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REVIEW

Apoptosis: A Current Molecular Analysis

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Apoptosis is a cell suicide program characterized by distinct morphological (cell shrinkage, membrane blebbing, pyknosis, chromatin margination, denser cytoplasmic images) and biochemical (e.g., DNA fragmentation into distinct ladders, degradation of apoptotic markers such as PARP and nuclear lamins) features. It is involved in multiple physiological processes exemplified by involution of mammary tissues, embryonic development, homeostatic maintenance of tissues and organs, and maturation of the immune system as well as in many pathological conditions represented by neurologic degeneration (Alzeimer's disease), autoimmune and inflammatory diseases, etiology of atherosclerosis, AIDS, and oncogenesis and tumor progression. Numerous molecular entities have been shown to regulate the apoptotic process. This review provides a concise summary of the recent data on the role of oncogenes/tumor suppressor genes, cytokines and growth factors/growth factor receptors, intracellular signal transducers, cell cycle regulators, reactive oxygen species or other free radicals, extracellular matrix, regulators of cell adhesion molecules, and specific endonucleases and cytoplasmic proteases (the ICE family proteins) in regulating cell survival and apoptosis. Elucidation of the molecular mechanisms regulating apoptosis bears tremendous impact on enhancing our understanding of many diseases inflicting the human beings and undoubtedly brings us hope for the cure of these diseases. (Pathology Oncology Research Vol 2, No 3, 147–157, 1996)

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Apoptosis, which represents a type of physiological cell death distinct from necrosis induced mostly by nonphysiological insults, has received tremendous attention from biomedical and clinical researchers due to its close involvement in multiple biological and pathological processes such as embryonic development, homeostatic maintenance of tissues and organs, maturation of the immune system, neurologic degeneration, autoimmune and inflammatory diseases, etiology of atherosclerosis, and oncogenesis and tumor progression. Apoptosis is an evolutionarily conserved process, which has been observed across various species from virus, bacteria, *Drosophila*, *C. elegans*, to mammalian cells and it serves primarily as a self-defense mechanism against hostile intruders (for microorganisms) or to eliminate aged, damaged or unwanted own cells (for multicellular organisms). Apoptosis is considered a genetically regulated process as seen in physiological events such as the involution of mammary gland. However, it can be triggered, especially in *in vitro* cultured cells, by physical (radiation, hyperthermia, disruption of cell-cell contact or cell-matrix interactions, growth factor deprivation) and toxic (various chemotherapeutic drugs, protein synthesis inhibitors, azide, H_2O_2) exposures, cellular effects of hormones, growth factors, and cytokines (e.g., glucocorticoids, TNF-α, IL-1, and TGF-β), viral infection, and immunologic mechanisms (Fas antigen, CTL-mediated killing, anti-Ig, and T cell receptor signaling).[1] A multitude of factors have been implicated in regulating/modulating apoptosis; these include: (i) oncogenes/tumor suppressor genes exemplified by p53, bcl-2 family (bcl-2, bcl-X_L, bcl-X_b, bcl-X_S, bax, BAG-1, Bad, bak, A1, Mcl-1), myc, ras, abl, raf, jun, Egr-1, Pim-1, Ets-1, WT1, Rb-1, and Waf-1;[2-10] (ii) hormones (e.g., estrogen, PTHrP), cytokines and growth factor/growth factor receptors (retinoid acid, interleukin-3, stem cell factor, interferon γ, erythropoietin, NGF/NGF

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receptor, TNF-α/Fas, steel factor/Kit receptor, TGF-β/TGF receptor, insulin, EGF/EGFR, IGF-1/IGF receptor, and PDGF/PDGF receptor);[17-27] (iii) intracellular signal transducers such as protein kinase C, PI-3 kinase, Ras and GTPase, PLC-γ, tyrosine kinases and protein phosphatases, lipid signaling molecules such as eicosanoids, sphingosine, ceramide, and Ca^{2+};[28-41] (iv) cell cycle regulators exemplified by cdc-2 and E2F;[42-46] (v) reactive oxygen species or other free radicals;[47-50] (vi) extracellular matrix regulators/cell adhesion molecules (extracellular matrix proteins such as fibronectin and transmembrane integrin receptors);[51-55] (vii) specific endonucleases such as Ca^{2+}- and Mg^{2+}-dependent DNase and cytoplasmic proteases typified by ICE (interleukin 1β-converting enzyme) family,[56-64] and (viii) global intracellular environment such as cytoplasmic pH.[65,66]

Many distinct morphological (cell shrinkage, membrane blebbing, pyknosis, chromatin margination, denser cytoplasmic images) and biochemical (e.g., DNA fragmentation into distinct ladders) features of apoptosis set it apart from other types of cell death such as oncosis.[1] Often apoptosis has been mistakingly exchanged for the programmed cell death, the latter of which in some cases (e.g., physiological spermatogenesis and cell death during nervous system development) does not evoke apoptosis.[1] Although apoptosis was once thought to proceed from nuclear collapse to cytoplasmic and membrane destruction, recent experimental data suggest that, at least in some forms of apoptosis such as those induced by Fas, UV and ceramide, cell death may be triggered by cytoplasmic constituents independent of nuclear activities.[67] In this review article we'll provide a concise summary of the molecules, as mentioned above, that have been shown to be involved in regulating cell survival and apoptosis, with emphasis on the signal transducers. A very important point to bear on mind is that different types of cells may utilize quite divergent molecular mechanisms for the regulation of their own fate: proliferation or demise. Therefore, one molecular entity may positively contribute to the suicide program in one cell type but has no effect on or even play a negative role in the apoptosis of another.

1. Oncogenes and Tumor Suppressor Genes Involved in Apoptosis

Of this class bcl-2 family and p53 are most extensively studied. ***Bcl-2*** has been convincingly demonstrated to suppress apoptosis induced by most, if not all, types of inducers, suggesting that it sits probably at a very distal end of the road leading to death. Two points deserve some discussion here. First, it has become very clear that Bcl-2 is only one member of a still-expanding family of proteins. This family now has more than ten members: Bcl-2 (B cell lymphoma 2), Bax (Bcl-2-associated X protein), Bcl-X_L, Bcl-X_s, several viral proteins (BHRF-1 and LMW5-HL), *ced*-9, Mcl-1, A1, Bad (bcl-X_L/bcl-2-associated death promoter homolog), Bak (Bcl-2 homologous antagonist/killer) and NR-13.[3,8] These proteins, via complex protein-protein interactions through BH1/BH2 (Bcl-2 homology) domains or other domains which are highly conserved among most Bcl-2 family members, regulate cell survival and apoptosis.[68,69] Early work has demonstrated that Bcl-2 protein inhibits apoptotic cell death induced by multiple stimuli, that Bax promotes cell death by complexing with Bcl-2 in a BH1 and BH2-dependent manner, and that the Bcl-2/Bax ratio dictates the fate of a cell (i.e., the higher the ratio the higher probability for a cell to survive). The identification of multiple new members of the Bcl-2 family, however, clearly makes this picture more complicated. Screening for protein-protein interactions has determined that Bcl-2, Bax, Bcl-X_L and Bcl-X_s are capable of homo-interactions and that Bcl-2 can heterodimerize with Bax, Bcl-X_s, A1 and Bad, Bax with Bcl-X_L, Mcl-1 and A1, and Bcl-X_L with Bax, Bad and Bcl-X_s.[3,68,69] Some of these homo- or hetero-interactions appear to require intact BH1 and/or BH2 domains. For instance, both BH1 and BH2 domains of Bcl-2 are needed for its heterodimerization with Bax.[69] In contrast, only the BH1 domain, together with the membrane-anchoring region at the C-terminal half of the Bcl-2 is required for its interaction with Bcl-Xs.[68] Similarly, Gly^{159} to Ala substitution in BH1 of Bcl-X_L has been shown to disrupt its heterodimerization with Bax.[69] On the other hand, a conserved domain (distinct from BH1 and BH2) in Bak and Bax appear to mediate protein binding functions and apoptotic cell death.[70] Among all Bcl-2 family members, Bcl-2, Bcl-X_L, NR-13, Mcl-1, BHRF1 and LMW5 are generally anti-apoptotic while Bcl-X_s, Bax, Bad, and Bak promote cell death. Another Bcl-2 binding protein, Bag-1 (Bcl-2-associated athanogene 1), which shares no significant homology with Bcl-2 family members, has been shown to possess anti-death activity.[3] Eukaryotic cells clearly demonstrate tremendous redundancy in the expression of the Bcl-2 family members and the balance between anti-apoptotic and pro-apoptotic members as a result of combinatorial interactions or feedback regulatory mechanisms will, to a large extent, determine the eventual outcome of the cell. However, some cells may preferentially utilize one member as their predominant survival factor. Examples include peripheral blood B lymphocytes which primarily express Mcl-1[71] and Reed-Sternberg cells of Hodgkin's lymphoma which primarily express Bcl-X_L.[72] Studies on many human tumors including neuroblastoma, glioma, lymphoma, breast carcinoma, colorectal adenocarcinoma, prostate adenocarcinoma, melanoma, and gastrointestinal malignancies have demonstrated a general correlation between increased expression of Bcl-2 (or Bcl-X_L) or decreased expression of Bax and uncontrolled tumor cell growth, and, in some cases, with tumor progression and a poor prognosis of cancer patients.[73-79]

Another important aspect regarding Bcl-2 is how it exerts anti-apoptosis effect and how its function is regulated. Early work revealed that Bcl-2 protein may play an anti-oxidant role thus preventing cells from free radical-elicited cytotoxicity. Recently, experimental data suggest that Bcl-2 as well as Bcl-X_L may exert an anti-cell death function by a mechanism unrelated to regulation of ROS (reactive oxygen species) activity since hypoxia can also induce apoptosis which still can be suppressed by Bcl-2 or Bcl-X_L.[80] As a matter of fact, Bcl-2 has been demonstrated to be a pro-oxidant.[81] The resolution of this controversy will be of great significance in helping delineate the molecular mechanisms of apoptosis. Suffice it to say, Bcl-2's function may be related to its physical localizations inside the cells (i.e., primarily at mitochondria membrane) and its ability to regulate oxygen tension. How Bcl-2 expression and function are regulated is also unclear, although down regulation by p53 and WT1 tumor suppressor genes and inactivation by phosphorylation have been demonstrated.[52,83]

p53, a phosphoprotein known to modulate gene transcription, police cell cycle checkpoints, control DNA replication and repair, and maintain genomic stability, also plays an important role in regulating apoptosis. Wild-type p53 activates apoptotic program while p53 gene mutations have been linked to attenuated apoptosis in multiple cancers represented by Wilms' tumor, colon cancer, cervical carcinoma and breast cancer.[84,85] A central point with respect to the relationship between p53 expression/function and apoptosis is that wild-type p53 can mediate apoptosis and that cell death triggered by certain stimuli (such as irradiation, ADA deficiency and genotoxic drugs) require wild-type p53 for the induction of apoptosis.[5,86] However, apoptosis induced in many cell types by multiple stimuli do not require the presence of functional p53 or can actually proceed irrespective of the status of p53, i.e., no matter whether it is wild-type or mutants.[87] Under certain circumstances, even mutant p53 can induce apoptosis.[88] The precise mechanism whereby p53 controls apoptosis is unknown. However, it must be connected to mechanisms regulating cell cycle progression, DNA repair, and apoptotic machinery since the transcriptional targets of p53 have been shown to include Gadd45, $p21^{WAF1}$, mdm-2, Bax, c-Myc, Bcl-2, PCNA (proliferating cell nuclear antigen) and IGF-BP3.[5,90]

Many other oncoproteins have been implicated in apoptosis. The ***c-Myc*** protein is a nuclear transcriptional activator with the bHLH/LZ (basic region helix-loop-helix/leucine zipper) motif located at the carboxyl terminus. It heterodimerizes with another bHLH/LZ protein partner, Max, and transactivates target gene expression upon binding to the DNA E-box elements. c-Myc is well-known to be involved in cell proliferation and tumorigenesis due to its prominent amplification in multiple tumors, in particular in some lymphomas and lung cancer. Recent work has also implicated c-Myc in apoptosis. For example, c-Myc mutants expressing only the HLH/LZ domains induce rapid apoptosis after microinjected into fibroblasts.[91] Other studies have shown that c-myc expression can either promote or suppress apoptosis, an effect clearly dependent on individual cell types, culture conditions, and the specific phase of the cell cycle during the experimental period. Ber-Abl, a chimeric oncogene formed by a chromosomal translocation that puts sequences from the ber gene upstream of the second exon of c-abl, is a deregulated tyrosine kinase expressed in Philadelphia chromosome-positive human leukemias. There are two naturally occuring forms of Ber-Abl: p185 is primarily associated with acute lymphocytic leukemia and p210 is found mainly in chronic myelogenous leukemia. Although it has long been postulated that prolonged hematopoietic cell survival as a result of reduced apoptosis may be causal to Ber-Abl induced chronic myelogenous leukemia, only very recently was experimental evidence provided for the Abl as a negative regulator of apoptosis.[9,92] Overexpression of Ber-Abl has been shown to confer on cells resistance to apoptosis induction by Fas, cycloheximide, actinomycin D, camptothecin, etoposide, ceramide and serum withdrawal.[9] Site-specific mutagenesis studies suggest that Ber-Abl may utilize different signaling pathways to elicit tumorigenic transformation and suppress apoptotic responses.[92] At least in the case of Ber-Abl-suppressed DNA damage-induced apoptosis, p53-dependent, $p21^{WAF1}$-mediated G_1 arrest or DNA repair appears not to be involved. Instead, a prolonged cell cycle arrest at the $G_{2/M}$ restriction point (thus allowing time to repair and complete DNA replication and chromosome segregation) was observed. $p21^{WAF1/Cip1}$ is a downstream target of wild type p53 and generally is considered as the actual mediator of the p53-induced cell cycle arrest at G_0/G_1. Although wild type 53 can activate apoptosis and many types of apoptosis occur at the growth-arrested cells, $p21^{WAF1/Cip1}$ may not be the mediator of apoptosis. This is best illustrated in the elegant experiments by Katayose et al[12] in which they observed that overexpression of p53 or $p21^{WAF1/Cip1}$ both leads to cell cycle arrest and overexpression of p53 can trigger apoptosis. However, overexpression of $p21^{WAF1/Cip1}$ alone does not cause cells to die, suggesting that p53-induced apotposis is not necessarily mediated by $p21^{WAF1/Cip1}$. A different scenario has been presented that may also suggest complicated roles of p53-$p21^{WAF1/Cip1}$ pathway in apoptosis. In response to treatment by Adriamycin, human T-cell leukemia virus type I-transformed lymphocytes are found to undergo prominent apoptosis regardless of the p53 status.[93] Also, the protein levels of $p21^{WAF1/Cip1}$ were found to be significantly elevated. These observations emphasize the point that different stimuli may trigger apoptosis by using quite different signaling mechanisms. This conclusion also gains support from studies on the involvement of Fos, Jun and Egr, a class of immediate early response genes closely associated

with cell growth, in apoptosis. Introduction of dominant negative mutants of c-jun and Egr-1 delays the onset of S-phase and reduces the cell survival[16], suggesting that these two oncogenes positively regulate cell survival. However, in thapsigargin-induced apoptosis of human melanoma cells, Egr-1 is found to be the mediator of apoptosis since the process can be blocked by either antisense oligos to Egr-1 or by a dominant negative construct.[94] Recent experimental evidence also points against a direct involvement of c-jun and c-fos in apoptosis.[95,96] Many other oncoproteins including WT1, Ets-1, Pim-1, Rb, and nm23 have been proposed to be involved in apoptotic process, however, their precise mechanisms of action (i.e., the cause-and-effect relationship) remain yet to be clarified.

2. *Growth Factors/Growth Factor Receptors, Hormones, and Cytokines Involved in Apoptosis*

In most cases, growth factors/growth factor receptors, hormones, cytokines serve as necessary survival factors for the target cells. Well-known examples include neuronal cell death after withdrawal of NGF and apoptosis of lymphocytes after following removal of appropriate cytokines such as IL-3. Blocking the EGF signaling using monoclonal antibodies also elicit apoptosis of cancer cells.[22] However, under certain circumstances, growth factors normally involved in supporting cell proliferation may trigger cell death, most probably as a result of confusing signals delivered to the treated cell. Thus, fibroblasts growth-arrested following serum-depletion can undergo apoptosis if restimulated by PDGF.[18] In this section, we will put our emphasis on three molecules, i.e., TNFα, TGFβ1 and insulin.

TNFα (tumor necrosis factor-α) has been shown to induce, either directly or in the presence of protein synthesis inhibitors such as cycloheximide, apoptosis of multiple cell types. It belongs to the TNF ligand superfamily which also includes LTa, LTb, CD27L, CD30L, CD40L, 4-1BBL, OX40L and Fas ligand.[97] These ligands are acidic, share ~20% overall homology, and exist mainly as membrane-bound forms. TNFα binds to the cell surface TNF receptor, a member of the TNF/NGF receptor superfamily which houses at least ten members: the p75 NGFR, p55 TNFR-I, p75 TNFR-II, TNF-RP/TNFR-III, CD27, CD30, CD40, 4-1BB, OX40, OX40 and Fas (APO-1 or CD95).[97] These are typical type I transmembrane proteins with multiple cysteine-rich domains in the extracellular (amino terminal) part which is involved in the ligand binding. The past two years has witnessed a significant progress in our understanding of the mechanisms of the death-inducing effects of this powerful cytokine. TNFR-I is responsible for most of the biological properties of TNFα, including apoptosis, antiviral activity and activation of NF-κB. TNFR-I shares a variety of physical and functional properties with Fas. When engaged by agonistic antibodies or by their respective ligands, both can transduce a death signal to the cell. They are homologous not only in the extracellular cysteine-rich domains, but also in the cytoplasmic "death domain" of a stretch of 65-80 aa. The death domain has been found in multiple proteins including the Drosophila death gene reaper, 34 kd TRADD (TNFR1-associated death domain protein), 23 kd FADD (Fas-associated death domain protein; also named MORT1 for mediator of receptor-induced toxicity), 74 kd RIP (receptor interacting protein),[98-100] as well as many other proteins including low-affinity NGFR, p105NF-κB, p100NF-κB, ankyrins, DAP kinase, and myD88.[101] Death domains, like many other protein-binding motifs such as SH domain and PH domain, have been proposed to mediate protein-protein interactions. Thus, The Fas/APO1 and TNFR1 death domains self associate and also bind to each other; FADD and RIP death domains interact with the Fas/APO1 death domain; the TRADD death domain binds to the corresponding region of TNFR1. Although overexpression of FADD, TRADD and RIP has been shown to trigger apoptosis in different cell types, not all death domain-possessing proteins described above have revealed a death-inducing effect. Besides, whereas the induction of cell death by TRADD or RIP requires only their death domains, apoptosis induced by FADD overexpression occurs independently of its C-terminal death domain. These observations suggest some other potential biological functions of this protein motif such as mediating general protein-protein recognition and binding in signal transductions. Very recently, it has been observed that TNF treatment leads to the association with TNFR1 of TRADD which might serve to recruit FADD through the respective domains.[102] Many other proteins such as FAF1 (Fas-associated protein factor 1),[103] FAP1 (Fas-associated protein tyrosine phosphatase),[104] and CAP3 and 4 (cytotoxicity-dependent APO-1-associated proteins 3 and 4)[105] have been reported to be associated with Fas, but whether they are also involved in the TNF-induced cytotoxicity remains unknown. The molecular mechanisms for TNFα-induced apoptosis are also unclear, although the following could be proposed: 1) TNF binding to TNFR1 results in the receptor oligomerization and subsequent recruitment of TRADD, FADD or some other proteins; 2) signal transduction triggered by this protein-protein "chain reaction", or by completely different mechanims, leads to PLA_2 activation and arachidonic acid metabolism, sphingomyelinase activation and ceramide production, and generation of ROIs (reactive oxygen intermediates;[97,106,107] and 3) all of the above mechanisms, either independently, sequentially, or in combination, activates CrmA-sensitive ICE family proteases,[108] leading to cell death.

TGF βI (transforming growth factor β1), a 25 kd homodimeric polypeptide that belongs to a family of multifunctional cytokines, could either promote or inhibit cell

growth depending on cell types. The diverse biological activities of TGFβ1 are mediated through two transmembrane serine/threonine kinases, type I and type II TGFβ receptors. The type II receptor is a constitutively active kinase, which, upon binding to TGFβ1, recruits and phosphorylates the type I receptor (which alone cannot bind to TGFβ1) and subsequently induces the dimeric complex formation between two receptors. The complex formation is required for signaling.[108] Recently, mutation and functional inactivation of TGFβ type II receptor has been implicated in the oncogenesis of colon cancer.[109] The molecular mechanisms whereby TGFβ1 inhibits cell growth may involve induction of cell cycle arrest at G1 and apoptosis. TGFβ1 has been observed to induce or enhance apoptosis of a variety of normal as well as transformed cells including ectocervical epithelial cells, ovarian carcinoma cells, hematopoietic progenitor cells and leukemia cells, vascular endothelial cells, and normal as well as adenomatous colon epithelial cells.[19,110,111] Growth inhibition due to apoptosis induction by vitamine E and retinoic acid has been found to be mediated by TGFβ.[20] Although the data with respect to TGFβ1 as an inducer or enhancer of apoptotic cell death is overwhelming, this cytokine may also function as a critical survival factor for certain types of cells such as T lymphocytes[112] by preventing apoptosis.

Another growth factor that has been consistently shown to provide protective effects from apoptosis is insulin, or, in some cases, insulin-like growth factor (*IGF*).[17] Insulin, together with EGF serves as a critical survival factor for mammary epithelial cells.[21] Treatment of human colorectal carcinoma cells with monoclonal antibodies to EGF receptor induced cell apoptosis, which could be delayed by insulin.[22] Spontaneous apoptosis of lens epithelial cells could be suppressed by insulin, an effect which might involve c-fos and c-jun. IGF-I and its cognate receptor have been shown to suppress apoptosis in vitro as well as in vivo.[17] The levels of IGF-I receptor expression in glioblastoma cells have been shown to be directly correlated with apoptosis and tumorigenesis in nude mice.[113] The above discussion, taken together, suggests that growth factors play a very important, yet complicated role in regulating cell growth and death.

3. *Intracellular Signal Transducers Involved in Apoptosis*

Cytoplasmic signal transducers relay the message from growth factors to nuclear oncogenes or tumor suppressor genes. Various signaling molecules including Ca^{2+}, protein kinase C (PKC), cAMP-dependent protein kinase, Wee1 kinase, PI-3 kinase, Ras and GTPase, PLC-γ, tyrosine kinases, protein phosphatases, lipid signaling molecules such as eicosanoids, sphingosine, and ceramide have all been implicated in apoptosis.[28-41] Although a cause-and-effect relationship has been established in many cell types between the Ca^{2+} concentration fluctuation and induction of apoptosis,[28] the precise roles of most other signal transducers in regulating apoptosis are much more complicated and no clean picture can be presented as yet. In this section we shall briefly update the role of protein phosphorylation mediated by PKC and the MAP kinase pathway (MEK/ERK, JNK, and p38), and of lipid biomolecules represented by eicosnaoids and ceramide in regulating cell survival and apoptosis. The readers are referred to many excellent recent review articles[25-40] related to these topics.

Reversible protein phosphorylation regulated by kinases and phosphatases represents one of the most important and versatile weapons that eukaryotic cells utilize to manipulate the protein functions. PKC is one of the first protein kinases reported to be involved in apoptosis. In general, PKC activation provides most cells a protective effect from apoptosis. Thus, activation of PKC by growth factors such bFGF protects endothelial cells from apoptosis induced by radiation. Similarly, phorbol esters have been observed to protect K562 cells from doxorubicin-induced apoptosis.[114] On the other hand, various PKC inhibitors such as calphostin C, safingol and bisindolylmaleimide GF109203X[31] have been observed to either induce or enhance apoptotic response. However, when the pattern of PKC isoform expression is altered, e.g., by overexpressing PKC-ζ, TPA may instead induce cell death by a PKC-dependent mechanism. This phenomenon has been observed in both leukemia cells (U973)[115] as well as solid tumor cells (MCF7 breast cancer cells).[32] Interestingly, in U937 cells undergoing a spontaneous apoptosis, reduced PKC-ζ and increased PKCβ were observed.[43] In thymocytes undergoing apoptosis induced by staphylococcal enterotoxin B, altered expression patten of the PKC subspecies was also observed. In this case, however, decreased mRNA levels of PKC-β and to a much lesser extent, PKC-α, but not PKC-ζ, were noticed.[116]

The most exciting development on the role of cytoplasmic signal transducers in regulating apoptosis is the recent demonstration that ERK and JNK-p38 MAP kinases appear to possess opposing regulatory effects on apoptosis, with the former being protective and the latter inducive.[36] The MAP kinase signaling pathway is one of the most critical in regulating cellular responses to extracellular signals. Three branches (cascades) of MAP kinase signaling leading to the activation of ERK (extracellular signal-regulated kinase; also called MAP kinase for mitogen activated protein kinase), JNK (c-Jun amino terminal kinase; also called SAPK for stress activated protein kinase) and p38 kinase (also called RK for reactivating kinase), respectively, have been identified.[117,118] The classical MAP kinase cascade includes MAPKKKK (MAP kinase kinase kinase kinase), MAPKKK (MAP kinase kinase kinase), MAPKK (MAP kinase kinase; dual specificity kinases), MAPK (MAP kinases or ERKs), and regulated substrates. In the ERK

cascade, extracellular growth related signals, through receptor tyrosine kinases, $G_{\beta\gamma}$ proteins (via interaction with PH domains), ras, mos, and raf (the MAPKKK) proteins, activate MEKs (MAPK or ERK kinases; the MAPKK) which in turn activate ERKs (the MAPK). In the JNK/SAPK cascade, various stress signals including UV irradiation, heat shock, cytokines and protein synthesis inhibitors, possibly through the Rho family small GTP-binding proteins such as RhoA, Rac and Cdc 42, activate PAK65 (the MAPKKK) which in turn activates MEKK1 (the MAPKK) which in turn activates JNKK/SEK/MKK4 (the MAPKK) which then activates JNK/SAPK (the MAPK). In the p38 MAP kinase cascade, stress signals lead to the activation of an unidentified MAPKKK which then activates MKK3/MKK4 which in turn activates p38 (HOG1).[117,118] There exist multiple isoforms (e.g., 5 isoforms for MEK) for many components in each cascade, thus making the whole signaling system even more complicated. The targets regulated by the MAP kinase pathway include a wide diversity of molecules encompassing cytoskeletal proteins (Tau, MAP1/2/4, caldesmon, etc), cytosolic proteins ($cPLA_2$, PTP2C, tyrosine hydroxylase, Raf-1, MAPKK, etc), membrane proteins (EGF-R, NGF-R, SOS, etc), and nuclear transcription factors (ATF2, Elk1, Ets2, c-Jun, c-Fos, c-Myc, TAL1, etc).[117,118] In general, the ERK cascade is thought to primarily mediate cell proliferation and differentiation while the JNK/p38 cascades are involved in stress responses and growth arrest. This conclusion is consistent with the observations that activation of JNK/p38 and concurrent inhibition of ERK mediate neuronal cell death after NGF withdrawal.[30] Also, γ radiation-triggered apoptosis of peripheral blood lymphocytes has been found to be correlated with the activation of JNK1.[119] However, caution needs to be executed in generalizing the potential roles of ERK and JNK/p38 MAP kinase pathways in regulating apoptosis since the effects could be cell type-dependent and very different with different apoptotic stimuli.

Eicosanoids are a large group of oxygenated molecules derived from arachidonic acid (AA), an essential component of the cell membrane phospholipids. Released AA, through the action of phospholipase A_2, is metabolized via three major biochemical pathways: (i) the cyclooxygenase (COX) pathway leading to the generation of prostaglandins, prostacyclin, and thromboxane; (ii) the lipoxygenase (LOX) pathway giving rise to various hydroperoxy (HPETEs) and hydroxy (HETEs) fatty acids as well as leukotrienes; and (iii) the P450-dependent epoxygenase pathway generating EETs. Various AA metabolites have been implicated in a wide variety of growth-related signaling pathways involving ras, interferon-α, EGF, cAMP, protein kinase C, MAP kinases and fos (reviewed in Ref. 120). Numerous studies have demonstrated a strong correlation between growth factor promoted cell proliferation and generation of various COX products, primarily prostaglandins. Similarly, eicosanoids derived from LOX pathways as well as epoxygenase pathways of AA metabolism also play an essential role in mediating the growth factor-stimulated cell growth.[120,121] On the other hand, both COX and LOX products of AA metabolism may also be involved in modulating cell survival and apoptosis. Many prostaglandins exogenously administered have been shown to induce apoptosis. The administration of a synthetic analog of PGE_1 in mice induces thymocyte apoptosis. Similarly, exogenous PGE_2 induces apoptosis of freshly isolated lymphocytes and immature normal as well as malignant B lymphocytes and a positive correlation has been observed between induction of lymphocyte apoptosis and PGE_2 production by macrophages infected with HIV. Also, apoptosis of ovarian surface epithelial cells is inhibited by indomethacin whose effect could be reversed by exogenous PGE_2 or $PGF2\alpha$ (reviewed in Ref. 120). The PGE_2-induced apoptosis may be mediated by elevating intracellular cAMP levels since agents which elevate cAMP concentrations have been shown to induce thymocyte apoptosis. Other effects such as increased c-Myc protein expression may also be involved. Cyclopentenone prostaglandins such as PGA_2 and Δ^{12}-PGJ_2 have also been reported to induce tumor cell apoptosis possibly through arresting cells in the G_2/M phase and synthesizing novel death-related genes, or by down-regulating $p21^{WAF1}$ and enhancing cyclin-dependent kinase 2 activity.[120,122] Interestingly, various COX inhibitors (NSAID) have been consistently demonstrated to trigger apoptosis of cultured cells.[123,124] Thus, indomethacin, sulindac sulfide and sulfone have been observed to inhibit colon carcinoma cell (HT-29) growth by inducing apoptosis.[124] Likewise, multiple NSAIDs including diflunisal, indomethacin, acemethacin, diclofenac, mefenamic acid, flufenamic acid, niflumic acid, ibuprofen, and carprofen cause apoptosis in chicken embryo fibroblasts.[123] The mechanisms of apoptosis induction by these NSAIDs are very complicated, but may not involve prostaglandin production. Induction of apoptosis in HT-29 cells by sulindac sulfide and some other COX inhibitors appear to involve inhibition of cell proliferation and cell cycle arrest caused by inhibition of activity of cyclin-dependent kinases.[124] Fibroblast apoptosis induced by indomethacin and other NSAIDs mentioned above could not be inhibited by PGE_2. Interestingly, the chronic treatment of the fibroblasts with these NSAIDs resulted in increased mRNA levels of both COX-1 and COX-2 and increased protein levels of COX-2. Based on the fact that all these NSAIDs inhibit the COX activity, the authors concluded that COXs and their products may play a key role in preventing apoptosis.[123] This conclusion recently gained support from the observation that overexpression of COX-2 confers on rat intestinal epithelial cells resistance to apoptosis induction.[125]

Similarly, LOXs and their products have also been implicated in regulating cell survival and apoptosis. Exogenous lipid hydroperoxides such as 15-HPETE

induces HIV-infected human T cells. The high sensitivity of these cells is due to their inability to convert 15-HPETE to 15-HETE owing to a marked reduction in glutathione peroxidase activity (reviewed in Ref 120). LOX inhibitors such as ETYA and NDGA have been shown to inhibit TNFα induced apoptosis of murine fibrosarcoma cells (L929).[29] Interestingly, lipoxygenase product 12(S)-HETE also provided a protective effect. On the other hand, 5-LOX inhibitors can cause apoptosis of human leukemia blast cells[130], suggesting that in some cells 5-LOX may function as a survival factor. Our recent work[43] demonstrated that 12-LOX and probably, 15-LOX, but not 5-LOX, play an essential role in regulating apoptosis of rat Walker carcinosarcoma cells of monocytoid origin (W256). These cells express platelet-type 12-LOX and synthesize 12(S)- and 15(S)-HETEs as their major AA lipoxygenase metabolites. Down-regulation of 12-lipoxygenase gene expression by antisense oligonucleotides triggered time- and dose-dependent apoptosis. The W256 cell death triggered by the antisense oligos could be partially blocked by exogenous 12(S)- or 15(S)-HETE but not by 5(S)-HETE. The antisense oligo treatment also resulted in a significant decerase in the ratio of Bcl-2/Bax, a critical determinant of apoptosis. As expected, overexpression of Bcl-2 protein provided partial death-sparing effect.[11,129] General inhibitors of the LOX activity (such as NDGA) as well as 12-LOX-selective inhibitors (such as baicalein and CDC) also caused significant W256 cell death. W256 cells are not the only cell type that responds to the above treatment by undergoing apoptosis. Other tumor cells such as RBL-1 (rat basophilic leukemia cells), MTLn-3 (rat mammary adenocarcinoma cells), and HEL (human erythroleukemia cell line) cells all undergo apoptosis following treatment with NDGA, although the doses required vary with different cell lines. In contrast, normal cells such as rat aortic endothelial cells (RAEC) do not undergo apoptosis upon treatment with antisense oligos or LOX inhibitors,[11,129] suggesting that tumor cells differ significantly from normal cells with respect their usage of LOXs in regulating cell survival and apoptosis. Based upon the above discussion, it is possible to present a model for the dual role of LOXs in regulating tumor cell survival and apoptosis. For some tumor cells or under certain circumstances, LOXs/LOX prodcuts are the actual mediato·s of the growth effect initiated by survival factors (growth factors, hormones, cytokines, etc). In this case, the LOX system is critical for the cell survival. Blocking the gene expression of LOXs or their enzymatic activities with inhibitors will trigger cell death through apoptosis. The prototypical example is W256 cells presented above. For other tumor cells or under different circumstances, LOXs/LOX products are the actual mediators of apoptosis triggered by death signals. The prototypical example for this case is the apoptosis induced by TNF-α. Whether LOXs/LOX products play a pro-survival or pro-apoptotic role will depend on the cell type and the immediate environmental milieu cells are in contact with (i.e., survival or death factors). This dual-function model also appears to apply to the COXs/COX products which can both induce cell death and protect cells from apoptosis.

Another potent bioactive lipid molecule that has received tremendous attention in the apoptosis field is ceramide derived from the sphingomyelin metabolism.[40] Similar to the mode of AA metabolism, the sphingomyelin pathway, which is initiated by hydrolysis of the membrane-concentrated phospholipid sphingomyelin via the action of either acidic or neutral sphingomyelinase, is a ubiquitous signaling system linking cell surface receptors to nucleus. Like many eicosanoids, ceramide could promote cell proliferation[135] as well as induce cell growth arrest and apoptosis.[40] Many growth factors such as neurotrophins and PDGF could modulate the activity of sphingomyelinase thus modulating the levels of ceramide. Exogenous, membrane-permeable ceramide analogs have been shown to induce apoptotic death of multiple cell types including U937 monoblastic, HL60 promyelocytic leukemia cells, Jurkat cells, and L929/LM and WEHI-164/13 human fibrosarcoma cells.[40,136] Endogenous generation of ceramide through sphingomyelinase activation has been proposed to mediate Fas/APO-1-, TNFα- and radiation-induced apoptosis[40,126] and de novo generation of ceramide via activation of ceramide synthase has been shown to be responsible for the daunorubicin-induced apoptosis of leukemia cells.[137] How ceramide is involved in triggering cell death is currently actively pursued by many groups world wide. Ceramide mimics serum withdrawal in inducing cell cycle arrest of MOLT-4 human leukemia cells,[138] an effect which may be mediated through dephosphorylation of Rb.[139] Downregulation of Bcl-2 protein levels has been proposed to be associated with ceramide-mediated apoptosis.[140] Many potential ceramide targets have been identified. These include: 1) ceramide-activated protein (CAP) kinase, a 97-kd proline-directed (minimal recognition sequence -T-L-P-) serine/threonine kinase which recently is shown to be a Raf1 kinase (it phosphorylates Raf1 at Thr 269 and activates Raf1 by increasing its activity towards MEK);[141] 2) SAPK/JNK;[142] 3) PKC-ζ;[143] 4) a 23-kd nuclear tyrosine phosphoprotein;[141] and 5) others such as Vav oncoprotein and CAPP (ceramide-activated protein phosphatase, a cation-independent heterotrimeric protein of the class PP2A).[40] What roles these divergent target molecules play in transducing the apoptotic signals clearly will be hot topics of future research.

4. Cell Cycle Regulators Involved in Apoptosis

Cell cycle progression and apoptotic process share many phenotypic similarities and apoptosis has oftentimes been considered as a consequence of "mitotic

catastrophe". Multiple oncoproteins involved in cell cycle regulation typified by p53, $p21^{WAF1}$, c-Myc and $p105^{Rb}$ have also been implicated in apoptosis (see discussion in the preceding sections). Many recent review articles (e.g. Ref. 46) have provided detailed accounts of the relationship between the cell cycle and apoptosis. Only a couple of important points will be summarized here. First, certain cell cycle regulator may be linked to certain types of apoptosis, however, the cause-and-effect relationship may not be universally applicable to all cases of apoptosis. This is illustrated by $p34^{cdc2}$ (cdk 1). Early studies indicate that premature activation of Cdc2 kinase is required for apoptosis. Apoptosis in 7-hydroxy-staurosporine-treated T lymphocytes correlates with activation of cdk1 and 2 with a concomitant increase in the H1 kinase activities of these proteins.[15] Withdrawal of NGF induces apoptosis of PC 12 cells which is accompanied by a significant increase in the amounts of cyclin B associated with $p34^{cdc2}$.[4] Also, apoptosis in HL60 cells induced by DNA damage is preceded by unscheduled activation of cyclin B1/Cdc2 kinase.[135] However, using a temperature sensitive expression system of $p34^{cdc2}$, Martin et al found that this kinase activity is not obligatory for the apoptosis induction in FT-210 cells by multiple inducers such as actinomycin D, cycloheximide, H_2O_2, UV irradiation, VP16, and ceramide.[136] These authors conclude that Cdc2 may only represent an upstream regulator of the apoptotic machinery instead of an integral part of this machinery.[136] This conclusion also gains support from recent studies showing that a form of mammalian Cdc2 actually can suppress apoptosis induced by DNA damage.[13] Second, apoptosis induction may occur in any phase of the cell cycle. Some forms of apoptotic cell death can take place only in a specific cell cycle phase while others can occur in any compartment of the cell cycle. For example, Fas ligation induced MML-1 cell death appears to occur specifically in G1B compartment.[137] HIV envelope-initiated CD4 cell death occurs specifically in G_2 phase.[138] X-irradiation triggered-apoptosis of p53-null HL60 cells is observed at the G_2 checkpoint.[140] In contrast to these cell cycle phase-specific apoptosis, programmed cell death induced by transfected wild-type p53 is observed in all phases.[140] Third, alterations in the cell cycle progression can result in apoptosis. E2F-1, a transcription factor which normally heterodimerizes with the DP family proteins and associate with pRb and pRb-related proteins p107 and p130 and thus is involved in the S-phase protein synthesis, can initiate apoptosis when the protein is mutated resulting in a lengthened S phase.[42] Also, point mutations in E2F-1 which disrupt its binding to RB (a negative regulator of cell proliferation) enhances S-phase entry and promotes apoptosis.[141] Even normal E2F1 expression can induce apoptosis in the absence of necessary survival factors and overexpression of the E2F-1/DP-1 complex can induce apoptosis in the presence of survival factors. These discussions illustrate the complex relationship between cell cycle and apoptosis and call for caution in making generalizations.

5. *Reactive Oxygen Species and Free Radicals Involved in Apoptosis*

Reactive oxygen intermediates (ROI) or species (ROS) and various free radicals are ubiquitous entities during physiological cell growth, aging process and response to stress as well as many pathological conditions involving organelles (such as mitochondria) and various organs (e.g., heart, lung, brain). They play an essential role in transducing signals leading to either cell proliferation or cell death. Numerous cellular targets have been reported for the oxidative signaling. Exmaples include various transcription factors such as AP-1, NF-κB, NF-AT and Oct-1, signaling molecules such as tyroine kinases $p56/59^{hck}$, $p72^{syk}$ and $p77^{btk}$, $p21^{ras}$ and ERK, and cell cycle regulator $p21^{WAF1}$.[142,143] Many growth factors including TNFα, bFGF, PDGF, angiotensin, LPA (lysophosphatidic acid) and TGFβ exert their signaling functions, at least partly, via ROS production.[144-146] Due to the potential detrimental effects of ROIs, mammalian cells have developed various defensive mechanisms which primarily include antioxidant enzymes such as superoxide dismutase and low molecular weight antioxidant biomolecules such as reduced glutathione and metallothionein. The evidence for the involvement of ROIs in apoptosis is overwhelming, compelling and unarguable. However, whether ROIs are absolutely required for apoptosis and whether they are the actual mediators of cell death are questionable due to the recent findings that apoptosis can be induced in hypoxic conditions[80] and Bcl-2, a protein claimed to protect cells from apoptotic death by serving as an antioxidant, can also exert its anti-death effect under hypoxic conditions and Bcl-2 may actually possess a pro-oxidant effect.[80,81]

6. *Role of Cell-Cell and Cell-Matrix Interactions in Apoptosis*

Most normal cells, especially epithelial cells, would require at least two signals for their survival: diffusible growth factors and survival signals derived from cell-cell contact and cell-matrix interactions. Although the critical role for soluble growth factors in maintaining cell survivability has been realized and investigated for the past several decades, the potential function for cell-cell/cell-matrix interactions, which are mediated by various adhesion molecules primarily represented by carbohydrates, integrins, cadherins, immunoglobulins and selectins, as survival parameters has only very recently come to the scene. Earlier studies observed that prevention or disruption of the cell-cell/cell-matrix associations of nor-

mal epithelial cells would cause these cells to undergo programmed cell death. In contrast, transformed cells are well-known for their disrupted cytoskeletal organizations, reduced fibronectin matrix assembly and diminished cell-cell/cell-matrix adhesions; however, they also have evolved mechanisms to bypass the requirement for the anchorage-dependent survival. Therefore, generally speaking, tumor cells have extended survivability in the absence of these two life-supporting parameters (i.e., growth factors and anchorage). Abnormal adhesion events have been observed in apoptotic cells. For example, neutrophil apoptosis has been observed to be accompanied by altered expression patterns of adhesion molecules with, specifically, reduced L-selectin/selectin ligand and increased CD11b/CD18 and CD11c/CD18.[74] Cell-cell/cell-matrix interactions could regulate apoptosis in versatile ways. In some cases, cell-cell communication is essential for the cell survival. Mouse malignant T-lymphoma cells (CS-21) undergo apoptosis when they are cultured alone. Coculture of these cells with lymph node stromal cells could block their death, suggesting that some factor(s) (in this case, cysteine) released from the stromal cells possess anti-apoptotic effects.[148] Analogously, normal tonsillar plasma cells, although Bcl-2$^+$, undergo swift programmed cell death which can be prevented by co-cultured bone marrow fibroblasts or rheumatoid synoviocytes.[149] Also, spontaneous apoptosis of mature lymphocytes in Bcl-2-deficient mice can be inhibited by T-B cell interactions.[150] Sometimes, conjugation of cell surface adhesion receptors is enough to deliver cell survival signals. For example, CD44 engagement, either by its ligand hyaluronic acid or anti-CD44 monoclonal antibodies, could significantly inhibit apoptosis of 3D0 T cells induced by dexamethasone or anti-CD3 monoclonal antibodies.[75] Cell-matrix interactions may also be critical for the cell survival. β1 integrins have been shown to be important for the survival of normal breast epithelial cells but not breast carcinoma cells.[54] Blocked expression of α2β1 in MDCK cells with antisense expression vectors results in programmed cell death.[76] Culture of human keratinocytes in suspension leads to apoptotic cell death which could be inhibited by TGFβ1.[151] The molecular mechanisms for adhesion regulated cell survival are not fully understood although enhanced Bcl-2 expression, suppression of ICE function, and ras signaling pathway[75,76] have been proposed.

7. *Role of Endonucleases and Proteinases in Apoptosis*

One of the cardinal biochemical features of apoptosis is the DNA fragmentation which is manifested as a distinct ladder on electrophoresis. This laddered pattern is thought to result from step-wise fragmentation of DNA (from 100 kb to 50 kb and finally to internucleosomal oligomers of ~180 bp) cleaved preferentially at nuclear matrix attachment sites. This led to the hypothesis that activation of specific endonucleases prior to apoptosis may be a critical event. Numerous experiments have provided evidence that this hypothesis may hold true. For instance, two major DNase activities (termed nuc 58 and nuc 40 based on their apparent m.w.) with cation-dependent characteristics have been found in CTLL2 lymphocytes undergoing apoptosis upon IL-2 withdrawal. Similarly, Burkitt lymphoma cell apoptosis induced by ionomycin has been found to be accompanied by the activation of a low m.w., Ca^{2+}-dependent nuclease, which may be a relatively late event since this endonuclease does not cleave DNA into 50 kb fragments.[77] Different types of cells possess a different repertoire of endonucleases and different apoptotic stimuli may activate different sets of endonucleases, even in the same type of cells. Peripheral PMNs and immature myeloid progenitors contain both Ca^{2+}/Mg^{2+}-dependent and DNase II-like acidic endonuclease activity in the nuclei while circulating $CD34^+$ cells possess Mg^{2+}-dependent, Ca^{2+}-independent endonuclease activity. When $CD34^+$ cells are induced to differentiate toward granulocytes, the Ca^{2+}/Mg^{2+}-dependent and acidic endonuclease re-emerge with concomitant disappearance of the Mg^{2+}-dependent endonuclease.[152] On the other hand, apoptosis induced by many stimuli may not involve endonucleases since the apoptotic process can reconstituted in nuclear-free extracts.[65] Or activation of endonuclease activity may proceed with fragmented DNA formation without morphological features of apoptosis (such as chromatin condensation and membrane blebbing).[153] Also, in vitro manipulations (such as SDS and proteinase K treatment) during isolation of fragmented DNA may artificially disrupt the integrity of genomic DNA. Therefore, as with many other situations, caution needs to be executed when exploring the relationship between endonuclease activity and apoptosis and interpreting data. The safest way to prove the nature of apoptotic cell death is to combine DNA fragmentation assays with some other approaches such as electron microscopy.

The observations that many forms of apoptosis can be reconstituted in the nuclei-free, cytosolic extracts suggest that molecular entities present in cytosol may be responsible for triggering cell death. This hypothesis has gained full support from one of the most exciting developments in the past year: the identification of ICE (interleukin-1β converting enzyme) and related molecules as essential triggers of apoptosis. The implication of ICE in regulating mammalian cell death should be attributed to the cloning of the cell death gene, *ced-3*, from *C. elgans*. Right after cloning of *Ced-3*, the related mouse protein *Nedd2* and the human homologue *ICH-1* were identified and characterized.[154] ICE is a cytoplasmic cysteine (cysteine 285 being the catalytic residue) protease which cleaves inactive 31 kd pro-IL-1β (at Asp116-Ala117) to generate the active 17.5 kd IL-1β. It is expressed in many tissues as a 45 kd

inactive propeptide of 404 aa. Active ICE is formed from proteolytic cleavage (at Asp103, Asp119, Asp297 and Asp316) of the proenzyme by, most probably, ICE itself, to generate p20 (residues 120-297) and p10 (residues 317-404) subunits which associate into a $(p20)_2/(p10)_2$ tetramer.[56,155] ICE has been shown to undergo alternative splicing to generate at least four isoforms with differential apoptotic activities.[81] ICE is a very unique cysteine protease in that its enzymatic activity requires Asp in the P_1 postion and a small hydrophobic aa residues at P_1', a property shared with otherwise unrelated serine proteses (granzyme B and fragmentin 2) of the granzyme B family. The optimal peptide sequence recognized by ICE is YVAD, thus laying foundation for the frequent use of synthetic peptide Ac-YVAD-CMK (chloromethylketone) as an effective ICE inhibitor.[155]

Multiple ICE family members have been reported. They include: 1) Nedd2/ICH-1 both of which can be alternatively spliced into the full-length protein and a shorter form;[155] 2) CPP32 (32 kd putative cysteine protease) which is synthesized also as pro-enzyme and then converted to the active form by proteolytic cleavage by some other enzymes instead of by autocleavage like ICE;[155] 3) prICE (protease resembling ICE), also termed Yama/CPP32β[58] or apopain[59]. This enzyme may represent an authentic "executioner" of apoptosis since it is different from ICE in that it does not cleave pro-IL-1β but instead cuts poly(ADP-ribose) polymerase (PARP), a marker protein that has been found to be degraded in nearly all forms of apoptosis; 4) TX/ICH-2/ICE_{rel}-II, which cleaves itself and p30 pro-ICE but not pro-IL-1β or PARP;[57,60,156] 5) ICE_{rel}-III;[156] 6) Mch2 (mammalian *Ced-3* homologue 2),[157] a protein identified using PCR with degenerate primers designed from the highly conserved pentapeptides QACRG and GSWFI. Mch-2α, which is the full-length protein, like CPP32, can cleave PARP and induce apoptosis when overexpressed;[157] 7) Mch3/ICE-LAP3, a protein highly related to CPP32 and also cleaves apoptotic markers such as PARP and lamins.[61,62]. These ICE family proteins, according to their overall homology, can be roughly categorized into three subfamilies: the ICE subfamily including ICE, ICE_{rel}II, ICE_{rel}III; the Nedd2 subfamily including Nedd2 and ICH-1; and the YAMA subfamily including Yama, Mch2a, *Ced-3*, and Mch3α.

There has presented compelling evidence that ICE family proteases play a key role in triggering apoptosis. Apoptosis induced by a wide diversity of agents including glucocorticoids, chemotherapeutic drugs (such as cisplatin), TNF-α, and Fas involves activation of one of more of the ICE family proteases.[158,159] How do these cysteine proteases execute cell killing has not been fully characterized. Neither is it known how these ICE family members are each individually regulated and what is the interactive relationship among this group of proteins. It appears that diverse apoptotic inducers, through signal transduction, trigger a cascade of protease activation. Thus, TX/ICH-2 can autocleave itself and process pro-ICE[60]; ICE processes both pro-ICE and pro-YAMA/CPP32β[58]; the in vivo proteolytic activation of Mch3α may depend on CPP32 activity;[61] and granzyme B induces apoptosis via cleaving the activating CPP32.[160] A hypothesis[58] has been proposed to determine whether an ICE family member is trully involved in mediating apoptosis by testing whether its pro-apoptotic activity can be inhibited by CrmA, a cowpox virus protein inhibitor of ICE-mediated apoptosis (such as in Fas- and TNF-triggered cell death),[108] and by whether it cleaves PARP. Fas- and TNF-induced apoptosis can also be inhibited by the baculovirus p35 protein,[161] possibly as a result of inhibition of ICE family proteases by p35. Potential substrates for the ICE cysteine proteases include cytoskeletal proteins such as fodrin and actin microfilaments, sterol regulatory element-binding proetins (SREBP-1 and 2), the 70 kd protein component of the U1, snRNP, PARP, lamins, DNA-dependent protein kinase, protein kinase C δ and retinoblastoma protein.[162-170] It can be safely predicted that more substrates for ICE and related proteases will be identified. The question remains whether the degradation of these target molecules is absolutely required for apoptosis or, in some instances, is just an accompanying event ensuing the initiation of the cell death program. Also it should be bore on mind that not all forms of apoptosis necessarily evoke the activation ICE and that many other proteases such as calpain and some novel serine proteases may also be involved in the execution of cell death.

Perspectives

There is no doubt that we've made striking progress towards understanding the molecular mechanisms regulating apoptosis, a bewilderingly complicated pre-programmed biological process which can be triggered by both physiological stimuli as well as pathological insults. The well-conserved families of apoptosis regulators such as Bcl-2 and ICE are still expanding in their numbers while new pro-apoptotic (such as *CSE1*) and anti-apoptotic genes (such as *dad-1* and IAPs) are constantly being reported. We firmly believe that elucidation of the molecular mechanisms regulating apoptosis will significantly enhance our understanding of many diseases inflicting the human beings. Development of novel, apoptosis-based therapeutic regimens will bring us hope to conquer these diseases.

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