Role of the *Bcl-2* Gene Family in Prostate Cancer Progression and Its Implications for Therapeutic Intervention

Khurram S. Chaudhary,[1] Paul D. Abel,[2] and El-Nasir Lalani[1]

[1]Department of Histopathology; [2]Department of Surgery, Imperial College School of Medicine, Hammersmith Campus, London, United Kingdom

Prostate cancer (PC) is an escalating health burden in the western world. A large number of patients still present with extraprostatic (i.e., T_3/T_4, N_0, M_0/M_1 or any T category and M_1 disease or involved lymph nodes) and therefore incurable disease. Since the work of Huggins in 1940, there have been no major therapeutic advances and androgen ablation remains the best treatment option for extraprostatic androgen-responsive PC. Eighty to ninety percent of PC patients respond well to this form of treatment initially. After a median time of approximately 2 years, however, relapse to an androgen-independent (AI) state occurs, followed by death after a further median 6 months. Androgen ablation is rarely curative. The major molecular defect in extraprostatic and AI PC is the inability of PC cells to initiate apoptosis in response to a variety of stimuli, including different forms of androgen ablation and cytotoxic agents. The balance between cellular proliferation and cell death is regulated by multiple genes or families of genes through the cell cycle. The exact mechanisms governing this intricate and complex process are as yet not fully understood. One family of genes involved in cell survival/death control is the *Bcl-2* gene family, which consists of homologous proteins that function to regulate distal and crucial commitment steps of the apoptotic pathway. The *Bcl-2* family constitutes both agonists and antagonists of apoptosis that function at least in part through protein–protein interactions between various members of the family. The final outcome depends on the relative ratio of death agonists and antagonists. Bcl-2 expression has been closely associated with the AI phenotype of PC. Cytotoxic chemotherapy may be used as palliative therapy in AI PC but has not been found effective. Most chemotherapeutic cytotoxic agents induce apoptosis in cancer cells by direct and indirect action on the cell cycle. *In vitro* and *in vivo* studies have established that Bcl-2 expression confers an antiapoptotic activity against androgen withdrawal and cytotoxic chemotherapy. It thus offers a tempting potential target for therapeutic manipulations of PC. — *Environ Health Perspect* 107(Suppl 1):49–57 (1999). *http://ehpnet1.niehs.nih.gov/docs/1999/Suppl-1/49-57chaudhary/abstract.html*

Key words: *Bcl-2* family, prostate cancer, apoptosis, therapeutics

Prostate Cancer

Prostate cancer (PC) is the most common cancer and the second leading cause of cancer-related death (after lung cancer) in men. In 1997, the estimated death toll due to PC in the United States alone was approximately 41,000 and the number of newly diagnosed cases exceeded 300,000 (*1*). Currently, one of every six men in the United States can expect to develop PC in his lifetime. PC shortens life dramatically, such that an individual suffering from metastatic PC may lose approximately 9 to 10 years of life expectancy (*2*). More than 90% of PCs are diagnosed between 45 and 89 years of age (average, 72 years of age). Because the risk of PC bears a strong relationship to age, the incidence [1 in 10,000 at ≤39 years of age to 1 in 6 for men > 80 years of age (*1*)] can be expected to rise because men are living longer than ever before (*3*). Other risk factors include family history (*4*), higher intake of dietary fat (*5,6*), high blood levels of male sex hormones, and Americans of African origin. Vitamins D and E and selenium are suggested to have a protective effect (*7,8*), whereas the role of vitamin A is controversial.

Manuscript received at *EHP* 9 July 1998; accepted 18 September 1998.

Address correspondence to E-N. Lalani, Department of Histopathology, Division of Investigative Sciences, Imperial College School of Medicine, Hammersmith Campus, London W12 0NN, United Kingdom. Telephone: 44 181 383 2430. Fax: 44 181 740 7417. E-mail: elalani@rpms.ac.uk

Abbreviations used: AI, androgen independent; AR, androgen receptor; ER, endoplasmic reticulum; HGPIN, high-grade prostatic intraepithelial neoplasia; PC, prostate cancer; PSA, prostate-specific antigen; PT, permeability transition.

Pathology of Prostate Cancer

Histopathologically, high-grade prostatic intraepithelial neoplasia (HGPIN) is considered the most likely precursor of invasive PC because of its peripheral zone location and immunohistochemical expression of biomarkers similar to that of PC (*9*). Prostatic intraepithelial neoplasia is present in 85% of PC samples (*10*). Histologically, almost all PCs are adenocarcinomas originating from the prostatic glandular epithelium. The most widely used system of grading PC is the Gleason system (*11,12*), which takes into account the degree of glandular differentiation of epithelial cells in relation to stroma at relatively low magnification. Grades range from 1 to 5 depending on tumor architecture, which varies from well differentiated (showing a preserved glandular pattern) to poorly differentiated (showing no definite glandular architecture). A combined Gleason's score or Gleason's sum (2–10) is determined by adding the most frequent primary and secondary pattern of cellular architecture in a heterogeneous tumor.

PC spreads to local as well as distant sites. Local spread can involve adjacent viscera including seminal vesicles, ejaculatory ducts, and rectum. The most frequent sites of metastatic spread are lymph nodes, bones, and lungs. Less common sites include bladder, liver, and adrenal glands (*13*).

Because of the gravity of extraprostatic disease, a screening program designed to detect PC at a curable stage appears logical. However, there are limitations with present screening methods; even after histologic diagnosis has been made, the biologic behavior of individual PC cannot be predicted. Identification of those cases of localized PC that require definitive treatment from those in which no treatment is the appropriate option remains an issue. At present no definite biologic or molecular marker exists that can predict the future clinical behavior of PC while it is still localized to the prostate [reviewed by Isaacs (*14*) and Lalani et al. (*15*)]. New genotypic and phenotypic markers are therefore urgently needed to classify the disease accurately and to guide its management (*16*). The underlying molecular and cellular mechanisms governing initiation and promotion of the malignant and

metastatic phenotypes are unknown in PC, although some clues may be gained from the study of other cancers. Of particular importance is the need to investigate factors governing hormone regulation that may be specific to PC [reviewed by Lalani et al. (*17*)].

Clinical Forms of Prostate Cancer

Prostate cancer can present in a number of clinical forms. These include latent, localized, locally advanced, and metastatic PC (Table 1).

Localized Clinically Significant and Latent Cancer

Part of the problem in managing PC lies in the fact that there is a latent form of the disease that is equally prevalent in communities throughout the world. Latent PC is generally an incidental finding, usually identified in postmortem studies or in prostates removed surgically for apparently benign disease or following prostate-specific antigen (PSA) testing and imaging-directed stereotactic biopsy. Latent PC is by definition small, generally well differentiated, and the clinical behavior is usually indolent. This cancer may be found in men as young as in their 30s and has been reported in approximately 30% of 50-year-olds and up to 80% of 80-year-olds at postmortem (*18*). This is in sharp contrast to clinically important cancer, which is rare in Asia (1:100,000) but reaches epidemic proportions in the West; the highest rates reported are in Americans of African origin (*19*).

For the clinician the challenge is to distinguish between patients with localized disease ($T_{1,2}N_0M_0$) that is *a*) clinically significant and that will metastasize if untreated, usually justifying radical therapy; *b*) clinically significant but is an apparently localized disease with unidentifiable occult metastases using current imaging techniques in whom there is no curative option (but these patients may be subjected to radical therapy in the hope of cure); and *c*) latent cancer that will not affect them during their lifetime. There are currently no clinical or molecular tests to distinguish between these three groups of patients.

Management of Prostate Cancer

Whitmore (*20*) asked, "Is cure possible in those for whom it is necessary, and is cure necessary in those for whom it is possible?" There is no doubt that a large number of patients require effective treatment for their disease, whether localized or extraprostatic. Treatment depends primarily on stage but also on the patient's age, general fitness, and comorbidity. Localized PC can be managed by watchful waiting, radiotherapy, or radical prostatectomy. During watchful waiting patients may be assessed regularly by monitoring serum PSA levels or by digital rectal examination to determine disease progression. By following patients conservatively, the morbidity of active treatment can be avoided. The mean age of PC at presentation is 72 years, at which age death due to intercurrent diseases is greater than that from PC itself. However, patients in younger age groups and especially those with high-grade disease may be considered for active treatment. With improvement in surgical techniques and increased knowledge of surgical anatomy of the prostate, radical prostatectomy has become a safe treatment for localized PC with apparent positive long-term results (*21*). However, it remains to be proven more effective than either radiotherapy or watchful waiting. Radiotherapy is especially used in older patients and in those presenting with intercurrent illnesses and who are therefore unsuitable for radical surgery. Patients best suited for radiotherapy have a low serum PSA level (< 20 ng/ml) and low-grade low-stage tumors (*22*). Endocrine (hormone) therapy is the mainstay of treatment in patients with locally advanced and/or disseminated metastatic PC. Hormone therapy may take the form of surgical castration, leutinizing-hormone releasing hormone analogues (medical castration), antiandrogens, or a combination of castration and antiandrogens that block both testicular and adrenal androgens—maximum androgen blockade. Cytotoxic chemotherapy in androgen-independent (AI) PCs has limited success.

Table 1. Clinical manifestations of prostate cancer.

Localized prostate cancer	Extra prostatic prostate cancer
Latent (clinically unimportant)	Locally advanced without metastases
Significant without local invasion or metastases (curable)	Locally advanced with metastases
Significant with undetected occult metastases (incurable)	Metastases but primary localized within prostate

Hormone and Chemoresistance in Prostate Cancer

Determinants of Treatment Failure in Extraprostatic Prostate Cancer

Because of the lack of definite curative therapy for extraprostatic PC, patients require palliative treatment such as hormone downregulation and cytotoxic chemotherapy to alleviate their disease-related symptoms and improve their general well being. These treatments aim to kill tumor cells *in situ* by inducing apoptosis, or if this cannot be achieved, by at least keeping them confined to their current boundaries by cytostasis. The exact underlying mechanisms responsible for the development of AI and chemoresistance in PC is unknown.

The normal prostatic epithelium requires androgens for mitogenesis, as is also true for prostatic adenocarcinomas. This is why most PCs respond initially to androgen ablation therapy, which causes tumor regression by inhibiting cellular proliferation and inducing apoptosis (*23*). The response is usually short-lived and results in an AI tumor. AI tumors are usually more aggressive and show little or no response to androgen-ablative measures. As the effects of androgen/antiandrogens are mediated through the androgen receptor (AR), much research effort is focused on the role of AR in tumor recurrence and progression (*24*), which may be a consequence of multiple genetic and epigenetic events, including mutations in and altered expression of the AR (*15*). Mechanisms by which PC cells circumvent endocrine therapy have thus far focused on loss of AR, amplification of wild-type AR and clonal expression of these cells, and mutations of AR resulting in either increased transactivation and/or loss of specificity for steroid hormones and their antagonists (*25–31*). Alternatively, the defects could occur in the AR down stream-signaling pathways, leading to AI in the presence of a normal AR. Whatever the cause of AI tumors, the final clinical outcome will be the uninhibited proliferation of PC cells even after androgen ablation. AI PCs also bear a close association with the expression of cell death inhibitors of the *Bcl-2* family (*32–34*).

Several cytotoxic agents, alone or in combination, have been used in the treatment of AI extraprostatic PC but with little benefit (*35*). Possible explanations for

the failure of cytotoxic chemotherapy include the slow proliferation rate of PC and the development of endogenous resistance to apoptosis by cancer cells due to dysregulated expression of apototic inhibitors (regulators) such as members of the *Bcl-2* family.

Molecular Regulation of Apoptosis

An Evolutionary Conserved Ordering from *Caenorhabditis elegans* to Mammals

Several diseases are associated with either impaired or excessive apoptosis including cancer, autoimmunity, HIV-associated immunodeficiency, and some neurodegenerative disorders (*36–38*). Apoptosis is an evolutionary conserved and genetically controlled mechanism through which cells are eliminated in both health and disease. Apoptosis constitutes distinct morphological and biochemical changes in the cell. Morphologically, it involves rapid condensation of chromatin, shrinkage of cells, and ultimately formation of membrane-enclosed apoptotic bodies that are engulfed by neighboring scavengers (*39–42*). Biochemically, there is relocation of phosphatidyl serine from the inner to the outer aspect of the plasma membrane and degradation of double-stranded nuclear DNA, leading to production of oligonucleosomal DNA fragments (Figure 1). The evolutionary conserved nature of genetic regulation of this process has become apparent from studies of *C. elegans*, a primitive nematode worm, through *Drosophila melanogaster* to mammals. Two important gene families, the *Bcl-2* gene family and the caspase family of cysteine proteases (previously known as the interleukin-1-β-converting enzyme family) play key roles in the regulation and execution of this program. The *Bcl-2* family, which consists of both inhibitors and promoters of apoptosis, constitutes mammalian homologs of *ced9*, which is an inhibitor of apoptosis in *C. elegans*. *ced9* functions upstream of two cell-death promoters, *ced4* and *ced3*. During the development of *C. elegans*, 131 of 1090 somatic cells normally die. However, these cells fail to die in response to gain of function mutations in *ced9* (*43*). Mammalian counterparts of *ced3* are caspase proteases, which can cleave a number of specific death substrates and execute the final stages of apoptotic pathways (Figure 2). Recently Zou et al. (*44*) identified another death-promoting protein, apoptotic protease-activating factor (Apaf1), which is considered a human homolog of *ced4*.

Bcl-2 Protein Family and Regulation of Apoptosis

In vitro studies have highlighted the role of Bcl-2 proteins as important regulators of the apoptotic pathway in several cell types (*38,45–49*). Bcl-2, the prototype of this family, was discovered by studies of t(14:18) chromosomal translocations, which are frequent in non-Hodgkin lymphomas and follicular lymphomas (*50–52*). The name Bcl-2 (B cell lymphoma/leukemia gene 2) signifies the close association of this gene to these malignancies in which enhanced expression was initially believed to arise solely as a result of these translocations, resulting in the juxtaposition of the *Bcl-2* gene to a potent enhancer element sequence of the *IgH* gene. Several genes have been identified and designated as the *Bcl-2* family based on their sequence homology to *Bcl-2*. These genes include both positive and negative regulators of apoptosis (Table 2). In contrast there is only a single homolog, *ced9*, in *C. elegans*.

Sequence analyses of Bcl-2 family proteins have identified up to four evolutionary conserved domains, recently named BH1 to BH4 (BH stands for Bcl-2 homology domain), as proposed by Oltavi et al.

Table 2. Bcl-2 protein family.

Cell death regulators	Reference
Death suppressors	
Bcl-2	See text
Bcl-X_L	Boise et al. (*135*)
Mcl-1	Zhou et al. (*136*)
Bcl-w	Gibson et al. (*137*)
A1	Lin et al. (*138*)
Death promoters	
Bax	Oltvai et al. (*53*)
Bak	Chittenden et al. (*139*)
Bad	Yang et al. (*140*)
Bcl-X_S	Boise et al. (*135*)
Bik	Boyd et al. (*141*)
Bid	Wang et al. (*142*)
Harakiri	Inohara et al. (*143*)

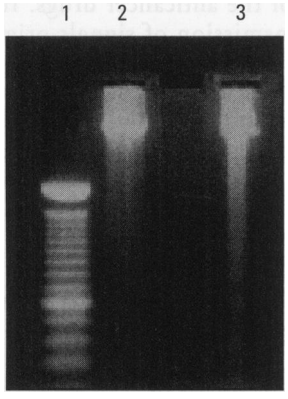

Figure 1. DNA electrophoresis on ethidium bromide-stained 1.8% agarose gel showing oligonucleosomal DNA ladder in thapsigargin-treated DU145 prostatic epithelial cells (lane 3). Gene transfer-mediated constitutive Bcl-2 expression in the same cells resulted in inhibition of apoptosis and DNA degradation (laddering) (lane 2). Lane 1 shows 100 bp DNA ladder (DNA marker).

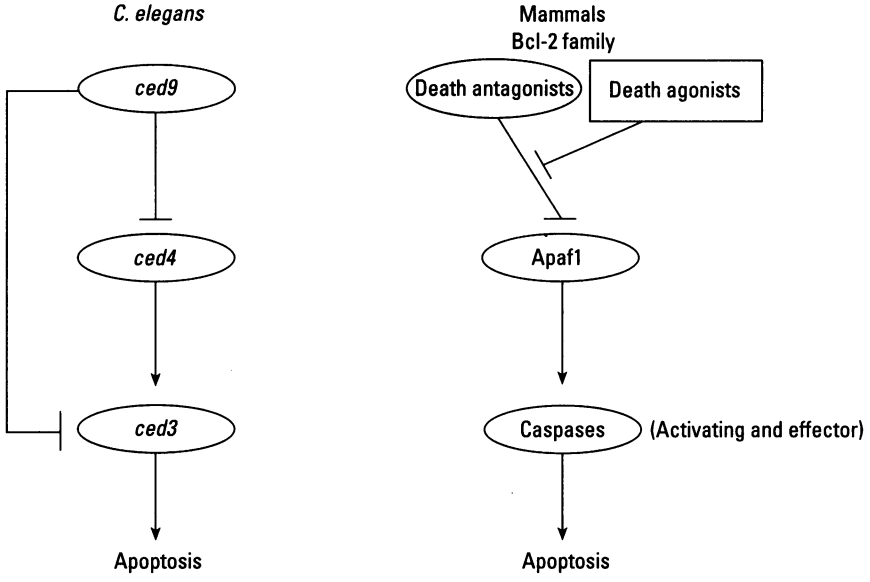

Figure 2. Molecular ordering of cell death regulators and effectors in *C. elegans* and mammals (see text).

(*53*). These domains may have functional significance, allowing Bcl-2 members to act as either death promoters or death inhibitors. Most Bcl-2 family members have a stretch of hydrophobic amino acids at their carboxy terminal, which is responsible for localization (anchorage) to membranes of cellular organelles.

Bcl-2 proteins interact with each other through homo- and heterodimerization to regulate apoptosis. For example, Bcl-2 can form homodimers by interaction with another molecule of Bcl-2 or a heterodimer with a molecule of Bax (*53*). These dimerizations are of functional significance. For example, a Bcl-2:Bax heterodimer will favor cell survival, whereas a Bax:Bax homodimer will favor cell death. Mutations in BH domains can effect dimerization potential and abrogate normal function (*54–57*). There is great complexity and cell specificity of homo- and heterodimerizations [reviewed by Reed (*58*)].

Biochemical Functions of the Bcl-2 Family Proteins

The Bcl-2 family proteins have been studied in relation to their localization to intracellular organelles, primarily the outer mitochondrial membrane (Figure 3A), nuclear envelope, and endoplasmic reticulum (*59,60*). A number of theories based on experimental observations have tried to explain how Bcl-2 functions as a death regulator. Initial observations suggested its role as an antioxidant (*61–64*), a regulator of intracellular calcium (*65–69*), and in transport of proteins across cellular membranes e.g., across the nuclear membrane (*68,70, 71*). Recently it has been proposed that Bcl-2 regulates activation of caspase proteases (*72–79*), which are responsible for the final executionary steps of apoptosis (Figure 3A, B). There is as yet no evidence that these functions are a result of the direct action of Bcl-2. These may, however, be consequences of Bcl-2 action upstream of these events. In the last 2 years, Bcl-2 function has been extensively studied in relation to its regulation of permeability transition (PT) in mitochondria. The induction of PT can result in loss of membrane potential (*80,81*), inhibition of oxidative phosphorylation, generation of reactive oxygen species, and release of proteins such as cytochrome *c*. Cytochrome *c* normally resides in the intermembrane space of the mitochondria and on its translocation to cytosol can induce activation of caspases (*81,82*) (Figure 3A, B). Bcl-2 expression can inhibit the release of cytochrome *c* from the mitochondrial space and stabilizes the mitochondrial potential ($\Delta\Psi$) in response to apoptosis-inducing agents, for example, stauroporine (*83*). Furthermore, a proposed pore-forming capability of Bcl-2, Bcl-X_L, and Bax in lipid membranes (*84–87*), similar to those of the bacterial toxins (e.g., diphtheria) and colicins suggests a novel function for these proteins as channels for ions, proteins, or both.

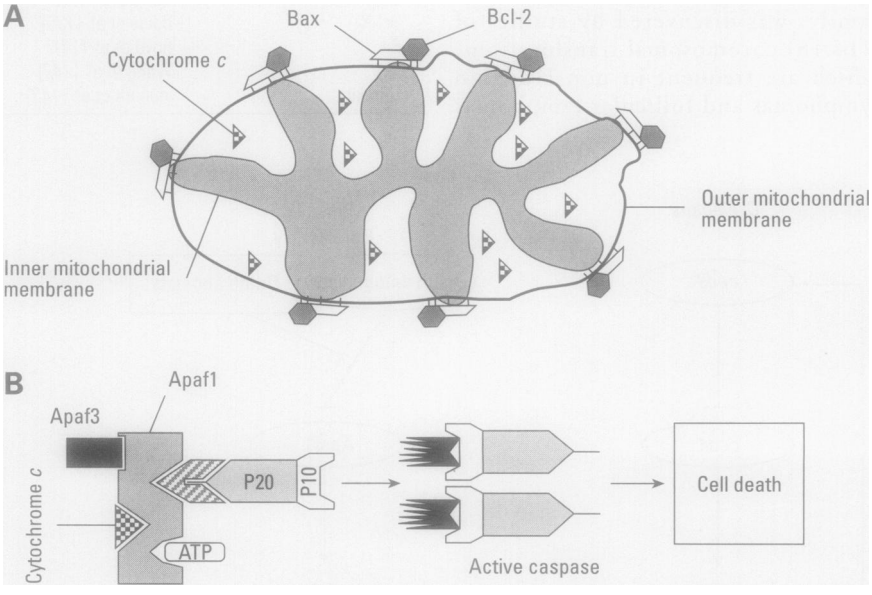

Figure 3. (*A*) Bcl-2 and Bax localize to the outer mitochondrial membrane, predominantly at sites where inner and outer mitochondrial membranes are in contact with each other. Bcl-2 inhibits the release of cytochrome *c* from the mitochondrial space (between the inner and outer membranes) and stabilizes mitochondrial potential in response to apoptotic stimuli. (*B*) Diagrammatic representation of caspase activation. The active form of a caspase is generated in the presence of cytochrome *c*, Apaf1, Apaf3 and dATP. The protein Apaf3 has not yet been characterized. P20 and P10 indicated here represent large and small subunits of a caspase. Bcl-2 blocks the release of cytochrome *c* from mitochondria and prevents the activation of caspases.

Bcl-2 Family Proteins—Determinants of Chemosensitivity

The survival advantage provided by Bcl-2 contributes not only to the development of cancer but also to resistance against a wide variety of anticancer agents including cyclophosphamide, cisplatin, etoposide (VP16), mitoxantrone, adriamycin, cytosine arabinoside, methotrexate, 5-fluorouracil, thapsigargin, stauroporine, dexamethasone, and radiation (*68,88–95*). Bcl-X_L, a death antagonist, also suppresses the apoptotic effect of a number of cytotoxic agents (*77,94*). Inhibition of apoptosis induced by a variety of anticancer agents by Bcl-2 and Bcl-X_L suggests a common potential pathway (*38,77,94,96*).

Downregulation of Bcl-2 protein can lead to a reversal of chemoresistance, rendering cancer cells sensitive to the cytotoxic effects of conventional cytotoxic chemotherapy (*97–100*). In contrast, upregulation of proapoptotic Bcl-2 members, such as Bax, Bcl-X_S, and Bak, can induce chemosensitization (*101–103*).

The function of Bcl-2 is unique because it does not affect the pharmacokinetics of the anticancer drugs. It blocks the transmission of signals originating from the damage to the effectors of apoptosis, allowing cells to survive in the presence of otherwise lethal damage, enhancing the chances of acquiring genetic alterations and favoring the development of a more malignant phenotype.

Role of Apoptosis in the Development and Progression of Prostate Cancer

Normal prostatic glandular cells have a low proliferation rate (< 0.3%/day) that is efficiently balanced by the same rate of apoptosis so that there is no net growth (*104*). During transformation to HGPIN, a net growth of malignant cells occurs because of the disturbance in equilibrium between cell proliferation and apoptosis. The growth kinetics of localized PC

indicate that there is a decrease in cell death (and no further increase in proliferation rate as compared to HGPIN) that is responsible for its continuous growth (*104*). A similar situation occurs in metastatic PCs in which the proliferation rate is also low (< 3%) (*104*). These cell kinetic data provide an explanation as to why conventional cytotoxic chemotherapy (which usually targets actively proliferating cancer cells) fails to kill PC cells and suggests that proliferation-independent therapeutic strategies may be more beneficial for the treatment of PC.

Androgen ablative therapies are the mainstay of treatment in extraprostatic PC. Androgen ablation has a dual effect i.e., inhibiting epithelial cellular proliferation and promoting apoptosis (*105*). Kyprianou et al. (*106*) demonstrated that castration of mice bearing PC82 prostatic epithelial cell xenografts resulted in tumor regression (*106*). Histologic analyses revealed a decrease in the number of cells undergoing mitosis and a dramatic increase in the percentage of cells undergoing apoptosis. *In vivo,* PC cells possessing the androgen-sensitive phenotype also show inhibited proliferation and apoptosis in response to androgen withdrawal (*23*). However, as AI develops, this response to androgen withdrawal is abolished. Several studies have demonstrated that androgen ablation does not induce apoptosis in AI PC cells. A close association of the Bcl-2 family antiapoptotic protein expression and the development of AI in PC has been found (*32,34*). This means that in AI PC, resistance to apoptotic-inducing cytotoxic chemotherapeutics is contributed both by slow proliferation and acquisition of the antiapoptotic potential owing to the dysregulated expression of antiapoptotic Bcl-2 homologs. Therefore, potential measures to overcome cytotoxic chemoresistance in AI PC can be achieved either by introducing proliferation-independent treatment strategies and/or restoring sensitivity to induce apoptosis in cancer cells. Thapsigargin, for example, could induce apoptotic cell death independent of cell proliferation (*107,108*). Thapsigargin is a specific inhibitor of endoplasmic reticulum (ER) Ca-ATPase and results in the depletion of the ER pool and the subsequent signaling of a sustained influx of extracellular calcium (*109*). Agents that could target Bcl-2 or related antiapoptotic proteins and therefore sensitize cancer cells to undergo apoptosis could also be useful as new therapeutic modalities in the management of PC.

Bcl-2 and Prostate Cancer Progression

De novo expression of *Bcl-2* has been reported in a number of epithelial malignancies including those arising from the breast, urinary bladder, thyroid, stomach, lung, intestine, and prostate (*32,34, 110–127*). In normal prostate and benign prostatic hypertrophy, Bcl-2 expression is limited to the androgen-insensitive basal cells of prostatic glandular epithelium. Hormone-responsive luminal cells do not express Bcl-2 (*32,34*). Krajewska et al. (*115*) reported Bcl-2, Bcl-X_L, and Mcl-1 expression in primary and metastatic PCs. Higher grade PCs and metastases expressed these antiapoptotic proteins more often and with greater intensity than lower grade tumors. A strong association of Bcl-2 expression and the development of AI in PC was reported in 1992 by McDonnel et al. (*34*). Colombel et al. (*32*) reported strong Bcl-2 expression in AI tumors obtained from metastatic PC patients after hormone treatment. Higher expression of *Bcl-2* has also been reported in patients kept on androgen-ablative treatment compared to a control group (*118*). These studies suggest a role for these antiapoptotic proteins in the progression of PC toward AI.

In vitro studies on PC cell lines suggest a role for Bcl-2 in the inhibition of apoptosis and progression to AI (*33*). Raffo et al. (*33*) have shown that *Bcl-2* transfection into LNCaP cells (a human androgen-sensitive PC cell line) resulted in the inhibition of apoptosis in response to serum (growth factors) withdrawal in contrast to apoptotic cell death in controls. Moreover, *Bcl-2* expression provided a growth advantage in the absence of androgens i.e., LNCaP/*Bcl-2* transfectant xenografts in nude mice continued their growth after castration, whereas tumors formed by the control transfectant regressed after castration and showed evidence of apoptotic cell death (*33*). McConkey et al. (*95*) reported close association of de novo *Bcl-2* expression with acquisition of the metastatic phenotype by LNCaP sublines derived by orthotopic implantation into rat prostates. These Bcl-2-expressing sublines were significantly resistant to adriamycin and thapsigargin-induced apoptosis (*95*). We have observed a similar chemoresistance against adriamycin and thapsigargin in the *Bcl-2* transfectants of the DU145 prostatic epithelial cell line expressing high levels of *Bcl-2* protein (*128*). Enforced Bcl-2 expression in these cells altered the expression of other cell-cycle and cell-death regulators including *p53* and proliferating cell nuclear antigen.

Studies utilizing antisense oligonucleotides directed against *Bcl-2* mRNA demonstrated the development of chemosensitivity to chemical agents in otherwise chemoresistant cells (*97–99*). A similar approach using ribozymes (enzyme coupled antisense nucleotides) against *Bcl-2* and *Bcl-X_L* has also been reported to favor apoptosis (*129,130*). Taken together, these observations suggest that ectopic *Bcl-2*/*Bcl-X_L* expression confers a death-resistant phenotype and imparts an ability to inhibit the initiation of the apoptotic cascade in response to several stimuli including chemical agents and radiation (*131*).

Potential for Therapeutic Intervention

Several studies, some of which have been cited in this review, demonstrated that inhibition of some of the death antagonists of the *Bcl-2* family can significantly potentiate apoptosis. This offers hope in developing potential therapeutic modalities to combat cancers in general and PC in particular. There are several other potential mechanistic targets involving *Bcl-2* that may offer additional hope, including antisense strategies utilizing oligonucleotides or ribozymes against *Bcl-2*/*Bcl-X_L* mRNA. Other potential mechanistic targets include therapies to alter Bcl-2/Bcl-X_L protein configuration inhibiting homo- and heterodimerization, for example, phosphorylating Bcl-2, rendering it nonfunctional (*132,133*), and upregulating the expression of death promoters e.g., Bax, Bak, etc.

Antisense strategies using anti-*Bcl-2* oligonucleotides or ribozymes may abolish *Bcl-2* expression by targeting *Bcl-2* mRNA and are effective at least *in vitro* (*129,130*). Another mechanism to prevent Bcl-2-mediated inhibition of cell death is to convert Bcl-2/Bcl-X_L protein configuration to nonfunctional forms. Phosphorylation of Bcl-2 renders it nonfunctional as a death inhibitor (*132,133*) and abolishes heterodimerization of Bcl-2 with death promoter partners, allowing death promoters to function uninhibited (*134*). Cytotoxic agents, for example, taxol, vinblastine, and vincristine result in phosphorylation and inactivation of Bcl-2 and can lead to apoptotic cell death in Bcl-2-expressing cells, including those of the prostate (*132,133*). Furthermore, selective upregulation of death promoters (e.g., Bax, Bak) through

gene therapy strategies may sensitize the cancer cells to conventional cytotoxics. Theoretically it is possible to design an approach utilizing a combination of gene therapy and chemotherapy that would effectively upregulate a death promoter via gene therapy and inhibit death suppression, such as offered by Bcl-2, by chemotherapy-induced phosphorylation. There are many challenges that need to be addressed before such therapies are realized. For example, thapsigargin can cause significant toxicity, thus limiting its use. Denmeade and Isaacs. (*107*) have attempted to address this by using an inactive prodrug form of thapsigargin that is enzymatically activated by tissue (prostate)-specific enzymes.

Summary

Current research into the molecular mechanism governing the cellular rheostat (cell growth/cell death) is unraveling a fascinating and complex array of molecules that both govern and regulate the balanced state. Future therapies will target specific molecules or signaling pathways by utilizing combination therapies. The exciting progress made in basic PC research offers opportunities in PC management.

REFERENCES AND NOTES

1. Wingo PA, Landis S, Ries LA. An adjustment to the 1997 estimate for new prostate cancer cases. Cancer 80:1810–1813 (1997).
2. Horm J, Sondik E. Person-years of life lost due to cancer in the United States 1970-1984. Am J Public Health 79:1490–1493 (1989).
3. Devesa SS, Blot WJ, Stone BJ, Miller BA, Tarone RE, Fraumeni JF Jr. Recent cancer trends in the United States. J Natl Cancer Inst 87:175–182 (1995).
4. Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC. Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci USA 89:3367–3371 (1992).
5. Giovannucci E, Rimm EB, Colditz GA, Stampfer MJ, Ascherio A, Chute CC, Willett WC. A prospective study of dietary fat and risk of prostate cancer. J Natl Cancer Inst 85:1571–1579 (1993).
6. Whittemore AS, Kolonel LN, Wu AH, John EM, Gallagher RP, Howe GR, Burch JD, Hankin J, Dreon DM, West DW, et al. Prostate cancer in relation to diet, physical activity, and body size in blacks, whites, and Asians in the United States and Canada. J Natl Cancer Inst 87:652–661 (1995).
7. Fair WR, Fleshner NE, Heston W. Cancer of the prostate: a nutritional disease? Urology 50:840–848 (1997).
8. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. N Engl J Med 330:1029–1035 (1994).
9. Epstein JI. Prostatic intraepithelial neoplasia. Adv Anat Pathol 1:123–134 (1994).
10. Bostwick DG. Is behaviour of prostate cancer understood? In: Questions and Uncertainties About Prostate Cancer (Peeling WB, ed). Oxford:Blackwell Science, 1996;30–49.
11. Gleason D. Classification of prostatic carcinomas. Cancer Chemother Rep 50:125–128 (1966).
12. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111:58–64 (1974).
13. Saitoh H, Hida M, Shimbo T. Metastatic patterns of prostatic cancer: correlation between sites and number of organs involved. Cancer 54:3078–3084 (1984).
14. Isaacs JT. Molecular markers for prostate cancer metastasis. Am J Pathol 150:1511–1521 (1997).
15. Lalani E, Stubbs A, Stamp GWH. Molecular genetic changes in prostate cancer. In: Progress in Pathology, Vol 4. New York:Churchill Livingstone, 1998;113–135.
16. Lalani EN, Stubbs A, Stamp GW. Prostate cancer; the interface between pathology and basic scientific research. Semin Cancer Biol 8:53–59 (1997).
17. Lalani E, Laniado M, Abel P. Molecular and cellular biology of prostate cancer. Cancer Metastasis Rev 16:29–66 (1997).
18. Schroder FH. Detection of prostate cancer [editorial]. Br Med J 310:140–141 (1995).
19. Boyle P, Maisonneuve P, Napalkov P. Incidence of prostate cancer will double by the year 2030: the argument for. Eur Urol 29(Suppl 2):3–9 (1996).
20. Whitmore WF Jr. Natural history of low-stage prostatic cancer and the impact of early detection. Urol Clin North Am 17:689–697 (1990).
21. Walsh PC, Partin AW, Epstein JI. Cancer control and quality of life following anatomical radical retropubic prostatectomy: results at 10 years. J Urol 152:1831–1836 (1994).
22. Hancock SL, Cox RS, Bagshaw MA. Prostate specific antigen after radiotherapy for prostate cancer: a reevaluation of long-term biochemical control and the kinetics of recurrence in patients treated at Stanford University. J Urol 154:1412–1417 (1995).
23. Westin P, Stattin P, Damber JE, Bergh A. Castration therapy rapidly induces apoptosis in a minority and decreases cell proliferation in a majority of human prostatic tumors. Am J Pathol 146:1368–1375 (1995).
24. Hakimi J, Rondinelli R, Schoenberg M, Barrack E. Androgen receptor gene structure and function in prostate cancer. World J Urol 14:329–337 (1996).
25. Brinkmann AO, Jenster G, Ris Stalpers C, van der Korput JA, Bruggenwirth HT, Boehmer AL, Trapman J. Androgen receptor mutations. J Steroid Biochem Mol Biol 53:443–448 (1995).
26. Suzuki H, Sato N, Watabe Y, Masai M, Seino S, Shimazaki J. Androgen receptor gene mutations in human prostate cancer. J Steroid Biochem Mol Biol 46:759–765 (1993).
27. Trapman J, Brinkmann AO. Mutations in the androgen receptor. Ann NY Acad Sci 684:85–93 (1993).
28. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi OP. *In vivo* amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 9:401–406 (1995).
29. Koivisto P, Visakorpi T, Kallioniemi OP. Androgen receptor gene amplification: a novel molecular mechanism for endocrine therapy resistance in human prostate cancer. Scand J Clin Lab Invest Suppl 226:57–63 (1996).
30. Ruizeveld de Winter JA, Janssen PJ, Sleddens HM, Verleun Mooijman MC, Trapman J, Brinkmann AO, Santerse AB, Schroder FH, van der Kwast TH. Androgen receptor status in localized and locally progressive hormone refractory human prostate cancer. Am J Pathol 144:735–746 (1994).
31. Elo JP, Kvist L, Leinonen K, Isomaa V, Henttu P, Lukkarinen O, Vihko P. Mutated human androgen receptor gene detected in a prostatic cancer patient is also activated by estradiol. J Clin Endocrinol Metab 80:3494–3500 (1995).
32. Colombel M, Symmans F, Gil S, O'Toole KM, Chopin D, Benson M, Olsson CA, Korsmeyer S, Buttyan R. Detection of the apoptosis-suppressing oncoprotein bcl-2 in hormone-refractory human prostate cancers. Am J Pathol 143:390–400 (1993).
33. Raffo AJ, Perlman H, Chen MW, Day ML, Streitman JS, Buttyan R. Overexpression of bcl-2 protects prostate cancer cells from apoptosis *in vitro* and confers resistance to androgen depletion *in vivo*. Cancer Res 55:4438–4445 (1995).
34. McDonnell TJ, Troncoso P, Brisbay SM, Logothetis C, Chung LW, Hsieh JT, Tu SM, Campbell ML. Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer. Cancer Res 52:6940–6944 (1992).
35. Yagoda A, Petrylak D. Cytotoxic chemotherapy for advanced hormone-resistant prostate cancer. Cancer 71:1098–1109 (1993).
36. Barr PJ, Tomei LD. Apoptosis and its role in human disease. Biotechnology NY 12:487–493 (1994).
37. Strasser A, Huang DC, Vaux DL. The role of the *bcl-2/ced-9* gene family in cancer and general implications of defects in cell death control for tumourigenesis and resistance to chemotherapy. Biochim Biophys Acta 1333:F151–F178 (1997).
38. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 267:1456–1462 (1995).
39. Kerr J, Wyllie A, Currie A. Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. Br J Cancer 26:239–257 (1972).
40. Kerr JF, Winterford CM, Harmon BV. Apoptosis. Its significance in cancer and cancer therapy. Cancer 73:2013–2026 (1994). [Published erratum appears in Cancer 73:3108 (1994).]
41. Wyllie A, Kerr J, Currie A. Cell death: the significance of apoptosis. Int Rev Cytol 68:251–306 (1980).
42. Wyllie A, Morris R, Smith A, Dunlop D. Chromatin cleavage in apoptosis: association

with condensed chromatin morphology and dependence on macromolecular synthesis. J Pathol 142:66–72 (1984).
43. Hengartner MO, Ellis RE, Horvitz HR. *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. Nature 356:494–499 (1992).
44. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3. Cell 90:405–413 (1997).
45. Vaux DL, Cory S, Adams JM. *Bcl-2* gene promotes haemopoietic cell survival and cooperates with c-*myc* to immortalize pre-B cells. Nature 335:440–442 (1988).
46. Hockenbery D, Nunez G, Milliman C, Schreiber RD, Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 348:334–336 (1990).
47. Reed JC, Cuddy M, Haldar S, Croce C, Nowell P, Makover D, Bradley K. *BCL2*-mediated tumorigenicity of a human T-lymphoid cell line: synergy with MYC and inhibition by *BCL2* antisense. Proc Natl Acad Sci USA 87:3660–3664 (1990).
48. Reed JC, Stein C, Subasinghe C, Haldar S, Croce CM, Yum S, Cohen J. Antisense-mediated inhibition of *BCL2* protooncogene expression and leukemic cell growth and survival: comparisons of phosphodiester and phosphorothioate oligodeoxynucleotides. Cancer Res 50:6565–6570 (1990).
49. Reed JC. *Bcl-2* and the regulation of programmed cell death. J Cell Biol 124:1–6 (1994).
50. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the *bcl-2* gene in human follicular lymphoma. Science 228:1440–1443 (1985).
51. Tsujimoto Y, Finger LR, Yunis J, Nowell PC, Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 226:1097–1099 (1984).
52. Cleary ML, Smith SD, Sklar J. Cloning and structural analysis of cDNAs for *bcl-2* and a hybrid *bcl-2*/immunoglobulin transcript resulting from the t(14;18) translocation. Cell 47:19–28 (1986).
53. Oltvai ZN, Milliman CL, Korsmeyer SJ. *Bcl-2* heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74:609–619 (1993).
54. Zha H, Aime Sempe C, Sato T, Reed JC. Proapoptotic protein Bax heterodimerizes with *Bcl-2* and homodimerizes with Bax via a novel domain (BH3) distinct from BH1 and BH2. J Biol Chem 271:7440–7444 (1996).
55. Han J, Sabbatini P, Perez D, Rao L, Modha D, White E. The E1B 19K protein blocks apoptosis by interacting with and inhibiting the *p53*-inducible and death-promoting Bax protein. Genes Dev 10:461–477 (1996).
56. Yin XM, Oltval ZN, Korsmeyer SJ. BH1 and BH2 domains of *Bcl-2* are required for inhibition of apoptosis and heterodimerization with Bax. Nature 369:321–323 (1994).
57. Hanada M, Aime Sempe C, Sato T, Reed JC. Structure-function analysis of Bcl-2 protein. Identification of conserved domains important for homodimerization with Bcl-2 and heterodimerization with Bax. J Biol Chem 270:11962–11969 (1995).
58. Reed J. Bcl-2 family proteins: strategies for overcoming chemoresistance in cancer. Adv Pharmacol 41:501–532 (1997).
59. Krajewski S, Tanaka S, Takayama S, Schibler MJ, Fenton W, Reed JC. Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes. Cancer Res 53:4701–4714 (1993).
60. Lithgow T, van Driel R, Bertram JF, Strasser A. The protein product of the oncogene *bcl-2* is a component of the nuclear envelope, the endoplasmic reticulum, and the outer mitochondrial membrane. Cell Growth Differ 5:411–417 (1994).
61. Kane DJ, Sarafian TA, Anton R, Hahn H, Gralla EB, Valentine JS, Ord T, Bredesen DE. *Bcl-2* inhibition of neural death: decreased generation of reactive oxygen species. Science 262:1274–1277 (1993).
62. Hockenbery DM, Oltvai ZN, Yin XM, Milliman CL, Korsmeyer SJ. *Bcl-2* functions in an antioxidant pathway to prevent apoptosis. Cell 75:241–251 (1993).
63. Shimizu S, Eguchi Y, Kosaka H, Kamiike W, Matsuda H, Tsujimoto Y. Prevention of hypoxia-induced cell death by Bcl-2 and Bcl-x_L. Nature 374:811–813 (1995).
64. Jacobson MD, Raff MC. Programmed cell death and Bcl-2 protection in very low oxygen. Natur 374:814–816 (1995).
65. Distelhorst CW, Lam M, McCormick TS. *Bcl-2* inhibits hydrogen peroxide-induced ER Ca^{2+} pool depletion. Oncogene 12:2051–2055 (1996).
66. Baffy G, Miyashita T, Williamson JR, Reed JC. Apoptosis induced by withdrawal of interleukin-3 (IL-3) from an IL-3-dependent hematopoietic cell line is associated with repartitioning of intracellular calcium and is blocked by enforced Bcl-2 oncoprotein production. J Biol Chem 268:6511–6519 (1993).
67. Lam M, Dubyak G, Chen L, Nunez G, Miesfeld RL, Distelhorst CW, van Ooteghem RB, Smit EM, Beishuizen A, Lambrechts AC, et al. Evidence that *BCL-2* represses apoptosis by regulating endoplasmic reticulum-associated Ca^{2+} fluxes. Proc Natl Acad Sci USA 91:6569–6573 (1994).
68. Marin MC, Fernandez A, Bick RJ, Brisbay S, Buja LM, Snuggs M, McConkey DJ, von Eschenbach AC, Keating MJ, McDonnell TJ. Apoptosis suppression by *bcl-2* is correlated with the regulation of nuclear and cytosolic Ca^{2+}. Oncogene 12:2259–2266 (1996).
69. Murphy AN, Bredesen DE, Cortopassi G, Wang E, Fiskum G. *Bcl-2* potentiates the maximal calcium uptake capacity of neural cell mitochondria. Proc Natl Acad Sci USA 93:9893–9898 (1996).
70. Ryan JJ, Prochownik E, Gottlieb CA, Apel IJ, Merino R, Nunez G, Clarke MF. c-*myc* and *bcl-2* modulate *p53* function by altering *p53* subcellular trafficking during the cell cycle. Proc Natl Acad Sci USA 91:5878–5882 (1994).
71. Meikrantz W, Gisselbrecht S, Tam SW, Schlegel R. Activation of cyclin A-dependent protein kinases during apoptosis. Proc Natl Acad Sci USA 91:3754–3758 (1994).
72. Hengartner MO, Horvitz HR. Programmed cell death in *Caenorhabditis elegans*. Curr Opin Genet Dev 4:581–586 (1994).
73. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature [letter]. Cell 87:171 (1996).
74. Boulakia CA, Chen G, Ng FW, Teodoro JG, Branton PE, Nicholson DW, Poirier GG, Shore GC. *Bcl-2* and adenovirus E1B 19 kDA protein prevent E1A-induced processing of CPP32 and cleavage of poly(ADP-ribose) polymerase. Oncogene 12:529–535 (1996).
75. Monney L, Otter I, Olivier R, Ravn U, Mirzasaleh H, Fellay I, Poirier GG, Borner C. *Bcl-2* overexpression blocks activation of the death protease CPP32/Yama/apopain. Biochem Biophys Res Commun 221:340–345 (1996).
76. Srinivasan A, Foster LM, Testa MP, Ord T, Keane RW, Bredesen DE, Kayalar C. *Bcl-2* expression in neural cells blocks activation of ICE/CED-3 family proteases during apoptosis. J Neurosci 16:5654–5660 (1996).
77. Ibrado AM, Huang Y, Fang G, Liu L, Bhalla K. Overexpression of *Bcl-2* or *Bcl-x_L* inhibits Ara-C-induced CPP32/Yama protease activity and apoptosis of human acute myelogenous leukemia HL-60 cells. Cancer Res 56:4743–4748 (1996).
78. Smyth MJ, Perry DK, Zhang J, Poirier GG, Hannun YA, Obeid LM. a downstream target for ceramide-induced apoptosis and for the inhibitory action of *Bcl-2*. Biochem J 316:25–28 (1996).
79. Messmer UK, Reimer DM, Reed JC, Brune B. Nitric oxide induced poly(ADP-ribose) polymerase cleavage in RAW 264.7 macrophage apoptosis is blocked by *Bcl-2*. FEBS Lett 384:162–166 (1996).
80. Zamzami N, Susin SA, Marchetti P, Hirsch T, Gomez Monterrey I, Castedo M, Kroemer G. Mitochondrial control of nuclear apoptosis. J Exp Med 183:1533–1544 (1996).
81. Susin SA, Zamzami N, Castedo M, Hirsch T, Marchetti P, Macho A, Daugas E, Geuskens M, Kroemer G. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease. J Exp Med 184:1331–1341 (1996).
82. Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome *c*. Cell 86:147–157 (1996).
83. Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: release of cytochrome *c* from mitochondria blocked. Science 275:1129–1132 (1997).
84. Antonsson B, Conti F, Ciavatta A, Montessuit S, Lewis S, Martinou I, Bernasconi L, Bernard A, Mermod JJ, Mazzei G, et al. Inhibition of Bax channel-forming activity by Bcl-2. Science 277:370–372 (1997).
85. Schlesinger PH, Gross A, Yin XM, Yamamoto K, Saito M, Waksman G, Korsmeyer SJ. Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. Proc Natl Acad Sci USA 94:11357–11362 (1997).
86. Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M, Reed JC. Channel formation by antiapoptotic protein Bcl-2. Proc Natl Acad Sci USA 94:5113–5118 (1997).

87. Minn AJ, Velez P, Schendel SL, Liang H, Muchmore SW, Fesik SW, Fill M, Thompson CB. Bcl-$x_{(L)}$ forms an ion channel in synthetic lipid membranes. Nature 385:353–357 (1997).
88. Miyashita T, Reed JC. Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. Blood 81:151–157 (1993).
89. Miyashita T, Reed JC. *bcl-2* gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. Cancer Res 52:5407–5411 (1992).
90. Walton MI, Whysong D, O'Connor PM, Hockenbery D, Korsmeyer SJ, Kohn KW. Constitutive expression of human Bcl-2 modulates nitrogen mustard and camptothecin induced apoptosis. Cancer Res 53:1853–1861 (1993).
91. Kamesaki S, Kamesaki H, Jorgensen TJ, Tanizawa A, Pommier Y, Cossman J. bcl-2 protein inhibits etoposide-induced apoptosis through its effects on events subsequent to topoisomerase II-induced DNA strand breaks and their repair. Cancer Res; 53: 4251–4256 (1993). [Published erratum appears in Cancer Res 54:3074 (1994).]
92. Fisher TC, Milner AE, Gregory CD, Jackman AL, Aherne GW, Hartley JA, Dive C, Hickman JA. *bcl-2* modulation of apoptosis induced by anticancer drugs: resistance to thymidylate stress is independent of classical resistance pathways. Cancer Res 53:3321–3326 (1993).
93. Tang C, Willingham MC, Reed JC, Miyashita T, Ray S, Ponnathpur V, Huang Y, Mahoney ME, Bullock G, Bhalla K. High levels of p26BCL-2 oncoprotein retard taxol-induced apoptosis in human pre-B leukemia cells. Leukemia 8:1960–1699 (1994).
94. Minn AJ, Rudin CM, Boise LH, Thompson CB. Expression of bcl-x_L can confer a multidrug resistance phenotype. Blood 86:1903–1910 (1995).
95. McConkey DJ, Greene G, Pettaway CA. Apoptosis resistance increases with metastatic potential in cells of the human LNCaP prostate carcinoma line. Cancer Res 56:5594–5599 (1996).
96. Vaux DL, Strasser A. The molecular biology of apoptosis. Proc Natl Acad Sci USA 93: 2239–2244 (1996).
97. Campos L, Sabido O, Rouault JP, Guyotat D. Effects of BCL-2 antisense oligodeoxynucleotides on *in vitro* proliferation and survival of normal marrow progenitors and leukemic cells. Blood 84:595–600 (1994).
98. Berchem GJ, Bosseler M, Sugars LY, Voeller HJ, Zeitlin S, Gelmann EP. Androgens induce resistance to *bcl-2*-mediated apoptosis in LNCaP prostate cancer cells. Cancer Res 55:735–738 (1995).
99. Kitada S, Takayama S, De Riel K, Tanaka S, Reed JC. Reversal of chemoresistance of lymphoma cells by antisense-mediated reduction of *bcl-2* gene expression. Antisense Res Dev 4:71–79 (1994).
100. Shirahama T, Sakakura C, Sweeney EA, Ozawa M, Takemoto M, Nishiyama K, Ohi Y, Igarashi Y. Sphingosine induces apoptosis in androgen-independent human prostatic carcinoma DU-145 cells by suppression of *bcl-*$X_{(L)}$ gene expression. FEBS Lett 407:97–100 (1997).
101. Bargou RC, Bommert K, Weinmann P, Daniel PT, Wagener C, Mapara MY, Dorken B. Induction of Bax-alpha precedes apoptosis in a human B lymphoma cell line: potential role of the *bcl-2* gene family in surface IgM-mediated apoptosis. Eur J Immunol 25:770–775 (1995).
102. Bargou RC, Wagener C, Bommert K, Mapara MY, Daniel PT, Arnold W, Dietel M, Guski H, Feller A, Royer HD, et al. Overexpression of the death-promoting gene bax-alpha which is downregulated in breast cancer restores sensitivity to different apoptotic stimuli and reduces tumor growth in SCID mice. J Clin Invest 97:2651–2659 (1996).
103. Sumantran VN, Ealovega MW, Nunez G, Clarke MF, Wicha MS. Overexpression of Bcl-X_S sensitizes MCF-7 cells to chemotherapy-induced apoptosis. Cancer Res 55:2507–2510 (1995).
104. Berges R. Implication of cell kinetic changes during the progression of human prostatic cancer. Clin Cancer Res 1:473–480 (1995).
105. Isaacs JT, Lundmo PI, Berges R, Martikainen P, Kyprianou N, English HF. Androgen regulation of programmed death of normal and malignant prostatic cells. J Androl 13:457–464 (1992).
106. Kyprianou N, English HF, Isaacs JT. Programmed cell death during regression of PC-82 human prostate cancer following androgen ablation. Cancer Res 50:3748–3753 (1990).
107. Denmeade SR, Isaacs JT. Activation of programmed (apoptotic) cell death for the treatment of prostate cancer. Adv Pharmacol 35:281–306 (1996).
108. Lin XS, Denmeade SR, Cisek L, Isaacs JT. Mechanism and role of growth arrest in programmed (apoptotic) death of prostatic cancer cells induced by thapsigargin. Prostate 33:201–207 (1997).
109. Thastrup O. Role of $Ca^{2(+)}$-ATPases in regulation of cellular Ca^{2+} signalling, as studied with the selective microsomal $Ca^{2(+)}$-ATPase inhibitor, thapsigargin. Agents Actions 29:8–15 (1990).
110. Gee JM, Robertson JF, Ellis IO, Willsher P, McClelland RA, Hoyle HB, Kyme SR, Finlay P, Blamey RW, Nicholson RI. Immunocytochemical localization of BCL-2 protein in human breast cancers and its relationship to a series of prognostic markers and response to endocrine therapy. Int J Cancer 59:619–628 (1994).
111. Lauwers GY, Scott GV, Karpeh MS. Immunohistochemical evaluation of bcl-2 protein expression in gastric adenocarcinomas. Cancer 75:2209–2213 (1995).
112. Bhargava V, Kell DL, van de Rijn M, Warnke RA. Bcl-2 immunoreactivity in breast carcinoma correlates with hormone receptor positivity. Am J Pathol 145:535–540 (1994).
113. Pollina L, Pacini F, Fontanini G, Vignati S, Bevilacqua G, Basolo F. bcl-2, p53 and proliferating cell nuclear antigen expression is related to the degree of differentiation in thyroid carcinomas. Br J Cancer 73:139–143 (1996).
114. Bubendorf L, Sauter G, Moch H, Jordan P, Blochlinger A, Gasser TC, Mihatsch MJ. Prognostic significance of Bcl-2 in clinically localized prostate cancer. Am J Pathol 148:1557–1565 (1996).
115. Krajewska M, Krajewski S, Epstein JI, Shabaik A, Sauvageot J, Song K, Kitada S, Reed JC. Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol 148:1567–1576 (1996).
116. Apakama I, Robinson MC, Walter NM, Charlton RG, Royds JA, Fuller CE, Neal DE, Hamdy FC. bcl-2 overexpression combined with p53 protein accumulation correlates with hormone-refractory prostate cancer. Br J Cancer 74:1258–1262 (1996).
117. Tu H, Jacobs SC, Borkowski A, Kyprianou N. Incidence of apoptosis and cell proliferation in prostate cancer: relationship with TGF-beta1 and bcl-2 expression. Int J Cancer 69:357–363 (1996).
118. Tsuji M, Murakami Y, Kanayama H, Sano T, Kagawa S. Immunohistochemical analysis of Ki-67 antigen and Bcl-2 protein expression in prostate cancer: effect of neoadjuvant hormonal therapy. Br J Urol 81:116–121 (1998).
119. Byrne RL, Horne CH, Robinson MC, Autzen P, Apakama I, Bishop RI, Neal DE, Hamdy FC. The expression of *waf-1*, *p53* and *bcl-2* in prostatic adenocarcinoma. Br J Urol 79:190–195 (1997).
120. Segal NH, Cohen RJ, Haffejee Z, Savage N. *BCL-2* proto-oncogene expression in prostate cancer and its relationship to the prostatic neuroendocrine cell. Arch Pathol Lab Med 118:616–618 (1994).
121. Ben Ezra JM, Kornstein MJ, Grimes MM, Krystal G. Small cell carcinomas of the lung express the Bcl-2 protein. Am J Pathol 145:1036–1040 (1994).
122. Fontanini G, Vignati S, Bigini D, Mussi A, Lucchi M, Angeletti CA, Basolo F, Bevilacqua G. Bcl-2 protein: a prognostic factor inversely correlated to *p53* in non-small-cell lung cancer. Br J Cancer 71:1003–1007 (1995).
123. Lu QL, Elia G, Lucas S, Thomas JA. *Bcl-2* proto-oncogene expression in Epstein-Barr-virus-associated nasopharyngeal carcinoma. Int J Cancer 53:29–35 (1993).
124. Lu QL, Abel P, Foster CS, Lalani EN. *bcl-2*: role in epithelial differentiation and oncogenesis. Hum Pathol 27:102–110 (1996).
125. Bronner MP, Culin C, Reed JC, Furth EE. The *bcl-2* proto-oncogene and the gastrointestinal epithelial tumor progression model. Am J Pathol 146:20–26 (1995).
126. Shiina H, Igawa M, Urakami S, Honda S, Shirakawa H, Ishibe T. Immunohistochemical analysis of bcl-2 expression in transitional cell carcinoma of the bladder. J Clin Pathol 49:395–399 (1996).
127. Chaudhary KS, Lu QL, Abel PD, Khandan Nia N, Shoma AM, el Baz M, Stamp GW, Lalani EN. Expression of bcl-2 and p53 oncoproteins in schistosomiasis-associated transitional and squamous cell carcinoma of urinary bladder. Br J Urol 79:78–84 (1997).
128. Chaudhary KS, Stamp GWH, Abel PD, Lalani EN. Bcl-2 transfection into DU145 adenocarcinoma cells alters cell cycle regulatory molecules (p53, PCNA and Bax) and confers chemo-resistance against adriamycin and thapsigargin. Eur Urol 34:261 (1998).
129. Dorai T, Goluboff ET, Olsson CA, Buttyan R. Development of a hammerhead ribozyme against BCL-2. II: Ribozyme treatment sensitizes hormone-resistant prostate cancer cells to apoptotic agents. Anticancer Res 17:3307–3312 (1997).
130. Dorai T, Olsson CA, Katz AE, Buttyan R.

Development of a hammerhead ribozyme against bcl-2. I: Preliminary evaluation of a potential gene therapeutic agent for hormone-refractory human prostate cancer. Prostate 32:246–258 (1997).
131. Kyprianou N, King ED, Bradbury D, Rhee JG. 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).
132. Haldar S, Chintapalli J, Croce CM. Taxol induces bcl-2 phosphorylation and death of prostate cancer cells. Cancer Res 56:1253–1255 (1996).
133. Haldar S, Basu A, Croce CM. Bcl2 is the guardian of microtubule integrity. Cancer Res 57:229–233 (1997).
134. Ibrado AM, Liu L, Bhalla K. Bcl-x_L overexpression inhibits progression of molecular events leading to paclitaxel-induced apoptosis of human acute myeloid leukemia HL-60 cells. Cancer Res 57:1109–1115 (1997).
135. Boise LH, Gonzalez Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G, Thompson CB. *bcl-x*, a *bcl-2*-related gene that functions as a dominant regulator of apoptotic cell death. Cell 74:597–608 (1993).
136. Zhou P, Qian L, Kozopas KM, Craig RW. Mcl-1, a Bcl-2 family member, delays the death of hematopoietic cells under a variety of apoptosis-inducing conditions. Blood 89:630–643 (1997).
137. Gibson L, Holmgreen SP, Huang DC, Bernard O, Copeland NG, Jenkins NA, Sutherland GR, Baker E, Adams JM, Cory S. *bcl-w*, a novel member of the *bcl-2* family, promotes cell survival. Oncogene 13:665–675 (1996).
138. Lin EY, Orlofsky A, Wang HG, Reed JC, Prystowsky MB. *A1*, a *Bcl-2* family member, prolongs cell survival and permits myeloid differentiation. Blood 87:983–992 (1996).
139. Chittenden T, Harrington EA, O'Connor R, Flemington C, Lutz RJ, Evan GI, Guild BC. Induction of apoptosis by the Bcl-2 homologue Bak. Nature 374:733–736 (1995).
140. 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 80:285–291 (1995).
141. Boyd JM, Gallo GJ, Elangovan B, Houghton AB, Malstrom S, Avery BJ, Ebb RG, Subramanian T, Chittenden T, Lutz RJ, et al. 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).
142. Wang K, Yin XM, Chao DT, Milliman CL, Korsmeyer SJ. BID: a novel BH3 domain-only death agonist. Genes Dev 10:2859–2869 (1996).
143. Inohara N, Ding L, Chen S, Nunez G. Harakiri, a novel regulator of cell death, encodes a protein that activates apoptosis and interacts selectively with survival-promoting proteins Bcl-2 and Bcl-$X_{(L)}$. EMBO J 16:1686–1694 (1997).