The pro-survival subfamily of Bcl-2 protein family

The discovery of Bcl-2

Bcl-2: B-cell lymphoma 2

Cloning of Bcl-2 as the oncogene which is deregulated at t(14;18) lymphomas

Pioneer works by Tsujimoto et al. (Science 229:1390, 1985), Bakhshi et al. (Cell 41:889, 1985), Cleary et al. (Cell 47:19, 1986)

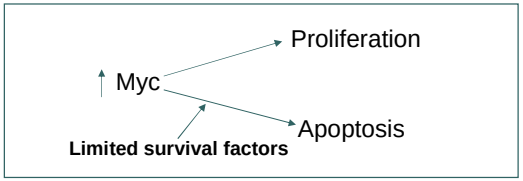
t(14;18) in 85% of follicular lymphomas and 20% of diffuse B-cell lymphomas

Translocation: Bcl-2 at chromosome segment 18q21 is juxtaposed with the immunoglobulin heavy chain locus at 14q32

Resulting in deregulated expression of Bcl-2

Bcl-2 and cell survival

Myc over-expression elicits apoptosis as well as proliferation

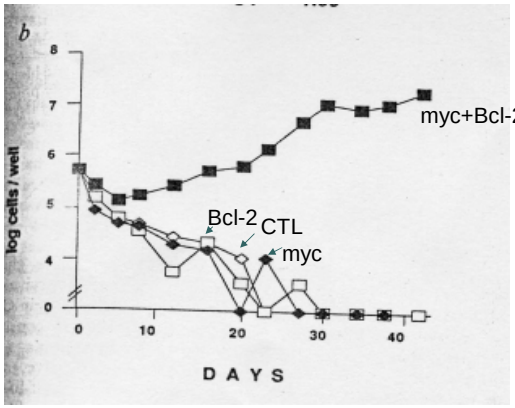


Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells.

Vaux, D. L., Cory, S. & Adams, J. M.
Nature (1988) 335, 440-442

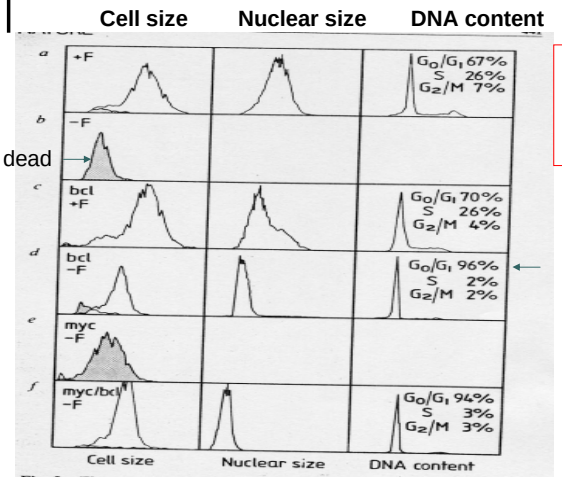
Bcl-2 cooperates with c-myc to promote proliferation of B-cell precursors

Retroviral vector of Bcl-2 transduced into Myc-transgenic B precusor



Nature 1988, 335:440-442

Bcl-2 promotes survival of pro-B cell line in the absence of IL-3



+F: +IL-3

-F: -IL-3

Bcl-2 over-expression results in cell survival and arrest in G0/G1

Nature 1988, 335:440-442

Bcl-2-overexpression leads to neoplasia which is synergized by c-myc overexpression

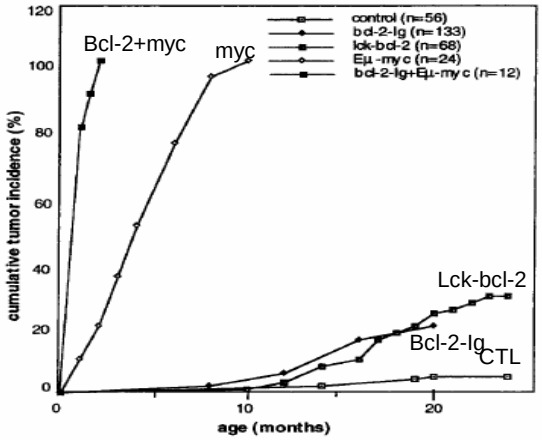


Fig 1. *BCL2* overexpression leads to neoplasia, which is synergized by *c-MYC* overexpression. Cumulative tumor incidence in *BCL2-Ig, lck^pr^-BCL2, and E$_\mu$-MYC transgenic mice and BCL2-Ig + E$_\mu$-MYC* double transgenic mice compared with littermate controls.[15-19,116]

Blood 1996, 88:386-401

The role of Bcl-2 in tumorigenesis

Discovery that Bcl-2 promotes cell survival. First recognition that cell survival is controlled *separately from cell proliferation* and that inhibition of apoptosis is a central step in tumorigenesis.

Bcl-2 represents a new class of proto-oncogene, unlike other oncogenes discovered earlier, functions in preventing programmed cell death.

Antiapoptotic BCL-2 is required for maintenance of leukemia

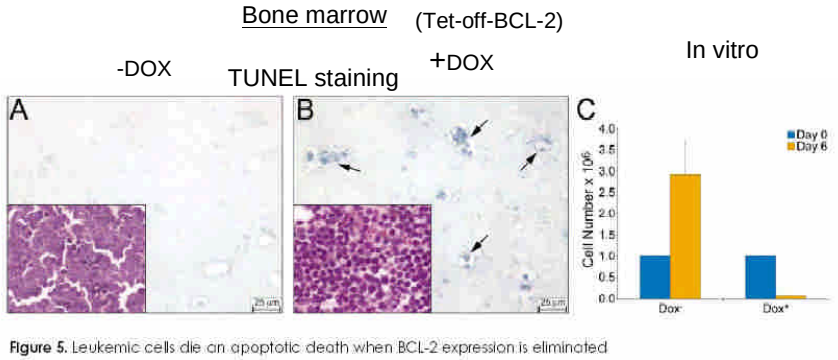


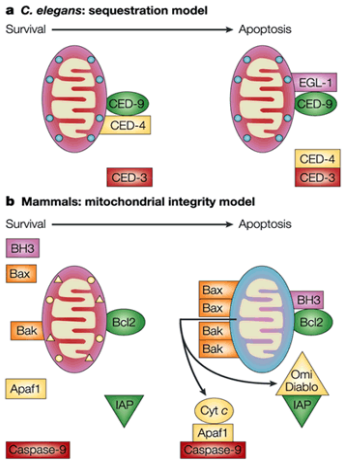
Figure 5. Leukemic cells die an apoptotic death when BCL-2 expression is eliminated

Cancer cell, 6, 241-249, 2004

Anti-apoptosis mechanisms of pro-survival molecules

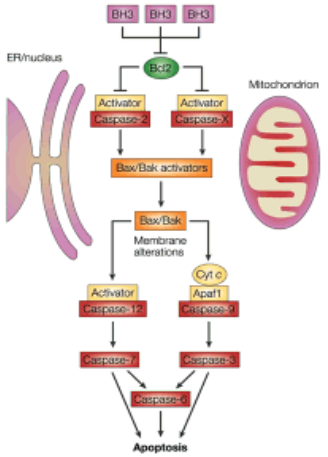
Sequestration model

Mitochondrial integrity model



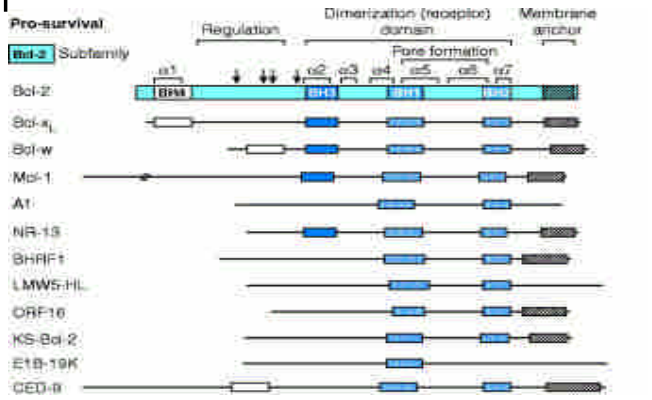
Nature Rev. Cancer 2002, 2:647-656

Anti-apoptosis mechanism of prosurvival molecules (e.g.,Bcl-2): Caspase inactivation



Nature Rev. Cancer 2002, 2:647-656

The pro-survival subfamily



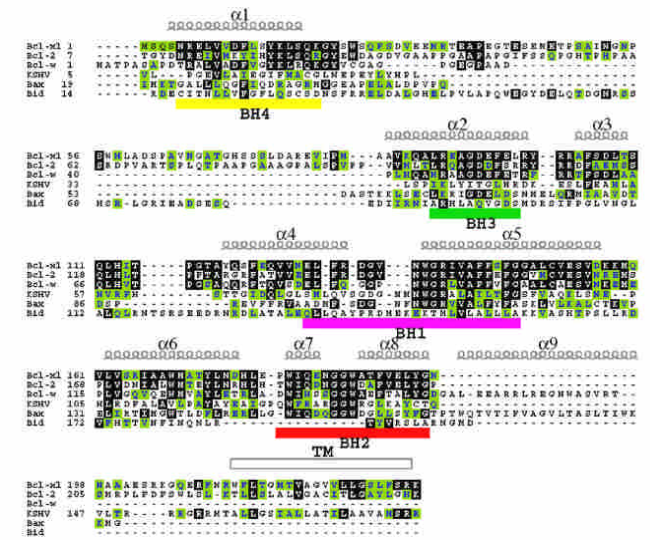
Science 1998, 281:1322-1326

BH: Bcl-2 homology

α1-7: 7 alpha helices have been found in both Bcl-2 and Bcl-x_L

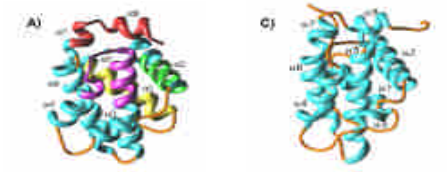
C-ternimal hydrophobic membrane domain

The structural homology among BCL-2 family members



Biochimica et Biophysica Acta 1644 (2004) 83–94

Structure and function of Bcl-2 and Bcl-x_L



Bcl-2 and Bcl-x_L show 44% identity in amino acid sequences

at least 7 α helices and a c terminal transmembrane domain

overall structure (particularly its α 5 and α 6 helices) resembles the membrane insertion domains of bacterial toxins

containing BH1 and BH2 (α 5 and α 6 helices) may form pores in membrane organelles

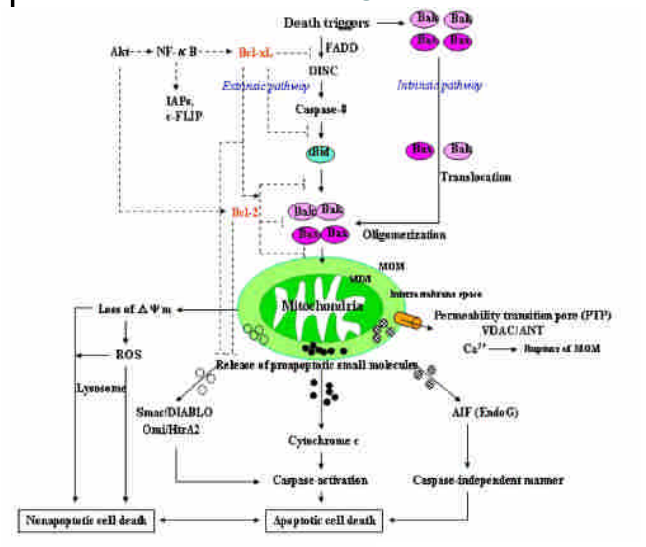
Bcl-2 and Bcl-x_L do form channels in lipid bilayers in vitro and have distinct ion selectivity

Biochimica et Biophysica Acta 1644 (2004) 83–94

Anti-apoptosis function of Bcl-2

Lymphoid cells
Growth/survival factor withdrawal (Il-2, IL-3, IL-4, IL-6, GM-CSF)
Glucocorticoid
γ -irradiation
Calcium
Neuronal
Growth/survival factor withdrawal (NGF, BDNF)
Serum withdrawal
Calcium
Infarction
Viral infections
Adenovirus
Sindbis virus
HTLV-1
Chemotheapeutic drugs
DNA synthesis inhibitors
Topoisomerase inhibitors
Microtubule inhibitors
Oxidant stress
Oncogene activation

Differential roles in regulating apoptosis by BCL-2 and BCL-xL



Biochemical and Biophysical Research Communications 333 (2005) 336–343

Lineage-specific roles for Bcl-2 and Bcl-x_L

Bcl-2	Bcl-x_L
B-cell memory	B-cell maturation
Mature B-cell survival	Double-positive thymocyte survival
Mature T-cell survival	T-cell activation
Kidney development	Brain development
Melanocyte survival	

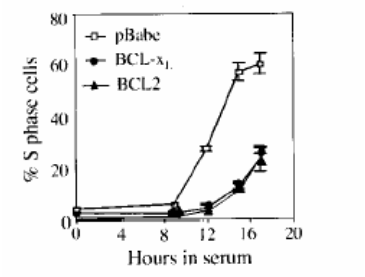
Physiological roles of BCL-2 and BCL-xL

Bcl-2 is widely expressed during embryogenesis but becomes much more restricted postnatally. Bcl-2 $^{-/-}$ mice develope normally but later exhibit marked lymphoid apoptosis, melanocyte, neuronal, and intestinal lesions and terminal kidney disease. Mice become ill and die at a few weeks of age.

Bcl-x_L $^{-/-}$ mice die in uterus around E13. They exhibit mass cell death in the central nervous system and reduced lymphoid maturation.

BCL-2 family and cell cycle regulation

Bcl-2 retards cell cycle progression

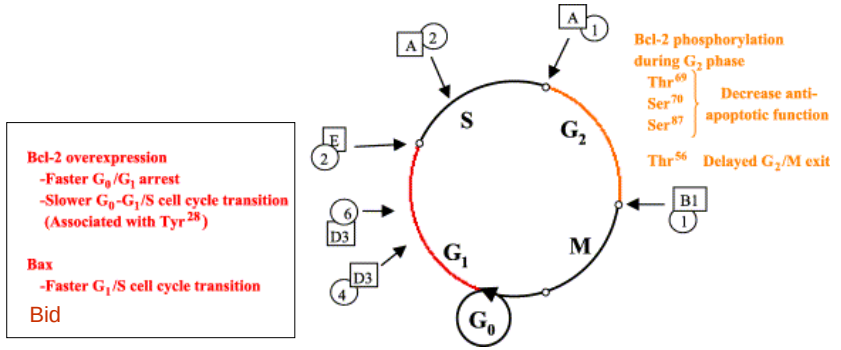


Oncogene 21, 7765-7775, 2002

Expression of a bcl-2 transgene reduced proliferation of thymocytes and delayed cell cycle entry of mitogen-stimulated B and T lymphocytes.

Overexpression of Bcl-2, Bcl-xL or adenovirus E1B19kD substantially delayed serum stimulation-induced S phase entry of quiescent NIH 3T3 fibroblasts.

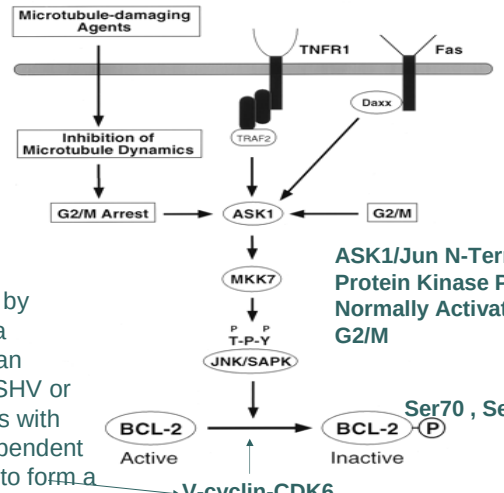
Interaction of BCL-2 family with cell cycle machinery



Biochim Biophys Acta. 2004 Mar 1;1644(2-3):159-68

Regulation of BCL-2

BCL-2 Is Phosphorylated and Inactivated

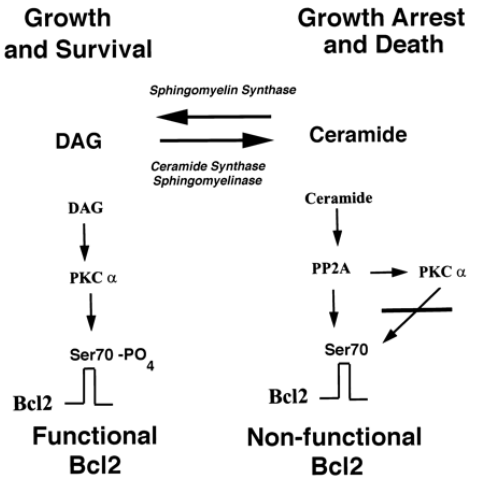


v-cyclin encoded by Kaposi's sarcoma herpesvirus/human herpesvirus 8 (KSHV or HHV8) associates with cellular cyclin-dependent kinase 6 (CDK6) to form a Bcl-2 kinase

ASK1/Jun N-Terminal Protein Kinase Pathway Normally Activated at G2/M

Mol. Cell Biol. 1999, 19:8469-8478

Phosphorylation of BCL-2 is required for its full anti-apoptotic function



J.Biol. Chem. 274, 20296-20300

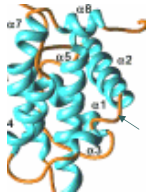
Conversion of Bcl-2 to a pro-apoptotic molecule by caspase cleavage

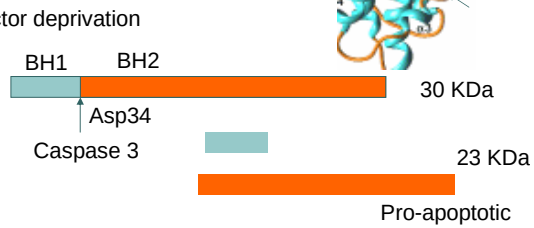
Triggered by death stimuli:

Fas ligand treatment

Sindbis virus infection

Survival factor deprivation





Cleaved Bcl-2 can behave like a pro-apoptotic molecule

As part of a amplification loop for further caspase activation

Mcl-1

Identified in a screen for differentiation-induced genes activated in the human monocytic leukemia cell line

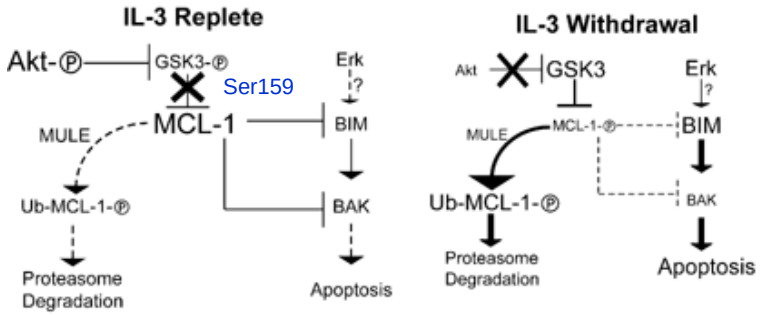
Induced expression and widely expressed

Intracellular membrane localized

Delay cell death in selected cell lines and not as potent as Bcl-2

Mcl-1 deficiency results in peri-implantation embryonic lethality

Growth factors inactivate GSK-3 and stabilize MCL-1



Cell Death and Differentiation (2006) **13,** 1260–1262

Development and maintenance of B and T lymphocytes requires anti-apoptotic MCL-1

Experimental model---
Conditional knock-out for Mcl-1

Loss of Mcl-1 displays a profound reduction of B and T lymphocytes

Loss of Mcl-1 during early lymphocyte differentiation increases apoptosis and arrests the development at pro-B-cell and double-negative T-cell stage

IL-2, IL-7, and IL-15 that promote lymphocytes survival during VDJ rearrangement induces Mcl-1 expression

Mcl-1 may selectively bind to Bim to maintain lymphocytes survival

Nature2003, 426:671-676
Opferman J. T. et al.

Bcl-w

Cloned out by PCR-based method

Confocal microscopy of HeLa cells has indicated that Bcl-w is located on the mitochondria

Surprisingly, Bcl-w did not behave like an organelle-associated protein on sub-cellular fractionation. Irrespective of the method used, only an insignificant amount of Bcl-w was recovered from membrane fractions

In healthy cells, Bcl-w was loosely attached to the mitochondrial membrane, but it was converted into an integral membrane protein by cytotoxic signals.

Structure and function of the pro-survival family

Study on the Bcl-w structure---

BH1-3 domains are in close proximity to each other and form a hydrophobic groove that is the docking site for BH3-only proteins

The binding groove is not freely accessible as predicted by previous structures of pro-survival Bcl-2-like molecules. Unexpectedly, the groove appears to be occluded by the C-terminal residues

Binding of the BH3-only proteins, critical for cell death initiation, is likely to displace the hydrophobic C-terminal region of Bcl-w and Bcl-xL

Biochimica et Biophysica Acta 1644 (2004) 83–94

Physiological roles of Bcl-w

In Bcl-w deficient mice:

the spermatogenesis is impaired.

The seminiferous tubules of adult males, however, were disorganized, contained numerous apoptotic cells, and produced no mature sperm.

In Bcl-w overexpressed transgenic (TG) mice:

Male Bcl-w TG mice developed normally but were infertile.

The adult TG testes displayed disrupted spermatogenesis

The adenoviral molecules modulate cell cycle and apoptosis

The oncogenes of adenovirus, *E1A* and *E1B*, play an essential role in establishing a productive virus infection in human cells, and are both necessary and sufficient for transformation of primary rodent epithelial cells

E1B encodes two proteins, E1B 55K and E1B 19K, which function in all or in part by inhibiting apoptosis

E1B 55K binds and inhibits the function of the p53 tumor suppressor protein, which has well-known roles in inhibiting cell cycle progression and stimulating apoptosis

Adenoviral E1A induces apoptosis

E1A stimulates entry into S phase to create the appropriate environment for the replication of viral DNA in productively infected human cells

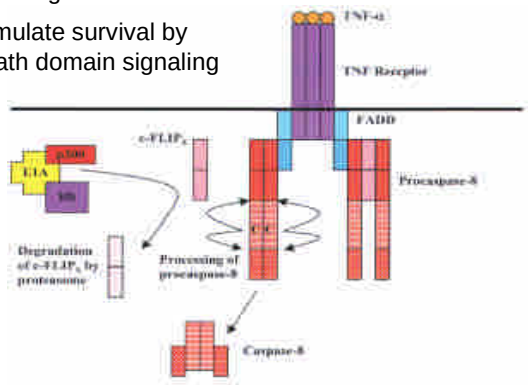
E1A accomplishes cell cycle deregulation by binding to and perturbing the normal function of key negative regulators of cell growth, including the retinoblastoma protein (Rb), and the transcriptional coactivator p300

Ironically, these very activities of E1A that are required for productive virus infection and oncogenic transformation also stimulate programmed cell death

E1A modulates death receptor signaling to induce apoptosis

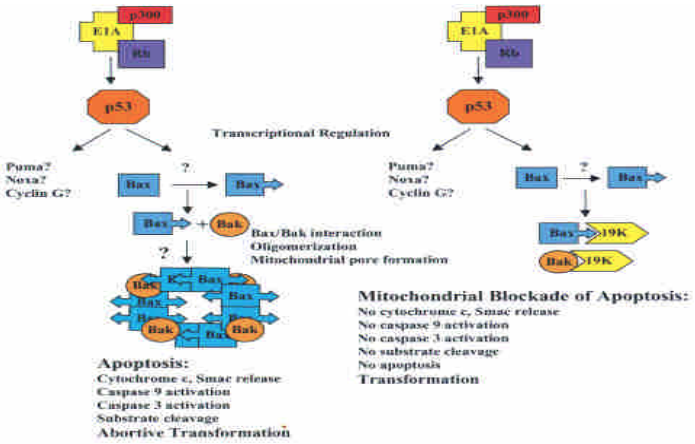
Increase c-FLIPs degradation

C-FLIPs stimulate survival by blocking death domain signaling



Oncogene 2001, 20:7836-7846

E1A increases p53 stability to induce apoptosis



Oncogene 2001, 20:7836-7846

The adenoviral E1B19K

The homology between E1B 19K and Bcl-2 includes
direct sequence homology,
functional interchangeability as apoptosis inhibitors,
and interaction with some of the same intracellular proteins, implying a related biochemical function

E1B 19K blocks apoptosis induced by E1A in productive infection, by signaling from death ligands such as TNF- α , Fas ligand (FasL), and TRAIL emanating from an immune response, and by p53 in the transformation setting

E1B 19K blocks death receptor-mediated apoptosis by preventing mitochondrial pore formation by Bax and Bak

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4. Cory S. and Adams J. M. :The BCL2 family: regulators of the cellular life-or-death switch. Nature Rev. Cancer 2002, 2:647-656.

5. Wilson-Annan J. et al.:Proapoptotic BH3-only proteins trigger membrane integration of prosurvival Bcl-w and neutralize its activity. J Cell Biol. 2003, 162:877-87.

**6. Bonnefoy-Berard N, Aouacheria A, Verschelde C, Quemeneur L, Marcais A, Marvel J.
Control of proliferation by Bcl-2 family members.Biochim Biophys Acta. 2004 Mar 1;1644(2-3):159-68**

7. Yin XM.:Bid, a BH3-only multi-functional molecule, is at the cross road of life and death. Gene. 2006 Mar 15;369:7-19.

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