

## Apoptosis: from Signalling Pathways to Therapeutic Tools

<sup>1,3\*</sup>Seyed Hadi Mousavi, <sup>1</sup> Zahra Tayarani-Najaran, <sup>2</sup> Peter Hersey

### Abstract

Apoptosis or programmed cell death is a gene regulated phenomenon which is important in both physiological and pathological conditions. It is characterized by distinct morphological features including chromatin condensation, cell and nuclear shrinkage, membrane blebbing and oligonucleosomal DNA fragmentation. Although, two major apoptotic pathways including 1) the death receptor (extrinsic) and 2) mitochondrial (intrinsic) pathway have been identified, recently endoplasmic reticulum and lysosomal pathways have been also recognized. Depending on both the cell type and the initiating factor, distinct pathways are activated. The pathways share a common final phase of apoptosis, consisting of activation of the executioner caspases and dismantling of substrates critical for cell survival. The important regulatory mechanisms include death receptors, caspases, mitochondria and Bcl-2 family proteins. Modulating of apoptosis is a novel therapeutic strategy in treatment of different diseases. These include situations with unwanted cell accumulation (cancer) and failure to diminish aberrant cells (autoimmune diseases) or diseases with an inappropriate cell loss (heart failure, stroke, AIDS and neurodegenerative diseases). Modulation of apoptosis is a novel therapeutic strategy in treatment of different diseases. Many approaches including gene therapy, antisense strategies and numerous apoptotic drugs to target specific apoptotic regulators, are currently being developed. The goal of this review is to provide a general overview of current knowledge on the process of apoptosis including morphology, biochemistry, signaling as well as a discussion of apoptosis in diseases and effective therapy.

**Keywords:** Apoptosis, Autoimmunity, Cancer, Intrinsic/Extrinsic pathway, Neurodegenerative diseases

---

1- Department of Pharmacology and Pharmacological Research Centre of Medicinal Plants, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

\*Corresponding author: Tel: +98- 9155199598; email: mousavih@mums.ac.ir

2- Immunology and Oncology Unit, Newcastle Mater Hospital, Newcastle, New South Wales, Australia

3- Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

## Introduction

Apoptosis, or cell suicide, is a form of cell death that is morphologically and biochemically distinct from necrosis. The term apoptosis (a-po-toe-sis) was first used by Kerr in 1972 (1-3). Apoptosis is a distinctive and important mode of “programmed” cell death, which involves the genetically determined elimination of cells. However, it is important to note that other forms of programmed cell death have been described and other forms of programmed cell death may yet be discovered (4-6). Apoptosis is a gene regulated phenomenon which is important in both physiological and pathological conditions. It plays an important role during development, metamorphosis and in many diseases including autoimmune, neurodegenerative, cancer and AIDS (7-9). Apoptosis plays an important role during development, metamorphosis and in many diseases (10). Failure to regulate apoptosis is a common feature in several diseases (11). In case of cancers, triggering of apoptosis in malignant cells will be a way of disease control. Alternatively, in a pathological process which induces cellular degeneration, apoptosis inhibition will be expected to be helpful. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents (12). Different injurious stimuli such as heat, radiation, hypoxia, reactive oxygen species (ROS) and cytotoxic anticancer drugs can induce apoptosis (13). Apoptosis-inducing compounds are good candidate in cancer chemotherapy.

## Morphological features of apoptosis

Apoptosis is characterized by distinct morphological features including; chromatin condensation, cell and nuclear shrinkage, membrane blebbing and oligonucleosomal DNA fragmentation (14). During the early process of apoptosis, cell shrinkage and pyknosis are visible by light microscopy which cells are smaller in size, the cytoplasm is dense and the organelles are more tightly packed (1). Pyknosis is the result of chromatin condensation and this is the most characteristic

feature of apoptosis. Extensive plasma membrane blebbing occurs followed by karyorrhexis and separation of cell fragments into apoptotic bodies during a process called “budding”. Apoptotic bodies consist of cytoplasm with tightly packed organelles with or without a nuclear fragment. The organelle integrity is still maintained and all of this is enclosed within an intact plasma membrane. These bodies are subsequently phagocytosed by macrophages, parenchymal cells, or neoplastic cells and degraded within phagolysosomes. Apoptotic cells do not release their cellular constituents into the surrounding interstitial tissue and there is no inflammatory reaction associated with apoptosis (15, 16).

## Biochemical features of apoptosis

Apoptotic cells exhibit several biochemical modifications such as protein cleavage, protein cross-linking, DNA fragmentation, and phagocytic recognition that together result in the distinctive structural pathology described previously (17). The central hydrolytic reactions of apoptosis are catalyzed by a family of proteases, now termed “caspases” for “cysteine proteases acting on aspartic acid”. At least 12 of these enzymes are known. Caspases are widely expressed in an inactive proenzyme form in most cells and once activated can often activate other procaspases, allowing initiation of a protease cascade. For example, when multiple procaspase-9 molecules assemble on the apoptosomes, they can cleave one another to remove a leader sequence and generate a short and long peptide (18). Caspases are often classified as initiators (caspase-2, 8, 9, 10), effectors or executioners (caspase-3, 6, 7) and inflammatory caspases (caspase-1, 4, 5) (19, 20). Effector caspases cleave and inactivate proteins that protect living cells from apoptosis, such as the DNA repair protein, poly (ADP-ribose) polymerase (PARP), ICAD/DFF45 (inhibitor of caspase-activated DNase, the nuclease responsible for DNA fragmentation), or the anti-apoptotic Bcl-2 proteins. Other actions of caspases in apoptosis include cleavage of cytoskeletal

proteins, including the lamins, proteins forming the nuclear lamina; cytoplasmic intermediate filaments (vimentin, cytokeratins), and several proteins involved in cytoskeleton regulation (gelsolin, focal adhesion kinase and p21-activated kinase 2). This results in disassembly of cell structures that depend on the cytoskeleton (18).

The other caspases that have been identified include caspase-11, which is reported to regulate apoptosis and cytokine maturation during septic shock, caspase-12, which mediates endoplasmic-specific apoptosis and cytotoxicity by amyloid- $\beta$ , caspase-13, which is suggested to be a bovine gene, and caspase-14, which is highly expressed in embryonic tissues but not in adult tissues (21-24).

Extensive protein cross-linking is another characteristic of apoptotic cells and is achieved through the expression and activation of tissue transglutaminase (25). DNA breakdown by  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -dependent endonucleases also occurs, resulting in DNA fragments of 180 to 200 base pairs (26). A characteristic "DNA ladder" can be visualized by agarose gel electrophoresis with an ethidium bromide stain and ultraviolet illumination.

Another biochemical feature is the expression of cell surface markers that result in the early phagocytic recognition of apoptotic cells by adjacent cells, leading to quick phagocytosis. This is achieved by the movement of the normal inward-facing phosphatidylserine of the cell's lipid bilayer to expression on the outer layers of the plasma membrane. Recent studies have shown that other proteins including Annexin I and calreticulin are also be exposed on the cell surface during apoptosis (27). Although externalization of phosphatidylserine is a well-known recognition ligand for phagocytes on the surface of the apoptotic cell, recent studies have shown that other proteins are also be exposed on the cell surface during apoptotic cell clearance. Annexin V is a recombinant phosphatidylserine-binding protein that interacts strongly and specifically with phosphatidylserine residues and can be used for the detection of apoptosis (28). Calreticulin

is a protein that binds to an LDL receptor related protein on the engulfing cell and is suggested to cooperate with phosphatidylserine as a recognition signal (28-30).

### **Distinguishing apoptosis from necrosis**

Necrosis is an uncontrolled, passive process and an energy-independent mode of death that usually affects large fields of cells whereas apoptosis is controlled and energy-dependent and can affect individual or clusters of cells (31, 32). Necrosis is mediated by two main mechanisms; interference with the energy supply of the cell and direct damage to cell membranes (33, 34). Some of the major morphological changes that occur with necrosis include cell swelling; formation of cytoplasmic vacuoles; distended endoplasmic reticulum; condensed, swollen or ruptured mitochondria; disaggregation and detachment of ribosomes; disrupted organelle membranes; swollen and ruptured lysosomes and eventually disruption of the cell membrane (1, 34, 35). This loss of cell membrane integrity results in the release of the cytoplasmic contents into the surrounding tissue, sending chemotatic signals with eventual recruitment of inflammatory cells (15, 16). It is also important to note that pyknosis and karyorrhexis are not exclusive to apoptosis and can be a part of the spectrum of cytomorphological changes that occurs with necrosis (36). Table 1 compares some of the major morphological features of apoptosis and necrosis. Whether a cell dies by necrosis or apoptosis depends in part on the nature of the cell death signal, the tissue type, the developmental stage of the tissue and the physiological milieu (32, 37). Two factors that will convert an ongoing apoptotic process into a necrotic process availability of caspases and intracellular ATP. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs can induce apoptosis but these same stimuli can result in necrosis at higher doses (38, 39).

Table 1. Comparison of morphological features of apoptosis and necrosis.

Apoptosis	Necrosis
Cell shrinkage and convolution	Cell swelling
Cytoplasm retained in apoptotic bodies	Cytoplasm released
Intact cell membrane	Disrupted cell membrane
No inflammation	Inflammation usually present

In addition to inducing apoptosis, a number of chemotherapeutic agents have been reported to induce non-apoptotic forms of cell death (40-42). For example, DNA alkylating agents kill cells resistant to apoptosis by inducing necrosis (42). In regard to melanoma, we demonstrated that Ingenol 3-angelate, one of the active ingredients in an extract from *Euphorbia peplus*, and rose bengal induce caspase-independent non-apoptotic cell death (43, 44). The significance of non-apoptotic forms of cell death in chemotherapy and the mechanism(s) by which they are induced by chemotherapeutic drugs remain, largely unclear. It is however noteworthy the non-apoptotic cell death is often observed

under conditions in which apoptosis is inhibited (44).

### Apoptotic pathways

To date, two major apoptotic pathways have been identified - the death receptor (extrinsic) and mitochondrial (intrinsic) pathway (45, 46). Although each pathway is initially mediated by different mechanisms, they share a common final phase of apoptosis, consisting of activation of the executioner caspases and dismantling of substrates critical for cell survival (47, 48). However, there is now evidence that these pathways are linked and that molecules in one pathway can influence the other (49) (Figure 1).

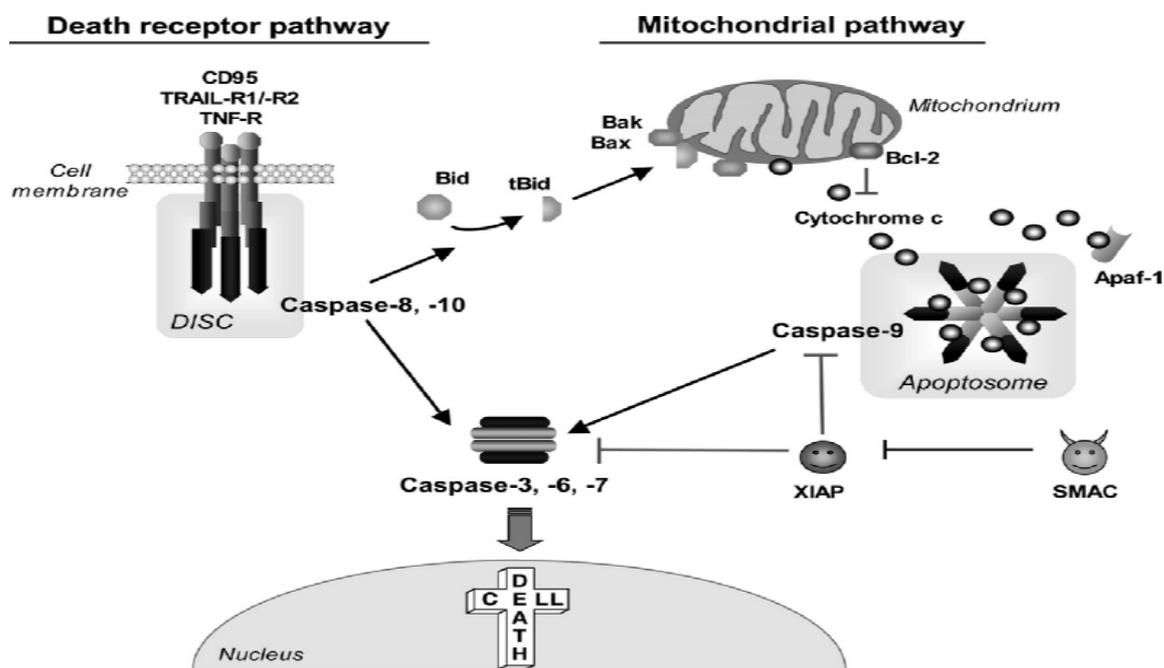


Figure 1. Two major pathways of apoptosis exist in mammalian cells. Left, the extrinsic cell death pathway is mediated by a subgroup of the TNF receptor superfamily called death receptors (CD95, TRAIL-R1/2, and TNF-R1). Receptor-mediated cell death results in the activation of caspase-8, which then directly cleaves and activates caspase-3, -6, or -7, the executioner enzymes of apoptosis.

Right, mitochondrial or intrinsic pathway, is initiated by multiple forms of cellular stress. Intrinsic pathway triggers the assembly of the apoptosome (Apaf-1 and caspase-9) and subsequent activation of caspase-3 and cell death. Pro-apoptotic Bcl-2 family members Bax and Bak translocate to the mitochondria. The BH3-only protein Bid activates Bax and Bak to mediate the release of cytochrome c in the cytosol. The inhibitory function of IAPs is countered by the SMAC (Adapted with permission) (50).

**Extrinsic Pathway**

The extrinsic signaling pathways that initiate apoptosis involve transmembrane receptor-mediated interactions. These involve death receptors that are members of the tumor necrosis factor (TNF) receptor gene superfamily (51). Members of the TNF receptor family share similar cyteine-rich extracellular domains and have a cytoplasmic domain of about 80 amino acids called the “death domain” (45). This death domain plays a critical role in transmitting the death signal from the cell surface to the intracellular signaling pathways. To date, the best-characterized ligands and corresponding death receptors include FasL/FasR, TNF- $\alpha$ /TNFR1, Apo3L/DR3, Apo2L/ DR4 and Apo2L/DR5 (Figure 1) (45, 52-55).

The sequence of events that define the extrinsic phase of apoptosis are best characterized with FasL/FasR and TNF- $\alpha$ /TNFR1 models. In these models, there is clustering of receptors and binding with the homologous trimeric ligand. Upon ligand binding, cytoplasmic adapter proteins are recruited which exhibit corresponding death domains that bind with the receptors. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD and the binding of TNF ligand to TNF receptor results in the binding of the adapter protein TRADD with recruitment of FADD and RIP (56, 57). FADD then associates with procaspase-8 via dimerization of the death effector domain. At this point, a death-inducing signaling complex (DISC) is formed, resulting in the auto-catalytic activation of procaspase-8 (58).

Once caspase-8 is activated, the execution phase of apoptosis is triggered. Death receptor mediated apoptosis can be inhibited by a protein called c-FLIP which will bind to FADD and caspase-8, rendering them ineffective (59, 60). Another point of potential apoptosis regulation involves a protein called Toso, which has been shown to block Fas-induced apoptosis in T cells via inhibition of caspase-8 processing (61). Table 2 lists the major extrinsic pathway proteins with common abbreviations and some of the alternate nomenclature used for each protein.

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a member of the tumor necrosis factor family, such as the tumor necrosis factor  $\alpha$  and Fas ligand, which is a type 2 membrane protein that can induce apoptotic cell death in a wide range of cultured malignant cells, but not normal tissues. Induction of apoptosis by TRAIL is believed to be mediated by its interaction with 2 death receptors on cells referred to as TRAIL-R1 (DR4) and TRAIL-R2 (DR5) (62). It is postulated that normal cells are protected from TRAIL-induced apoptosis by their expression of TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2), which lack cytoplasmic death domains and act to sequester TRAIL (decoy receptors, DcRs) or to mediate anti-apoptotic signals. (43, 62-74).

**Mitochondrial Pathway**

The intrinsic signaling pathways that initiate apoptosis involve a diverse array of non-receptor-mediated stimuli that produce intracellular signals that act directly on targets within the cell and are mitochondrial-initiated events.

Table 2. Some of proteins involved in extrinsic pathway (74, 75).

Abbreviation	Protein Name
Apo2L	Apo2 ligand
Apo3L	Apo3 ligand
Caspase 8	Cysteiny1 aspartic acid-protease 8
DED	Death effector domain
DR3	Death receptor 3
DR4	Death receptor 4
DR5	Death receptor 5
FADD	Fas-associated death domain
FasL	Fatty acid synthetase ligand
FasR	Fatty acid synthetase receptor
RIP	Receptor-interacting protein
TNFR1	Tumor necrosis factor receptor 1
TNF- $\alpha$	Tumor necrosis factor alpha
TRADD	TNF receptor-associated death domain

The stimuli that initiate the intrinsic pathway produce intracellular signals that may act in either a positive or negative fashion. Negative signals involve the absence of certain growth factors, hormones and cytokines that can lead to failure of suppression of death programs, thereby triggering apoptosis. In other words, there is the withdrawal of factors, loss of apoptotic suppression, and subsequent activation of apoptosis. Other stimuli that act in a positive fashion include, but are not limited to, radiation, toxins, hypoxia, hyperthermia, viral infections, and free radicals.

All of these stimuli cause changes in the inner mitochondrial membrane that result in an opening of the mitochondrial permeability transition (MPT) pore, loss of the mitochondrial transmembrane potential and release of two main groups of normally sequestered pro-apoptotic proteins from the intermembrane space into the cytosol (77).

The first group consists of cytochrome C, Smac/DIABLO, and the serine protease HtrA2/Omi (78-80). These proteins activate the caspase-dependent mitochondrial pathway. Cytochrome C binds and activates Apaf-1 as well as procaspase-9, forming an "apoptosome" (81, 82).

The clustering of procaspase-9 in this manner leads to caspase-9 activation. Smac/DIABLO and HtrA2/Omi are reported to promote apoptosis by inhibiting IAP (inhibitors of apoptosis proteins) activity (83, 84). Additional mitochondrial proteins have also been identified that interact with and suppress the action of IAP however gene knockout experiments suggest that binding to IAP alone may not be enough evidence to label a mitochondrial protein as "pro-apoptotic" (85). Apoptosis induced by TRAIL in melanoma cell lines is also caspase-dependent (86).

The second group of pro-apoptotic proteins, AIF, endonuclease G and CAD, are released from the mitochondria during apoptosis, but this is a late event that occurs after the cell has committed to die. AIF translocates to the nucleus and causes DNA

fragmentation into ~50–300 kb pieces and condensation of peripheral nuclear chromatin (87). This early form of nuclear condensation is referred to as "stage I" condensation (88).

Endonuclease G also translocates to the nucleus where it cleaves nuclear chromatin to produce oligonucleosomal DNA fragments (89). AIF and endonuclease G both function in a caspase-independent manner. CAD is subsequently released from the mitochondria and translocates to the nucleus where, after cleavage by caspase-3, it leads to oligonucleosomal DNA fragmentation and a more pronounced and advanced chromatin condensation (90). This later and more pronounced chromatin condensation is referred to as "stage II" condensation (88). In our previous studies we also report that apoptosis induced by Staurosporine and rose bengal in melanoma cells is both caspase-dependent and in-dependent (13, 91).

#### *Bcl-2 family proteins*

The control and regulation of these apoptotic mitochondrial events occurs through members of the Bcl-2 family of proteins (92). The tumor suppressor protein p53 has a critical role in regulation of the Bcl-2 family of proteins; however the exact mechanisms have not yet been completely elucidated (93). The Bcl-2 family of proteins governs mitochondrial membrane permeability and can be either pro-apoptotic or anti-apoptotic.

To date, a total of 25 genes have been identified in the Bcl-2 family. Some of the anti-apoptotic proteins include Bcl-2, Bcl-x, Bcl-XL, Bcl-XS, Bcl-w, BAG, and some of the pro-apoptotic proteins include Bcl-10, Bax, Bak, Bid, Bad, Bim, Bik, and Blk.

It is thought that the main mechanism of action of the Bcl-2 family of proteins is the regulation of cytochrome C release from the mitochondria via alteration of mitochondrial membrane permeability. In response to apoptotic stimuli, several pro-apoptotic proteins are translocated to the mitochondria, where they can interact with membranebound anti-apoptotic proteins, thereby inhibiting the survival functions of the latter (94). Bcl-2,

Bcl-XL, and Bax can form ion channels in artificial membranes, suggesting regulation of apoptosis via the formation of pores.<sup>56,61</sup> Other hypotheses for the inhibition of apoptosis by Bcl-2 include participation in an anti-oxidant pathway<sup>62</sup> and blockage of the release of cytochrome C. (94) In the absence of a death signal, pro-apoptotic Bcl-2 family members are often sequestered by cytoskeletal elements or cytoplasmic proteins (*e.g.*, sequestration of phosphorylated Bad by 14-3-3 proteins) or are only loosely associated with membranes. In contrast, anti-apoptotic Bcl-2 family members are often integral membrane proteins found in the mitochondrial membrane, the nuclear envelope, and the endoplasmic reticulum (94) The activity of Bcl-2-related proteins is regulated through several mechanisms, including their levels of expression, sequestration, and post-translational modifications, such as phosphorylation, cleavage, and translocation Mitochondrial damage in the Fas pathway of apoptosis is mediated by the caspase-8 cleavage of Bid (95, 96). This is one example of the “cross-talk” between the death-receptor (extrinsic) pathway and the mitochondrial (intrinsic) pathway (49). Serine phosphorylation of Bad is associated with 14-3-3, a member of a family of multifunctional phosphoserine binding molecules. When Bad is phosphorylated, it is trapped by 14-3-3 and sequestered in the cytosol but once Bad is unphosphorylated, it will translocate to the mitochondria to release cytochrome C (97). Bad can also heterodimerize with Bcl-XL or Bcl-2, neutralizing their protective effect and promoting cell death (98). When not sequestered by Bad, both Bcl-2 and Bcl-XL inhibit the release of cytochrome C from the mitochondria although the mechanism is not well understood. Reports indicate that Bcl-2 and Bcl-XL inhibit apoptotic death primarily by controlling the activation of caspase proteases (99). An additional protein designated “Aven” appears to bind both Bcl-XL and Apaf-1, thereby preventing activation of procaspase-9 (100). There is evidence that

overexpression of either Bcl-2 or Bcl-XL will down-regulate the other, indicating a reciprocal regulation between these two proteins.

Puma and Noxa are two members of the Bcl-2 family that are also involved in pro-apoptosis. Puma plays an important role in p53-mediated apoptosis. It was shown that, *in vitro*, overexpression of Puma is accompanied by increased Bax expression, Bax conformational change, translocation to the mitochondria, cytochrome C release and reduction in the mitochondrial membrane potential (101). Noxa is also a candidate mediator of p53-induced apoptosis. Studies show that this protein can localize to the mitochondria and interact with anti-apoptotic Bcl-2 family members, resulting in the activation of caspase-9 (102). Since both Puma and Noxa are induced by p53, they might mediate the apoptosis that is elicited by genotoxic damage or oncogene activation. The Myc oncoprotein has also been reported to potentiate apoptosis through both p53-dependent and – independent mechanisms (103).

The ratio of pro- to anti-apoptotic members has been suggested to regulate cell life or death. In our studies increased Bax/Bcl-2 expression has been shown in glucose- and lead-induced apoptosis in PC12 cells (104, 105).

Further elucidation of these pathways should have important implications for tumorigenesis and therapy. Table 3 lists the major intrinsic pathway proteins with common abbreviations and some of the alternate nomenclature used for each protein.

### ***Endoplasmic reticulum and Lysosomal pathways***

It has become clear that each of the main cellular organelles including endoplasmic reticulum (ER) and lysosome can participate in cell death signaling pathways. Recent advances have highlighted the importance of the ER in cell death processes (106). The efficient functioning of the endoplasmic reticulum (ER) is essential for most cellular activities and survival.

Table 3. Some of proteins involved in intrinsic pathway (74).

Abbreviation	Protein Name
AIF	Apoptosis Inducing Factor
Apaf-1	Apoptotic protease activating factor
BAD	Bcl-2 antagonist of cell death
BAG	Bcl-2 associated athanogene
BAK	Bcl-2 antagonist killer 1
BAX	Bcl-2 associated X protein
Bcl-10	B-cell lymphoma protein 10
Bcl-2	B-cell lymphoma protein 2
Bcl-w	Bcl-2 like 2 protein
Bcl-x	Bcl-2 like 1
Bcl-XL	Bcl-2 related protein, long isoform
Bcl-XS	Bcl-2 related protein, short isoform
BID	BH3 interacting domain death agonist
BIK	Bcl-2 interacting killer
BIM	Bcl-2 interacting protein
Blk	Bik-like killer protein
CAD	Caspase-Activated DNase
Caspase-9	CysteinyI aspartic acid-protease-9
IAP	Inhibitor of Apoptosis Proteins
Myc	Oncogene Myc
Noxa	Phorbol-12-myristate-13-acetate-induced protein 1
Puma	Bcl-2 binding component 3
Smac/DIAB	Second mitochondrial activator of caspases/direct IAP binding protein with low

Conditions that interfere with ER function lead to the accumulation and aggregation of unfolded proteins (107). ER transmembrane receptors detect the onset of ER stress and initiate the unfolded protein response (UPR) to restore normal ER function. If the stress is prolonged, or the adaptive response fails, apoptotic cell death ensues (107, 108). Many studies have focused on how this failure initiates apoptosis, as ER stress-induced apoptosis is implicated in the pathophysiology of several neurodegenerative and cardiovascular diseases including alzheimer disease, parkinson disease, and type 2 diabetes (109-111). Recent work has shown that the Bcl-2 family of proteins plays a central role in regulating this form of cell death, both locally at the ER and from a distance at the mitochondrial membrane. The existence of Bcl-2-regulated initiator procaspase activation complexes at the ER membrane has also been described (110, 112). In addition to propagating death-inducing stress signals itself, the ER also contributes in a fundamental way to Fas-mediated apoptosis and to p53-dependent pathways resulting from DNA damage and oncogene expression. Mobilization of ER calcium stores can initiate the activation of cytoplasmic death pathways

as well as sensitize mitochondria to direct pro-apoptotic stimuli (113).

Lysosomes may function as death signal integrators. Rupture of lysosomes, leading to the release of their cathepsin content, has long been recognized as potentially harmful to the cell (114). Strong evidence is now accumulating for the involvement of alternative proteases, such as cathepsin B (CB), in apoptosis (115), but the molecular identity of the mediators and the necessity of activation of the apoptotic pathways remain to be elucidated in most cases and may vary on the type of cells and the applied death stimulus (116). CB has been reported to contribute to apoptosis via induction of mitochondrial membrane permeabilization, possibly via cleavage of Bid, in some systems, thereby acting upstream of the caspase cascade (117-119). Recent evidence has suggested that cathepsin D is involved in apoptosis induced by a number of conventional anti-cancer agents, including etoposide, cisplatin and 5-fluorouracil. The mechanisms leading to release of cathepsin from the lysosomes after treatment with these agents are unclear as is the relative importance of the lysosomal pathway for the cytotoxicity of these compounds (108, 120-124).

## Apoptosis: from Signalling Pathways to Therapeutic Tools

Table 4. Some of proteins involved in execution phase (74, 75).

Abbreviation	Protein name
CAD	Caspase-activated DNase
Caspase-10	Cysteiny aspartic acid-protease-10
Caspase-3	Cysteiny aspartic acid-protease-3
Caspase-6	Cysteiny aspartic acid-protease-6
Caspase-7	Cysteiny aspartic acid-protease-7
ICAD	Inhibitor of CAD
PARP	Poly (ADP-ribose) polymerase

### Apoptosis and Pharmacotherapy

Modulating of apoptosis is a novel therapeutic strategy in treatment of different diseases. These include situations with unwanted cell accumulation (cancer) and failure to eradicate aberrant cells (autoimmune diseases) or disorders with an inappropriate loss of cells (heart failure, stroke, AIDS, neurodegenerative diseases, and liver injury). Many approaches including gene therapy, antisense strategies and numerous apoptotic drugs to target specific apoptotic regulators, are currently being developed (50).

#### Apoptosis and cancer

Defects in apoptosis play important roles in tumor pathogenesis, allowing neoplastic, as well as genetically unstable cells, to survive (125). Moreover, deregulation of apoptosis affects chemo- and radioresistance, increasing the threshold for cell death and facilitating metastasis (126-128). Apoptotic strategies to kill tumor cells can involve direct induction of pro-apoptotic molecules, modulation of anti-apoptotic proteins, or restoration of tumor suppressor gene functions. Death receptors have been pursued as potential targets for cancer therapy. Candidates such as TNF death receptor family have been investigated after observing promising anti-tumor activity *in vitro*. However, TNF was shown to be ineffective in triggering cancer cell killing *in vivo*, in addition to its toxic side effects. The problems seen with TNF and Fas were overcome when TRAIL (Apo2) emerged as potential anticancer agent. TRAIL and agonist antibodies against TRAIL are well tolerated *in vivo*. Indeed, a phase I trial has been recently completed in humans, raising the possibility of using these biological agents as a novel approach in cancer treatment (Table 1).

Other routes, such as protein kinase C (PKC) may be important in leukemia, as it has been

demonstrated that some PKC modulators stimulate myeloid leukemia cell lines to produce TNF, resulting in apoptosis induction. A great part of the solid tumors overexpress growth factor receptors such as EGFR (epidermal growth factor receptor). Herceptin (Hoffmann-La Roche), an antibody blocking the EGF-R type 2 (Her2/neu), was one of the first rationally designed drugs that is now successfully applied in metastatic breast cancer (129). In addition, Gefitinib/Iressa (AstraZeneca) has been approved for treatment of non-small-cell lung cancer as a potent, selective ATP-competitive inhibitor of EGF-R tyrosine kinase, which inhibits growth of many different cell lines. As well as inhibiting tumor cell proliferation, Gefitinib treatment increases apoptosis (130, 131), reduces invasiveness (132, 133) and decreases angiogenesis in some tumor cells (134). The degradation and elimination of cells in apoptosis is dependent on the degradation of cellular proteins by caspases.

Active caspases have been engineered by fusing one or more chemically inducible dimerization domains. These engineered molecules are named artificial death switches. This synthetic activation of caspases has been shown to be effective in prostate cancer cell lines (135). Tumor cells may be preferentially sensitive to agents that trigger the lysosomal apoptosis pathway (136). The degree of lysosomal permeabilization may determine the amounts of cathepsins released into the cytosol: a complete breakdown of all lysosomes will result in necrosis, whereas partial breakdown may trigger apoptosis (137). Lysosomal cathepsins including cathepsins B, D, and L translocate from the lysosomal lumen to the cytosol in response to a variety of signals such as TNF receptor ligation, p53 activation, oxidative stress, and the lipid second messenger sphingosine. Such a

translocation can also be induced by lysosomotropic agents such as ciprofloxacin, norfloxacin, and hydroxychloroquine. We also proposed lysosome as a proposed target for rose bengal in inducing cell death in melanoma cells (138).

We have previously found that rose bengal (a xanthine dye) could induce dual modes of cell death (apoptotic and non-apoptotic cell death) in melanoma cells and has clinical activity against melanoma (44). Recently, we showed apoptogenic properties of saffron (*Crocus sativus* L.), an Iranian medicinal plant, in human cancer cell lines and proposed saffron as a promising chemotherapeutic agent in cancer treatment (139).

Lysosomes and the endoplasmic reticulum (ER) hold promise as drug targets and mediators of apoptosis signaling which may be less affected by intrinsic or chemotherapy-induced resistance mechanisms. Tumor cell lysosomes contain increased levels of cathepsins, and the release of these enzymes into the cytosol may result in apoptosis or necrosis, as has been reported for TNF- $\alpha$ . It is also reported that tumor transformation leads to increased sensitivity to cathepsin B-dependent apoptosis (140).

Tumor cells often show evidence of constitutive ER stress, possibly due to hypoxia and glucose depletion. Various anticancer drugs, including cisplatin, tunicamycin and proteasome inhibitors, have been shown to induce ER stress. Manipulating the ER stress response of tumor cells is an interesting therapeutic strategy (73). We conclude that organelle damage responses can be used to trigger tumor cell death, and that the response to such damage may be triggered in cells that are resistant to conventional DNA-damaging agents (68).

#### ***Apoptosis and autoimmunity***

T-cell mediated cytotoxicity is a variant of type IV hypersensitivity where sensitized CD8<sup>+</sup> cells kill antigen-bearing cells. These cytotoxic T lymphocytes (CTLs) are able to kill target cells via the extrinsic pathway and the FasL/FasR interaction is the predominant method of CTL-induced apoptosis (141). Autoimmunity represents a diverse set of

diseases defined by the target organ destroyed. Apoptosis plays a prominent role in autoimmune diseases in two different ways. First, controlled regulation of apoptosis is a normal part of T cell selection and education. Interruption of this process could lead to auto-reactive cells and second, cell death can represent a lymphocyte-independent mechanism causing premature death of certain organs.

#### ***Systemic lupus erythematosus***

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the involvement of multiple organs and the presence of auto-antibodies against various nuclear and cytoplasmic antigens in serum (142). One common feature of SLE is the generation of antiphospholipid antibodies (143). Lymphocytes from lupus patients undergo accelerated apoptosis compared with normal individuals. Deficiencies in components of the complement cascade have been shown to predispose humans and mice to lupus-like diseases (144-147), as well as defects in certain pro- and anti-apoptotic molecules such as Fas and members of the Bcl-2 family (148;149), CD28 and CD40 (150-152). For example, the BH3-only protein Bim promotes apoptosis by binding to, and antagonizing Bcl-2 and Bcl-XL. In Bim<sup>-/-</sup> mice, plasma cells accumulate, inducing autoimmune kidney diseases (153). Moreover, DNase 1 deficient mice developed also lupus-like autoimmune disease (154).

#### ***Rheumatoid arthritis***

Rheumatoid arthritis (RA) is a systemic autoimmune disease whose hallmark is the destruction of the synovial membrane due to inflammatory and proliferative processes. At the cellular level, there is a remarkable hyperplasia of synoviocytes, in addition to local secretion of pro-inflammatory cytokines such as IL-1, IL-6 and TNF- $\alpha$ . Several reports suggest that defects in apoptosis regulation are implicated in the pathogenesis of RA. The RA synovium has an abnormal high proliferative rate, which may be explained by altered expression of anti-apoptotic genes (Bcl-2, Bcl-XL and survivin), oncogenes or tumor

## Apoptosis: from Signalling Pathways to Therapeutic Tools

suppressor genes (p53). In addition, the Fas/FasL pathway may also be implicated in RA, since both are constitutively expressed in RA (155-158). Various promising drugs such as Pralnacasan, HMR-3480, AZQs or SPC-839 that target either different types of caspases or the inhibitor of kappaB kinase (IKK) are currently tested in cellular models of RA and in clinical phase II trials (Table 5).

### *Autoimmune diabetes*

Autoimmune diabetes, or insulin-dependent

diabetes mellitus (IDDM), is characterized by selective destruction of insulin-producing cells. Infection-associated molecular mimicry has been a popular hypothesis for the development of autoimmunity in Type 1 diabetes. However, there is evidence that early developmental remodeling and/or homeostasis of  $\beta$ -cell mass involves  $\beta$ -cell apoptosis which might trigger autoimmunity. There is emerging evidence that T cell-induced apoptosis is a dominant effector mechanism in Type 1 diabetes.

Table 5. Novel promising therapeutics modulating apoptosis signaling pathways in inflammation, neurodegenerative diseases and cancer (11, 50).

Drug	Molecular target	Target disease
Pralnacasan	Caspase-1/-4 inhibitor	Rheumatoid arthritis (phase II)
AZQs (AstraZeneca)	Caspase-3 inhibitor	Rheumatoid arthritis and other inflammatory diseases
HGS-ETR1	Agonistic TRAIL-R1 mAb	Apoptosis induction in various tumor cell lines and tumor xenografts, synergistic with anticancer drugs (phase II)
HGS-ETR2	Agonistic TRAIL-R2 mAb	Apoptosis induction in tumor cell lines (phase 1)
HGS-TR2J	Agonistic TRAIL-R2 mAb	Apoptosis induction in tumor cell lines (phase 1)
PRO1762	Soluble human Apo2L/TRAIL	Apoptosis induction in tumor cell lines, no side effects in cynomolgus monkeys and mice, synergistic with anticancer drugs (phase 1)
AEG35156/GEM640	XIAP antisense oligonucleotide	Exhibits antitumor activity alone or in combination with chemotherapeutics in cancer xenograft models (phase 1)
LY2181308	Survivin antisense construct	Preclinical studies show antitumor activity in a broad range of cancers (phase 1 clinical trials started November 2004)
Cladribine	Direct disruption of mitochondrial membrane potential	Approved for chronic lymphocytic and hairy cell leukemia
Arsenite	Oxidative disruption of mitochondrial membrane and proteins	Approved for acute promyelocytic leukemia
IDN6556	Pan-caspase inhibitor	Prevents from cold- and ischemia-induced damage of donor liver organ transplants (phase II); multiple sclerosis; Hepatitis C (phase II)
SPC-839	IKK inhibitor	Arthritis
Minocycline	Inhibits cytochrome <i>c</i> release, NO-synthetase and casp-3 mRNA upregulation	ALS (phase III), HD (phase II), PD, multiple sclerosis
Pifithrin	p53 inhibitor	Nervous system trauma, stroke
Recombinant Trail	Activation of DR4 and DR5	Cancer (phase I)
Genasense	Bcl-2 antisense	Malignant melanoma (phase III), chronic lymphocytic leukemia (phase III), multiple myeloma (phase III)
Herceptin	Antibody blocking EGF-R (Her2/neu)	Metastatic breast cancer (approved)
INGN201	p53-expressing adenovirus	Apoptosis induction in tumor cell lines and xenograft models, head and neck cancer (phase III), clinical trials for other advanced solid tumors
SCH58500	p53-expressing adenovirus	Apoptosis induction in tumor cell lines and xenograft models, advanced ovarian cancer (phase III)
ONYX-015	p53 delivery with mutant adenovirus	Combination therapy of advanced squamous cell cancer (phase II and III);
IDN13389	XIAP antagonist	Cancer
Zarnestra	Farnesyltransferase blocking Ras function inhibitor	Acute myeloid leukemia (continuous marketing application in-process), multiple myeloma (phase II)

If clinical stage is not mentioned, drugs are at preclinical stage.

In this regard, pancreatic  $\beta$ -cells derived from newly diagnosed patients with Type 1 diabetes were found to have increased cell surface expression of Fas (CD95) as compared to  $\beta$ -cells from healthy subjects that did not constitutively express detectable Fas (CD95) (159). A murine model of IDDM, the nonobese diabetic mouse (NOD) spontaneously develops IDDM in two phases: infiltration of T and B cells, macrophages and dendritic cells and finally, destruction of  $\beta$  cells by CD8 and CD4 cells. Apoptosis of  $\beta$  cells has been clearly demonstrated in mouse models of IDDM. The proposed mechanism to explain the observed apoptosis included perforin-dependent cytotoxicity as well as TNF- $\alpha$  and Fas/FasL pathways (155, 156, 160-164). In addition, disruption of STAT4 activation completely prevents the development of spontaneous diabetes in NOD mice, suggesting an important role of STAT4 in autoimmune diabetes pathogenesis (165).

#### *Multiple sclerosis*

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) in which myelin and myelin-producing cells become the target of an inflammatory response leading to apoptotic cell death (166, 167). One of the most important progresses in the treatment of MS has been the development of interferon- $\beta$  as a therapy. This therapy improves MS, possibly by lowering IFN- $\gamma$  secretion and inhibiting responses to IFN- $\gamma$ . IFN- $\beta$  acts primarily on blood cells with probable selectivity for functionally different lymphocyte subpopulations, monocytes and granulocytes by downregulating FLIP expression (168). In addition, upregulation of Bcl-2, CD95 and CD95L has been observed in MS patients. Abnormal Bcl-2 expression may promote apoptotic resistance of potentially pathogenic autoreactive lymphocytes and may allow for continuing cellular proliferation and tissue destruction. (169, 170). It has demonstrated that members of the inhibitor of apoptosis (IAP) family of anti-apoptotic genes are elevated in peripheral blood immune cells (monocytes, T cells) of patients with aggressive forms of MS. These findings

suggest that the IAPs may be novel diagnostic markers for distinguishing subtypes of MS. Moreover, antisense-mediated knockdown of the IAP family member known as X-linked IAP (XIAP) reverses paralysis in an animal model of MS suggesting that treatments targeting XIAP may be useful in the treatment of MS (171).

#### *Apoptosis and neurodegenerative disorders*

Neurodegenerative diseases include a variety of progressive disorders resulting in cognitive and/or motor deterioration. For such disease, it is clear that apoptosis mechanisms are the candidates for cell death. Excessive death of one or more populations of neurons results in disease or injury. For example, death of hippocampal and cortical neurons results in Alzheimer's disease (AD), death of mid brain neurons results in Parkinson's disease (PD), death of neurons in the striatum results in Huntington's disease (HD) and finally, death of lower motor neurons results in amyotrophic lateral sclerosis (ALS). We have reported that glucose could induce apoptosis in PC12 cells as a possible mechanism of glucose-induced neuropathy in diabetes (104). In an other study using PC12 cells, we have shown lead could cause PC12 cell death, in which apoptosis or programmed cell death plays an important role (105).

#### *Parkinson's disease*

PD patients suffer from degeneration of dopaminergic neurons in their substantia nigra. Increase oxidative stress and mitochondria dysfunction seem to be the central key of this disease (172). Evidence for apoptosis in PD has been observed in human tissues, as well as in animal models (173-175). Dopaminergic neurons die by apoptosis as shown by histochemical evidences (176) and increased expression of apoptosis-related genes encoding p53, CD95 and Bax, as well as Par-4, has been observed in brain tissue from PD patients (177, 178). Furthermore, it has been shown that delivery of an Apaf-1 dominant negative mutant using an adenovirus vector in a mouse model of PD inhibits mitochondrial apoptotic signaling pathways

preventing neuronal cell death. This report suggests that gene therapy may be an encouraging approach for treatment of neurodegenerative disorders (173). In addition, Cephalon Inc. and Lundbeck have discovered a novel drug named CEP-1347 offering great benefits for PD patients in phase II trials by blocking mixed-lineage kinases of the JNK pathway.

### *Alzheimer's disease*

Alzheimer's disease (AD) correlates with synaptic degeneration and death of neurons in limbic structures (179, 180). A defining feature of AD is the accumulation of amyloid plaques formed by aggregates of the amyloid- $\beta$  peptide (80, 181, 182), a fragment generated by processing of amyloid precursor protein (APP). Increased DNA damage, caspase activity and altered expression of Bcl-2 family members have been demonstrated in neurons associated with amyloid deposits (183, 184). Moreover, caspase-mediated cleavage of APP results in the release a carboxy-terminal peptide, which is a potent inducer of apoptosis (185-187). Recent advances in the molecular genetics of AD have led to the identification of four specific genes involved in the disease:  $\beta$ -amyloid precursor protein ( $\beta$ -APP), presenilin-1 (PS1), presenilin-2 (PS2) and apolipoprotein E (185, 186). It is now clear that mutations, or unfavorable forms of these genes results in increased P-amyloid plaques in the brain. Studies of human tissues of AD prove that expression of the anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bax are regulated in areas of the brain showing increased apoptosis (188). Addition of  $\beta$ - amyloid peptide to PC12 cells, neuroblastoma cells, rodent cortical and

hippocampal neurons results in apoptotic cell death (189-191). Altered proteolytic processing of APP is shown to be an important alteration contributing to the neurodegenerative cascade. Oxidative stress and perturbed regulation of intracellular calcium levels are central to the neuronal death in AD. Additional biochemical data have proposed that mitochondrial function is compromised in brain cells of AD patients (192). Analyses of post-mortem brain tissues from AD patients have provided evidence for nuclear DNA fragmentation. Immunohistochemical studies revealed elevated levels of caspase activity (193), increased expression of apoptosis-related gene Bax (194), as well as elevated levels of Par-4. Similarly, Huntington's disease (HD) is associated with mitochondrial dysfunction and increased caspase-2 activation (195).

### **Prospects of Apoptosis-Targeted Therapies**

Depending on the molecular target, different strategies are being employed. Recombinant biologicals including death ligands or agonistic and antagonistic antibodies that inhibit or trigger death receptor signaling have proven efficacy in various animal models. Although, new drugs are currently being designed the relatively low rate of clinical entry associated with these molecules is related to the lack of specificity, low efficacy, or development to drug resistance. Application of various mechanisms at which apoptosis can be targeted offers hope that apoptosis-based therapies and improved clinical outcome for a wide range of diseases may be not far from realization (196).

### **References**

1. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972; 26: 239-357.
2. Kerr JFR. History of the events leading to the formulation of the apoptosis concept. *Toxicology* 2002; 181-182: 471-474.
3. Paweletz N, Walther Flemming: Pioneer of mitosis research. *Nat Rev Mol Cell Biol* 2001; 2: 72-75.
4. Debnath J, Baehrecke EH, Kroemer G. Does autophagy contribute to cell death? *Autophagy* 2005; 1: 66-74.
5. Formigli L, Papucci L, Tani A, Schiavone N, Tempestini A, Orlandini GE, *et al.* Aponecrosis: Morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. *J Cell Physiol* 2000; 182: 41-49.

6. Sperandio S, De Belle I, Bredesen DE. An alternative, nonapoptotic form of programmed cell death. *Proc Natl Acad Sci USA* 2000; 97: 14376-14381.
7. Elmore S. Apoptosis: A Review of Programmed Cell Death. *Toxicol Pathol* 2007; 35: 495-516.
8. Steller H. Mechanisms and genes of cellular suicide. *Science* 1995; 267: 1445-1449.
9. Williams GT, Smith CA. Molecular regulation of apoptosis: Genetic controls on cell death. *Cell* 1993; 74: 777-779.
10. Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. *Cell* 1997; 88: 347-354.
11. Fleischer A, Ghadiri A, Dessauge F, Duhamel M, Rebollo MP, varez-Franco F, *et al*. Modulating apoptosis as a target for effective therapy. *Mol Immunol* 2006; 43: 1065-1079.
12. Norbury CJ, Hickson ID. Cellular responses to DNA damage. *Annu Rev Pharmacol Toxicol* 2001; 41: 367-401.
13. Mousavi SH, Hersey P. Role of caspases and reactive oxygen species in rose bengal-induced toxicity in melanoma cells. *Iran J Basic Med Sci* 2007; 10: 210-215.
14. Hacker G. The morphology of apoptosis. *Cell Tissue Res* 2000; 301: 5-17.
15. Kurosaka K, Takahashi M, Watanabe N, Kobayashi Y. Silent Cleanup of Very Early Apoptotic Cells by Macrophages. *J Immunol* 2003; 171: 4672-4679.
16. Savill J, Fadok V. Corpse clearance defines the meaning of cell death. *Nature* 2000; 407: 784-788.
17. Hengartner MO. The biochemistry of apoptosis. *Nature* 2000; 407: 770-776.
18. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X. Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 1999; 15: 269-290.
19. Cohen GM. Caspases: The executioners of apoptosis. *Biochem J* 1997; 326: 1-16.
20. Rai NK, Tripathi K, Sharma D, Shukla VK. Apoptosis: A basic physiologic process in wound healing. *Int J Low Extrem Wounds* 2005; 4: 138-144.
21. Hu S, Snipas SJ, Vincenz C, Salvesen G, Dixit VM. Caspase-14 is a novel developmentally regulated protease. *J Biol Chem* 1998; 273: 29648-29653.
22. Kang SJ, Wang S, Kuida K, Yuan J. Distinct downstream pathways of caspase-11 in regulating apoptosis and cytokine maturation during septic shock response. *Cell Death Differ* 2002; 9: 1115-1125.
23. Koening U, Eckhart L, Tschachler E. Evidence that caspase-13 is not a human but a bovine gene. *Biochem Biophys Res Commun* 2001; 285: 1150-1154.
24. Nakagawa T, Zhu H, Morishima N, Li E, Xu J, Yankner BA, *et al*. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid- $\beta$ . *Nature* 2000; 403: 98-103.
25. Nemes J, Friis RR, Aeschlimann D, Saurer S, Paulsson M, Fesus L. Expression and activation of tissue transglutaminase in apoptotic cells of involuting rodent mammary tissue. *Eur J Cell Biol* 1996; 70: 125-133.
26. Bortner CD, Oldenburg NBE, Cidlowski JA. The role of DNA fragmentation in apoptosis. *Trends Cell Biol* 1995; 5: 21-26.
27. Bratton DL, Fadok VA, Richter DA, Kailey JM, Guthrie LA, Henson PM. Appearance of phosphatidylserine on apoptotic cells requires calcium-mediated nonspecific flip-flop and is enhanced by loss of the aminophospholipid translocase. *J Biol Chem* 1997; 272: 26159-26165.
28. Arur S, Uche UE, Rezaul K, Fong M, Scranton V, Cowan AE, *et al*. Annexin I is an endogenous ligand that mediates apoptotic cell engulfment. *Dev Cell* 2003; 4: 587-598.
29. Gardai SJ, McPhillips KA, Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, *et al*. Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. *Cell* 2005; 123: 321-334.
30. Jimenez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, Bouck N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nature Med* 2000; 6: 41-48.
31. Hirsch T, Marchetti P, Susin SA, Dallaporta B, Zamzami N, Marzo I, *et al*. The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. *Oncogene* 1997; 15: 1573-1581.

## Apoptosis: from Signalling Pathways to Therapeutic Tools

32. Zeiss CJ. The apoptosis-necrosis continuum: Insights from genetically altered mice. *Vet Pathol* 2003; 40: 481-495.
33. Levin S, Bucci TJ, Cohen SM, Fix AS, Hardisty JF, LeGrand EK, *et al.* The nomenclature of cell death: Recommendations of an ad hoc Committee of the Society of Toxicologic Pathologists. *Toxicol Pathol* 1999; 27: 484-490.
34. Majno G, Joris I. Apoptosis, oncosis, and necrosis: An overview of cell death. *Am J Pathol* 1995; 146: 3-15.
35. Trump BF, Berezsky IK, Chang SH, Phelps PC. The pathways of cell death: Oncosis, apoptosis, and necrosis. *Toxicol Pathol* 1997; 25: 82-88.
36. Cotran RS, Kumar V, Robbins SL. Cellular injury and cellular death. *Pathologic Basis of Disease* 1994; 1-34.
37. Fiers W, Beyaert R, Declercq W, Vandenaabeele P. More than one way to die: Apoptosis, necrosis and reactive oxygen damage. *Oncogene* 1999; 18: 7719-7730.
38. Denecker G, Vercammen D, Declercq W, Vandenaabeele P. Apoptotic and necrotic cell death induced by death domain receptors. *Cell Mol Life Sci* 2001;58: 356-370
39. Leist M, Single B, Castoldi AF, Hnle S, Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: A switch in the decision between apoptosis and necrosis. *J Exp Med* 1997; 185: 1481-1486.
40. Broker LE, Kruyt FAE, Giaccone G. Cell death independent of caspases: A review. *Clin Cancer Res* 2005; 11: 3155-3162.
41. Edinger AL, Thompson CB. Death by design: Apoptosis, necrosis and autophagy. *Curr Opin Cell Biol* 2004; 16:663-669.
42. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB. Alkylating DNA damage stimulates a regulated form of necrotic cell death. *Genes Dev* 2004; 18: 1272-1282.
43. Gillespie SK, Zhang XD, Hersey P. Ingenol 3-angelate induces dual modes of cell death and differentially regulates tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in melanoma cells. *Mol Cancer Ther* 2004; 3: 1651-1658.
44. Mousavi SH, Zhang X, Gillespie S, Wachter E, Hersey P. Rose Bengal induces dual modes of cell death in melanoma cells and has clinical activity against melanoma. *Melanoma Res* 2006; 16: S8.
45. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. *Science* 1998; 281: 1305-1308.
46. Green DR, Reed JC. Mitochondria and apoptosis. *Science* 1998; 281: 1309-1312.
47. Cryns V, Yuan J. Proteases to die for. *Genes Dev* 1998; 12: 1551-1570.
48. Thornberry NA, Lazebnik Y. Caspases: Enemies within. *Science* 1998; 281:1312-1316.
49. Igney FH, Krammer PH. Death and anti-death: Tumour resistance to apoptosis. *Nat Rev Cancer* 2002; 2: 77-88.
50. Fischer U, Schulze-Osthoff K. New approaches and therapeutics targeting apoptosis in disease. *Pharmacol Rev* 2005; 57:187-215.
51. Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: Integrating mammalian biology. *Cell* 2001; 104: 87-501.
52. Chicheportiche Y, Bourdon PR, Xu H, Hsu YM, Scott H, Hession C, *et al.* TWEAK, a new secreted ligand in the tumor necrosis factor family that weakly induces apoptosis. *J Biol Chem* 1997; 272: 32401-32410
53. Peter ME, Krammer PH. Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. *Curr Opin Immunol* 1998; 10: 45-51.
54. Rubio-Moscardo F, Blesa D, Mestre C, Siebert R, Balasas T, Benito A, *et al.* Characterization of 8p21.3 chromosomal deletions in B-cell lymphoma: TRAIL-R1 and TRAIL-R2 as candidate dosage-dependent tumor suppressor genes. *Blood* 2005; 106: 214-22.
55. Suliman A, Lam A, Datta R, Srivastava RK. Intracellular mechanisms of TRAIL: Apoptosis through mitochondrial-dependent and -independent pathways. *Oncogene* 2001; 20: 2122-2133
56. Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF- $\kappa$ B activation. *Cell* 1995; 81:495-504.
57. Wajant H. The Fas signaling pathway: More than a paradigm. *Science* 2002; 296:635-636.

58. Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, *et al*. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. *EMBO J* 1995; 14: 55-88.
59. Kataoka T, Ter MHahne M, Schneider P, Irmeler M, Thome M, *et al*. FLIP prevents apoptosis induced by death receptors but not by perforin/granzyme B, chemotherapeutic drugs, and gamma irradiation. *J Immunol* 1998; 161:3936-3942.
60. Scaffidi C, Schmitz I, Krammer PH, Peter ME. The role of c-FLIP in modulation of CD95-induced apoptosis. *J Biol Chem* 1999; 274:1541-1548.
61. Hitoshi Y, Lorens J, Kitada SI, Fisher J, LaBarge M, Ring HZ, *et al*. Toso, a cell surface, specific regulator of Fas-induced apoptosis in T cells. *Immunity* 1998; 8:461-471.
62. Leblanc V, Delumeau I, Tocque B. Ras-GTPase activating protein inhibition specifically induces apoptosis of tumour cells. *Oncogene* 1999; 18:4884-4889.
63. Zhang XD, Borrow JM, Zhang XY, Nguyen T, Hersey P. Activation of ERK1/2 protects melanoma cells from TRAIL-induced apoptosis by inhibiting Smac/DIABLO release from mitochondria. *Oncogene* 2003; 22:2869-2881.
64. Gillespie S, Borrow J, Zhang XD, Hersey P. Bim plays a crucial role in synergistic induction of apoptosis by the histone deacetylase inhibitor SBHA and TRAIL in melanoma cells. *Apoptosis* 2006; 11:251-265.
65. Bron LP, Scolyer RA, Thompson JF, Hersey P. Histological expression of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) in human primary melanoma. *Pathology* 2004; 36:561-565.
66. Zhang XD, Wu JJ, Gillespie S, Borrow J, Hersey P. Human melanoma cells selected for resistance to apoptosis by prolonged exposure to tumor necrosis factor-related apoptosis-inducing ligand are more vulnerable to necrotic cell death induced by cisplatin. *Clin Cancer Res* 2006; 12:1355-1364.
67. Zhuang L, Lee CS, Scolyer RA, McCarthy SW, Zhang XD, Thompson JF, *et al*. Progression in melanoma is associated with decreased expression of death receptors for tumor necrosis factor-related apoptosis-inducing ligand. *Hum Pathol* 2006; 37:1286-1294.
68. Hersey P, Zhang XD. Resistance of follicular lymphoma cells to chemotherapy is more than just Bcl-2. *Cancer Biol Ther* 2003; 2:541-543.
69. Wu JJ, Zhang XD, Gillespie S, Hersey P. Selection for TRAIL resistance results in melanoma cells with high proliferative potential. *FEBS Lett* 2005; 579:1940-1944.
70. Chen LH, Jiang CC, Kiejda KA, Wang YF, Thorne RF, Zhang XD, *et al*. Thapsigargin sensitizes human melanoma cells to TRAIL-induced apoptosis by up-regulation of TRAIL-R2 through the unfolded protein response. *Carcinogenesis* 2007; 28:2328-2336.
71. Zhang XD, Gillespie SK, Borrow JM, Hersey P. The histone deacetylase inhibitor suberic bishydroxamate: A potential sensitizer of melanoma to TNF-related apoptosis-inducing ligand (TRAIL) induced apoptosis. *Biochem Pharmacol* 2003; 66:1537-1545.
72. Zhang XY, Zhang XD, Borrow JM, Nguyen T, Hersey P. Translational Control of Tumor Necrosis Factor-related Apoptosis-inducing Ligand Death Receptor Expression in Melanoma Cells. *J Biol Chem* 2004; 279:10606-10614.
73. Chen CJ, Li HC, Gillespie S, Kiejda KA, Mhaidat N, Yu FW, *et al*. Tunicamycin sensitizes human melanoma cells to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by up-regulation of TRAIL-R2 via the unfolded protein response. *Cancer Res* 2007; 67:5880-2885.
74. Gillespie S, Zhang XD, Hersey P. Variable expression of protein kinase CE in human melanoma cells regulates sensitivity to TRAIL-induced apoptosis. *Mol cancer ther* 2005; 4:668-676.
75. Human Protein Reference Database. 2008. Available at: <http://www.hprd.org>
76. ExpASY Proteomics Server. 2008. Available at: <http://ca.expasy.org>
77. Saelens X, Festjens N, Vande Walle L, Van Gurp M, Van Loo G, Vandenaabeele P. Toxic proteins released from mitochondria in cell death. *Oncogene* 2004; 23:2861-2874.
78. Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. *Cell* 2000; 102:33-42.
79. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* 2006; 13:1423-1433.

## Apoptosis: from Signalling Pathways to Therapeutic Tools

80. Loo DT, Copani A, Pike CJ, Whittemore ER, Walencewicz AJ, Cotman CW. Apoptosis is induced by  $\beta$ -amyloid in cultured central nervous system neurons. *Proc Natl Acad Sci USA* 1993; 90:7951-7958.
81. Chinnaiyan AM. The apoptosome: heart and soul of the cell death machine. *Neoplasia* (New York, NY) 1999; 1:5-15.
82. Hill MM, Adrain C, Duriez PJ, Creagh EM, Martin SJ. Analysis of the composition, assembly kinetics and activity of native Apaf-1 apoptosomes. *EMBO J* 2004; 23:134-145.
83. Schimmer AD. Inhibitor of apoptosis proteins: Translating basic knowledge into clinical practice. *Cancer Res* 2004; 64:7183-7190.
84. Van Loo G, Van Gurp M, Depuydt B, Srinivasula SM, Rodriguez I, Alnemri ES, *et al.* The serine protease Omi/HtrA2 is released from mitochondria during apoptosis. Omi interacts with caspase-inhibitor XIAP and induces enhanced caspase activity. *Cell Death Differ* 2002; 9:20-26.
85. Ekert PG, Vaux DL. The mitochondrial death squad: Hardened killers or innocent bystanders? *Curr Opin Cell Biol* 2005; 17:626-630.
86. Zhang XD, Franco A, Myers K, Gray C, Nguyen T, Hersey P. Relation of TNF-related apoptosis-inducing ligand (TRAIL) receptor and FLICE-inhibitory protein expression to TRAIL-induced apoptosis of melanoma. *Cancer Res* 1999; 59:2747-2753.
87. Joza N, Susin SA, Daugas E, Stanford WL, Cho SK, Li CYJ, *et al.* Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* 2001; 410:549-554.
88. Susin SA, Daugas E, Ravagnan L, Samejima K, Zamzami N, Loeffler M, *et al.* Two distinct pathways leading to nuclear apoptosis. *J Ex Med* 2000; 192:571-579.
89. Li LY, Luo X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. *Nature* 2001; 412:95-99.
90. Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S. A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature* 1998; 391:43-50.
91. Zhang XD, Gillespie SK, Hersey P. Staurosporine induces apoptosis of melanoma by both caspase-dependent and -independent apoptotic pathways. *Mol cancer ther* 2004; 3:187-197.
92. Cory S, Adams JM. The Bcl-2 family: Regulators of the cellular life-or-death switch. *Nat Rev Cancer* 2002; 2:647-656.
93. Schuler M, Green DR. Mechanisms of p53-dependent apoptosis. *Biochem Soc Trans* 2001; 29: 684-688.
94. Gross A, McDonnell JM, Korsmeyer SJ. Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev* 1999; 13:1899-1911.
95. Esposti MD. The roles of Bid. *Apoptosis* 2002;7:433-440
96. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 1998; 94:491-501.
97. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not Bcl-X(L). *Cell* 1996; 87:619-628.
98. Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. Bad, a heterodimeric partner for Bcl-x(L), and Bcl-2, displaces Bax and promotes cell death. *Cell* 1995; 80:285-291.
99. Newmeyer DD, Bossy-Wetzel E, Kluck RM, Wolf BB, Beere HM, Green DR. Bcl-x(L) does not inhibit the function of Apaf-1. *Cell Death Differ* 2000; 7:402-407.
100. Chau BN, Cheng EHY, Kerr DA, Hardwick JM. Aven, a novel inhibitor of caspase activation, binds Bcl-X(L) and Apaf-1. *Mol Cell* 2000; 6:31-40.
101. Liu FT, Newland AC, Jia L. Bax conformational change is a crucial step for PUMA-mediated apoptosis in human leukemia. *Biochem Biophys Res Commun* 2003; 310:956-962.
102. Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, *et al.* Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 2000; 288:1053-1058.
103. Meyer N, Kim SS, Penn LZ. The Oscar-worthy role of Myc in apoptosis. *Semin Cancer Biol* 2006; 16:275-287.
104. Sharifi AM, Mousavi SH, Farhadi M, Larijani B. Study of high glucose-induced apoptosis in PC12 cells: Role of bax protein. *J Pharm Sci* 2007; 104:258-262.

105. Sharifi AM, Mousavi SH. Studying the effects of lead on DNA fragmentation and proapoptotic Bax and antiapoptotic Bcl-2 protein expression in PC12 cells. *Toxicol Mech Methods* 2008; 18:75-79.
106. Szegezdi E, Logue SE, Gorman AM, Samali A. Mediators of endoplasmic reticulum stress-induced apoptosis. *EMBO Reports* 2006; 7: 880-885.
107. Faitova J, Krekac D, Hrstka R, Vojtesek B. Endoplasmic reticulum stress and apoptosis. *Cell Mol Biol Lett* 2006; 11: 488-505.
108. Chen CJ, Li HC, Gillespie S, Yu FW, Kiejda KA, Xu DZ, *et al*. Inhibition of MEK sensitizes human melanoma cells to endoplasmic reticulum stress-induced apoptosis. *Cancer Res* 2007; 67: 9750-9761.
109. Lipson KL, Fonseca SG, Urano F. Endoplasmic reticulum stress-induced apoptosis and autoimmunity in diabetes. *Curr Mol Med* 2006; 6: 71-7.
110. Oakes SA, Lin SS, Bassik MC. The control of endoplasmic reticulum-initiated apoptosis by the Bcl-2 family of proteins. *Curr Mol Med* 2006; 6: 99-109.
111. Takuma K, Yan SS, Stern DM, Yamada K. Mitochondrial dysfunction, endoplasmic reticulum stress, and apoptosis in Alzheimer's disease. *J Pharmacol Sci* 2005; 97: 312-316.
112. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, *et al*. BAX and BAK regulation of endoplasmic reticulum Ca<sup>2+</sup>: A control point for apoptosis. *Science* 2003; 300: 135-139.
113. Breckenridge DG, Germain M, Mathai JP, Nguyen M, Shore GC. Regulation of apoptosis by endoplasmic reticulum pathways. *Oncogene* 2003; 22: 8608-8618.
114. Turk V, Turk B, Turk D. Lysosomal cysteine proteases: Facts and opportunities. *EMBO J* 2001; 20: 4629-4633.
115. Wyllie AH, Golstein P. More than one way to go. *Proc Natl Acad Sci USA* 2001; 98: 11-3.
116. Herr I, Debatin KM. Cellular stress response and apoptosis in cancer therapy. *Blood* 2001; 98: 2603-2614.
117. Boya P, Andreau K, Poncet D, Zamzami N, Perfettini JL, Metivier D, *et al*. Lysosomal membrane permeabilization induces cell death in a mitochondrion-dependent fashion. *J Experiment Med* 2003; 197: 1323-1334.
118. Guicciardi ME, Deussing J, Miyoshi H, Bronk SF, Svingen PA, Peters C, *et al*. Cathepsin B contributes to TNF- $\alpha$ -mediated hepatocyte apoptosis by promoting mitochondrial release of cytochrome c. *J Clin Invest* 2000; 106: 1127-1137.
119. Stoka V, Turk B, Schendel SL, Kim TH, Cirman T, Snipas SJ, *et al*. Lysosomal protease pathways to apoptosis: Cleavage of Bid, not pro-caspases, is the most likely route. *J Biol Chem* 2001; 276: 3149-3157.
120. Emert-Sedlak L, Shangary S, Rabinovitz A, Miranda MB, Delach SM, Johnson DE. Involvement of cathepsin D in chemotherapy-induced cytochrome c release, caspase activation, and cell death. *Mol Cancer Ther* 2005; 4: 733-742.
121. Hersey P, Zhang XD. Adaptation to ER stress as a driver of malignancy and resistance to therapy in human melanoma. *Pigment Cell Melanoma Res* 2008; 21: 358-367.
122. Li HC, Chen CJ, Watts R, Thorne RF, Kiejda KA, Xu DZ, *et al*. Inhibition of endoplasmic reticulum stress-induced apoptosis of melanoma cells by the ARC protein. *Cancer Res* 2008; 68: 834-842.
123. Ekegren T, Grandstrom E, Lindholm D, Aquilonius SM. Upregulation of Bax protein and increased DNA degradation in ALS spinal cord motor neurons. *Acta Neurol Scand* 1999; 100: 317-321.
124. Mousavi SH, Mousavi SH. Lysosome: as a proposed target for rose bengal in inducing cell death in melanoma cells. *Iran J Med Hypotheses and Ideas* 2008; 2(12):79-92.
125. Ionov Y, Yamamoto H, Krajewski S, Reed JC, Perucho M. Mutational inactivation of the proapoptotic gene BAX confers selective advantage during tumor clonal evolution. *Proc Nat Acad Sci USA* 2000; 97: 10872-10877.
126. Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD, *et al*. Proteasome inhibitors: a novel class of potent and effective antitumor agents. *Cancer Res* 1999; 59: 2615-2622.

## Apoptosis: from Signalling Pathways to Therapeutic Tools

127. Frisch SM, Screaton RA. Anoikis mechanisms. *Curr Opin Cell Biol* 2001; 13: 555-562.
128. Makin G, Hickman JA. Apoptosis and cancer chemotherapy. *Cell Tissue Res* 2000; 301: 143-152.
129. Albanell J, Codony J, Rovira A, Mellado B, Gascoñ P. Mechanism of action of anti-HER2 monoclonal antibodies: Scientific update on trastuzumab and 2C4. *Adv Exp Med Biol* 2003; 532: 253-268.
130. Ciardiello F, Caputo R, Bianco R, Damiano V, Fontanini G, Cuccato S, *et al.* Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. *Clin Cancer Res* 2001; 7: 1459-1465.
131. Normanno N, Campiglio M, De Luca A, Somenzi G, Maiello M, Ciardiello F, *et al.* Cooperative inhibitory effect of ZD1839 (Iressa) in combination with trastuzumab (Herceptin) on human breast cancer cell growth. *Ann Oncol* 2002; 13: 65-72.
132. Barnes CJ, Bagheri-Yarmand R, Mandal M, Yang Z, Clayman GL, Hong WK, *et al.* Suppression of epidermal growth factor receptor, mitogen-activated protein kinase, and Pak1 pathways and invasiveness of human cutaneous squamous cancer cells by the tyrosine kinase inhibitor ZD1839 (Iressa). *Mol Cancer Ther* 2003; 2: 345-351.
133. Fujimura M, Hidaka T, Saito S. Selective inhibition of the epidermal growth factor receptor by ZD1839 decreases the growth and invasion of ovarian clear cell adenocarcinoma cells. *Clin Cancer Res* 2002; 8: 2448-2454.
134. Hirata A, Ogawa SI, Kometani T, Kuwano T, Naito S, Kuwano M, *et al.* ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. *Cancer Res* 2002; 6: 2554-2560.
135. MacCorkle RA, Freeman KW, Spencer DM. Synthetic activation of caspases: artificial death switches. *Proc Natl Acad Sci USA* 1998; 95: 3655-3660.
136. Yan S, Sameni M, Sloane BF. Cathepsin B and human tumor progression. *Biol Chem* 1998; 379: 113-123.
137. Bursch W. The autophagosomal-lysosomal compartment in programmed cell death. *Cell Death Differ* 2001;8: 569-581.
138. Mousavi SH, Zhang XD, Sharifi AM, Hersey P. Study of rose bengal-induced cell death in melanoma cells in the absence of light. *Iran J Basic Med Sci* 2008; 9:216-222.
139. Tavakkol Afshari J, Brook A, Mousavi SH. Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. *Food Chem Toxicol* 2008; 8:3443-3447.
140. Linder S, Shoshan MC. Lysosomes and endoplasmic reticulum: targets for improved, selective anticancer therapy. *Drug Resist Updat* 2005; 8: 199-204.
141. Brunner T, Wasem C, Torgler R, Cima I, Jakob S, Corazza N. Fas (CD95/Apo-1) ligand regulation in T cell homeostasis, cell-mediated cytotoxicity and immune pathology. *Semin Immunol* 2003; 15:167-76.
142. Elkon K. Autoantibodies in systemic lupus erythematosus. *Curr Opin Rheumatol* 1995; 7:384-388.
143. Casciola-Rosen L, Rosen A, Petri M, Schlissel M. Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: Implications for coagulation events and antigenic spread in systemic lupus erythematosus. *Proc Natl Acad Sci USA* 1996; 93:1624-9.
144. Botto M, Dell'Agnola C, Bygrave AE, Thompson EM, Cook HT, Petry F, *et al.* Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat Gene* 1998; 19:56-59.
145. Morgan BP, Walport MJ. Complement deficiency and disease. *Immunol Today* 1991; 12: 301-306.
146. Rordorf C, Schnebli HP, Baltz ML. The acute-phase response in (NZB x NZW) F1 and MRL/l mice. Contrasting patterns resembling those in human systemic lupus erythematosus and rheumatoid arthritis, respectively. *J Ex Med* 1982; 156:1268-73.
147. Taylor PR, Carugati A, Fadok VA, Cook HT, Andrews M, Carroll MC, *et al.* A hierarchical role for classical pathway complement proteins in the clearance of apoptotic cells *in vivo*. *J Exp Med* 2000; 192:359-366.
148. Mysler E, Bini P, Drappa J, Ramos P, Friedman SM, Krammer PH, *et al.* The apoptosis-1/Fas protein in human systemic lupus erythematosus. *J Clin Invest* 1994; 93:1029-1034.

149. Ohsako S, Hara M, Harigai M, Fukasawa C, Kashiwazaki S. Expression and function of Fas antigen and Bcl-2 in human systemic lupus erythematosus lymphocytes. *Clin Immunol Immunopathol* 1994; 73: 109-114.
150. Chu EB, Hobbs MV, Wilson CB, Romball CG, Linsley PS, Weigle WO. Intervention of CD4+ cell subset shifts and autoimmunity in the BXSB mouse by murine CTLA4Ig. *J Immunol* 1996; 156: 1262-1268.
151. Davis JC, Totoritis MC, Rosenberg J, Sklenar TA, Wofsy D. Phase I clinical trial of a monoclonal antibody against CD40-ligand (IDEC-131) in patients with systemic lupus erythematosus. *J Rheumatol* 2001; 28: 95-101.
152. Mihara M, Tan I, Chuzhin Y, Reddy B, Budhai L, Holzer A, *et al*. CTLA4Ig inhibits T cell-dependent B-cell maturation in murine systemic lupus erythematosus. *J Clin Invest* 2000; 106:91-101.
153. Bouillet P, Metcalf D, Huang DCS. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 1999; 286: 1735-1738.
154. Napirei M, Karsunky H, Zevnik B, Stephan H, Mannherz HG, Moroy T. Features of systemic lupus erythematosus in Dnase1-deficient mice. *Nat Genet* 2000; 25: 177-181.
155. Hayashida K, Shimaoka Y, Ochi T, Lipsky PE. Rheumatoid arthritis synovial stromal cells inhibit apoptosis and up-regulate Bcl-X(L) expression by B cells in a CD49/CD29-CD106-dependent mechanism. *J Immunol* 2000; 164: 1110-1116.
156. Matsumoto S, Iler-Ladner U, Gay RE, Nishioka K, Gay S. Ultrastructural demonstration of apoptosis, Fas and Bcl-2 expression of rheumatoid synovial fibroblasts. *J Rheumatol* 1996; 23: 1345-1352.
157. Pap T, Franz JK, Kuchen S. Expression of Survivin, a novel anti-apoptotic molecule, in the synovium of patients with rheumatoid arthritis (RA). *Arthritis Rheum* 1998; 41.
158. Sioud M, Mellbye O, Førre Ø. Analysis of the NF- $\kappa$ B p65 subunit, Fas antigen, Fas ligand and Bcl-2-related proteins in the synovium of RA and polyarticular JRA. *Clin Exp Rheumatol* 1998; 16: 125-134.
159. Lee SC, Pervaiz S. Apoptosis in the pathophysiology of diabetes mellitus. *Int J Biochem Cell Biol* 2007; 39: 497-504.
160. Heimbros DC, Oliff A. Therapeutic intervention and signaling *Curr Opin Cell Biol* 1998; 10: 284-288.
161. Itoh N, Imagawa A, Hanafusa T, Waguri M, Yamamoto K, Iwahashi H, *et al*. Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. *J Exp Med* 1997; 186: 613-618.
162. Kagi D, Odermatt B, Ohashi PS, Zinkernagel RM, Hengartner H. Development of insulinitis without diabetes in transgenic mice lacking perforin-dependent cytotoxicity. *J Exp Med* 1996; 183: 2143-2152.
163. Yang XD, Tisch R, Singer SM, Cao ZA, Liblau RS, Schreiber RD, *et al*. Effect of tumor necrosis factor  $\alpha$  on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J Exp Med* 1994; 180: 995-1004.
164. Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J Exp Med* 2004; 199: 1467-1477.
165. Yang Z, Chen M, Ellett JD, Fialkow LB, Carter JD, McDuffie M, *et al*. Autoimmune diabetes is blocked in Stat4-deficient mice. *J Autoimmun* 2004; 22: 191-200.
166. Dowling P, Shang G, Raval S, Menonna J, Cook S, Husar W. Involvement of the CD95 (APO-1/Fas) receptor/ligand system in multiple sclerosis brain. *J Exp Med* 1996; 184: 1513-1518.
167. Hisahara S, Okano H, Miura M. Caspase-mediated oligodendrocyte cell death in the pathogenesis of autoimmune demyelination. *Neurosci Res* 2003; 46: 387-397.
168. Sharief MK, Semra YK, Seidi OA, Zoukos Y. Interferon- $\beta$  therapy downregulates the anti-apoptosis protein FLIP in T cells from patients with multiple sclerosis. *J Neuroimmunol* 2001; 120: 199-207.

## Apoptosis: from Signalling Pathways to Therapeutic Tools

169. Kuhlmann T, Lucchinetti C, Zettl UK, Bitsch A, Lassmann H, et al. Bcl-2-expressing oligodendrocytes in multiple sclerosis lesions. *GLIA* 1999; 28: 34-39.
170. Sharief MK, Matthews H, Noori MA. Expression ratios of the Bcl-2 family proteins and disease activity in multiple sclerosis. *J Neuroimmunol* 2003; 134: 158-165.
171. Hebb ALO, Moore CS, Bhan V, Robertson GS. Targeting apoptosis to treat multiple sclerosis. *Curr Drug Discov Technol* 2008; 5:75-7.
172. Jenner P, Olanow CW. Understanding cell death in Parkinson's disease. *Ann Neurol* 1998; 44: (SUPPL. 1): S72-84
173. Mochizuki H, Hayakawa H, Migita M, Shibata M, Tanaka R, Suzuki A, et al. An AAV-derived Apaf-1 dominant negative inhibitor prevents MPTP toxicity as antiapoptotic gene therapy for Parkinson's disease. *Proc Nat Acad Sci USA* 2001; 98: 10918-10923.
174. Anglade P. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. *Histol Histopathol* 1997; 12: 25-31.
175. Tatton NA, Kish SJ. In situ detection of apoptotic nuclei in the substantia nigra compacta of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice using terminal deoxynucleotidyl transferase labelling and acridine orange staining. *Neuroscience* 1997; 77: 1037-1048.
176. Tatton NA, Lean-Fraser A, Tatton WG, Perl DP, Olanow CW. A fluorescent double labelling method to detect and confirm apoptotic nuclei in Parkinson's disease. *Ann Neurol* 1998; 44: S142-8. Review.
177. Hassouna I, Wickert H, Zimmermann M, Gillardon F. Increase in bax expression in substantia nigra following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment of mice. *Neurosci Lett* 1996; 204: 85-88.
178. Tatton WG, Chalmers-Redman R, Brown D, Tatton N, Schapira, Hunot, et al. Apoptosis in Parkinson's disease: signals for neuronal degradation. *Ann Neurol* 2003; 53: (SUPPL. 3): S61-70
179. Cummings JL, Vinters HV, Cole GM, Khachaturian ZS. Alzheimer's disease: Etiologies, pathophysiology, cognitive reserve, and treatment opportunities. *Neurology* 1998;51(1 SUPPL.): 2-17.
180. Su JH, Anderson AJ, Cummings BJ, Cotman CW. Immunohistochemical evidence for apoptosis in Alzheimer's disease. *NeuroReport* 1994; 5: 2529-2533.
181. Forloni G, Bugiani O, Tagliavini F, Salmona M. Apoptosis-mediated neurotoxicity induced by  $\beta$ -amyloid and PRP fragments. *Mol Chem Neuropathol* 1996; 28: 163-171.
182. Gschwind M, Huber G. Apoptotic cell death induced by  $\beta$ -amyloid1-42 peptide is cell type dependent. *J Neurochem* 1995; 65: 292-300.
183. Martin LJ, Price AC, Kaiser A, Shaikh AY, Liu Z. Mechanisms for neuronal degeneration in amyotrophic lateral sclerosis and in models of motor neuron death (Review). *Int J mol med* 2000; 5: 3-13.
184. Masliah E, Mallory M, Alford M, Tanaka S, Hansen LA. Caspase dependent DNA fragmentation might be associated with excitotoxicity in Alzheimer disease. *J Neuropathol Exp Neurol* 1998; 57: 1041-1052.
185. Mattson MP, Partin J, Begley JG. Amyloid P-peptide induces apoptosis-related events in synapses and dendrites. *Brain Res* 1998; 807: 167-176.
186. Mattson MP, Guo Q, Furukawa K, Pedersen WA. Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J Neurochem* 1998; 70: 1-14.
187. Weidemann A, Paliga K, Rrwang U, Reinhard FBM, Schuckert O, Evin G, et al. Proteolytic processing of the Alzheimer's disease amyloid precursor protein within its cytoplasmic domain by caspase-like proteases. *J Biol Chem* 1999; 274: 5823-5829.
188. Su JH, Deng G, Cotman CW. Bax Protein Expression Is Increased in Alzheimer's Brain: Correlations with DNA Damage, Bcl-2 Expression, and Brain Pathology. *J Neuropathol Exp Neurol* 1997; 56:86-93.
189. Cotman CW. Apoptosis decision cascades and neuronal degeneration in Alzheimer's disease. *Neurobiol Aging* 1998; 19 (Suppl. 1):29-32
190. Kruman I, Bruce-Keller AJ, Bredesen D, Waeg G, Mattson MP. Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. *J Neurosci* 1997; 17:5089-50100.

191. Mattson MP, Lindvall O. Neurotrophic factor and cytokine signaling in the aging brain. *Aging Brain* 1997; 2:299-345.
192. Gibson GE, Park LCH, Zhang H, Sorbi S, Calingasan NY. Oxidative stress and a key metabolic enzyme in Alzheimer brains, cultured cells, and an animal model of chronic oxidative deficits. *Ann NY Acad Sci* 1999; 893:79-94.
193. Chan SL, Griffin WST, Mattson MP. Evidence for caspase-mediated cleavage of AMPA receptor subunits in neuronal apoptosis and Alzheimer's disease. *J Neurosci Res* 1999; 57: 315-323.
194. Tortosa A, pez E, Ferrer I. Bcl-2 and Bax protein expression in Alzheimer's disease. *Acta Neuropathol* 1998; 95: 407-412.
195. Sawa A, Wiegand GW, Cooper J, Margolis RL, Sharp AH, Lawler J, *et al*. Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. *Nat Med* 1999; 1194-1198.
196. Zaidman BZ, Yassin M, Mahajna J, Wasser SP. Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Appl Microbiol Biotechnol* 2005; 67: 453-468.