[Frontiers in Bioscience, 3, d365-375, March 23, 98]

SIGNAL TRANSDUCTION DURING APOPTOSIS; IMPLICATIONS FOR CANCER THERAPY

Michael Story[1] and Reinhard Kodym[2]

[1]Department of Experimental Radiation Oncology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, [2] Klinic fur Strahlentherapie und Strahlenbiologie, Wahringer Gurtel 18-20, A-1090, Vienna, Austria

Received 2/15/98 Accepted 2/20/98

TABLE OF CONTENTS

1. ABSTRACT

Programmed cell death is a fundamental aspect of organismal development and stasis through its role in the maintenance of the balance between cell growth and cell death. If the balance is tipped, e.g. by unregulated cell growth, the result can be cancer. If tipped the other direction, e.g. by dysregulated cell death, and the result can again be cancer. The concept of dysregulated cell death, which until recently was not considered by oncologists, has forced a shift in the paradigm of cancer development. Along with this shift in thinking comes the likelihood that radiation and chemotherapy, both major modalities of cancer therapy, can benefit from strategies that modulate programmed cell death. One form of programmed cell death that is distinguished by its morphological features is called apoptosis. This form of programmed cell death is an energy dependent biochemically regulated process that is contingent upon a set of factors: the initial stimulatory event or in some cases a cellular insult, the organism, the cell type, the cellular environment, and other factors. This biochemical process is the result of the expression of a number of genes. In this review, the roles of several genes and gene families considered to be critical to the signal transduction cascade of apoptosis are described. Growth factors and cytokines are also discussed in the context of their interaction with these genes. We also discuss how these genes and their protein products are being used as prognostic indicators for cancer and cancer therapy and/or how they are the focus of strategies that either cause apoptosis or alter a cancer cell's propensity to initiate apoptosis when insulted by chemotherapy agents or radiation in the course of cancer therapy.

2. INTRODUCTION

The development, maintenance, or growth of any tissue is determined by the relationship between cell loss and cell replacement, whether the tissue is normal or malignant. Developmental biologists very early on noted the growth of tissues followed by the loss of those same cells as a tissue undergoes differentiation (1). Originally denoted as programmed cell death, the process was described as the developmentally regulated death of specific larval muscle cells in emerging adult moths and represented a mechanism whereby individual cells that are responsible for a tissue's architecture are cleared without injuring adjacent cells so as to modify tissue architecture.

Necrosis, another mechanism of cell death, is the result of exposure to exogenous agents resulting in a loss of osmotic balance. Endoplasmic reticulum and mitochondria swell as a result of the influx of cations, which also poison respiration. Ca^{2+} in particular may activate Ca^{2+}-dependent degradative enzymes such as phospholipases and endonucleases that disrupt cellular membranes and randomly degrade DNA. Ultimately, the osmolytic imbalance results in a cell's bursting and releasing a cache of lysosomal and degradative enzymes that attack and severely injure or kill adjacent cells. In tissues this can result in a zone of necrotic cells radiating from a necrotic center. Most important, necrosis, unlike programmed cell death, is not under biological control.

The focus of this article is on a specific form of programmed cell death referred to as apoptosis. There has been some confusion amongst the scientific disciplines as to the differences in programmed cell death and apoptosis. Programmed cell death is generally considered a deliberate process involved in organismal development, whereas apoptosis is a morphological description of a specific death process. Apoptosis can be the specific mode of death that occurs during development and as such can also be referred to as programmed cell death. However, there are other forms of programmed cell death during development that are distinct from apoptosis.

Identified in 1972 by Kerr *et al*., (2) the word "apoptosis" was chosen to represent the natural ordered elimination of cells from a tissue in a fashion analogous to the classic Greek definition of apoptosis, which describes the loss

of leaves from a tree during autumn. Apoptotic cell death is characterized by distinct morphologic changes as cells die by this mechanism. These changes include alterations of the plasma membrane due to the severing of junctions with neighboring cells, and the loss of microvilli. Blebs also appear on the cell surface. Chromatin condenses, forming aggregates along the nuclear membrane followed by a segmentation of the nucleus, which along with a convolution of the plasma membrane results in the formation of apoptotic bodies, which can contain intact organelles and normal mitochondria. These bodies are recognized by macrophages and adjacent cells and are rapidly phagocytosed.

In some cases, the DNA is digested by a Ca^{2+}/Mg^{2+}-dependent endonuclease between the nucleosomes such that when separated on an agarose gel, the DNA separates in sizes that are multiples of 180-190 base pairs (3). These oligonucleosome-sized DNA fragments, referred to as a DNA ladder, are not always associated with apoptosis. This was pointed out by Collins *et. al*.(4), who exposed cells to DEAE-dextran to induce necrosis by a rapid influx of Ca^{2+} ions and showed the distinct cleavage pattern that had until then been exclusively associated with apoptosis. In addition, there is a step preliminary to the production of DNA laddering where DNA is digested into multiples of 300 and 50 kilobases that likely represent higher order chromatin structure. In some cases the fragmentation of DNA stops at this higher order stage, which means that the endonucleases responsible for the ordered DNA cleavage associated with apoptosis are likely to be different and that the production of oligonucleosome-sized DNA fragments is not a necessary step for cell death (5). Blocking the production of oligonucleosome-sized DNA fragments does not necessarily mean that cells are spared their apoptotic fate either (6). Nevertheless, in most cases DNA laddering can be used as a surrogate marker for apoptosis but it should be confirmed by the morphological endpoints that originally defined apoptosis.

Little enthusiasm was originally shown for research in apoptosis, but as the central role of apoptosis became more and more evident in normal tissue development and in the onset of certain diseases, interest grew to the point where today apoptosis research is one of the most competitive areas of biology. Research on apoptosis has caused a fundamental shift in how biologists and oncologists view the onset of cancer. At one time cancer could be explained as the dysregulation of cell growth as cells no longer remain growth arrested but entered the cell cycle to grow and divide uncontrollably. Now, in some cases, cancer can be described as the dysregulation of cell death in that cells, possibly injured or mutated, that should have died do not. This survival advantage likely allows these cells to accumulate mutations that result in a malignant phenotype. Apoptosis also occurs spontaneously in many tumors. Why apoptosis should occur in tumor cells is unknown, but there are a number of possible explanations. The apoptosis seen may simply be the tumor cell following the same programmed response of the normal cell from which it is derived, or it may be responding to stimuli produced in the tumor microenvironment. This cell loss by apoptosis may be a significant factor in the rate of tumor growth, depending on tumor type (2,3). Some tumors have high mitotic indices but because cell loss factors may be as high as 95% the tumor growth rate is modest. Small alterations in the rate of cell loss may, therefore, shift the balance in tumor physiology from growth to regression. Oncologists and basic scientists are pursuing this notion.

A number of genes already linked to cancer onset or progression through their association with cell cycle regulation or DNA repair, like p53 or poly-ADP ribose polymerase (PARP), respectively, have also been implicated in apoptotic regulation and thus have newly defined functional roles. Growth factors, cytokines, and a number of newly cloned genes have also been shown to have a considerable role in the instigation or repression of apoptotic signaling. The following sections will focus on some of the more prominent genes that have been identified with apoptosis and how they fit into the apoptosis signal transduction cascade.

3. CERAMIDE

One upstream regulator of PKC and a number of other enzymes is N-acyl-sphingosine, or ceramide. Ceramide is the metabolite of sphingomyelin hydrolysis. Hydrolysis is achieved by either a neutral or acid sphingomyelinase (SMase). Upon hydrolysis, ceramide acts as a second messenger, stimulating or repressing the activity of a number of molecules, the result being cellular differentiation, senescence, cell proliferation, or apoptosis. For a review see (7).

The SMase activated is dependent on the biological agent to which a cell is exposed. Ionizing radiation can stimulate the acid form of Smase, and radiation-induced apoptosis is blocked in acid SMase knockout mice (8). Neutral SMase, which is membrane bound and as its name implies is active at physiological pH as opposed to the acidic pH of its counterpart, can be activated after daunorubicin, TNF-alpha, or radiation exposure, particularly when the cells have been enucleated (9,10). Binding of the CD95 ligand, fas/APO1, also stimulates neutral SMase (11).

Depending on the cell type, all of the previous conditions result in apoptosis and in some cases that apoptosis can be abrogated by agents that stimulate PKC activity (12,13). On the other hand, in cells that lack ceramide production after radiation, such as WEHI-231 cells, where sphingomyelinase is not activated after radiation, apoptosis is abrogated, resulting in increased radioresistance (14). Introduction of exogenous ceramide or inhibitors of sphingosine kinase restores both the apoptotic potential and radiosensitivity of these cells.

Interestingly, neutral SMase is also upregulated in cells undergoing senescence (15), suggesting that other downstream targets and/or other effector proteins determine which path is chosen. This is analogous to the decision that is made through the p53 pathway in that after an initial cellular insult a path is chosen that leads to either p21 or bax upregulation and thus to dramatically different cell fates. In their report, Kolesnick and Fuks (16) discuss the pleiotropic nature of ceramide second messenger signaling.

Once sphingomyelin is cleaved, the ceramide generated can act on at least two target molecules. One target

is a membrane-bound serine/threonine kinase, referred to as CAP kinase (ceramide-activated protein kinase). CAP kinase activation has been linked to receptor activation events such as IL-1 and TNF receptor binding and to Raf activation, which stimulates the extracellular signal-regulated kinase (ERK) (17). However, ceramide likely does not stimulate CAPK directly because ceramide did not phosphorylate a partially purified preparation of CAPK (18). Some intermediary or cofactor seems to be required.

A direct target of ceramide is CAPP, or ceramide-activated protein phosphatase, which is a serine/threonine protein phosphatase (19). Ceramide, via CAPP activation, downregulates c-myc in a manner analogous to TNF-alpha, that is, by blocking transcription elongation. Furthermore, okadaic acid, a phosphatase inhibitor, is capable of blocking c-myc down-regulation caused by ceramide exposure (20).

Related to c-myc down-regulation by ceramide via CAPP are the inactivation of PKC-alpha (21) and possibly the translocation of PKC isoforms delta and epsilon. While the role of PKC alpha is less clear regarding apoptosis, PKC delta and epsilon translocation from the membrane to the cytosol after TNF-alpha or spingomyelinase exposure is mediated by ceramide production (22) in leukemia cells. These isoforms, unlike alpha, do not require Ca^{2+} for activation but are influenced by diacyl glycerol. Because the isoforms of PKC vary with cell type, inactivation of PKC via ceramide might explain why some cells require an influx of Ca^{2+} for apoptosis and others do not.

Ceramide is indirectly responsible for the cleavage and activation of a Ced-3/ICE-like cysteine protease called CPP32 (23), (recently renamed caspase-3) a member of the caspase family of Ced-3-like proteases, which is specific for the apoptosis pathway. Caspase-3 has been shown to cleave poly(ADP-ribose) polymerase (PARP) (24) and DNA-dependent protein kinase (25, 26) during apoptosis. What intermediate steps result in caspase-3 activation via ceramide are unknown. Another indirect target of ceramide is the stress-activated protein kinase (SAPK/JNK) pathway (27), however, SAPK/JNK signaling does not always lead to apoptosis, and what role SAPK/JNK plays in apoptosis signaling is also unknown.

Finally, an alternative pathway for ceramide production and apoptosis signaling was identified. De novo synthesis of ceramide by ceramide synthase was shown to be responsible for an increase in ceramide after daunorubicin exposure in U937 cells that led to apoptosis (28). And while fumonisin B1, a specific inhibitor of ceramide synthase, blocked daunorubicin-induced apoptosis in these same cells, another study (9) showed no inhibition of daunorubicin-induced apoptosis by Fumonisin B1 and suggested that neutral sphingomyelinase was responsible for the increased ceramide production.

4. PROTEIN KINASE C

An extensive list of genes has been identified as belonging to the signal transduction pathway that leads to apoptosis, a much longer list than can be addressed here. A number of these genes, such as diacyl glycerol (DAG), protein kinase C (PKC), cyclic AMP-dependent protein kinase (PKA), and cyclic AMP (cAMP) are common intermediaries of signal transduction in general. All have roles in the up- or down-regulation of apoptosis. PKC and PKA, both serine/threonine-specific kinases, have opposing roles in apoptosis signaling. PKC as a phospholipid-dependent kinase requires DAG and, depending on the PKC isoform, may require Ca^{2+} for activation. Which isoform is activated is highly dependent on the ligand and the cell type. (For a general review of PKC and its isoforms, see (29)).

Transient downregulation of PKC has been shown to render a cell susceptible to apoptosis (30-33) after addition of Ca^{2+}, upregulation of p21ras, addition of phospholipase A2, and to radiation exposure. Consistent with this, upregulation of PKC has been shown to inhibit apoptosis, as is the case with Peyer's patch B cells maintained in culture with agents that stimulate PKC activation (34). Similarly, upregulation of cAMP which upregulates PKA results in apoptosis upon appropriate stimulation (35). PKA is upregulated during the signaling cascade of apoptosis; however, PKC activation is likely agonistic to the action of PKA (30, 36).

DAG and cAMP represent second messengers that, along with cofactors, activate PKA and PKC. Kinases such as PKC and PKA are intermediaries in the apoptotic pathway and not the primary genes responsible for regulating apoptosis. For example, bcl-2 expression will override the apoptotic signal generated by down-regulation of PKC by ras expression (31) as it does with a number of different apoptotic stimuli. Instead, PKC or PKA activate a number of genes via phosphorylation. Whether a cell continues down the apoptotic pathway, undergoes differentiation, or undergoes cell cycle arrest is highly dependent upon interaction with other signaling pathways, which are likely to vary with cell type.

5. NF-KAPPAB

The transcription factor NF-kappaB is activated downstream of PKC (37). In its active form NF-kappaB is a hetero- or homo-dimer belonging to a family of proteins partially homologous to the oncogene product c-Rel. The homology between the members of the Rel family is through the Rel homology domain, which is about 300 amino acids in size and constitutes the DNA-binding domain of these proteins. The 'classic' NF-kappaB is a heterodimer formed by the smaller p50 subunit, which is essentially a Rel homology domain, and the larger RelA (p65) subunit which consists of an N-terminal Rel homology domain and two C-terminal transactivation domains. NF-kappaB is found in its inactive form in the cytoplasm, where it is bound to the 43-kDa protein I-kappaB that covers the nuclear localization signal region of the RelA/p50 dimer. Activation of NF-kappaB starts with the proteolytic destruction of I-kappaB followed by the transport of the RelA/p50 complex into the nucleus, where it binds to its recognition site on the DNA and activates transcription of target genes. (For a timely review of the NF-kappaB family see (38)).

Depending on the cell system used and the signal transduction pathway studied, the activation of NF-kappaB either induces a reaction which protects cells against apoptosis

or activates the cellular suicide program. Data supporting an anti-apoptotic effect and showing an involvement of NF-kappaB in the apoptotic signal transduction pathway came from mice in which the RelA protein had been knocked out (39). These mice showed embryonic lethality due to apoptotic degeneration of the liver. In two other cases, inhibition of the nuclear translocation of NF-kappaB after exposure of cells to TNF-alpha, ionizing radiation, or daunorubicin, enhanced apoptotic cell death (40, 41) and similar results have been reported for rat neuroblast-like PC12 cells (42) and for murine B-cells (43, 44). Finally, Liu et al. (45) demonstrated that NF-kappaB activation but not JNK activation via TNF-alpha receptor type 1 stimulation protects against TNF-alpha-induced apoptotic cell death.

There is equally strong evidence that the activation of NF-kappaB is a required element in the chain of events leading to apoptotic cell death. In one study (46) it was shown that Sindbis-virus-induced apoptosis of a prostate carcinoma cell line is dependent on the binding of NF-kappaB to its recognition sequence on the nuclear DNA. In another study a transdominant negative mutant RelA was able to partially prevent apoptosis of human embryonic kidney cells induced by serum starvation (47). It has also been demonstrated recently that glutamate-induced neuronal apoptosis can be prevented by blocking NF-kappaB activation with aspirin (48).

Other correlative evidence linking NF-kappaB activation to the induction of apoptosis are the observations of ischemia-induced increases in the levels of hydrogen peroxide, TNF-alpha, and IL-1-beta in fetal brain, which is followed by NF-kappaB activation and evidence of apoptotic cell death (49). Also, some cell lines, after ionizing radiation exposure at either low dose (0.5 Gy) (50) or high dose (20 Gy) (51) activate NF-kappaB, and it is very likely that this response influences the susceptibility of the cells to apoptosis. It is difficult, however, to decide whether this activation is a futile effort to make use of the anti-apoptotic properties of NF-kappaB or the successful initiation of a chain of events finally leading to apoptotic cell death.

6. p53

The first indication of the involvement of p53 in apoptosis resulted from the analysis of M1 leukemic cells, which lack functional p53, after introduction of a vector with a p53 gene containing a temperature sensitive mutation (52). At the permissive temperature, where the mutant p53 protein behaved as wild-type, cells died rapidly by apoptosis. At the non-permissive temperature the p53 did not behave as wild-type and the M1 cells did not die by apoptosis. Addition of IL-6 to the culture medium spared cells from their apoptotic fate, thus linking the loss of apoptotic potential to the generation of a pretransformed cell population that is waiting for oncogenic transformation, in a manner analogous to that identified with the blc-2 gene (see later discussion) (53-55).

The requirement for p53 in the signal transduction pathway of radiation-induced apoptosis was determined by the irradiation of excised thymocytes from transgenic mice that were either null, heterozygous, or wild-type for p53 (56). The extent of apoptosis was dependent on p53 status, with wild-type > heterozygous > homozygous. p53 was not, however, required for glucocorticoid-induced apoptosis. The implications for p53 regulation of apoptosis during multi-step carcinogenesis became evident when it was shown that the lack of p53 expression allowed mouse fibroblast cells to become tumorigenic via E1A (adenovirus early region 1A) transfection (57). Oncogenic transformation was gene dosage dependent for p53 in that suppression of the transformed phenotype was through apoptosis. Furthermore, tumor environment , in particular hypoxic regions common to solid tumors, exerts a selective pressure on cells within a tumor that eliminates cells that have wild-type p53 by causing apoptosis via the low oxygen tension or nutritional environment (58). This encourages the growth of cells deficient in p53 that may be susceptible to oncogenic transformation, (if not already transformed) and are likely to have escaped cell cycle control because of the lack of functional p53. This same interaction between hypoxia and p53 is likely true for bcl-2 as well.

7. BCL-2 AND FAMILY MEMBERS

Bcl-2 is a protein of about 26 kDa that is localized in the outer mitochondrial membrane, nuclear envelope, and the endoplasmic reticulum. Bcl-2 was discovered in 1984 and is a representative member of a family of partially homologous proteins that includes bcl-2, bcl-X_L, mcl-1 and BAG-1 which act as inhibitors of cell death (59-62) and bad, bax, bak, and bik, which act as promoters of apoptosis (63-66). Interestingly, the Bcl-X gene is responsible for both bcl-X_L and bcl-X_S via alternative splicing of the Bcl-X RNA (62). The members of the bcl-2 protein family form homo- or heterodimers and depending upon whether the monomers belong to the group of pro- or anti- apoptotic bcl-2 family members, the final dimeric molecule has either pro- or anti-apoptotic properties. How these molecules exert their influence on the decision as to whether or not a cell is to undergo apoptosis is not yet fully known. Four important clues, however, have been recently discovered:

1. The anti-apoptotic proteins bcl-2 and bcl-XL, when overexpressed, inhibit the release of cytochrome c from the mitochondria (67, 68). Cytochrome c is a mediator of apoptosis via the activation of caspase (69).

2. In the nematode caenerabditis elegans the protein CED-9 (which is homologous to the bcl-2 family) directly interacts with CED-4 (the mammalian homologue of which has not yet been discovered), which in turn can directly interact with CED-3 (homologous to the caspases) (70, 71). It seems likely then that bcl-2 family proteins influence caspase activity directly. This assumes, however, that there is a mammalian protein functionally equivalent to CED-3.

3. Bcl-2 is intimately involved in cellular redox status. Bcl-2 is found in areas of O_2-free radical generation such as the mitochondria, endoplasmic reticulum, and nuclear membrane. It has been argued that bcl-2 acts in an antioxidant pathway (72) or as a pro-oxidant (73). Regardless of the mechanism, overexpression of bcl-2 results in increased cellular concentrations of reduced

glutathione (73-76). Cells are therefore likely to have better radical scavenging abilities, which may be important when they are first exposed to particular anti-neoplastic agents, or perhaps the reduced glutathione is important to the maintenance of important signal transduction elements some time after the initial apoptotic stimulus.

4. The bcl-2 family members also appear to be responsible for the maintenance of the mitochondrial membrane permeability transition. Disruption of this membrane potential is seen early in apoptosis and results in the uncoupling of respiration, the generation of superoxides, and a release of calcium and glutathione from the mitochondria (77, 78).

A mammalian cell is dependent upon mitochondria for the energy requirements necessary to sustain itself, and it has been recognized that cells can die through the depletion of energy in the form of ATP. From the mitochondrial point of view that is a passive process. So there is a certain irony in the four points made above and that is that mitochondria, life sustaining as they are, are also intimately involved in the apoptotic death process, murder if you will, of its symbiotic host.

There are several observations showing that the protein expression levels of apoptosis-promoting or -inhibiting members of the bcl-2 family influence radiation-induced programmed cell death. The acquired radiation resistance and loss of apoptotic potential of a murine B-cell lymphoma cell line, LY-ar, has been attributed to its overexpression of bcl-2 and concomitant increase in reduced cellular glutathione (75, 79). Furthermore, transfection experiments in murine erythroleukemia cell lines showed that high levels of bcl-2 protein inhibit apoptotic cell kill normally induced by ionizing radiation (80). For human prostate carcinoma cells which were transfected with bcl-2, a similar decrease in radiation-induced apoptosis was reported (81); remarkably it was not accompanied by a change in clonal cell survival. In the case of the apoptosis-promoting molecule bax it was shown (82) in the human breast cancer cell line MCF-7 that its overexpression led to an increase in radiosensitivity. Similar results were reported in experiments with transgenic mice, where overexpressing bax (83) in T cells resulted in an accelerated apoptotic response to gamma-irradiation.

How the signal originating from the initial radiation-induced damage to cellular structures is transmitted to the bcl-2 family proteins is not yet clearly understood. There is evidence that radiation influences these proteins on the transcriptional level, or as recent data suggest, on a post-transcriptional level as well (see below). It has been shown that the transcription factor p53, which is responsive to DNA damage, is a direct transcriptional activator of both the bcl-2 and bax genes (84, 85) and that ionizing radiation up-regulates bax protein in mouse lymphoid and small intestinal epithelial cells (86). p53 also influences the transcription of other members of the bcl-2 family including bcl-XL (87). Further evidence for a radiation-induced transcriptional modulation of bcl-2 family proteins is provided by the observations that after the irradiation of fetal rat brains, the levels of bcl-2 and bcl-XL mRNAs are reduced while the mRNAs of p53 and bax are increased (88). The same scenario is played out in the human lymphoid cell lines HL60 and U937 after C2-ceramide or TNF-alpha exposure, that is, the downregulation of bcl-2 (89).

Post-translational modification of the bcl-2 family of proteins by phosphorylation may play an important role in their activity. This has been demonstrated for bcl-2, where phosphorylation increases the inhibition of apoptosis (90). Phosphorylation of the pro-apoptotic bad protein (91) seems to be a mechanism by which IL-3 promotes cell survival by stimulating the phosphorylation of bad, which sequesters bad in the cytosol where it can no longer bind to and counter the effects of mitochondrial bcl-2. One of the kinases that phosphorylates bad is Raf-1 (92), which in turn can be targeted to the mitochondrial membrane by bcl-2. Raf-1 kinase can be activated by ionizing radiation (93), and the resulting expression and activation increases cellular survival. These phosphorylation events may be part of another pathway leading from initial cellular damage inflicted by ionizing radiation to initiation or inhibition of the apoptotic response via the activity of the respective bcl-2 family member at the mitochondrial and/or nuclear membrane level.

8. THERAPEUTIC APPLICATIONS

There is ample evidence from in vitro studies that susceptibility to the apoptotic mode of cell death strongly influences the efficiency of tumor and normal cell killing by radio- or chemotherapeutic drug exposure. Apoptosis could also explain the dramatic response seen in tissues considered dose limiting such as the parotid gland, which is often irradiated during treatment for head and neck cancer. Whether tumors respond in general in this manner, or whether a particular tumor would respond in this manner, was unknown. Early examinations of tumors showed clearly that some tumors would respond in a manner analogous to that seen in vitro, e.g. they responded to low doses of toxic agent and responded in a few hours (94, 95). A very telling aspect of the study by Meyn *et. al*. (95) was the fact that the best prognostic indicator for apoptotic response among a group of 15 different tumor types was the background level of apoptosis seen in untreated tumor tissue. The greater the level of apoptosis seen in untreated samples, the greater the apoptosis seen upon treatment. Furthermore, increases beyond a certain dose of radiation did not result in increases in apoptosis.

These, and other studies, highlight two strategies that may offer therapeutic advantage when treating cancer. The first is to take advantage of the prognostic potential of apoptosis. Could apoptotic potential provide clinicians with a sense of the sensitivity of a tumor to therapy? Secondly, the plateau of apoptotic response may well be an advantage when considering cancer treatment regimens. In general, cells respond to apoptotic stimuli when they are presented at relatively low concentrations. When the stimulus is too large, e.g. the cell injury is too great, exposed cells die indiscriminately by necrosis. Necrosis can result in massive tissue destruction of adjacent “innocent” cells. If tumor cells could be manipulated into participating in their demise by

Table 1. Recent applications of apoptotic endpoints in cancer and cancer therapy

SOURCE	TUMOR	MARKER(S)	FINDINGS
Elledge, *et al*., (96)	Breast cancer (estrogen positive) treated with tamoxifen	bcl-2 and p53	Treatment failure not correlated with p53 status, better treatment response and higher survival in patients with high bcl-2 expression
Keen *et al*., (97)	Breast cancer	bcl-2	High bcl-2 expression found in patients with good clinical tumor regression
Silvestrini *et al*., (98)	Breast cancer treated by surgery and radiation	p53 and bcl-2	Risk of local recurrence after surgery is higher in patients with elevated p53, bcl-2 not clearly connected to outcome
Grignon *et al*., (99)	Prostate cancer (locally advanced) treated with radiation	p53	Abnormal p53 expression correlates with a high rate of distant metastasis and decreased survival
Cardillo *et al*., (100)	Prostate cancer treated with endocrine therapy	Bcl-2, bax, bcl-X, bak	Treatment related apoptosis in both normal and malignant prostate cells, bcl-2 expressing prostate hyperplasias did not show apoptosis
Buttitta *et al*., (101)	Ovarian carcinoma treated with chemotherapy	p53	Alterations in p53 status in patients showing a low treatment response rate
Puglisi *et al*., (102)	Squamous cell carcinoma of the esophagus treated by radiotherapy	p53 and bcl-2	No correlation of p53 or bcl-2 with the response rate
Wheeler *et al*., (103)	Stage 1B cervical carcinoma treated by radiation	Visually scored apoptotic cells	Baseline level of apoptosis above median score predicted for survival
Levine *et al*., (104)	Cervical carcinoma, stages I-IV, treated by radiation	Visually scored apoptotic cells	Higher survival with apoptotic score less than median value
Komaki *et al*., (105)	Non-small cell lung cancer	Visually scored apoptotic cells	A high apoptotic fraction of tumor cells correlates with increased 5 year survival with overall survival unaffected
Ha *et al*., (107)	Follicular lymphoma, stages I-III	PCR analysis of bcl-2 breakpoint	Conversion of detection of bcl-2 molecular breakpoint from positive to negative after radiation treatment in peripheral blood and bone marrow samples

Examples of recent efforts to analyze particular aspects of apoptosis in the prognosis of cancer and cancer therapy.

strategies that made them permissive to apoptotic signaling, which would occur at low exposure levels of the anti-neoplastic agent, then the likelihood of tumor destruction and normal tissue sparing is enhanced.

It is no surprise, therefore, that there is an ever-growing number of studies investigating the susceptibility of tumor cells to apoptosis and the signal transduction molecules associated with apoptosis for use as prognostic markers or as targets for therapeutic intervention. A sampling of recent studies of the prognostic value of either apoptosis per se or of the expression of members of the bcl-2 family or p53 are listed in table 1 (96-105, 107). It is obvious from these studies that the prognostic value of apoptosis or of two of its major signal transduction components is not clear cut and like many prognostic factors are likely to be very specific to the type of tumor. For instance, observation of the overexpression of bcl-2 would lead one to expect a tumor to be chemo- or radiation-resistant. However, in two reports (96) and (97) bcl-2 expression was more likely to predict a better treatment response. Visually scoring apoptosis also provided seemingly contradictory evidence. The study of Wheeler *et. al*. (103) demonstrated that the baseline level of apoptosis in Stage 1B cervical adenocarcinoma predicted survival, that is, patients whose biopsy specimens of pretreatment samples contained greater than 2% apoptotic bodies (the median value) had better overall survival. However, Levine et al. (104) who examined carcinomas of the cervix, grades I–IV, for apoptotic figures before treatment found that apoptotic ratios below the cutoff figure of 0.71% (median score) predicted for better survival. Somewhere in the middle are the interesting results of Komaki *et. al*. (105), where a high apoptotic fraction correlated with increased 5-year survival, but the correlation did not hold up for overall survival.

At the molecular level, studies of follicular and B-cell lymphoma have demonstrated that molecular diagnosis of treatment efficacy by PCR amplification of the bcl-2 molecular break point that is associated with lymphomas, particularly follicular lymphomas, is predictive of treatment response (106, 107) in that a negative amplification was indicative of a lower probability of recurrence. Yet, in a study by the Southwest Oncology Group, bcl-2 positivity identified a metastatic breast cancer phenotype that was responsive to tamoxifen and predictive of longer survival. Clearly, one molecule like bcl-2 is not going to be the only determinant for either the level of tumor cell apoptosis or the extent of treatment response for every cancer.

In parallel with the growing interest in apoptosis as a prognostic parameter, there are a number of clinical studies where cellular susceptibility to apoptosis is modulated by inducing the overexpression of p53 via the introduction of vectors that express wild-type p53 directly into the tumor or by downregulating tumor bcl-2 protein expression by antisense technology. A review of recent proposals to the Recombinant DNA Advisory Committee (RAC) (www.nih.gov/od/orda/) reflects this. However, there is only one published study known to us. In this preliminary report in table 2, on the use of antisense technology against the bcl-2 protein, disease-related symptoms improved and a measurable tumor response was associated with the downregulation of bcl-2 protein in patients with non-Hodgkin's lymphoma (108).

Table 2. Recent applications of apoptotic endpoints in cancer and cancer therapy

SOURCE	TUMOR	MARKER(S)	FINDINGS
Webb *et. al*., (108)	Non-Hodgkin's lymphoma	Bcl-2 antisense oligonucleotide gene therapy	Reduction in tumor size in 2 of 9 patients, decreased LDH levels in 4 of 9 patients

Recent attempt to use genetic technology to manipulate apoptosis as an approach to enhance cancer therapy.

Bringing to the bedside such laboratory technology as small pharmacological molecules that inhibit or stimulate the activity of particular apoptosis or gene therapy, is a formidable task. There are numerous questions to be answered. What mechanisms of delivery, such as direct injection into the tumor or systemic delivery are best? What is the target specificity? Should or can sense, antisense, ribozyme or single chain antibodies technologies be used? These are all issues before the RAC. Whether such strategies are effective when therapeutic resistance is due to multiple drug resistance genes is unresolved. Like strategies aimed at the cell cycle machinery, apoptosis-directed strategies may have to be specific to the type of cancer being treated. In any case it is likely not the magic bullet but rather one more bullet in the armamentarium of therapeutic strategies designed to cure cancer.

9. REFERENCES

1. Lockshin R. A. & Williams: Programmed Cell Death. III. Neural control of the breakdown of the intersegmental muscles. *J. Insect Physiol.* 11, 605-610, (1965)

2. Kerr J. F. R., A. H. Wyllie & A. R. Currie: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26, 239-257, (1972)

3. Wyllie A. H.: The biology of cell death in tumours. *Anticancer Res* 5, 131-136, (1985)

4. Collins R. J., B. V. Harmon, G. C. Gobé & J. F. R. Kerr: Internucleosomal DNA cleavage should not be the sole criterion for identifying apoptosis. *Int J Radiat Biol* 61, 451-453, (1992)

5. Oberhammer F., J. W. Wilson, C. Dive, I. D. Morris, J. A. Hickman, A. E. Wakeling, P. R. Walker & M. Sikorska: Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragmentation. *EMBO J* 12, 3679-3684, (1993)

6. Voehringer D. W., M. D. Story, R. G. O'Neil & R. E. Meyn: Modulating Ca Ca^{2+}, in radiation-induced apoptosis suppresses DNA fragmentation but does not enhance clonogenic survival. *Int J Radiat Biol* 71, 237-243, (1997)

7. Hannun Y. A.: Functions of ceramide in coordinating cellular responses to stress. *Science* 274, 1855-1859, (1996)

8. Santana P., L. A. Peña, A. Haimovitz-Friedman, S. Martin, D. Green, M. McLoughlin, D. Green, C. Cordon-Cardo, E. H. Schuchman, Z. Fuks & R. Kolesnick: Acid sphingomyelinase-deficient human lymphoblasts and mice are defective in radiation-induced apoptosis. *Cell* 86, 189-198, (1996)

9. Jaffrézou J.-P., T. Levade, A. Bettaïeb, N. Andrieu, C. Bezombees, N. Maestre, S. Vermeersch, A. Rousse & G. Laurent: Daunorubicin-induced apoptosis: Triggering of ceramide generation through sphingomyelin hydrolysis. *EMBO J* 15, 2417-2424, (1996)

10. Haimovitz-Friedman A., C.-C. Kan, D. Ehleiter, R. S. Persaud, M. McLoughlin & Z. Fuks: Ionizing radiation acts on cellular membranes to generate ceramide and initiate apoptosis. *J Exp Med* 180, 525-535, (1994)

11. Cifone M. G., P. Roncaioli, R. De Maria, G. Camarda, A. Santoni, G. Ruberti & R. Testi: Multiple pathways originate at the Fas/APO-1 (CD95) receptor: sequential involvement of phosphatidylcholine-specific phospholipase C and acidic sphingomyelinase in the propagation of the apoptotic signal. *EMBO J* 14, 5859-5868, (1995)

12. Obeid L. M., C. M. Linardic, L. A. Karolak & Y. A. Hannun: Programmed cell death induced by ceramide. *Science* 259, 1769-1771, (1993)

13. Jarvis W. D., F. A. J. Fornari, R. S. Traylor, H. A. Martin, L. B. Kramer, R. K. Erukulla, R. Bittman & S. Grant: Induction of apoptosis and potentiation of ceramide-mediated cytotoxicity by sphingoid bases in human myeloid leukemia cells. *J Biol Chem* 271, 8275-8284, (1996)

14. Chmura S. J., E. Nodzenski, M. A. Beckett, D. W. Kufe, J. Quintans & R. R. Weichselbaum: Loss of ceramide production confers resistance to radiation-induced apoptosis. *Cancer Res* 57, 1270--1275, (1997)

15. Venable M. E., J. Y. Lee, M. J. Smythe, A. Bielawska & L. M. Obeid: Role of ceramide in cellular senescence. *J Biol Chem* 270, 30701-30708, (1995)

16. Kolesnick R. & Z. Fuks: Ceramide: a signal for apoptosis or mitogenesis? *J Exp Med* 181, 1949-1952, (1995)

17. Yao B., Y. Zhang, S. Delikat, S. Mathias, S. Basu & R. Kolesnick: Phosphorylation of Raf by ceramide-activated protein kinase. *Nature* 378, 307-310, (1995)

18. Liu J., S. Mathias, Z. Yang & R. N. Kolesnick: Renaturation and tumor necrosis factor-alpha stimulation of a 97-kDa ceramide-activated protein kinase. *J Biol Chem* 269, 3047-3052, (1994)

19. Dobrowsky R. T. & Y. A. Hannun: Ceramide stimulates a cytosolic protein phosphatase. *J Biol Chem* 267, 5048-5051, (1992)

20. Wolff R. A., R. T. Dobrowsky, A. Bielawska, L. M. Obeid & Y. A. Hannun: Role of ceramide-activated protein phosphatase in ceramide-mediated signal transduction. *J Biol Chem* 269, 19605-19609, (1994)

21. Lee J. Y., Y. A. Hannun & L. M. Obeid: Ceramide inactivates cellular protein kinase Cα. *J Biol Chem* 271, 13169-13174, (1996)

22. Sawai H., T. Okazaki, Y. Takeda, M. Tashima, H. Sawada, M. Okuma, S. Kishi, H. Umehara & N. Domae: Ceramide-induced translocation of protein kinase C-δ and -ε to the Cytosol. *J Biol Chem* 272, 2452-2458, (1997)

23. Mizushima N., R. Koike, H. Kohsaka, Y. Kushi, S. Handa, H. Yagita & N. Miyasaka: Ceramide induces apoptosis via CPP32 activation. *FEBS Lett* 395, 267-271, (1996)

24. Tewari M., L. T. Quan, K. O'Rouke, S. Desnoyers, Z. Zeng, D. R. Beidler, G. G. Poirier, G. S. Salvesen & V. M. Dixit: Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell* 81, 801-809, (1995)

25. Song Q., S. P. Lees-Miller, S. Kumar, Z. Zhang, D. W. Chan, G. C. Smith, S. P. Jackson, E. S. Alnemri, G. Litwack, K. K. Khanna & M. F. Lavin: DNA-dependent protein kinase catalytic subunit: a target for an ICE-like protease in apoptosis. *EMBO J* 15, 3238-3246, (1996)

26. Casciola-Rosen L. A., G. J. Anhalt & A. Rosen: DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J Exp Med* 182, 1625-1634, (1995)

27. Verheij M., R. Bose, X. H. Lin, B. Yao, W. D. Jarvis, S. Grant, M. J. Birrer, E. Szabo, L. I. Zon, J. M. Kyriakis, A. Haimovitz-Friedman, Z. Fuks & R. N. Kolesnick: Requirement for ceramide-initiated SAPK/JNK signaling in stress-induced apoptosis. *Nature* 380, 75-79, (1996)

28. Bose R., M. Verheij, A. Haimovitz-Friedman, K. Scotto, Z. Fuks & R. Kolesnick: Ceramide synthase mediates daunorubicin-induced apoptosis: An alternative mechanism for generating death signals. *Cell* 82, 405-414, (1995)

29. Hug H. & T. F. Sarre: Protein kinase C isoenzymes; divergence in signal transduction? *Biochem J* 291, 329-343, (1993)

30. McConkey D., M. Hartzell, M. Jondal & S. Orrenius: Inhibition of DNA fragmentation in thymocytes and isolated thymocyte nuclei by agents that stimulate protein kinase C. *J Biol Chem* 264, 13399-13402, (1989)

31. Chen C. Y. & D. V. Faller: Direction of p21ras-generated signals towards cell growth or apoptosis is determined by protein kinase C and Bcl-2. *Oncogene* 11, 1487-1498, (1995)

32. Shaposhnikova V. V., O. R. Dobrovinskaya, L. K. Eidus & Y. N. Korystov: Dependence of thymocyte apoptosis on protein kinase C and phospholipase A_2. *FEBS Lett* 348, 317-319, (1994)

33. Ojeda F., M. I. Guarda, C. Maldonado, H. Folch & H. Diehl: Role of protein kinase-C in thymocyte apoptosis induced by irradiation. *Int J Radiat Biol* 61, 663-667, (1992)

34. Motyka B., P. J. Griebel & J. D. Reynolds: Agents that activate protein kinase C rescue sheep ileal Peyer's patch B cells from apoptosis. *Eur. J. Immunol.* 23, 1314-1321, (1993)

35. McConkey D. J., S. Orrenius, S. Okret & M. Jondal: Cyclic AMP potentiates glucocorticoid-induced endogenous endonuclease activation in thymocytes. *FASEB J* 7, 580-585, (1993)

36. Marti A., B. Jehn, E. Costello, N. Keon, G. Ke, F. Martin & R. Jaggi: Protein kinase A and AP-1 (c-Fos/JunD) are induced during apoptosis of mouse mammary epithelial cells. *Oncogene* 9, 1213-1223, (1994)

37. Uckun F. M., G. L. Schieven, L. M. Tuel-Ahlgren, I. Dibirdik, D. E. Myers, J. A. Ledbetter & C. W. Song: Tyrosine phosphorylation is a mandatory proximal step in radiation-induced activation of the protein kinase C signaling pathway in human B-lymphocyte precursors. *Proc Natl Acad Sci USA* 90, 252-256, (1993)

38. Gomez J., D. Garcia-Domingo, C. Martinez-A. & A. Rebollo: Role of NF-KappaB in the control of apoptotic and proliferative responses in IL-2-responsive T cells. *Frontiers in Bioscience* 2, 49-60, (1997)

39. Begg A. A., W. C. Sha, R. T. Bronson, S. T. Ghosh & D. Baltimore: Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 376, 167-170, (1995)

40. Van Antwerp D. J., S. J. Martin, T. Kafri, D. R. Green & I. M. Verma: Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* 274, 787-789, (1996)

41. Wang C. Y., M. W. Mayo & A. S. J. Baldwin: TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. *Science* 274, 784-787, (1996)

42. Taglialatela G., R. Robinson & J. R. Perez-Polo: Inhibition of nuclear factor kappa B (NF-kappaB) activity induces nerve growth factor-resistant apoptosis in PC12 cells. *J Neurosci Res* 47, 155-162, (1997)

43. Wu M., H. Lee, R. E. Bellas, S. L. Schauer, M. Arsura, D. Katz, M. J. Fitzgerald, T. L. Rothstein, D. H. Sherr & G. E. Sonenshein: Inhibition of NF-kappaB/Rel induces apoptosis of murine B cells. *EMBO J* 15, 4682-4690, (1996)

44. Arsura M., M. Wu & G. E. Sonenshein: TGF beta 1 inhibits NF-kappaB/Rel activity inducing apoptosis of B cells: transcriptional activation of I kappa B alpha. *Immunity* 5, 31-40, (1996)

45. Liu Z. G., H. Hsu, D. V. Goeddel & M. Karin: Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. *Cell* 87, 565-576, (1996)

46. Lin K. I., S. H. Lee, R. Narayanan, J. M. Baraban, J. M. Hardwick & R. R. Ratan: Thiol agents and Bcl-2 identify an alphavirus-induced apoptotic pathway that requires activation of the transcription factor NF-kappa B. *J Cell Biol* 131, 1149-1161, (1995)

47. Grimm S., M. K. Bauer, P. A. Baeuerle & K. Schulze-Osthoff: Bcl-2 down-regulates the activity of transcription factor NF-kappaB induced upon apoptosis. *J Cell Biol* 134, 13-23, (1996)

48. Grilli M., M. Pizzi, M. Memo & P. Spano: Neuroprotection by aspirin and sodium salicylate through blockade of NF-kappaB activation. *Science* 274, 1383-1385, (1996)

49. Clemens J. A., D. T. Stephenson, E. B. Smalstig, E. P. Dixon & S. P. Little: Global ischemia activates nuclear factor-kappa B in forebrain neurons of rats. *Stroke* 28, 1073-1080, (1997)

50. Mohan N. & M. l. Meltz: Induction of nuclear factor kappa B after low-dose ionizing radiation involves a reactive oxygen intermediate signaling pathway. *Radiat Res* 140, 97-104, (1994)

51. Brach M. A., R. Hass, M. I. Sherman, H. Gunji, R. Weichselbaum & D. Kufe: Ionizing radiation induces expression and binding activity of the nuclear factor kappa B. *J Clin Invest* 88, 691-695, (1991)

52. Yonish-Rouach E., D. Resnitzky, J. Lotem, L. Sachs, A. Kimchi & M. Oren: Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature (London)* 352, 345-347, (1991)

53. Nunez G., L. London, D. M. Hockenbery, M. Alexander, J. P. McKearn & S. J. Korsmeyer: Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. *J Immunol* 144, 3602-3610, (1990)

54. Vaux D. L., H. L. Aguila & I. L. Weissman: Bcl-2 prevents death of factor-deprived cells but fails to prevent apoptosis in targets of cell mediated killing. *Int Immunol* 4, 821-824, (1992)

55. Hockenberry D., G. Nunez, R. D. Schreiber & S. J. Korsmeyer: *Bcl-2* is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 348, 334-336, (1990)

56. Lowe S. W., E. M. Schmitt, S. W. Smith, B. A. Osborne & T. Jacks: p53 is required for radiation-induced apoptosis in mouse thymocytes. *Nature (London)* 362, 847-849, (1993)

57. Lowe S. W., T. Jacks, D. E. Housman & H. E. Ruley: Abrogation of oncogene-associated apoptosis allows transformation of p53-deficient cells. *Proc Natl Acad Sci USA* 91, 2026-2030, (1994)

58. Graeber T. G., C. Osmanian, T. Jacks, D. E. Housman, C. J. Koch, S. W. Lowe & A. J. Giaccia: Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 379, 88-91, (1996)

59. Pegoraro L., A. Palumbo, J. Erikson, M. Falda, B. Giovanazzo, B. S. Emanuel, G. Rovera, P. C. Nowell & C. M. Croce: A 14;18 and an 8;14 chromosome translocation in a cell line derived from an acute B-cell leukemia. *Proc Natl Acad Sci USA* 81, 7166-7170, (1984)

60. Takayama S., T. Sato, S. Krajewski, K. Kochel, S. Irie, J. A. Millan & J. C. Reed: Cloning and functional analysis of BAG-1: a novel Bcl-2-binding protein with anti-cell death activity. *Cell* 80, 279-284, (1995)

61. Reynolds J. E., T. Yang, L. Qian, J. D. Jenkinson, P. Zhou, A. Eastman & R. W. Craig: Mcl-1, a member of the Bcl-2 family, delays apoptosis induced by c-Myc overexpression in Chinese hamster ovary cells. *Cancer Res* 54, 6348-6352, (1994)

62. Boise L. H., M. Gonzalez-Garcia, C. E. Postema, L. Ding, T. LIndsten, L. A. Turk, X. Mao, G. Nunez & C. B. Thompson: bcl-x, a bcl-2 related gene that functions as a dominant regulator of apoptotic cell death. *Cell* 74, 597, (1993)

63. Oltvai Z. N., C. L. Milliman & S. J. Korsmeyer: Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74, 609-619, (1993)

64. Farrow S. N., J. H. White, I. Martinou, T. Raven, K. T. Pun, C. J. Grinham, J. C. Martinou & R. Brown: Cloning of a bcl-2 homologue by interaction with adenovirus E1B 19K. *Nature* 374, 731-733, (1995)

65. Yang E., J. Zha, J. Jockel, L. H. Boise, C. B. Thompson & S. J. Korsmeyer: Bad, a heterodimeric partner for Bcl-XL and Bcl-2, displaces Bax and promotes cell death. *Cell* 80, 285-291, (1995)

66. Boyd J. M., G. J. Gallo, B. Elangovan, A. B. Houghton, S. Malstrom, B. J. Avery, R. G. Ebb, T. Subramanian, T. Chittenden & R. J. Lutz: Bik, a novel death-inducing protein shares a distinct sequence motif with Bcl-2 family proteins and interacts with viral and cellular survival-promoting proteins. *Oncogene* 11, 1921-1928, (1995)

67. Yang J., X. Liu, K. Bhalla, C. N. Kim, A. M. Ibrado, J. Cai, T. I. Peng, D. P. Jones & X. Wang: Prevention of apoptosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* 275, 1129-1132, (1997)

68. Kluck R. M., E. Bossy-Wetzel, D. R. Green & D. D. Newmeyer: The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 275, 1132-1136, (1997)

69. Liu X., C. N. Kim, J. Yang, R. Jemmerson & X. Wang: Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* 86, 147-157, (1996)

70. Spector M. S., S. Desnoyers, D. J. Hoeppner & M. O. Hengartner: Interaction between the C. elegans cell-death regulators CED-9 and CED-4. *Nature* 385, 653-656, (1997)

71. Chinnaiyan A. M., K. O'Rourke, B. R. Lane & V. M. Dixit: Interaction of CED-4 with CED-3 and CED-9: a molecular framework for cell death. *Science* 275, 1122-1126, (1997)

72. Hockenbery D. M., Z. N. Oltvai, X. M. Yin, C. L. Milliman & S. J. Korsmeyer: Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75, 241-251, (1993)

73. Steinman H. M.: The Bcl-2 oncoprotein functions as a pro-oxidant. *J Biol Chem* 270, 3487-3490, (1995)

74. Hedley D. W. & E. A. McCulloch: Generation of reactive oxygen intermediates after treatment of blasts of acute myeloblastic leukemia with cytosine arabinoside: role of bcl-2. *Leukemia* 10, 1143-1149, (1996)

75. Mirkovic N., D. W. Voehringer, M. D. Story, D. J. McConkey, T. J. McDonnell & R. E. Meyn: Resistance to radiation-induced apoptosis in Bcl-2-expressing cells is reversed by depleting cellular thiols. *Oncogene* 15, 1461-1470, (1997)

76. Ellerby L. M., H. M. Ellerby, S. M. Park, A. L. Holleran, A. N. Murphy, G. Fiskum, D. J. Kane, M. P. Testa, C. Kayalar & D. E. Bredesen: Shift of the cellular oxidation-reduction potential in neural cells expressing Bcl-2. *J Neurochem* 67, 1259-1267, (1996)

77. Zamzami N., T. Hirsch, B. Dallaporta, P. X. Petit & G. Kroemer: Mitochondrial implication in accidental and programmed cell death: apoptosis and necrosis. *J Bioenerg Biomembr* 29, 185-193, (1997)

78. Hirsch T., I. Marzo & G. Kroemer: Role of mitochondrial permeability transition pore in apoptosis. *Biosci Rep* 17, 67-76, (1997)

79. Story M. D., D. W. Voehringer, C. G. Malone, M. L. Hobbs & R. E. Meyn: Radiation-induced apoptosis in sensitive and resistant cells isolated from a mouse lymphoma. *Int J Radiat Biol* 66, 659-668, (1994)

80. Fukunaga-Johnson N., J. J. Ryan, M. Wicha, G. Nunez & M. F. Clarke: Bcl-2 protects murine erythroleukemia cells from p53-dependent and -independent radiation-induced cell death. *Carcinogenesis* 16, 1761-1767, (1995)

81. Kyprianou N., E. D. King, D. Bradbury & J. G. Rhee: bcl-2 over-expression delays radiation-induced apoptosis without affecting the clonogenic survival of human prostate cancer cells. *Int J Cancer* 70, 341-348, (1997)

82. Sakakura C., E. A. Sweeney, T. Shirahama, Y. Igarashi, S. Hakomori, H. Nakatani, H. Tsujimoto, T. Imanishi, M. Ohgaki, T. Ohyama, J. Yamazaki, A. Hagiwara, T. Yamaguchi, K. Sawai & T. Takahashi: Overexpression of bax sensitizes human breast cancer MCF-7 cells to radiation-induced apoptosis. *Int J Cancer* 67, 101-105, (1996)

83. Brady H. J., G. Salomons, R. C. Bobeldijk & A. J. Berns: T cells from bax-alpha transgenic mice show accelerated apoptosis in response to stimuli but do not show restored DNA damage-induced cell death in the absence of p53 gene product. *EMBO J* 15, 1221-1230, (1996)

84. Miyashita T., S. Krajewski, M. Krajewska, H. G. Wang, H. K. Lin, D. A. Liebermann, B. Hoffman & J. C. Reed: Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene* 9, 1799-1805, (1994)

85. Miyashita T. & J. C. Reed: Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 80, 293-299, (1995)

86. Kitada S., S. Krajewski, T. Miyashita, M. Krajewska & J. C. Reed: Gamma-radiation induces upregulation of Bax protein and apoptosis in radiosensitive cells in vivo. *Oncogene* 12, 187-192, (1996)

87. Zhan Q., I. Alamo, K. Yu, L. H. Boise, B. Cherney, G. Tosato, P. M. O'Connor & A. J. Fornace: The apoptosis-associated gamma-ray response of BCL-X(L) depends on normal p53 function. *Oncogene* 13, 2287-2293, (1996)

88. Borovitskaya A. E., V. I. Evtushenko & S. I. Sabol: Gamma-radiation-induced cell death in the fetal rat brain possesses molecular characteristics of apoptosis and is associated with specific messenger RNA elevations. *Brain Res Mol Brain Res* 35, 19-30, (1996)

89. Chen M., J. Quintans, Z. Fuks, C. Thompson, D. W. Kufe & R. R. Weichselbaum: Suppression of Bcl-2 messenger RNA production may mediate apoptosis after ionizing radiation, tumor necrosis factor alpha, and ceramide. *Cancer Res* 55, 991-994, (1995)

90. Chang B. S., A. J. Minn, S. W. Muchmore, S. W. Fesik & C. B. Thompson: Identification of a novel regulatory domain in Bcl-X(L) and Bcl-2. *EMBO J* 16, 968-977, (1997)

91. Zha J., H. Harada, E. Yang, J. Jockel & S. J. Korsmeyer: Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). *Cell* 87, 619-628, (1996)

92. Wang H. G.: Bcl-2 targets the protein kinase Raf-1 to mitochondria. *Cell* 87, 589-592, (1996)

93. Kasid U., S. Suy, P. Dent, S. Ray, T. I. Whiteside & T. W. Sturgill: Activation of Raf by ionizing radiation. *Nature* 382, 813-816, (1996)

94. Lennon S. V., S. J. Martin & T. G. Cotter: Dose-dependent induction of apoptosis in human tumor cell lines by widely diverging stimuli. *Cell Prolif* 24, 203-214, (1991)

95. Meyn R. E., L. C. Stephens, K. K. Ang, N. R. Hunter, W. A. Brock, L. Milas & L. J. Peters: Heterogeneity in the development of apoptosis in irradiated murine tumours of different histologies. *Int J Radiat Biol* 64, 583-591, (1993)

96. Elledge R. S., S. Green, L. Howes, G. M. Clark, M. Berardo, D. C. Allred, R. Pugh, D. Ciocca, P. Ravdin, J. O'Sullivan, S. Rivkin, S. Martino & C. K. Osborne: Bcl-2, p53 and response to tamoxifen in estrogen receptor-positive metastatic breast cancer: A Southwest Oncology Group Study. *J Clin Oncol* 15, 1916-1922, (1997)

97. Keen J. C., J. M. Dixon, E. P. Miller, D. A. Cameron, U. Chetty, A. Hanby, C. Bellamy & W. R. Miller: The expression of Ki-S1 and BCL-2 and the response to primary tamoxifen therapy in elderly patients with breast cancer. *Breast Cancer Res Treat* 44, 123-133, (1997)

98. Silvestrini R., S. Veneroni, E. Benini, G. M. Daidone, A. Luisi, M. Leutner, A. Maucione, R. Kenda, R. Zucali & U. Veronesi: Expression of p53, glutathione S-transferase-pi, and Bcl-2 proteins and benefit from adjuvant radiotherapy in breast cancer. *J Natl Cancer Inst* 89, 639-645, (1997)

99. Grignon D. J., R. Caplan, F. H. Sarkar, C. A. Lawton, E. H. Hammond, M. V. Pilepich, J. D. Forman, J. Mesic, K. K. Fu, R. A. Abrams, T. F. Pajak, W. U. Shipley & J. D. Cox: p53 status and prognosis of locally advanced prostatic adenocarcinoma: a study based on RTOG 8610. *J Natl Cancer Inst* 89, 158-165, (1997)

100. Cardillo J., G. Berchem, M. A. Tarkington, S. Krajewski, M. Krajeewski, J. C. Reed, T. Tehan, L. Ortega, J. Lage & E. P. Gelmann: Resistance to apoptosis and up regulation of Bcl-2 in benign prostatic hyperplasia after androgen deprivation. *Urology* 158, 212-216, (1997)

101. Buttitta F., A. Marchetti, A. Gadducci, S. Pellegrini, M. Morganti, V. Carnicelli, S. Cosio, O. Gagetti, A. R. Genazzani & G. Bevilacqua: p53 alterations are predictive of chemoresistance and aggressiveness in ovarian carcinomas: a molecular and immunohistochemical study. *Br J Cancer* 75, 230-235, (1997)

102. Puglisi F., C. Di Loreto, R. Panizzo, C. Avellini, S. Fongione, V. Cacitti & C. A. Beltrami: Expression of p53 and bcl-2 and response to preoperative chemotherapy and radiotherapy for locally advanced squamous cell carcinoma of the esophagus. *J Clin Pathol* 49, 456-459, (1996)

103. Wheeler J. A., L. C. Stephens, P. Eifel, K. K. Ang, L. Milas, P. K. Allen & R. E. Meyn: ASTRO Research Fellowship: Apoptosis as a predictor of tumor response to radiation in stage IB cervical carcinoma. *Int J Radiat Oncol Biol Phys* 32, 1487-1493, (1995)

104. Levine E. L., S. E. Davidson, S. A. Roberts, C. A. Chadwick, C. S. Potten & C. M. L. West: Apoptosis as predictor of response to radiotherapy in cervical carcinoma. *Lancet* 344, 472, (1994)

105. Komaki R., T. Fujii, P. Perkins, J. Y. Ro, P. K. Allen, K. A. Mason, C. F. Mountain & L. Milas: Apoptosis and mitosis as prognostic factors in pathologically staged N1 nonsmall cell lung cancer. *Int J Radiat Oncol Biol Phys* 36, 601-605, (1996)

106. Cabanillas F., M. S. Lee, P. McLaughlin, A. Majlis, F. B. Hagemeister, F. Swan, M. A. Rodriguez, J. Romaguera, A. Sarris & A. Younes: Polymerase chain reaction (PCR): an effective way of monitoring response in follicular lymphomas (FL). *Proc Am Soc Clin Oncol* 12, 365, (1993)

107. Ha C. S., F. C. Cabanillas, M.-S. Lee, P. C. Besa, P. McLaughlin & J. D. Cox: Serial determination of the bcl-2 gene in the bone marrow and peripheral blood after central lymphatic irradiation for stages I-III follicular lymphoma: a preliminary report. *Clin Cancer Res* 3, 215-219, (1997)

108. Webb A., D. Cunningham, F. Cotter, P. A. Clarke, F. di Stefano, P. Ross, M. Corbo & Z. Dziewanowska: BCL-2 antisense therapy in patients with non-Hodgkin lymphoma. *Lancet* 349, 1141, (1997)

Key Words: Apoptosis, Cancer therapy, p53, bcl-2, Ceramide

Send correspondence to: Michael Story, Ph. D., Department of Experimental Radiation Oncology-66, University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, Tel: (713)-792-3424, Fax:: (713)-794-5369, E-mail: mstory@odin.mdacc.tmc.edu