REVIEW ARTICLE

ROLE OF CASPASES IN APOPTOSIS AND DISEASE

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Abstract: Apoptosis, a genetically governed process of eliminating cells in response to a variety of stimuli provides protection against cancer and viral infections as well as maintains homeostasis. Recent studies using both molecular and cloning approaches, and in vitro systems have identified a class of highly specific proteases, termed caspases, that appear to have an important role in apoptotic execution. Caspases are synthesized as precursor molecules that require processing at specific aspartate residues to produce the active enzyme which in turn leads to the cleavage of various death substrates that lead to morphological changes typical of apoptosis. This review discusses caspases, their inhibitors and regulators. Since cytotoxic drugs used in chemotherapy of leukemia’s and solid tumors cause apoptosis in target cells, elucidating the consequences of proteolytic activity occupies a central role for understanding of the molecular mechanism of apoptosis which can help us to use the caspase inhibitors as targets of therapy.

Key words: caspases apoptosis Bcl-2

INTRODUCTION

Caspase mediated apoptotic cell death plays an important role in both physiological and pathological processes. During embryo development, surplus cells die as part of the process that balances growth and differentiation with death. In case of central and peripheral nervous systems, apoptosis occurs extensively during development. Inappropriate apoptosis however, underlies the etiology of many of the most intractable of human diseases, for e.g. suppression of apoptosis is involved in the proliferation of preneoplastic and/or neoplastic cells and in the development of autoimmune diseases. Increased apoptosis may be involved in neurodegenerative diseases, during retroviral infection (HIV-1), and perhaps in the development of diabetes mellitus (1-6).

Apoptosis, which is conserved throughout evolution comprises of four stages. Priming is triggered either by removal of survival factors or by a cellular insult such as genotoxic damage caused by irradiation, mitotic defect or by reactive oxygen species. A commitment step follows, when cells activate the apoptotic machinery
e.g. cell death induced by Fas/TNF α. Commitment renders the process irreversible. The cell then enters an execution phase where the morphological characteristics of apoptosis become apparent: i.e., membrane blebbing, cytoplasmic and nuclear shrinkage, internucleosomal DNA fragmentation and chromatin condensation. Finally, the removal of apoptotic cells occurs by either macrophages or neighboring cells without initiating an inflammatory response (7–8).

The biochemical machinery involved in the killing and degradation of the cells is expressed constitutively and is accessible for activation by various signals. It appears that although distinct pathways leading to apoptosis are induced by different signals they finally converge to a common effector pathway. At the heart of this pathway are a family of cysteine proteases, the “caspases” that are related to mammalian ICE (Interleukin 1–β converting enzyme) and Ced-3 (cell death abnormal), the product of a gene that is absolutely necessary for apoptotic suicide in the nematode C. elegans (9–12).

As ICE – like cysteine proteases do play a fundamental role in apoptosis, it may be possible to regulate cell death by using specific protease inhibitors. This may have important therapeutic implications in the treatment of the diseases that arise from excess or premature cell death.

CASPASES

The cysteine proteases, caspases which cleave after aspartic acid appear to play a critical role in initiating and sustaining the biochemical events that result in apoptotic cell death. Much of the knowledge on the genetic regulation of cell death has emerged from C. elegans as a model organism. Three genes Ced-3, Ced-4 and Ced-9 play crucial roles in executing and regulating cell death while others are needed for engulfment and disposal of dead cells (13–15). Ced-3 was found to exhibit significant homology to ICE (16), a cysteine protease which converts the 33 kD protease form of IL-1β to active 17.5 kDa form, once again by cleaving after aspartate residues. These observations have been closely followed by the discovery of several more ICE /Ced-3 homologs whose overexpression in various cell types under the influence of diverse stimuli results in apoptosis, thereby suggesting that programmed cell death is conserved widely in phylogeny from nematode C. elegans to humans.

In humans, the caspase family of proteases consists of 10 members that can be subdivided into 3 subfamilies: ICE subfamily (caspase 1, 4, 5), CPP32 subfamily (caspase 3, 6, 7, 8, 10) and Ich-1 subfamily (caspase 2 and caspase 9) (17). All these caspases are normally present in cells as catalytically inactive forms (zymogens) and contain cysteine residues and the conserved pentapeptide QACRG at the active site. They are proteolytically processed and activated through cleavage at aspartic acid by other caspases or possibly through autoprocessing in a proteolytic cascade similar to complement activation or blood clotting (18–22).

The currently known family members in humans participate in one of the two distinct signalling pathways.
1) activation of proinflammatory cytokines and

2) promotion of apoptotic cell death.

Caspase 1 (and probably 4 and 5) which is required for activation of IL-1β and IFN-γ IF belongs to procytokine activation family and other caspases notably caspase 2, 3, 6, 7, 8, and 10 are considered to promote pathways to apoptosis. This conclusion is based on the fact that these caspases endoproteolytically cleave a select group of cellular proteins involved in DNA repair, replication, RNA splicing (PARP, lamin, a fodrin, 70 kDa U1 snRNP, gelsolin) etc. thereby causing nuclear and cytoplasmic alterations that typify apoptosis (22-25).

CASPASE INHIBITORS

Based on their substrate specificity, synthetic substrates and inhibitors (with YVAD specific for the ICE subfamily and DEVD specific for CPP32 subfamily) have been designed. Aldehyde derivatives of these peptides are reversible inhibitors whereas chloromethyl, fluoro methyl and acyloxy methyl ketones are irreversible inhibitors (26).

In addition, several proteins encoded by viral genes are known to inhibit members of the ICE family. These include crmA, a cytokine response modifier gene encoded by cowpox virus and p35 encoded by baculovirus (27-30). These viral proteins seem to inhibit protease activity by forming a stable complex. p35 has a broader specificity for ICE family members than crmA, i.e. crmA preferentially inhibits caspase 1 over caspase 3 while p35 inhibits both caspase 1 and caspase 3 equally well. IAP (inhibitor of apoptosis) proteins were identified by their ability to compensate for loss of function of caspase inhibitor protein p35 in Autographa California nuclear polyhedrosis virus (AcNPV) mutants. Last year, several cellular homologues of the virus iap were reported namely XIAP, cIAP1, cIAP2 etc. whose overexpression can suppress cell death induced by a variety of stimuli. The first mammalian homologue XIAP found was identified as the product of a gene mutated in several forms of spinal muscular atrophy, a disease in which motor neurons die prematurely, whereas it has been reported that cIAP1, cIAP2 (murine homologues) are rapidly induced in cells which are resistant to TNF α mediated death but not in cells sensitive to TNFα.

Although new proteases and their mechanisms in bringing about cell death have emerged, basic issues relating to the connection between ICE family of proteases and the Bcl-2 family of proteins still remain unresolved. Overexpression of Bcl-2 and Bcl-xL in Jurkat cells inhibited staurosporine induced apoptosis and abrogated activation of CPP32 and ICE-LAP3 but had no effect on Fas mediated apoptosis thereby suggesting that two apoptosis pathways are present in Jurkat cells with Bcl-2 and Bcl-xL functioning upstream of ICE like proteases in one of the pathways. On the other hand E1B 19k and Bcl-2 prevent processing of CPP32 and cleavage of downstream substrates during E1A induced apoptosis. Thus cellular context and cell type specificity may be factors in the regulation of apoptosis. Recently a model has been proposed to explain the
relationship between Bcl-2 and ICE like proteases. According to this model Bcl-2 molecules transduce constitutive survival signals by forming ion channels or by some other mechanism to suppress ICE like protease activation (31-34). This activity is antagonized by Bax like proteins which activate ICE like proteases by forming dimers and inducing death promoting signal. Both pathways could be working simultaneously in a cell to activate or inhibit apoptosis as may be required. Although Bcl-2 is presumed to inhibit caspase activation by acting upstream of caspases, a report by Cheng et al (35) suggested that Bcl-2 can also be a downstream death substrate of caspases, suggesting the existence of a feedback loop between caspases and Bcl-2. The observation that Bcl-2 cannot inhibit apoptosis in some situations implies that specific caspases may by pass the pathway inhibited by Bcl-2. In this scenario, activation of a subset of caspases that are insensitive to Bcl-2 may also promote cleavage of Bcl-2, not only inactivating its antiapoptotic function but also enhancing cell death.

Apoptosis is of critical importance both to pathogenesis of cancers and to their likelihood of resistance to conventional antineoplastic treatments. Mutations in p53 gene and its regulators (mdm2) are extremely common, occurring in perhaps 55-75% of human cancers. In response to DNA damage, the p53 protein induces apoptosis by acting as a transcription factor, activating expression of numerous apoptosis mediating genes. A current model proposes that DNA damage causes the p53 protein to turn on genes whose products generate free radicals that, in turn damage the cells mitochondria, whose contents (such as cytochrome c) leak out into the cytoplasm and activate apoptotic caspases. Apart from this, the p53 protein can also induce apoptosis by upregulating expression of Bax, a proapoptotic Bcl-2 family member. Tumors exhibit varying numbers of apoptotic cells; a high proportion of apoptotic cells correlates with slower tumor growth. Conversely, mice that lack the genes for Bax develop fast growing tumors than similar tumors from mice with normal Bax genes. In summary, mutations in genes that lead directly or indirectly to reduced apoptosis are generally associated with poor prognosis in a variety of tumor types (36), since conventional chemotherapy and radiation therapy rely primarily on induction of apoptosis in cancer cells for therapeutic effect. New cancer therapies that aim to induce apoptosis specifically in cancer cells are the source of much excitement and renewed hopes for cures.

CASPASES AND APOPTOSIS

The caspases implicated in apoptosis are currently divided into initiators and executioners. The exact order of the executioners and the place of other caspases in the pathway are still controversial but at least in Fas pathway, signalling of death is transmitted in part by sequential caspase activators.

Receptor aggregation either by Fas ligand or by antibody crosslinking induces the formation of a death inducing signalling complex (DISC) of proteins comprising Fas itself, an adaptor protein FADD (Fas associated death domain protein) and the
inactive zymogen form of caspase 8 (37-40). A similar DISC involving TNFR1 (Tumor necrosis factor receptor), TRADD (TNF receptor associated death domain), FADD, and procaspase 8, is thought to mediate TNF induced apoptosis. Once activated, caspase 8 is thought to activate other downstream caspases in a hierarchy by proteolytic cleavage of their zymogen forms, thus amplifying the caspase signal. Recently it has been found that, apart from FADD dependent pathway (Figure 1), CD95 can also trigger apoptosis by a FADD-independent pathway which involves initiator (lacks DD homology) and Jun kinase (JNK). Differential usage of FADD and Daxx pathways is perhaps due to differences in the levels of FADD or the ratio of membrane bound versus soluble CD95 L and may explain why Bcl-2 is a poor antidote to CD95 induced apoptosis of lymphocytes, yet it can inhibit Fas induced apoptosis of hepatocytes. Moreover, the Fas death effector system is a double edged...

Fig. 1: Interrelationship between caspases, apoptosis and diseases. Fas L, fas ligand; Fas, fas receptor; TNF, tumor necrosis factor; TNFR, TNF receptor; DISC, death inducing signalling complex; FADD, fas associated death domain; JNK, Jun N terminal kinase; FLICE, FADD-like ICE (Interleukin 1 beta converting enzyme); ROS, reactive oxygen species, NFkB, Nuclear factor kappa B; YVAD, DEVD, tetra peptide inhibitors for caspases.
sword. If this system is properly regulated, it is useful for downregulating the immune reaction and for removing virally infected as well as cancerous cells; but, if this system is exaggerated, it can cause tissue destruction. It means this system can be modulated to be applied to human diseases. It can be applied in the killing of tumor cells, since some cancer cells, particularly some lymphoid tumors, express functional Fas. However, since the systemic treatment of patients with Fas L will cause deleterious side affects, methods of local administration and/or proper targeting of Fas L to the tumor should be devised. Fas L can also be used as immunosuppressive agent since the rejection of grafts is mediated by activated T cells. If a transplanted tissue is engineered to express Fas L or is cotransplanted with Fas L-expressing cells, the transplant may be tolerated. The other application of this system is to block Fas L induced tissue destruction. As Fas is shown to play a role in human diseases, therefore neutralizing antibodies against Fas or Fas L, or other inhibitors of Fas mediated apoptosis, would have potential as therapeutic agents.

Literature available suggests that TNF receptor shares a signal cascade with Fas in one apoptotic pathway, but it also activates additional signalling pathways including one that activates a survival signal. It will be interesting to examine what kinds of survival genes are activated by NF-kB and how these molecules inhibit apoptosis. Identification of these survival genes may provide clues as to why some tumor cells are resistant to various apoptosis inducing agents including Fas L, TNF, and other anticancer drugs. Report by Wissing et al (41) has proved that TNF-induced activation of caspases results in the cleavage and activation of cytosolic phospholipase A₂ (cPLA₂) and the activated cPLA₂ contributes to apoptosis (a mechanism similar to the one used by non steroidal anti inflammatory drugs, [NSAID], which are widely being used in medications).

**DYSREGULATED CELL DEATH AND APOPTOSIS BASED THERAPIES.**

There are several diseases/disorders in which apoptosis is dysfunctional. Huntington's disease (HD) was the first disorder in which caspase cleavage of a specific substrate has been linked to a disease pathology. In HD polyglutamine tract of Huntington (ubiquitously expressed protein of unknown function) is cleaved in cells or apoptotic extracts by caspase 3 and the rate of cleavage increases with the length of its polyglutamine tract.

Apoptotic cell death and its attendant molecular mediators also appear to play a role in many neurodegenerative disorders, including Alzheimer's disease. Mutations in the presenilin 2 gene have recently been associated with familial Alzheimer disease and is hypothesized to function in a pathway downstream of Fas. In primary cultures of human neurons, peptide fragments of amyloid β can down regulate antiapoptotic Bcl-2 and up regulate proapoptotic Bax expression, thus making the neurons more prone to die especially in response to oxidative stress. Apart from this, involvement of ICE like proteases has been proved in the bone marrow of patients with Myelodysplastic syndrome, neuroblastomas,
myelomas, heart failure, autoimmune/lymphoproliferative syndromes (ALPS) etc. (42-45).

Thus the ability to block apoptosis represents an exciting possibility for extending the productive lifetimes of cells. Experiments have already been performed in some cases for e.g. it has been reported that the use of ICE like and CPP32 like protease inhibitors reduces ischemic and excitotoxic neuronal damage which is implicated in stroke and neurodegenerative disorders (46–47). T Leukemia cells and peripheral blood mononuclear cells exposed to HIV-1 undergo enhanced viral replication in the presence of cell death inhibitor (z VAD-fmk), thereby suggesting that programmed cell death may serve as a beneficial host mechanism to limit HIV spread (48).

Apoptosis also plays an important role in cancer therapy. Most leukemic cells are well known to undergo apoptosis by several events, and the basic strategy of leukemia therapy is the induction of apoptosis. For example, many chemotherapeutic agents such as cytosine arabinoside and VP16 induce apoptosis and kill leukemic cells. On the other hand, Fas ligand and TNF α, which physiologically exist in the body, are also inducers of apoptosis in leukemia cells and other cells. Although previous reports have shown that Bcr-Abl expression confers resistance against the antileukemic drug induced DNA fragmentation and morphological features of apoptosis, a recent report by Amarante et al (49) using AML (acute myelogenous leukemia) HL-60 cells has proved that treatment with antileukemic drugs causes the release of cytochrome C, induces the activity of DFF (DNA fragmentation factor), while Ber-Abl expression results in the inhibition of the preapoptotic mitochondrial perturbation thereby blocking the generation of caspase activity and apoptosis.

Many of the events in Fas signalling are regulated by protein – protein interactions. In a recent report by MacCorkle et al (50), they have used caspases (caspase 1 and caspase 3) as ideal targets for designing conditional alleles based on chemically induced dimerization. In both cases aggregation of the target protein is achieved by a nontoxic lipid permeable dimeric FK506 analog that binds to the attached FK506 binding proteins, FKBP. It has also been found that crosslinking of caspase-1 and caspase-3 is sufficient to trigger rapid apoptosis in a Bcl-xL independent manner, suggesting that these conditional proapoptotic molecules can bypass intracellular checkpoint genes, such as Bcl-xL that limit apoptosis. Because these chimeric molecules are derived from autologous proteins, they are nonimmunogenic and thus ideal for long-lived gene therapy vectors, which will be useful for developmental studies, for treating hyperproliferative disorders, and for developing animal models to a wide variety of diseases.

With new information on programmed cell death constantly being elucidated, cell death promises to be a lively area of future innovation. The long term challenge in basic research is to understand the course of caspase activity during animal development, homeostasis and pathology, in therapy to
develop drugs targeted to specific cells and tissues, and to block acute and chronic diseases through intervention in caspase network.

REFERENCES


