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Apoptosis and Aging

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I. Introduction

Since its initial discovery by Kerr *et al.* (1972) as a mechanism of cell death distinct from necrosis, the process of "apoptosis" has been the subject of intense scientific interest, and deregulation of apoptosis has been implicated as a fundamental pathogenic mechanism in a myriad of aging-related human diseases. For example, inefficient elimination of malignant or autoreactive cells can result in the development of cancer or autoimmune diseases. On the other hand, excessive apoptotic cell death may result in aberrant cell loss and organ atrophy, pathological events that underlie neurodegenerative diseases, cardiovascular dysfunction, muscle atrophy, intestinal disorders, and kidney disease. Indeed, the numerous and wide-ranging manifestations associated with dysregulation of apoptosis reflect the complexity of apoptotic signaling and the importance of its role in organismic homeostasis.

Unlike necrosis, which usually results from severe trauma to the cell and is manifested by an uncontrolled breakdown of cellular and organelle structure, cell lysis, and an inflammatory response, apoptosis can be induced by mild signals and occurs through an ATP-dependent, gene-driven, non-inflammatory process (Majno & Joris, 1995). Morphologically, necrosis exhibits cell swelling and loss of membrane integrity, whereas apoptosis is characterized by cell shrinkage, formation of membrane-enclosed bodies, preservation of organelles, and maintenance of membrane integrity. Phagocytosis of the apoptotic bodies by macrophages prevents an inflammatory response. Apoptosis is also characterized by chromatin condensation and margination and by fragmentation of the nuclear DNA into integer multiples of the internucleosomal length (approximately 180 bp) (Wyllie, 1980). Biochemically, apoptosis is accompanied by the *de novo* expression of a spectrum of genes that facilitate the execution of the cellular suicide program and/or the processing of preexisting gene/protein entities (such as caspase) into functional mode.

Apoptosis is necessary during development when excess cells need to be removed, for example, during organ morphogenesis. However, with age, the

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exquisite control of gene expression for apoptotic events may not be finely tuned, resulting in either retaining cells that should be eliminated or losing cells that should be retained. We hypothesize here that cells that inappropriately fail to die (apoptosis-resistant cells) and cells inappropriately prone to apoptotic death (apoptosis-susceptible cells) are dangerous to the tissues in which they reside. The former cells may act as seeds for transformation and neoplastic growth, whereas the absence of the latter may compromise tissue function. The activation or repression of "killer" or "survival" factors that modulate the regularity of apoptotic activity may be crucial to understanding the etiology of some age-dependent debility. Investigation of the molecular mechanisms leading to dysregulated apoptosis may provide tools necessary to the prognosis, diagnosis, and eventual treatment of age-related diseases such as cancer, Alzheimer's disease, and cardiovascular degeneration.

The past decade has seen an explosion of research into the biochemical signaling pathways of apoptosis. In this review, we provide an overview outlining important findings in the molecular mechanisms of apoptosis in general. We further discuss how these pathways may be initiated or circumvented in cells that exhibit apoptotic sensitivity or resistance, respectively, and the relevance of these phenotypes to aging and aging-associated diseases.

II. General Molecular Mechanism of Apoptosis

A. Caspases

A common end event in the execution of apoptotic cell death is the activation of a family of proteases called caspases [reviewed in Cohen (1997)], a family of cysteine proteases that mediate proteolysis of a variety of intracellular proteins at specific aspartate residues. Among the many caspase substrates that have been identified are poly(ADP-ribose) polymerase (PARP) (Lazebnik *et al.*, 1994), lamin A (Orth *et al.*, 1996), U1 70-kDa small nuclear ribonucleoprotein (Casciola-Rosen *et al.*, 1996), actin (Mashima *et al.*, 1995), fodrin (Martin *et al.*, 1995), and the retinoblastoma tumor suppressor Rb (Janicke *et al.*, 1996). The first mammalian caspase to be described, a protein previously known as interleukin–1β converting enzyme (ICE, now designated caspase 1), was identified as playing a role in apoptosis by virtue of its homology with the proapoptotic *Caenorhabditis elegans* protein, *ced-3* (Yuan *et al.*, 1993). Ten caspase family members have since been identified; caspases 3 and 7 are the key effector caspases upon which divergent apoptotic signaling pathways appear to converge. Overexpression of caspase 1 or 3 is sufficient to trigger apoptosis (Miura *et al.*, 1993), and caspase 3 knockout mice exhibit an embryonic lethal phenotype due to abnormal brain formation caused by the inability to diminish neuronal numbers during development (Kuida *et al.*, 1996).

Caspases are synthesized as proenzymes with variable prodomains and an enzymatic domain. Activation occurs by posttranslational cleavage within the enzymatic domain at specific aspartate residues, yielding large and small subunits; two large and two small subunits subsequently associate into an active heterotetrameric complex. Removal of the prodomain is not necessary for proteolytic activation, nor is its presence necessary for enzymatic activity (Salvesen & Dixit, 1997; Villa *et al.*, 1997). Because activation requires cleavage at specific aspartate residues, an ability unique to the caspase family, activation can only be mediated through autoactivation or by another caspase.

Once the activation of caspases is effected, cell death appears to be inevitable. Therefore, researchers have turned to studying the upstream mechanisms that initiate the activation of caspases. A plethora of data now suggests that activation of caspases can be mediated through proapoptotic receptor signaling complexes at the cell surface, by mitochondrion-dependent processes within the cytosol, or by p53-dependent processes originating in the nucleus (Fig. 1).

B. Receptor-Dependent Caspase Activation

Proapoptotic signals can be transmitted from the extracellular milieu by associating extracellular death signal ligands with their respective receptors on the cell surface. Among the best characterized of the cell-surface-initiated apoptotic pathways is that initiated by the tumor necrosis factor receptor family, of which five members have been identified. These receptors include Fas, TNFR1, DR3, DR4,

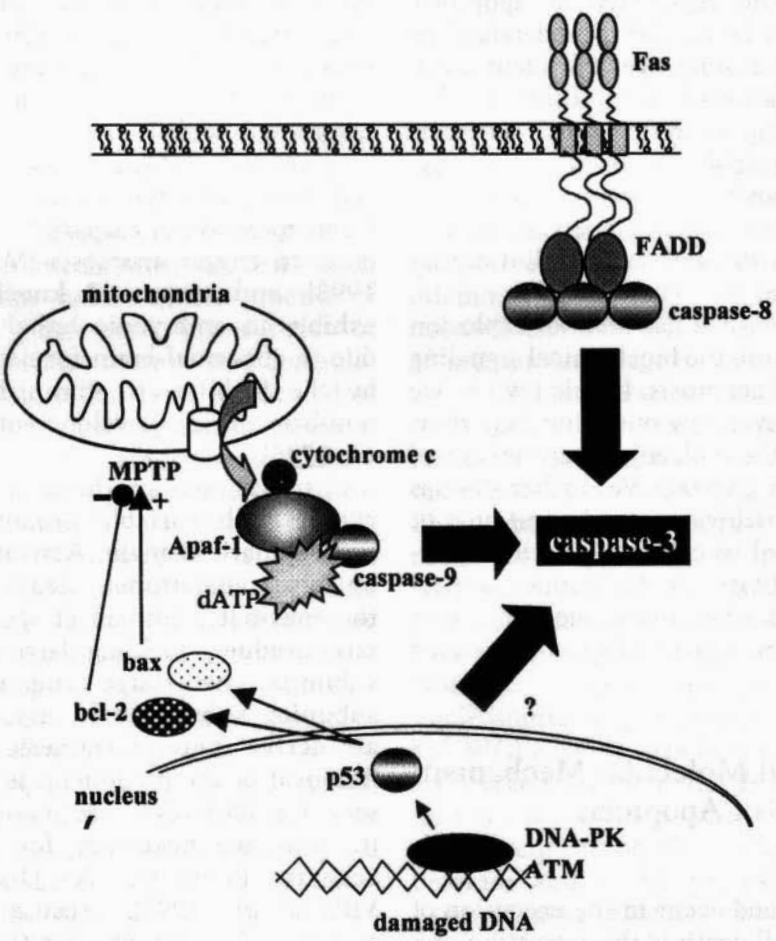

Figure 1. Apoptotic signaling pathways to caspase activation. The machinery involved in the induction of apoptotic signals and the activation of apoptotic effectors, such as caspases, is complex. Three well-studied mechanisms capable of leading to the apoptosis of cells include (i) mitochondrion-dependent pathways, (ii) Fas(receptor)-dependent pathways, and (iii) p53-dependent pathways. The pathways are not completely distinct, but exhibit a limited capacity of crosstalk. See text for details.

and DR5 [reviewed by Haunstetter and Izumo, (1998)]. These death receptors share a motif termed the "death domain," an approximately 80-amino acid stretch that resides in the cytoplasmic domain of these receptors and is necessary for their proapoptotic function. Signal transduction through these receptors requires binding of the appropriate ligand, such as Fas ligand (FasL), which associates with the Fas receptor, TNF-α, which binds to TNFR1, and Apo–3L, which interacts with DR3. The TRAIL ligand has been shown to interact with both DR4 and DR5. Receptor–ligand interaction results in the formation of a homotrimeric complex, facilitating the recruitment to the "death domain" of an intracellular adaptor protein by protein–protein interactions. In the case of TNFR1 and DR3, this adaptor protein is the TNFR-associated death domain protein (TRADD), whereas Fas and DR4 recruit the Fas-associated death domain protein, FADD. The adaptor protein involved in DR5 signaling remains unknown, suggesting the existence of hitherto undiscovered adaptor proteins.

FADD has been shown to interact directly with caspase 8, leading to its autoactivation (Boldin *et al.*, 1996; Muzio *et al.*, 1996), which is followed by the activation of caspase 3 and apoptosis (Fernandes-Alnemri *et al.*, 1996).

C. Mitochondrion-Dependent Caspase Activation

Another potential mechanism by which apoptosis is initiated and caspases are activated is through mitochondrion-dependent pathways. Some apoptotic stimuli evidence a cascade of events that occur at the mitochondria and lead to cell death. These events include (i) opening of the mitochondrial permeability transition pore (MPTP) and (ii) release of cytochrome *c* from the intermitochondrial matrix, which induces (iii) the activation of caspases (Cai *et al.*, 1998; Gross *et al.*, 1999; Halestrap *et al.*, 1998; Zoratti & Szabò, 1995).

The mitochondrial permeability transition pore (MPTP) is a proteinacous megachannel complex that spans the intermitochondrial matrix. It consists of several inner and outer mitochondrial membrane proteins, including the adenine nucleotide translocator (ANT), the voltage-dependent anion channel (VDAC), the peripheral benzodiazepine receptor (PBR), cyclophilin D, hexokinase, creatine kinase, and perhaps other unidentified components (Zoratti & Szabò, 1995). The MPTP functions primarily to control the efflux of mitochondrial matrix solutes with molecular masses <1500 Da from the mitochondria to the cytoplasm, thereby maintaining a mitochondrial matrix environment distinct from that of the cytoplasm (Zamzami *et al.*, 1998). One of the first steps in the apoptotic process is the opening of the MPTP (Kroemer *et al.*, 1997), followed by mitochondrial depolarization, organelle swelling, and uncoupling of oxidative phosphorylation.

Opening of the MPTP provides a channel for expulsion of key components of the mitochondrial electron transport chain, which is necessary for the activation of several downstream apoptotic executors. In particular, the intermitochondrial matrix protein cytochrome *c* is released from mitochondria following the initiation of apoptosis (Liu *et al.*, 1996) and functions in the formation of an "apoptosome," a complex that also contains apoptosis protease activation factor 1 (Apaf-1) and procaspase 9. This complex subsequently catalyzes the autoactivation of caspase 9 by a dATP- or ATP-dependent mechanism (Li *et al.*, 1997). Active caspase 9 subsequently activates caspase 3, precipitating cell death.