Programmed cell death and its clinical implications

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Cell death is a highly regulated process that is ubiquitous in all eukaryotes. Programmed cell death (PCD) is an integral part of both animal and plant development. Studies on apoptosis, the well-characterized form of programmed cell death led to the identification of a central tripartite death switch i.e. apoptosome consisting of Apaf-1, Apaf-2 and Apaf-3. The caspases, a family of cysteine-dependent aspartate directed-proteases, constitute the central executioners of apoptosis. Much of the attention on programmed cell death is focused on caspases, however, cell death can still occur even when the caspase cascade is blocked, revealing the existence of nonapoptotic alternative pathway(s) of cell death. The mitochondrial release of cytochrome C following a PCD inducing stimulus in both plants and animals suggests the evolutionary conservation of death pathways. Dysregulation of apoptosis may be related to the development of several disease states as well as ageing. Excessive apoptosis is associated with neurodegenerative disorders, AIDS etc., whereas deficient apoptosis is associated with cancer, auto-immunity, viral infections etc. Understanding the regulation of programmed cell death would throw light in designing drugs and gene therapies that can target specific molecules in the apoptotic pathway opening the vistas for new therapeutic endeavors in many areas of medicine.

As much as the definition of life may be controversial, the definition of death also may prove problematic. The death of a living cell may result from an external physical injury or it may be an outcome of activating an internal pathway for cell suicide i.e. programmed cell death (PCD). This suggests that cells can participate in their own demise and certain types of cell death that are thought to be caused by extrinsic factors such as pathogens or drugs may in fact result from the activation of a programmed death pathway that is normally dormant in cells. PCD was coined initially to explain the cell death occurring during development, whereas apoptosis was used to describe the cell death that exhibits a set of morphological features. The process of programmed cell death and apoptosis are distinct, however, apoptosis often has been used interchangeably with programmed cell death. While many instances of apoptosis represent true programmed cell death, nonapoptotic caspase independent forms of PCD such as paraptosis, oncrosis, magenesis etc. are also seen in some eukaryotes. The biochemical basis for these alternative forms of cell death is yet to be understood.

Necrosis, Apoptosis and Paraptosis

Cell death occurs either by necrosis or apoptosis. Necrosis results from physical injury, metabolic block mutations, toxic substances etc. and is not genetically controlled. It is characterized by cell swelling, mitochondrial dilation, dissolution of other organelles, non-caspase proteolytic cascades depending on serine proteases, calpains or cathepsins, plasma membrane rupture and spillage of the cytoplasmic contents ultimately leading to the inflammatory response (Table 1)1. By contrast, apoptosis is a genetically controlled process exhibiting a constellation of structural and functional changes including calcium flux, cytochrome C redistribution, caspase activation, loss of plasma membrane asymmetry, reduction in cell volume, selective proteolysis of a subset of cellular proteins, chromatin condensation, nucleosomal DNA fragmentation and ultimately breakdown into apoptotic bodies that are rapidly phagocytosed (Table 1). Inhibition of the classical caspase dependent apoptotic pathway may lead to necrotic cell death suggesting that the same death stimulus can result in either apoptotic or necrotic cell death, depending on the availability of activated caspases. Paraptosis,1 a caspase independent programmed cell death that often exists in mammalian as well as non mammalian model systems in parallel with apoptosis is distinct from it by the criteria of morphology, biochemistry as well as response to apoptotic inhibitors (Table 1). Paraptosis is dependent on RNA and protein synthesis and its molecular mechanism is yet to be understood. Oncrosis and magenesis are the other forms of nonapoptotic PCD whose mechanisms are not clear.

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Evolutionary Origin of PCD

The primitive cell death programs are seen in prokaryotes where they arose as a result of competition between bacteria themselves or between viruses. The molecular mechanisms involved are varied involving cleavage of RNA, DNA or specific proteins. There are some similarities between the suicide program of E.coli and PCD of animal cells. A constitutively expressed proteolytic enzyme cascade which gets activated in response to different stress conditions, cleaves the translational elongation factor leading to its arrest and thereby killing the cell. Thus, the cell commits suicide in response to infection for protecting its neighboring cells. It could be possible that mechanisms like this in the ancestral prokaryote may have provided the starting point for the initiation of death programs in animal cells.

Eukaryotic organisms that remain unicellular throughout their life, for example, Trypanosomes elicit death programs with characteristics of nonapoptotic PCD. Interestingly, yeast under normal circumstances does not display any death program, but the expression of *bax*, a death gene kills the cells by nonapoptotic PCD and the same can be blocked by co-expressing death suppressor genes. The recent discovery indicating the presence of the basic mechanism of apoptosis in yeast and its activation by reactive oxygen species suggests that apoptosis originated in unicellular organisms as an altruistic response to severe oxidative damage. Later, cells developed mechanisms to purposely produce reactive oxygen species as regulators of apoptosis. *Dictyostelium discoideum*, a cellular slime mold, exhibits both unicellular and multicellular forms in its life cycle. It displays two terminally differentiated cell types, the viable spore cells and the stalk cells that die as a normal part of their differentiation program and display features quite similar to nonapoptotic PCD.

Interestingly, *D. discoideum* also exhibits oxidative stress induced cell death that can be intercepted by antioxidants such as reduced glutathione, selenium, spermine, etc. However, the molecular mechanism of developmental as well as oxidative stress induced cell death in *D. discoideum* is yet to be understood.

In animals, model systems such as *Caenorhabditis elegans*, *Drosophila melanogaster*, and mice have shown a general cell death profile of induction, caspase activation, apoptotic body formation and phagocytosis. The ordering of cell death genes i.e. *ced-3, 4* and *9*, the involvement of mitochondria, identification of caspase-activated DNase (CAD) that degrades nuclear DNA during PCD and signal transduction pathways involving caspases are some of the findings of immense value in understanding animal PCD.

Cell death plays a critical role in plant development and shares similarities in certain morphological features such as chromatin condensation and oligonucleosomal DNA fragmentation with PCD in animals. However, the dead cells cannot be phagocytosed in plants because of the presence of thick cell wall. In plant system the key elements that orchestrate cell demise remain elusive. To date neither caspase nor Bcl-2 homologue has been cloned from plants. Interestingly, caspase-specific peptide inhibitors baculoviral anti-apoptotic genes i.e. p35 and IAP could abrogate bacterial/viral/chemical induced plant PCD. Oxidative stress induced apoptosis in tobacco protoplasts exhibited the release of cytochrome C from mitochondria suggesting its central role in plant apoptosis. Cloning of plant caspase-like protease genes and elucidation of the mechanism through which mitochondria may regulate cell death would throw light on the evolution of cell death control in eukaryotes and may help to identify essential components that are highly conserved in eukaryotes.

Apoptosis, a programmed process for which the molecular cascade is increasingly understood, is addressed in detail hereafter.

Table I — Apoptosis, necrosis and paraposis at a glance

<table>
<thead>
<tr>
<th>A Morphology</th>
<th>Apoptosis</th>
<th>Necrosis</th>
<th>Paraposis</th>
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<tr>
<td>4 Cytoplasmic vacuolation</td>
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<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>5 Mitochondrial swelling</td>
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<tr>
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<th>Apoptosis</th>
<th>Necrosis</th>
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<tr>
<td>1 DNA fragmentation</td>
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<table>
<thead>
<tr>
<th>C Biochemical Features</th>
<th>Apoptosis</th>
<th>Necrosis</th>
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<td>a Caspase Activity</td>
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</tr>
<tr>
<td>1 DEVD-cleaving activity</td>
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<td>2 Caspase-3 processing</td>
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<td>Absent</td>
</tr>
<tr>
<td>3 PARP cleavage</td>
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<th>b Inhibitory Studies</th>
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<th>Necrosis</th>
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<tr>
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<td>Variable</td>
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<td>2 Cycloheximide</td>
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<td>Present</td>
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<tr>
<td>3 p35</td>
<td>Present</td>
<td>Absent</td>
<td>Absent</td>
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<tr>
<td>4 Bel-XL (mitochondrial)</td>
<td>Present</td>
<td>Absent</td>
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Regulation of Apoptotic Pathways

The genetically conserved intrinsic cell suicide program can be divided into three phases. The initiation phase comprises the exposure of healthy cells to various extrinsic/intrinsic death signals including physiological, genetic and environmental factors that trigger apoptosis. During the effector phase, release of cytochrome C from the mitochondrial compartment into the cytosol occurs, which is regulated by the Bcl-2 family proteins. Execution phase is characterized by the activation of constitutive caspases in the presence of cytochrome c and the activated caspases play a crucial role in the proteolysis of specific proteins resulting in the apoptotic phenotype.

There are three distinct apoptotic pathways that exist in the cell. Of these, the two major pathways i.e. the extrinsic one activated through ligand-dependent death receptor oligomerization (Fig. 1), the intrinsic pathway (Fig. 1) acting through mitochondrial involvement and the third minor one induced through stress-mediated events involving the endoplasmic reticulum have been identified. These pathways may interact and amplify weak apoptotic signals and shorten cellular execution time. The intrinsic apoptotic cascade involves formation of "apoptosome" consisting of Apaf-1, Apaf-2 (cytochrome c) and Apaf-3 (procaspases-9). The binding of dATMP to Apaf-1 induces the formation of a multimeric Apaf-1 that interacts with cytochrome C and procaspase-9. Apoptosome formation leads to the activation of caspase-9, which in turn activates the downstream caspases including caspase-3 that orchestrate the biochemical execution of cells.

The extrinsic apoptotic pathway involves the ligand-receptor interactions triggering the activation of proteases whose actions culminate in the destruction of cell structure. A complex containing several components forms at the receptor. Fas ligand activates Fas receptor on the cell membrane followed by FADD recruitment; TNF receptor (TNF R1) activation leads to TRADD binding which in turn interacts with FADD and in either case, FADD binds caspase-8 with a death domain as well as protease activity and may trigger a common pathway. The death signals mediated by Fas/TNF-R1 receptors usually activate caspases directly, bypassing the need for mitochondria. However, Bid (a pro-apoptotic Bcl-2 family protein) is cleaved by caspase-8 in response to Fas/TNF-R1 death receptor signals and translocated to mitochondria and induces cytochrome c release, which in turn activates the downstream caspases. Such a connection between the two-apoptotic pathways could be important for induction of apoptosis in certain types of cells and is responsible for the pathogenesis of a number of human diseases.

Fas ligand and ultraviolet light/Ras can also induce apoptosis by activating the c-Jun N-terminal kinase (JNK), a MAP kinase and this pathway is mediated by death domain associated adapter protein (DAXX). The TNF receptor can also activate JNK by means of another distinct adapter protein. Also JNK is activated by several forms of stress, independent of the Fas-activated pathway that is not inhibited by Bel-2 explaining the variable ability of cells to resist apoptosis in response to Bel-2. Cytotoxic T lymphocytes kill the target cells by a process involving the release of granules containing granzymes and perforin.

Two alternative hypotheses have been proposed to explain the mechanism of apoptotic execution, either activation of caspase cascade or the loss of mitochondrial function could contribute to the cell death.

Caspases and Apoptosis

The caspases, first discovered almost a decade ago, are considered essential for almost all the forms of metazoan PCD. Recently two families of caspase-like proteins i.e. paracaspases and metacaspases have been identified. Interestingly, caspases seem to be limited to metazoa, paracaspases are seen in metazoa and Dictyostelium, whereas metacaspases are found in plants, fungi, protozoa and bacteria suggesting that metacaspase is the ancient form of this superfamily. Caspases derived their name from their characteristic property of being cysteine proteases with aspartate residue specificity. Mature caspase has a heterotetrameric structure with two active sites that may function independently. Based on their substrate specificities and DNA sequence homologies, the 14 currently identified mammalian caspases can be divided into three groups: apoptotic initiators, apoptotic executioners, and inflammatory mediators. They are synthesized as procaspases that are converted to active proteases during apoptosis through an intracellularly regulated proteolytic cascade with upstream and downstream caspases. Upstream caspases are characterized by long amino-terminal prodomains that carry specific protein-protein interaction domains, which mediate their oligomerization, often assisted by specific adapter molecules. Oligomerization appears to be sufficient for autocatalytic activation of these
Fig. 1—An overall view of major apoptotic pathways in a mammalian cell
caspases, which further process downstream caspases initiating a cascade of amplifying events. While the upstream procaspases are activated by oligomerization, downstream procaspases are often activated by other proteases i.e. trans activation\textsuperscript{21}. The activated caspases then cleave various cellular proteins leading to apoptotic phenotype. To date, more than 60 proteins are known to act as substrates for mammalian caspases, which may be activated or inactivated but not degraded by caspase processing. The substrates may fall into two general groups i.e. a large group of proteins involved in regulation and execution of apoptosis, and a small group of pro-inflammatory cytokine precursors (Table 2)\textsuperscript{21}. The evolutionary conservation of caspase substrate proteins, in particular their caspase cleavage sites, suggests that caspases were first employed for apoptosis and later co-opted for cytokine processing in mammals. Among others, the PARP (poly ADP-ribose polymerase) constitutes one of the targets whose degradation is not essential, but offers a useful diagnostic marker for apoptosis. Other caspase substrates include structural proteins such as keratins and nuclear lamins, transcription factors or their regulatory proteins, kinases and other signaling proteins.

The molecular mechanism that leads to DNA fragmentation has also been identified. At least two redundant parallel pathways may lead to chromatin processing during apoptosis. One of these pathways involves Apaf-1, caspases as well as caspase activated DNase (CAD), and leads to oligonucleosomal DNA fragmentation and advanced chromatin condensation. Caspase-3 cleaves ICAD of the dimer i.e. ICAD-CAD (Fig. 1) activating the CAD that degrades DNA\textsuperscript{26}. Another pathway is mediated by AIF exhibiting large-scale DNA fragmentation and peripheral chromatin condensation without caspase activation\textsuperscript{27}. Several recent observations suggest that caspases and apoptosis regulatory molecules exert important functions beyond that of cell death, including the control of T-cell proliferation and cell-cycle progression\textsuperscript{28}.

As apoptosis is a highly regulated process, numerous strategies for the activation and inhibition of these proteases have evolved, including the regulation of caspase expression and function at the transcriptional and post-translational levels, as well as the expression of viral and cellular inhibitors of caspases.

**Natural inhibitors**

Apoptosis and inflammatory responses are the major host defense mechanisms against viruses. Hence, viruses imply inhibitors of caspases, the central components of the apoptotic machinery, to prolong the life of host cell for maximal viral replication. These viral inhibitors either directly inhibit caspases, as exemplified by the cowpox virus protein Crm A (cytokine response modifier A), baculoviral protein p35 and IAPs, or inhibit procaspase-adapter interactions as exemplified by v-FLIP\textsuperscript{21}.

Crm A is a serpin that directly targets the active site of mature caspases and granzyme B. Similar to Crm A, the baculovirus protein p35 also targets mature caspases and serves as a suicide inhibitor. p35 is a broad spectrum caspase inhibitor and it inhibits human caspases -1, -3, -6, -7, -8 and -10, however, it does not inhibit granzymes B\textsuperscript{13}. Interestingly, p35 gene is able to intercept oxidative stress induced apoptosis and functionally complement Bcl-2 despite having no sequence homology with the well-known Bcl-2 family\textsuperscript{29}. We have recently demonstrated the upstream action of p35 in the pathway of apoptosis induced by oxidants using insect cells as a model system\textsuperscript{29,30}. Our results demonstrate that p35 acts as an antioxidant by directly sequestering free radicals and prevents reactive oxygen species-mediated cell death\textsuperscript{29}. Thus, the dual role of p35 makes it a potential
therapeutic molecule. In contrast, to Crm A and p35, IAPs are not active site specific inhibitors and their inhibition of apoptosis does not require cleavage by caspases. The baculoviral IAPs are identified by their ability to functionally replace p35. Evolutionarily ancient IAPs such as survivin may also be involved in the cytokinesis and link caspase activation to cell cycle progression. The level and activity IAPs may determine the sensitivity of cells to apoptotic stimuli and they are down regulated during apoptosis by proteosome mediated degradation to ensure effective cell killing. v-FLIP represents another group of viral apoptotic inhibitors and it has two death effector domains (DEDs) that are similar to those in the N-terminal region of procaspase-8. v-FLIP inhibits apoptosis mediated by death receptors through competition with procaspases for recruitment to the death receptor complex.

**Heat shock proteins**

The cellular-stress response can mediate cellular protection through expression of heat-shock proteins and recently heat-shock proteins (Hsp) have been shown to act as inhibitors of apoptosis. Hsp70, an antiapoptotic chaperone is highly expressed in certain types of tumors and acts as a strong suppressor of apoptosis acting downstream of cytochrome c release and upstream of caspase-3 activation. Another chaperone i.e. Hsp90 acts as a negative cytosolic regulator of cytochrome c-dependent apoptosis by inhibiting cytochrome c-mediated oligomerization of Apaf-1 and thereby activation of procaspase-9. Hsp27 binds to cytochrome c and prevents its interaction with Apaf-1 and procaspase-9, thus interfering specifically with the mitochondrial pathway of caspase-dependent cell death. Interestingly, alpha B-crystallin, a small Hsp abrogates both the mitochondrial and death receptor pathways by inhibiting the autophosphorylization of caspase-3.

**Phosphorylation and nitrosylation**

Phosphorylation, a posttranslational modification is also employed to modulate caspase activity either affecting the assembly of caspase tetramer or caspase allosteric activity. Phosphorylation of caspase-9 inhibits its activity in vitro and in vivo. S-nitrosylation also modifies caspase activity as nitric oxide (NO) or related molecules have been found to inhibit apoptosis. In healthy human cells, the active site of endogenous procaspase-3 seems to be S-nitrosylated, however, during Fas-mediated apoptosis, it becomes denitrosylated. Further, the denitrosylation is found to enhance mature caspase-3 activity without affecting procaspase-3 processing.

**Compartmentalization**

Different procaspases may be present at different intracellular compartments, and their localization may change during apoptosis. One of the best examples is that during Fas mediated apoptosis, procaspase-8 is recruited from the cytosol to the Fas receptor complex leading to its activation. Procaspases may normally be compartmentalized away from their substrates to prevent accidental apoptosis and during apoptosis, the activation and coordinate translocation of caspases allow them to move close to their targets.

**Mitochondria and Apoptosis**

Mitochondrion plays an important role during apoptotic cell death, integrating different pro- and antiapoptotic stimuli. The apoptotic process exhibits the swelling and the formation of megamitochondria due to the opening of the permeability transition pores. This is followed by a series of events including the collapse of its transmembrane potential and release of several apoptotic factors such as cytochrome c, apoptosis-inducing factor (AIF), Smac/DIABLO, deoxyguanosine kinase (dGK), IKB-a/NF-kB etc. into the cytosol. Cytochrome c and Smac/DIABLO synergistically activate caspases by activating Apaf-1 and relieving the apoptotic inhibition by IAPs respectively. Deoxyguanosine kinase may be of importance for the activation of apoptotic purine nucleoside cofactors such as dATP. Interestingly the mitochondrial IKB-a/NF-kB pool appears to participate in the regulation of apoptosis.

Alterations in cellular metabolism, intracellular pH, redox potential and ion transport can also lead to proapoptotic changes. Selective elimination of the entire cohort of mitochondria from cells also results in apoptosis, which is completely prevented by the expression of the antiapoptotic protein Bcl-2. As cells without mitochondria are irreversibly committed to death, prevention of mitochondrial loss may be crucial during the long-term regeneration therapies adapted for tissues.

**Bcl-2: Regulator of apoptosis**

The oncogene bcl-2 has attracted recent research attention because of its control over apoptosis. It belongs to an expanding multigene family of proteins
that regulate apoptosis during development and homeostasis. Ten mammalian and three viral homologues of Bcl-2 have been cloned to date\textsuperscript{11}. Interestingly, Bel-2-expressing cells exhibit enhanced antioxidant capacities and suppress oxidative stress signals generated during the initiation phase of many apoptotic pathways. Death signals influence mitochondria during apoptosis, yet the critical initiating event for mitochondrial dysfunction \textit{in vivo} is unclear. Pro and antiapoptotic members of the Bcl-2 family may regulate mitochondrial participation in cell death by controlling cellular redox pathways\textsuperscript{39}, voltage dependent anion channel (VDAC) function and also form multimeric channels large enough to release apoptosis promoting proteins from the intermembrane space\textsuperscript{20}. Alternatively, Bcl-2 family proteins may coordinate the permeability of both the outer and inner mitochondrial membranes through the permeability transition (PT) pore. Pro-apoptotic members increase mitochondrial membrane permeability by favoring Bcl-2/Bax heterodimer formation, whereas homodimerization of anti-apoptotic Bcl-2 members prevent the same. Bak and BID may release cytochrome c from mitochondria, whereas Bid may also activate additional downstream molecules and PKCdelta. tBID, the caspase-activated form of Bid triggers the homo oligomerization of Bak or Bax, resulting in the release of cytochrome c from mitochondria. Thus Bak or Bax appears to be important for mitochondrial dysfunction leading to cell death in response to diverse stimuli\textsuperscript{41}. Bcl-rambo shows no interaction with either anti-apoptotic (Bcl-2, Bcl-XL, Bcl-w, A1, MCL-1, EIB-19K, BHRFl) or pro-apoptotic (Bax, Bak, Bik, Bid, Bim and Bad) members of the Bcl-2 family. However, its overexpression induces apoptosis that is specifically blocked by the caspase inhibitors, while inhibitors controlling upstream events of either the death receptor or the mitochondrial pathway have no effect\textsuperscript{42}. Thus the Bcl-2 family proteins seem to regulate cell survival, but the exact mechanism is still unknown. Phosphorylation of Bcl-2 has also been implicated as an important regulatory mechanism for its functioning\textsuperscript{43}. Current investigations indicate that different signaling pathways may be involved in Bcl-2 phosphorylation, dependent on the kinases activated by the various stress stimuli. Since deregulation of the expression of Bcl-2 proteins occurs in a variety of human tumors, a better understanding of the molecular mechanisms by which Bcl-2 family proteins regulate apoptosis could help in designing approaches to enhance the susceptibility to drug-induced cell death in anti tumor chemotherapy.

**p53: Regulator of apoptosis**

The human p53 tumor suppressor is a multifunctional phosphoprotein involved in coupling DNA damage to cell cycle arrest and DNA repair or apoptosis\textsuperscript{17}. p53 is the most commonly mutated gene in human cancer. p53 arrests the cell cycle by inducing transcription of cyclin-dependent kinase inhibitor p21\textsuperscript{44}. The most recently identified members of the p53 family, p63 and p73, share certain structural and functional similarities with p53. Both p63 and p73 can bind to p53 promoters, transactivate p53 target genes and induce apoptosis\textsuperscript{45}. Studies in human colon cancer cell line suggest that p53 transactivation dependent apoptosis is due to upregulation of the death receptors Fas, TRAIL-R1 and TRAIL-R2, and activation of caspase-9 and caspase-3\textsuperscript{46}. Interestingly, p53 also induces caspase-1, Apaf-1, Bik, Bak and cFLIP (the death receptor-inhibitory protein) proteins and down-regulates Bcl-XL protein. Thus p53 seems to mediate the activation of the mitochondrial pathway of apoptosis. p53 may also promote apoptosis through activation of Cdc42 and inactivation of Bcl-2. Cdc42 (a Ras-like GTPase) promotes apoptosis via PAK1 and JNK1 activation which is inhibited by Bcl-XL but not Bcl-2, as Bcl-2 is inactivated by JNK1-induced phosphorylation.

Thus, in contrast to p53-mediated cell cycle arrest, the mechanism of p53-mediated apoptosis in response to DNA damage, has just begun to be understood. The therapeutic interest in p53 as the molecular target of anticancer intervention rests mainly on its powerful apoptotic capability. Recent evidence of a direct proapoptotic role of p53 protein at mitochondrial level suggests a synergistic effect with its transcriptional activation function and brings an unexpected new level of complexity into p53\textsuperscript{47}. Nevertheless, a wild type p53 can induce both G1 and G2/M arrest, in a p21 dependent manner, a p53 mutant defective in transactivation elicits apoptosis without inducing cell cycle arrest. Hence, the use of a transactivation deficient p53 in gene therapy trials or employing drugs that convert mutant p53 to a transactivation independent mediator of apoptosis may be a promising approach than current approaches that employ wild type p53.

**Apoptosis in the Pathogenesis and Therapeutic Implications**

Apoptosis is a general biological mechanism responsible for maintenance of cell number, tissue
growth and development. Impairment of apoptosis regulation entails disorders in homeostasis and various diseases. Defective apoptosis is implicated in the pathogenesis of a variety of human diseases including cancer, autoimmune disorders and viral infection, whereas excess of cell death results in a wide number of diseases characterized by cell loss, such as neurodegenerative disorders, AIDS, osteoporosis etc. The control of apoptosis forms the basis in addressing the apoptosis-associated diseases. Therapies that enhance the susceptibility of cancer and autoimmune cells to undergo immune-mediated apoptosis will be useful in the treatment of these diseases. Drugs or therapies that decrease the apoptotic threshold by modulating the intracellular regulatory mechanisms will be helpful in the treatment of neurodegenerative disorders, AIDS etc.

**Ageing**

Ageing, a general decline in various biochemical and physiological functions is due to increased DNA damage, decreased capacity to repair or both. Immunosenesence, the deterioration of the immune system due to ageing, is characterized by decreased tendency to undergo apoptosis. It is due to the adaptation of the system to lifelong exposure to reactive oxygen species and may lead to modifications of humoral and cellular immunity resulting in increased morbidity and mortality from infections and possibly autoimmune diseases and cancer. Among others, the most profound changes involve effector and immunoregulatory T cell functions. The main functions of T cells i.e. activation, anergy and apoptosis are affected during ageing. Ageing is also associated with mitochondrial dysfunction in excitable tissues such as nerve and muscle as well as in lymphocytes. Lymphocytes during ageing exhibit enhanced activation of mitochondrial membrane potential that may inhibit energy metabolism and enhance apoptosis, thus contributing to immunosenesence. The neurohormone melatonin unites the functions of nervous, endocrine and immune systems and displays immunomodulating, radioprotective and antitumour activities. The progressive decline in melatonin secretion during ageing has been suggested to be one of the main factors that enhance the effects of oxidative stress causing cellular damage resulting in senescence and age associated diseases. Suitable antioxidant therapy involving melatonin or natural plant products would probably be the best approaches to control ageing and age associated diseases.

<table>
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<th>Process/Disease</th>
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<th>Defect</th>
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<tbody>
<tr>
<td><strong>Ageing</strong></td>
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<td>Increased DNA damage</td>
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<tr>
<td><strong>Immunosenescence</strong></td>
<td>Increase/decrease</td>
<td>Changes in T-cell function</td>
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<tr>
<td><strong>Cancer</strong></td>
<td>Decrease</td>
<td>Overexpression of Bcl-2 and survivin gene</td>
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<tr>
<td><strong>Autoimmunity</strong></td>
<td>Decrease</td>
<td>Failure to remove autoreactive lymphocytes</td>
</tr>
<tr>
<td>a. Systemic lupus erythematosus</td>
<td>Decrease (Fas-mediated apoptosis)</td>
<td>Elevated levels of soluble Fas</td>
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<tr>
<td>b. Hereditary autoimmunity</td>
<td>Decrease (Fas-mediated apoptosis)</td>
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<td>c. Rheumatoid arthritis, psoriasis, autoimmune diabetes mellitus</td>
<td>Decrease</td>
<td>Failure to remove autoreactive lymphocytes</td>
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<td><strong>Neurodegenerative disorders</strong></td>
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<td><strong>Virus induced lymphocyte depletion</strong></td>
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<td>Myelodysplastic syndrome, Aplastic anaemia</td>
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<td>Activation of genes promoting apoptosis</td>
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<td>Acquired deficiency in hematopoietic survival factors</td>
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Cancer

Abnormal cell survival is the hallmark of cancer cells. Activation of the apoptotic program has been implicated in the response of cancer cells to chemotherapy and radiotherapy. However, chemotherapy-resistant cancers may develop lesions in the apoptosis signal transduction cascade. The molecular basis of increased resistance of tumor cells to apoptosis may be due to overexpression among others, antiapoptotic genes such as bel-2 and apoptotic inhibitor protein, survivin. Oncogene transformation also leads to dysregulation of genes that control cell division such as cyclins and cyclin dependent kinases or p53. The survivin gene is a novel apoptotic inhibitor, which is believed to play a pivotal role in cancer and therapeutic targeting of survivin may be beneficial to patients with recurrent or metastatic diseases. The transfer of apoptosis-inducible genes such as Apaf-1, caspases, Fas-ligand, FADD etc. and the restricted induction of apoptosis in tumor cells, afforded by the human telomerase catalytic subunit (hTERT) gene promoter may be a promising targeting approach for the treatment of tumors with telomerase activity.

Autoimmunity

Failure to remove autoimmune cells that arise during development or that develops during somatic mutations as a result of immune response can result in autoimmune diseases. Hereditary autoimmune diseases may arise due to alteration in Fas mediated apoptosis. Systemic lupus erythematosus is characterized by elevated levels of soluble Fas that competitively inhibits Fas-ligand – Fas-receptor interactions and thus decrease in Fas-mediated apoptosis resulting in accumulation of autoimmune cells in this disorder. Alterations in the susceptibility of autoreactive lymphocytes to die by apoptosis in vitro have been reported in several diseases like rheumatoid arthritis, psoriasis, inflammatory bowel disease, autoimmune diabetes mellitus etc. Selective depletion of autoreactive lymphocytes by caspase activation and Fas-induced apoptosis by systemic administration of bis-indolylmaleimide VIII may ameliorate autoimmune diseases.

Neurodegenerative disorders

A number of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis (ALS), Retinitis Pigmentosa, and Spinal Muscular Atrophy are characterized by neuronal loss by apoptosis. Caspase inhibition by p35, CrmA or peptide caspase inhibitors can abrogate neuronal apoptosis and ameliorate neurodegenerative diseases. However, broad caspase inhibitors that can prevent neuronal loss in neurodegeneration may also lead to chronic caspase inhibition and block homeostatic apoptosis and risk autoimmunity and cancer. Selective interference with caspase-12 that may not be required for developmental or homeostatic apoptosis could be a better target for treating neurodegenerative diseases.

Virus induced lymphocyte depletion

Development of AIDS induced by HIV is mainly due to depletion of CD4+ T cells which act as receptors for viral attachment. Recent evidence suggests that stimulation of CD4 receptor; by its binding to soluble viral product gp120 results in enhanced susceptibility of uninfected T cells to undergo apoptosis. In chronic HIY infection there is a rapid depletion of CD4+ T cells and subsequent loss of protective cell mediated immune response against a wide variety of viral infection. Inhibitors of caspases are able to block T cell apoptosis induced by HIV infection, however, increases the rates of viral production and host cell infection. Anti HIV therapy based on the sensitivity of HIV infected cells to selectively undergo apoptosis can be attempted. The transfer of apoptosis-inducible gene i.e. an engineered procaspase 3 with HIY-I protease cleavage specificity that can specifically induce apoptosis in HIY infected cells and decrease virus spread may be a promising targeting approach for the treatment of HIV infection. Similar strategies may be helpful in the treatment of other chronic infections.

Hematological disorders

Mature blood cells are constantly being produced from hematopoietic stem cells located in the bone marrow. The regulation of hematopoiesis is influenced by a number of growth factors and thrombopoietin. Hematopoietic progenitors rapidly undergo apoptosis in vitro if deprived of growth factors. Hematopoietic growth factors are also important in regulating the survival of postmitotic blood cells such as neutrophils. A number of hematological diseases including aplastic anaemia, chronic neutropenia and the myelodysplastic syndrome are associated with a decreased production of blood cells and increased apoptotic cell death within the bone marrow. These disorders could result from the activation of genes that promote apoptosis, acquired deficiencies in stromal cells or hematopoietic survival factors. Caspase
Caspases, the major executioners of cell death are being extensively studied. Understanding the mechanism and effector molecules controlling apoptotic cell death is evolving. Caspases, the major executioners of cell death are synthesized as inactive zymogens that become activated by adapter protein mediated oligomerization or transactivation by upstream proteases in an intracellular cascade. Regulation of caspase activation as well as activity occurs at several different levels. Unraveling the biochemical pathways for apoptotic and nonapoptotic programmed cell death provides new insights for the understanding of neurodegeneration biology, cancer therapies, development and evolutionary aspects of cell death programs. Further, the mitochondria may play a central role for cell death activation in both animal and plant cells. The molecular mechanisms involved in the nuclear-mitochondrial crosstalk that are essential for cell life and death are yet to be understood. Caspase based therapies may be promising for apoptosis associated diseases as well as gene and cell based therapies. Apoptosis of recombinant gene expressing cells, by small molecule mediated oligomerization and activation of chimeric caspases offers a fail-safe mechanism to terminate gene therapy and can also be exploited in tissue engineering. Based on the intense research over the past few decades, the coming years may witness exciting discoveries of the net working of cell death biology, facilitating the design of novel therapeutic strategies for cancer and other disease states in which apoptosis plays a pivotal role.

Conclusion

The field of research on apoptosis is rapidly growing. Understanding the mechanism and effector molecules controlling apoptotic cell death is evolving. Caspases, the major executioners of cell death are synthesized as inactive zymogens that become activated by adapter protein mediated oligomerization or transactivation by upstream proteases in an intracellular cascade. Regulation of caspase activation as well as activity occurs at several different levels. Unraveling the biochemical pathways for apoptotic and nonapoptotic programmed cell death provides new insights for the understanding of neurodegeneration biology, cancer therapies, development and evolutionary aspects of cell death programs. Further, the mitochondria may play a central role for cell death activation in both animal and plant cells. The molecular mechanisms involved in the nuclear-mitochondrial crosstalk that are essential for cell life and death are yet to be understood. Caspase based therapies may be promising for apoptosis associated diseases as well as gene and cell based therapies. Apoptosis of recombinant gene expressing cells, by small molecule mediated oligomerization and activation of chimeric caspases offers a fail-safe mechanism to terminate gene therapy and can also be exploited in tissue engineering. Based on the intense research over the past few decades, the coming years may witness exciting discoveries of the net working of cell death biology, facilitating the design of novel therapeutic strategies for cancer and other disease states in which apoptosis plays a pivotal role.

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