bcl-2 in cancer, development and apoptosis

David M. Hockenbery

Fred Hutchinson Cancer Research Center, Seattle, WA, USA

SUMMARY

The bcl-2 gene provides a window on the basic cellular machinery of apoptosis or programmed cell death, a process involved in virtually all biologic events in multicellular organisms, but particularly relevant to neoplasia and development. bcl-2 gene function supports cell survival and appears to lie at a nodal point in pathways leading to activation or execution of apoptosis. Carcinogenesis may involve several steps at which cell death programs are normally activated and are bypassed in cancer cells, including apoptotic pathways activated by several oncogenes. Functional redundancy and the complexity of the regulation of cell survival are demonstrated by the less than expected phenotype of bcl-2 knockout mice and the cloning of several bcl-2 related genes, some of which promote cell death. The molecular function for bcl-2 is unknown, but several lines of evidence support a role in protection from oxidative stress. These studies suggest that many environmental perturbations and genetic pathways converge to disrupt a metabolic balance between oxidant generation and anti-oxidant defenses.

Key words: oncogene, cell death, oxidative stress

INTRODUCTION

As has been the case for genes involved in cellular proliferation and activation, an important cellular gene controlling programmed cell death was discovered by a search for novel oncogenes. bcl-2 was identified as an oncogene involved in the t(14;18) translocation that is characteristic of follicular lymphoma, a human B cell malignancy (Tsujimoto et al., 1984; Bakhshi et al., 1985; Cleary and Sklar, 1985). bcl-2 on chromosome 18q and the immunoglobulin heavy chain gene locus on chromosome 14q are broken and rejoined in a balanced translocation, creating a chromosomal context in which transcriptional deregulation of bcl-2 occurs.

Initial assays for cell transforming properties were disappointing and a potential role for bcl-2 in oncogenesis was lacking until the experiments of Vaux et al. (1988) were reported. Introduction of bcl-2 into an interleukin-3-(IL-3)-dependent cell line led, not to factor-independent growth, but to factor-independent survival. Subsequent experiments confirmed the ability of bcl-2 to inhibit the expression of an apoptotic cell death program following factor withdrawal independent of cell cycle effects (Nunez et al., 1990; Hockenbery et al., 1990). The overexpression of a normal bcl-2 protein is the important oncogenic event in human follicular lymphoma and can be partially recreated in transgenic models. The generation of bcl-2-transgenic mice using the bcl-2-promoter and cDNA and immunoglobulin heavy chain enhancer demonstrated the consequences in the B cell lineage of deregulated cell survival (McDonnell et al., 1989). Dramatic polyclonal expansion of mature B lymphocytes precedes development of diffuse immunoblastic B cell lymphomas in the second year of life. A high proportion of these tumors have rearrangements of c-myc as a second genetic alteration (McDonnell and Korsmeyer, 1991).

Although several groups have reported similar results in the B cell lineage (Strasser et al., 1991b; Katsumata et al., 1992), oncogenic effects of bcl-2 in other cell types have not been as evident. Targeted overexpression of bcl-2 in thymic lymphocytes, mammary and intestinal epithelium, and myeloid populations has not produced tumors, although occasional T cell neoplasms have been reported in mice bearing immunoglobulin enhancer-bcl-2 transgenes (Sentman et al., 1991; Lagasse and Weissman, 1993; D. M. Hockenbery, unpublished observations).

bcl-2 FUNCTION IN CANCER

One model for bcl-2 action in oncogenesis relies on the dual effects of proliferation and cell death in homeostatic regulation of cell populations, suggesting that abnormalities in both pathways may lead to clonal selection and neoplasia (Korsmeyer, 1992). Documentation of an extended lifespan of tumor cells is lacking in most cases, however, and is presumably not the case in tumors with a demonstrably high cell turnover, such as Burkitt's lymphoma. The requirement for a deregulated cell survival mechanism may be temporally restricted to the period of early acquisition of several genetic lesions, when the constraints of physiologic cell turnover may be a limiting factor on clonal evolution. B lymphocytes, which survive only several days in the peripheral circulation without antigenic stimulation, may be one lineage and differentiation stage with this requirement.

Another model would propose a more targeted role for bcl-2 in neoplasia in which bcl-2 is highly synergistic with specific cellular oncogenes. This concept follows the recognition that several oncogenes that function in cell activation and prolifer-

ation pathways have a paradoxical effect of promoting cell death by apoptosis. This tendency is unmasked by adverse growth conditions or blockade of cell proliferation. This connection between cell proliferation and apoptosis was originally demonstrated for c-myc, and has been shown to be relieved by overexpression of bcl-2 (Evan et al., 1992; Bissonnette et al., 1992; Fanidi et al., 1992). Tumorigenesis in cell lines and transgenic models is dramatically accelerated by co-expression of c-myc and bcl-2 (Strasser et al., 1990). Progression of human lymphoid tumors with deregulated c-myc and bcl-2 suggests that these observations hold true in naturally occurring tumors (Lee et al., 1989). Additional cellular oncogenes have been found that can predispose cells to undergo apoptosis, including c-rel and c-fos (Smeyne et al., 1993; Abbadie et al., 1993).

Deregulation of bcl-2 may also function as a survival mechanism in cancer cells predisposed to cell death by environmental factors. bcl-2 acts as a broad anti-apoptotic factor and opposes cell deaths following ionizing radiation, cancer drugs and hormonal manipulations (Sentman et al., 1991; Strasser et al., 1991a; Miyashita and Reed, 1993). Apoptosis may also be triggered by withdrawal of extracellular matrix interactions, suggesting that tumor growth pattern and metastasis may impose an apoptosis-inducing stress (see article by C. Streuli, this issue). Several non-lymphoid neoplasms, including prostate, lung, colon and breast express high levels of bcl-2, although a genetic alteration in bcl-2 has not been demonstrated (McDonnell et al., 1992; Pezzella et al., 1993; Bronner et al., 1994; D. M. Hockenbery, unpublished observations).

bcl-2 FUNCTION IN DEVELOPMENT

The identification of an anti-apoptotic function of bcl-2 in cancer and in in vitro assays has produced much interest in the potential normal in vivo functions of this gene. Apoptotic cell deaths are abundant in developmental processes of many organisms (Sulston and Horvitz, 1977; Abrams et al., 1993). These appear to be essential events in higher organisms, leading to important morphogenetic changes such as loss of interdigital webs, and functional matching of cell populations in the nervous system, resulting in the death of up to 85% of neurons that are born (Cowan et al., 1984). bcl-2 is expressed in a limited number of tissues in the adult, including T and B lymphocytes, hemopoietic cells, epithelia, and neurons (Hockenbery et al., 1991). Predictions of a determinative role for bcl-2 in life or death decisions in vivo are best supported by study of germinal centres in lymphoid follicles. Germinal centres are sites with high rates of apoptotic cell death of B cells during humoral immune responses to T cell-dependent antigens (MacLennan and Gray, 1986). Cell death operates as a selective mechanism for expansion of B cell clones bearing high-affinity immunoglobulin antigen receptors. Diversity in immunoglobulin receptors is created by hypermutation of the rearranged immunoglobulin genes of one or two B cells that are recruited to the germinal centre, where expansion and hypermutation take place in the centroblastic zone. A need for survival cues becomes evident as these cells cease proliferating and accumulate in the centrocytic zone. Limiting amounts of cognate antigen are localized within the germinal centre on dendritic cells, provoking a competition for antigen-antigen receptor interactions won by B cells bearing mutated, high-affinity receptors. The evidence suggests that these cells are the survivors of germinal centre reactions, emigrating as memory B cells or plasma cells characteristic of the mature immune response. The remainder of B cells generated in the germinal centre succumb to apoptosis. The primary role of bcl-2 in this selection scheme is suggested by the vigorous regulation of bcl-2 expression that is observed. B lymphocytes that are recruited to the germinal centre and undergo expansion as centroblasts have down-regulated the abundant bcl-2 expression characteristic of circulating B cells to undetectable levels (Hockenbery et al., 1991; Pezzella et al., 1990). This remains true for the majority of centrocytes in the area of the germinal centre with high rates of apoptotic death, with the exception of rare single cells that stain positively for bcl-2.

In vitro study of centrocytes reveals the probable course of events. Germinal centre lymphocytes undergo an accelerated apoptotic death in vitro unless specific signals are present. These include immunoglobulin receptor stimulation and the co-stimulatory ligands anti-CD40 antibody or rCD23 protein plus interleukin-1 (Liu et al., 1991). These factors likely represent antigen-antibody binding in the context of cell-cell and cytokine-receptor interactions present in the germinal centre environment. bcl-2 protein, significantly, is induced by both sets of stimuli. Thus, in the germinal centre reaction, down-regulation of bcl-2 appears to set up an apoptotic reaction and is the target of positively-selecting stimuli to rescue cells from an apoptotic end. The apparent complexity of bcl-2 regulation in germinal centres is further increased by in situ RNA hybridization studies, which revealed abundant bcl-2 RNA throughout the germinal centre, suggesting a predominant post-transcriptional mechanism (Chleq-Deschamps et al., 1993).

bcl-2 is expressed more widely in the developing fetus, notably in many neuronal populations, the retina, and limb buds. The expression pattern of bcl-2 is, in some instances, inversely related to topographically-restricted sites of apoptotic death, such as interdigital webs (Veis et al., 1993).

FUNCTIONAL REDUNDANCY AND bcl-2-RELATED GENES

Transgenic mice with deletions of single genes by homologous recombination provide a stringent test for the essential function of genes during development. Two gene 'knockout' models of bcl-2 have been reported (Veis et al., 1993; Nakayama et al., 1993). Surprisingly, except for a slight growth retardation, development proceeds normally in these mice and normal births are reported with no excess mortality for mice with homozygously deleted bcl-2. Three tissue-specific phenotypes are evident post-natally. Lymphoid organs undergo massive involution by apoptosis within the first 2 months of life, although thymic and B cell maturation initially proceed normally. These mice undergo early deaths, probably more related to a polycystic kidney lesion apparent soon after birth. Finally, a pattern of progressive graying during the second hair follicle cycle develops, although hair pigmentation at birth is normal (Veis et al., 1993).

The results in mice with deleted bcl-2 suggests a level of functional redundancy for bcl-2, particularly during develop-

mental stages. Recently, several genes homologous to bcl-2 have been cloned, which may explain models based on redundant gene function. Two genes, *bax* and *bcl-x*, encode proteins that lack one or more regions evolutionarily conserved in bcl-2 (Oltvai et al., 1993; Boise et al., 1993). Both of these, when co-expressed with bcl-2, produce inhibition of bcl-2 function in survival assays. bax was isolated by co-immuno-precipitation with bcl-2 and has been demonstrated to form het-erodimeric complexes with bcl-2 (Oltvai et al., 1993). The ratio of bcl-2 to bax protein within a cell appears to act as a 'rheostat' predicting cell survival following apoptotic triggers.

bcl-x, cloned by low-stringency hybridization of a cDNA library, produces alternatively-spliced mRNAs, one of which encodes an inhibitor of bcl-2 function, bcl-x_S, lacking 2 domains conserved in bcl-2. A larger protein, bcl-x_L, is encoded by a second mRNA and retains all conserved domains identified in bcl-2. This bcl-x product inhibits apoptotic death and appears to be functionally equivalent to bcl-2. bcl-x_L and bcl-x_S appear to be regulated independently and are expressed in different cell types. Additional bcl-2 family members, mcl-1 and A1, have recently been cloned from hematopoietic cells, but have not been functionally characterized (Kozopas et al., 1993; Lin et al., 1993). bcl-2 is part of a still larger family of homologous proteins, including cell death inhibitors found in the DNA viruses adenovirus, Epstein-Barr virus, African swine fever virus and herpesvirus saimiri (White, 1993; Cleary et al., 1986; Neilan et al., 1993; Albrecht et al., 1992). Sequence

homology is strongest in two short regions, BH1 and BH2 (Fig. 1). Homologous genes have also been found in mouse, chicken, rat and *Caenorhabditis elegans* (Negrini et al., 1987; Eguchi et al., 1992; Reed, 1994; Hengartner and Horvitz, 1994).

Knowledge of redundancy in bcl-2 function may help to explain one conundrum in the literature. Down-regulation of bcl-2 expression precedes cell death in many models and, if prevented in gene transfection experiments, cell death is impeded. These results suggest that some stimuli that induce apoptosis have bcl-2 down-regulation as their target. However, attempts to induce cell death by decreasing bcl-2 function, using anti-sense oligonucleotides or overexpression of an inhibitor such as bax, still require an apoptotic trigger (Oltvai et al., 1993; Reed et al., 1990). The intracellular changes produced by an apoptotic stimulus, such as serum deprivation, are therefore poorly understood. If more than a single bcl-2-like protein is expressed and involved in maintaining cellular viability, attempts to reproduce the events of serum depriva-tion by down-regulating one member of this family may be insufficient. It may only increase the apoptotic response to serum deprivation, if the effect of serum deprivation is to down-regulate all bcl-2-like proteins below some threshold level. This is, in fact, the sensitization response that is observed after bcl-2 down-regulation, including assays of cells from bcl-2 'knockout' mice.

Why are there multiple bcl-2 family members? One potential
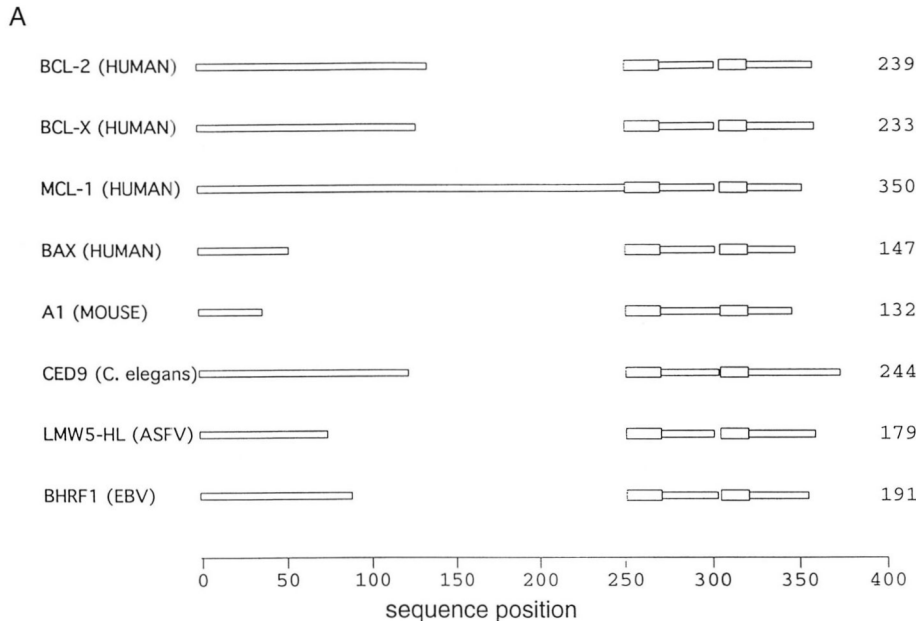
Fig. 1. (a) Schematic representation of regions of homology among bcl-2 family members. Blocks of aligned sequences were analyzed with the progam MACAW (Schuler et al., 1991). (b) Conserved nucleotide sequences in the BH1 region are shown. Amino acids identical to human bcl-2 are in black and conservative substitutions are shaded. (c) Conserved nucleotide sequences in the BH2 region are shown.
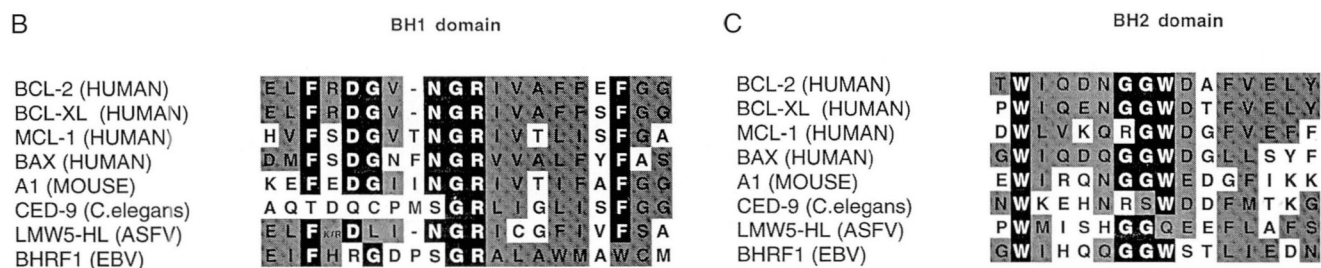
explanation is suggested by the pattern of bcl-2 expression within a cell lineage. Hemopoietic precursors of myeloid, erythroid and megakaryotic lineage express bcl-2, but more mature, post-mitotic progeny lack bcl-2. We have recently demonstrated that quiescent, pluripotent hemopoietic cells with 'stem cell' features also lack bcl-2. This pattern of bcl-2 expression within a limited number of differentiation or developmental stages of a lineage is seen in other cell types, such as complex epithelia. If some bcl-2-like proteins have non-overlapping distributions, perhaps each stage in a lineage of a cell will have a member(s) of this family of proteins actively maintaining cell viability. This would mean that cellular transitions would result in down-regulation of one bcl-2 family member and up-regulation of another. This might provide an efficient check on any cells that fail to execute normal developmental or differentiation programmes. These cells, which may fail to exit the cell cycle in G_0 or fail to express the correct receptor for a trophic factor, would automatically undergo apoptosis by losing expression of one family member and failing to induce another. A family of genes would increase the complexity of regulatory strategies that could be used in different lineages or stages of differentiation. This model would also suggest that in vitro models of apoptotic cell death after various exogenous treatments may be variations on a theme used in vivo, in which cell cycle arrest, DNA damage or factor-withdrawal could converge to down-regulate intracellular survival factors.

APOPTOTIC MECHANISMS AND bcl-2 FUNCTION

Mechanisms of apoptotic cell death must be closely linked to the function of bcl-2, since bcl-2 has been shown in experimental models to block almost all examples of apoptotic death. There is also room for those cell deaths not responsive to bcl-2 overexpression to remain part of the larger category of bcl-2-responsive deaths, if high levels of inhibitory proteins like bax are present.

The subcellular localization of bcl-2 may provide a clue to its function. bcl-2 has been found associated with mitochondria, nuclear membrane, and smooth endoplasmic reticulum (Hockenbery et al., 1990: Jacobson et al., 1993; Monaghan et al., 1992). These sites have in common an oxidation-reduction function that can result in oxygen-free radical generation. Several stimuli that can produce apoptosis are linked to oxidative stress, including ionizing radiation, tumor necrosis factor and phorbol esters.

To investigate the possibility that reactive oxygen species (ROS) play a wider role in apoptosis, we examined the model of interleukin-3 (IL-3)-withdrawal-induced cell death (Hockenbery et al., 1993). Pretreatment of IL-3-dependent FL5.12 cells with either N-acetylcysteine, a direct free radical scavenger that increases intracellular glutathione levels, or vitamin E, a chain-terminating antioxidant, gave partial protection from factor withdrawal-induced apoptosis. To achieve enhanced antioxidant effects, stable transfectants of FL5.12 cells were made with glutathione peroxidase or manganese superoxide dismutase (MnSOD) cDNAs and evaluated for response to factor withdrawal. While overexpression of MnSOD had no effect in this model, glutathoine peroxidase provided substantial protection. These results suggested that ROS participated in factor withdrawal-induced cell death. In

view of the recognized effects of oxidative stress in gene induction and modification of protein function, a role in cell signaling or a more direct role in cell damage could be envisaged. The specificity for peroxides activity suggests that peroxidase are the relevant ROS involved in apoptosis.

A useful assay of ROS-mediated cellular damage is the analysis of lipid peroxidation. A fluorescent lipid analog, cis-parinaric acid (CPA) has been used as an indirect measure of lipid peroxidation in flow cytometric assays. CPA is distributed to all cellular membranes and remains stably fluorescent unless it is peroxidized, which involves a molecular rearrangement with loss of fluorescent properties. We examined the model of dexamethasone-induced apoptosis of a T cell hybridoma, 2B4 cells, for the advantage of a more synchronized apoptotic response. 2B4 cells that are bcl-2-transfected remained viable after dexamethasone treatment and showed no loss of CPA fluorescence. To the contrary, control 2B4 cells died by apoptosis and showed an early diminution of CPA fluorescence by 4 hours after dexamethasone that progressively increased during the incubation. An early marker of apoptotic cell death in this model, DNA fragmentation, occurred at 10-11 hours. This result suggests that early ROS-mediated cell damage occurs and increases during the time period when other cellular events in apoptosis take place. Finally, we asked if the effect of bcl-2 in preventing lipid peroxidation was at the level of ROS generation or at a downstream point. Menadione is a toxic vitamin K derivative that causes intracellular formation of superoxide radical by redox cycling. Cells treated with menadione undergo apoptosis, which is blocked by bcl-2 at lower menadione doses. Generation of superoxide radical can be measured by oxygen consumption in the presence of cyanide, an inhibitor of oxidative phosphorylation. bcl-2 protected cells demonstrated no change in the rate of superoxide production as measured in this assay, implying a downstream, antioxidant function for bcl-2. Further studies using a flow cytometric indicator of peroxide generation, dichlorofluorescein, confirmed that bcl-2 did not inhibit the generation of ROS. Interestingly, the effect of dexamethasone as a trigger of apoptotic death does not appear to be mediated by an increase in ROS generation. As pro-oxidant effects can be achieved by decreases in antioxidant levels, this mechanism may be operative in dexamethasone-induced apoptosis. If bcl-2 directly or indirectly acts as an antioxidant, the models of apoptosis in which bcl-2 down-regulation occurs might also fit this model.

Why would cells use oxidative damage as a general effector mechanism in programmed cell deaths? ROS have been shown to stimulate gene transcription, regulate transcription factors, and activate components of signal transduction pathways. As described for the role of nitric oxide as a second messenger, ROS may be tightly regulated within a cell to achieve desired physiologic effects and avoid pathologic effects. Global cellular programs, such as cell proliferation and differentiation, respond to changes in redox environment. If variations in ROS levels regulate some of these programs, a novel view of cellular oxidant/antioxidant balance as a labile, dynamic control element may be required. Exaggerated responses or prolonged down-regulation of balancing antioxidants could readily result in apoptotic cell death.

REFERENCES

Abbadie, C., Kabrun, N., Bouali, F. et al. (1993). High levels of c-rel expression are associated with programmed cell death in the developing avian embryo and in bone marrow cells in vitro. *Cell* **75**, 899-912.

Abrams, J. M., White, K., Fessler, L. I. and Steller, H. (1993). Programmed cell death during *Drosophila* embryogenesis. *Development* **117**, 29-43.

Albrecht, J. C., Nicholas, J., Cameron, K. R., Newman, C., Fleckenstein, B. and Honess, R. W. (1992). Herpesvirus saimiri has a gene specifying a homologue of the cellular membrane glycoprotein CD59. *Virology* **190**, 527-530.

Bakhshi, A., Jensen, J. P., Goldman, P. et al. (1985). Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around J_H on chromosome 14 and near a transcriptional unit on chromosome 18. *Cell* **41**, 899-906.

Bissonnette, R. P., Echeveri, F., Mahboubi, A. and Green, D. (1992). Apoptotic cell death induced by c-*myc* is inhibited by *bcl-2*. *Nature* **359**, 552-554.

Boise, L. H., Gonzalez-Garcia, M., Postema, C. E. et al. (1993). bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* **74**, 597-608.

Bronner, M. P., Culin, C., Reed, J. and Furth, E. (1994). Bcl-2 protooncogene and the gastrointestinal mucosal epithelial tumor progression model. *Am. J. Pathol.* (in press).

Chleq-Deschamps, C. M., LeBrun, D. P., Huie, P. et al. (1993). Topographical dissociation of BCL-2 messenger RNA and protein expression in human lymphoid tissues. *Blood* **81**, 293-298.

Cleary, M. L. and Sklar, J. (1985). Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. *Proc. Nat. Acad. Sci. USA* **82**, 7439-7443.

Cleary, M. L., Smith, S. D. and Sklar, J. (1986). 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.

Cowan, W. M., Fawcett, J. W., O'Leary, D. D. M. and Stanfield, B. B. (1984). Regressive events in neurogenesis. *Science* **225**, 1258-1265.

Eguchi, Y., Ewart, D. L. and Tsujimoto, Y. (1992). Isolation and characterization of the chicken bcl-2 gene: expression in a variety of tissues including lymphoid and neuronal organs in adult and embryo. *Nucl. Acids Res.* **20**, 4187-4192.

Evan, G. I., Wyllie, A. H., Gilbert, C. S. et al. (1992). Induction of apoptosis in fibroblasts by c-myc protein. *Cell* **69**, 119-128.

Fanidi, A., Harrington, E. A. and Evan, G. (1992). Cooperative interaction between c-*myc* and *bcl-2* proto-oncogenes. *Nature* **359**, 554-556.

Hengartner, M. O. and Horvitz, H. R. (1994). C. elegans cell survival gene *ced-9* encodes a functional homolog of the mammalian proto-oncogene *bcl-2*. *Cell* **76**, 665-676.

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

Hockenbery, D. M., Zutter, M., Hickey, W., Nahm, M. and Korsmeyer, S. J. (1991). BCL2 protein is topographically restricted in tissues characterized by apoptotic death. *Proc. Nat. Acad. Sci. USA* **88**, 6961-6965.

Hockenbery, D. M., Oltvai, Z. N., Yin, X.-M. et al. (1993). Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* **75**, 241-251.

Jacobsen, M. D., Burne, J. F., King, M. P. et al. (1993). Bcl-2 blocks apoptosis in cells lacking mitochondrial DNA. *Nature* **361**, 365-369.

Katsumata, M., Siegel, R. M., Louie, D. C. et al. (1992). Differential effects of Bcl-2 on T and B cells in transgenic mice. *Proc. Nat. Acad. Sci. USA* **89**, 11376-11380.

Korsmeyer, S. J. (1992). Bcl-2 initiates a new category of oncogenes: regulators of cell death. *Blood* **80**, 879-886.

Kozopas, K. M., Yang, T., Buchan, H. L., Zhou, P. and Craig, R. W. (1993). *MCL1*, a gene expressed in programmed myeloid differentiation, has sequence similarity to *BCL2*. *Proc. Nat. Acad. Sci. USA* **90**, 3516-3520.

Lagasse, E. and Weissman, I. L. (1993). bcl-2 inhibits apoptosis of neutrophils but not their engulfment by macrophages. *J. Exp. Med.* **179**, 1047-1052.

Lee, J. T., Innes, D. J. and Williams, M. E. (1989). Sequential bcl-2 and c-myc oncogene rearrangements associated with the clinical transformation of non-Hodgkin's lymphoma. *J. Clin. Invest.* **84**, 1454-1459.

Lin, E., Orlofsky, A., Berger, M. and Prystowsky, M. (1993). Characterization of A1, a novel hemopoietic-specific early-response gene with sequence similarity to *bcl-2*. *J. Immunol.* **151**, 1979-1988.

Liu, Y.-J., Mason, D. Y., Johnson, G. D. et al. (1991). Germinal center cells express bcl-2 protein after activation by signals which prevent their entry into apoptosis. *Eur. J. Immunol.* **21**, 1905-1910.

MacLennan, I. C. M. and Gray, D. (1986). Antigen-driven selection of virgin and memory B cells. *Immunol. Rev.* **91**, 61-65.

McDonnell, T. J., Deane, N., Platt, F. M. et al. (1989). bcl-2-immunoglobulin transgenic mice demonstrate extended B cell survival and follicular lymphoproliferation. *Cell* **57**, 79-88.

McDonnell, T. J. and Korsmeyer, S. J. (1991). Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). *Nature* **349**, 254-256.

McDonnell, T. J., Troncoso, P., Brisbay, S. M. et al. (1992). Expression of the protooncogene *bcl-2* in the prostate and its association with emergence of androgen-independent prostate cancer. *Cancer Res.* **523**, 6940-6944.

Miyashita, T. and Reed, J. C. (1993). Bcl-2 oncoprotein blocks chemotherapy-induced apoptosis in a human leukemia cell line. *Blood* **81**, 151-157.

Monaghan, P., Robertson, D., Amos, T. A. S. et al. (1992). Ultrastructural localization of Bcl-2 protein. *J. Histochem. Cytochem.* **40**, 1819-1825.

Nakayama, K., Nakayama, K., Nagashi, I. et al. (1993). Disappearance of the lymphoid system in *bcl-2* homozygous mutant chimeric mice. *Science* **261**, 1584-1588.

Negrini, M., Silini, E., Kozak, C., Tsujimoto, Y. and Croce, C. M. (1987). Molecular analysis of mbcl-2: structure and expression of the murine gene homologous to the human gene involved in follicular lymphomas. *Cell* **49**, 455-463.

Neilan, J., Lu, Z., Afonso, C., Kutish, G., Sussman, M. and Rock, D. (1993). An African swine fever virus gene with similarity to the proto-oncogene *bcl-2* and the Epstein-Barr virus gene *BHRF1*. *J. Virol.* **67**, 4391-4394.

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

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

Pezzella, F., Tse, A. G. D., Cordell, J. L., Pulford, K. A. F., Guter, K. C. and Mason, D. Y. (1990). Expression of the Bcl-2 oncogene protein is not specific for the 14;18 translocation. *Am. J. Pathol.* **137**, 225-232.

Pezzella, F., Turley, H., Kuzu, I. et al. (1993). bcl-2 protein in non-small-cell lung carcinoma. *New Eng. J. Med.* **329**, 690-694.

Reed, J. C., Cuddy, M., Haldar, S. et al. (1990). BCL2-mediated tumorigenicity of a human T-lymphoid cell line: synergy with MYC and inhibition by BCL2 antisense. *Proc. Nat. Acad. Sci. USA* **87**, 3600-3664.

Reed, J. C. (1994). Bcl-2 and the regulation of programmed cell death. *J. Cell Biol.* **124**, 1-6.

Schuler, G. C., Altschul, S. F. and Lipman, D. J. (1991). A workbench for multiple alignment construction and analysis. *Prot. Struct. Funct. Genet.* **9**, 180-190.

Sentman, C. L., Shutter, J. R., Hockenbery, D., Kanegawa, O. and Korsmeyer, S. J. (1991). bcl-2 inhibits multiple forms of apoptosis but not negative selection in thymocytes. *Cell* **67**, 879-888.

Smeyne, R. J., Vendrell, M., Hayward, M. et al. (1993). Continuous c-*fos* expression precedes programmed cell death *in vivo*. *Nature* **363**, 166-169.

Strasser, A., Harris, A. W., Bath, M. L. and Cory, S. (1990). Novel primitive lymphoid tumors induced in transgenic mice by cooperation between myc and bcl-2. *Nature* **348**, 331-333.

Strasser, A., Harris, A. W. and Cory, S. (1991a). bcl-2 transgene inhibits T cell death and perturbs thymic self-censorship. *Cell* **67**, 889-899.

Strasser, A., Whittingham, S., Vaux, D. L. et al. (1991b). Enforced BCL2 expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc. Nat. Acad. Sci. USA* **88**, 8661-8665.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110-156.

Tsujimoto, Y., Finger, L. R., Yunis, J., Nowell, P. C. and Croce, C. M. (1984). Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* **226**, 1097-1099.

Vaux, D. L., Cory, S. and Adams, J. M. (1988). Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**, 440-442.

Veis, D. J., Sorenson, C. M., Shutter, J. R. and Korsmeyer S. J. (1993). Bcl-2 deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* **75**, 241-251.

White, E. (1993). Death-defying acts: a meeting review on apoptosis. *Genes Dev.* **7**, 2277-2284.