Suicidal Tendencies: Apoptotic Cell Death by Caspase Family Proteinases*  

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Caspase-11 knockout mice have defective interleukin-1. The caspase-2 isoforms. By contrast, caspase-1 and motes melanotic tumor development (14). Caspase-2-deficient mice are not merely degradative enzymes but are highly regulated signaling molecules that control critical biological processes via specific limited proteolysis. Caspase proteinases and their central role in apoptotic cell death provide a prime example of this concept. These cysteine proteinases exist as latent zymogens; however, once activated by apoptotic signals, they systematically dismantle and package the cell by cleaving key cellular proteins solely after apoptotic residues. Here we review caspase proteinases with an emphasis on their structure, activation, and critical role in the apoptotic mechanism.

Caspases and Apoptosis

In 1993, researchers discovered that the Caenorhabditis elegans cell death gene, ced-3, had remarkable sequence similarity to interleukin-1β-converting enzyme (caspase-1), a mammalian proteinase responsible for proteolytic maturation of pro-interleukin-1β (1–3). This seminal finding delineated the first two members of the caspase family and suggested that these proteinases might function during apoptosis. Subsequent studies identified over a dozen caspase family members important for apoptosis and/or inflammation (Table I) (4–6) (reviewed in Refs. 7 and 8).

Several lines of evidence indicate that caspases are important for apoptosis. First, caspase activation correlates with the onset of apoptosis and caspase inhibition attenuates apoptosis (7–10). Second, C. elegans mutants lacking the worm caspase, CED-3, have a complete absence of developmental programmed cell death (11). More recently, targeted deletion of caspase genes has shown a definitive role for caspases in apoptosis and inflammation (reviewed in Ref. 12). Animals deficient in caspase-3, caspase-8, or caspase-9 die perinatally because of profound defects in developmental programmed cell deaths (12, 13). Similarly, deletion of the gene for Drosophila caspase-1 causes larval lethality and also promotes melanotic tumor development (14). Caspase-2-deficient mice develop normally, but cells from these animals show diminished or enhanced apoptosis, depending on their tissue of origin (15). These differences may relate to tissue-specific expression of pro- and anti-apoptotic caspase-2 isoforms. By contrast, caspase-1 and caspase-11 knockout mice have defective interleukin-1β production but develop normally and have minimal apoptotic defects (12, 16).

Caspase Structure

Sequence analysis and X-ray crystallography data suggest that all caspases share a common structure (4–8, 17–20). Each zymogen contains an N-terminal prodomain, a large subunit containing the active site cysteine within a conserved QACXG motif, and a C-terminal small subunit. An aspartate cleavage site separates the prodomain from the large subunit, and an interdomain linker containing one or two aspartate cleavage sites separates the large and small subunits. Activation accompanies proteolysis of the interdomain linker and usually results in subsequent removal of the prodomain. The active enzymes function as tetramers, consisting of two large/small subunit heterodimers (17–20). The heterodimers each contain an active site composed of residues from both the small and large subunits. Each active site contains a positively charged S1 subsite that binds the substrate’s negatively charged P1 aspartate (17–20). This S1 binding site is highly conserved; therefore, all caspases cleave solely after aspartate residues.

The individual caspases have two major structural differences. First, the predicted S2–S3 substrate binding sites vary significantly, resulting in varied substrate specificity in the P2–P4 positions, despite an absolute requirement for aspartate in the P1 position (4–8, 17–20). Thornberry et al. (21) recently defined the optimal tetrapeptide substrate specificity for 10 caspases using a synthetic combinatorial peptide library. The sequence preferences generally correlate with caspase function as apoptotic initiators, apoptotic executioners, and cytokine processors (Table I). Note that the tetrapeptide preferences listed in Table I are not absolute and that the preferences do not represent kinetic values that can be directly compared. For example, caspase-3 and caspase-7 both prefer DEDX-based peptides; however, the kinetics of the individual hydrolysis reactions may differ significantly.

Second, caspase prodomains vary in length and sequence (Table I). Long prodomain caspases function as signal integrators for apoptotic or pro-inflammatory signals and contain sequence motifs that promote their interaction with activator molecules (Table I) (22, 23). The apoptotic initiators (caspase-2, -8, -9, and -10) generally act upstream of the small prodomain apoptotic executioners (caspase-3, -6, and -7) (7, 8, 23). By contrast, caspase-1 and caspase-11 function predominantly as cytokine processors (12, 16). Less is known about caspase-4, -5, -12, -13, and -14; however, these caspases demonstrate a higher degree of sequence similarity to caspase-1 than to the apoptotic caspases (4–8). Therefore, these caspases are grouped with the cytokine processors. Overall, caspase substrate specificity, prodomain length, and prodomain sequence determine caspase function.

Caspase Activation

Because caspases exist as latent zymogens, the question remains as to how the zymogens are activated. Current evidence suggests that activation may proceed by autoactivation, transactivation, or proteolysis by other proteinases. Affinity-labeling experiments demonstrate that caspase zymogens have low but detectable proteolytic activity, suggesting the potential for autoactivation under certain circumstances (24, 25). Furthermore, overexpression of wild type caspases, but not catalytically inactive mutants, results in caspase processing and activation, indicating that autoactivation may occur at high enzyme concentration (26). Forced oligomerization of procaspase-8, procaspase-9, or CED-3 facilitates zymogen autoactivation and promotes apoptosis (25, 27–30). This process may approximate zymogens and restrict their mobility, thereby increasing the local enzyme concentration and promoting autoactivation. In vivo, adapter molecules mediate oligomerization of long prodomain procaspases.

Adapter molecules link apoptotic sensors such as death receptors and mitochondria to procaspases. To accomplish this, adapters generally contain one domain that couples the adapter to the sensor and another that binds to long prodomain procaspases. These domains include death domains (DDs), death effector domains (DEDs), and caspase recruitment domain (CARD). FADD, Fas-associated death domain; RAIDD, RIP-associated ICH-1/CED-3 homologous protein with a death domain; APAF-1, apoptotic protease-activating factor-1; CARDIAK,
(DEDs), and caspase recruitment domains (CARDs) (Table I). DDs, DEDs, and CARDs all contain six anti-parallel \( \alpha \)-helices arranged in a similar three-dimensional fold and associate via like-like interactions (31–33). However, hydrophobic interactions are important for DED-DED interactions, whereas electrostatic interactions are critical for CARD-CARD interactions (31–33).

The adapter molecule FADD couples the Fas death receptor to procaspase-8. FADD contains a DD that interacts with a similar domain on Fas and also contains a DED that binds to the DEDs of procaspase-8 (23). Fas activation stimulates binding of the receptor's DD to the corresponding domain in FADD, which in turn recruits procaspase-8 by a homophilic interaction involving DEDs. Subsequent oligomerization then promotes procaspase-8 autoactivation (Fig. 1) (23, 34, 35). FADD probably activates procaspase-10 through a similar mechanism (36). Interestingly, FLIP, a catalytically inactive caspase-8-like molecule with two DEDs, inhibits Fas-FADD-procaspase-8 interactions and thereby inhibits apoptosis (37).

Mitochondria sense apoptotic signals and convey them to the activation adapter APAF-1 via the release of cytochrome \( c \). Cytochrome \( c \) binds to APAF-1, and in the presence of adenine nucleotides, the APAF-1-cytochrome \( c \) complex promotes activation of procaspase-9 (38). Cytochrome \( c \) and adenine nucleotides likely induce a conformational change that exposes the APAF-1 CARD domain. The exposed APAF-1 CARD domain can in turn recruit procaspase-9 by a homophilic interaction involving CARDs. Subsequent procaspase-9 oligomerization then facilitates caspase autoactivation. Bel-\( X_1 \), an anti-apoptotic Bel-2 family protein, may inhibit apoptosis by blocking these interactions (39). A second CARD-containing adapter, RAIDD, couples procaspase-2 to death receptors via CARD-CARD interactions (40, 41). Thus, adapter-mediated protein-protein interactions are widespread among the apoptotic caspases.

Less is known about activation of pro-inflammatory caspases. CARDIAK, a CARD-containing kinase, promotes procaspase-1 activation in vitro via a CARD-CARD interaction, suggesting that CARD-mediated oligomerization could function in procaspase-1 activation (42). Ligation of the CD40 receptor also promotes procaspase-1 activation (43); however, whether CARDIAK facilitates this activation is unknown. Finally, caspase-11 does not directly process procaspase-1 but may facilitate zymogen activation by a non-proteolytic interaction (16).
Once activated, caspases transactivate other procaspases, providing the opportunity for cascade amplification and positive feedback. Caspase-8 for example efficiently activates procaspase-3 ($k_{\text{cat}}/K_m = 8.7 \times 10^6 \text{ M}^{-1} \text{s}^{-1}$) (44), and active caspase-3 in turn may activate procaspase-8. Although this positive feedback loop is theoretically possible, it has not yet been demonstrated. Additionally, because caspases have varied substrate specificity, a single activated caspase may not directly activate all other family members. For example, caspase-9 activates procaspase-9 and procaspase-7 but cannot activate procaspase-6 (29). Propagation of a caspase cascade will thus depend on which caspases a cell expresses, the relative concentrations of each caspase, and the kinetic efficiency of the individual transactivation reactions.

Caspase activation by non-caspase proteinases represents another mechanism for activation. The cytoxic T cell proteinase, granzyme B, an aspartate-specific serine proteinase, provides the best example of this concept as this enzyme can activate several caspases and potently induces apoptosis (44–48). This proteinase is an extremely efficient activator of procaspase-3 and procaspase-7 ($k_{\text{cat}}/K_m = 4.8 \times 10^3 \text{ M}^{-1} \text{s}^{-1}$; $8.6 \times 10^3 \text{ M}^{-1} \text{s}^{-1}$, respectively) (44, 48). Remarkably, granzyme B activates procaspase-3 5.5 times faster than caspase-8 and 17 times faster than caspase-10 (44). A second caspase, caspase-8, also activates procaspase-3 5.5 times faster than caspase-8 and 17 times faster than caspase-10 (44, 48). As the apoptotic signal propagates, caspases activate other components of the apoptotic machinery (Table II). These components likely include ICAD, gelsolin, PAR2, MEKK1, and PKCδ (7, 8, 54). Proteolysis of these proteins directly impacts upon the apoptotic phenotype. For example, caspase-3 cleaves ICAD, prompting release of active CAD, which then cleaves DNA and prompts chromatin condensation. Caspase-3 also cleaves and activates gelsolin, a protein that regulates actin dynamics. Interestingly, activated gelsolin promotes both cytoplasmic and nuclear apoptosis, including DNA fragmentation. Caspase-dependent activation of kinases including PAR2, MEKK1, and PKCδ also promotes cytoplasmic and nuclear apoptosis. Interestingly, MEKK1 activation enhances caspase activation, suggesting that kinases may amplify or initiate the caspase cascade.

Caspases also cleave structural proteins of the nucleus and cytoskeleton (Table II) (7, 8, 54, 57). Proteolysis of lamins, NuMa, and SAF-A likely promotes nuclear dissolution and packaging. Similarly, caspases may disrupt cytoskeletal integrity by proteolysis of fodrin, Gas2, keratins, Rabaptin-5, and actin. Cleavage of β-catenin and FAK may interrupt cell-cell contacts and cell-matrix focal adhesions. Altogether, proteolysis of these caspase substrates may promote cellular packaging and subsequent engulfment by phagocytes. It should be noted, however, that the functions of these cleavage events are largely speculative and await a more rigorous evaluation.

Another class of caspase substrates includes proteins important for cellular signaling, cellular repair, and macromolecular synthesis. These proteinases include kinases, other enzymes, and factors necessary for protein and nucleic acid synthesis (Table II) (7, 8, 54). Caspase-dependent proteolysis also promotes degradation of Akt-1 and Raf-1, kinases important for cell growth and survival (58). However, these proteinases are not direct caspase substrates, suggesting that caspases may activate other proteinases that participate in apoptotic events (58). Overall, proteolysis of these proteinases could disrupt cellular homeostasis and terminate survival signals; however, this has not yet been formally demonstrated.

Caspases also cleave presenilins, huntingtin, atrophin-1, and other proteins implicated in neurodegenerative disease (Table II) (7, 8, 54, 59–61). Some of these proteins are cleaved during apoptosis; however, the significance of these proteolytic events is not clear. Wellington et al. (59), however, suggest that caspase zymogens may cleave neurodegenerative proteins at a low level, generating toxic fragments that initiate apoptosis and perhaps neurodegenerative diseases.

### Conclusion

Numerous studies establish caspases as essential mediators of apoptosis. Because disordered apoptosis can promote human disease, these findings have broad implications. Insufficient apoptosis because of caspase inactivation may promote oncogenesis by allowing cell accumulation (62). Recent evidence supports this hypothesis, and careful study of the caspase knockout animals should provide more definitive answers to this question (12, 63). On the other hand, caspase over-reactivity promotes cellular suicide, and this may be the basis for degenerative diseases such as Huntington’s disease and Alzheimer’s disease (59–61). Net increases in apoptosis could result from a decreased apoptotic threshold, enhanced apoptotic stimulation, or a combination of these factors. Regardless of the inciting factor(s), enhanced caspase activity should result. On the molecular level, increased expression/activity of caspases, apoptotic sensors, or adapters or diminished expression/activity of caspase inhibitors could increase caspase activity.
Similarly, loss of survival signals, mitochondrial dysfunction, or diminished stress responses might prompt caspase activation and apoptosis. Further study of the caspase system will prove or disprove these theories and will one day offer treatments for disorders of apoptosis.

Acknowledgments—Because of space limitations, it was not possible to include a comprehensive list of references for all the work discussed. We apologize to those authors whose important contributions could not be described or properly cited.

Note Added in Proof—Since this Minireview was accepted for publication, key studies concerning caspase activation and caspase involvement in man disease have been published. First, Stennicke et al. (64) established that caspase-9 activation does not require proteolytic processing of the zymogen and that the zymogen itself has significant activity in the presence of cyto-
colic factors (presumably APAF-1). Second, we demonstrated that calpain may regulate caspase-9 activation by removing the procaspase-9 CARD domain (65). Third, Yuan and colleagues (66) demonstrated that polyglu-
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