingly, intracerebro-ventricular administration of the caspase-3 inhibitor (z-DEVD-fmk) before and after the injury reduced post-traumatic cell apoptosis and significantly improved neurological recovery. Involvements of the mitochondrial and death receptors pathways are implicated. Activation of mitochondrial apoptotic signaling is indicated in cold injury-induced brain trauma (89), traumatic axonal injury (90), and controlled cortical impact (91). The findings of increased Fas immunoreactivity in cortical astrocytes and neurons as well as increased FasL expression in cortical neurons, astrocytes, and microglia after unilateral cortical impact injury (92) are evidence of death receptor involvement. The presence of apoptotic cells or activated caspases in spinal white matter in animal models (93), and in human patients (94) is consistent with elevated apoptotic activity in spinal cord injury. Treatment with caspase inhibitor or overexpression of the caspase-1 dominant negative mutation in transgenic mice results in significant improvement of motor function and reduction of lesion size (95). Mitochondrial apoptotic signaling is implicated that involves Ca^{2+} elevation and calpain up-regulation, increase in Bax: BcL-2 ratio, release of cytochrome *c*, and caspase-3 activation (96).

5. SUMMARY AND PERSPECTIVE

Recent interest in cerebral apoptosis comes from the recognition of its role in disease pathogenesis in neurodegeneration and trauma or ischemia-induced cerebral injury. The notion that neuronal apoptotic death may play a center role in disease or injury progression has spurred research efforts into development of intervention strategies directed at apoptosis regulation in the neuronal apoptotic death program. On the basis of existing evidence and their defined roles in the apoptotic pathway, much attention has focused on interventions directed against caspases and the BcL-2 protein family. Our more recent understanding of redox control and delineation of redox sensitive molecular components of the apoptotic death machinery should provide an important conceptual challenge for future research. Additionally, the documented involvement of astrocytes, oligodendrocytes and endothelial cells in models of acute cerebral injury suggests new research avenues to investigate the contribution of non-neuronal cell types to cerebral pathophysiology of a variety of acute and chronic neurological disorders.

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Send correspondence to: Tak Yee Aw, PhD, Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport Louisiana 71130-3932, Tel: 318-675-6032, Fax: 318-675-4217, E-mail: taw@lsuhsc.edu