BCL2 (B-Cell Leukemia/Lymphoma 2)

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Abstract

BCL2 is the milestone of apoptosis-regulatory genes. It contributes to tumorigenesis by blocking programmed cell death as such, promoting cell survival. The aberrant expression of BCL2 gene is strongly associated with resistance to chemotherapy and radiation. This review outlines the structure, function, and role of BCL2 gene in cancer.

Keywords
BCL2, apoptosis, antiapoptotic proteins, proapoptotic proteins, t(14;18), follicular lymphoma, IGH, BH3-only protein, Bcl-2 family proteins

Identity

Other names
Apoptosis Regulator, Protein Phosphatase 1, Regulatory Subunit 50, PPP1R50
HGNC (Hugo)
BCL2
Location
18q21.33
Local order
telomere to centromere orientation.

Figure 1 BCL2 (18q21) PAC 248E24 - Courtesy Mariano Rocchi

Note

BCL2 is a proto-oncogene, initially cloned as the result of its consistent involvement by t(14;18)(q32;q21) in lymphoma where its transcription becomes driven by the immunoglobulin heavy chain (IGH) gene enhancer on chromosome 14q32, subsequently leading to constitutive expression of BCL2 in B-cell clones (Tsujimoto et al, 1984, Bakhshi et al, 1985). In contrast to many oncogenes, BCL2 does not trigger cell proliferation but promotes cell survival specifically by preventing programmed cell death, apoptosis (Vaux and Adams 1988).

DNA/RNA

Description

The human BCL2 gene has a three exon structure with an untranslated first exon, a facultative 220 bp intron I, and a large 370 kb intron II. The native BCL2 gene has a long 3’ untranslated regions and
two distinct promoters, P1 and P2. The main promoter region, P1, is a TATA-less, GC-rich promoter containing multiple transcriptional start sites and located 1386-1423 bp upstream of the translation start site. The use of alternative promoter results in mRNAs comprised of exons II/III or I/II/III. The t(14;18) translocation in B-cells generates heterogeneous 4.2 - 7.2 kb BCL2-IGH chimeric mRNAs resulting from alternative BCL2 5’ exons and varied IGH 3’ untranslated regions. The t(14;18) does not interrupt the BCL2 open reading frame, however, inappropriately high levels of BCL2-IGH mRNAs are present. The native BCL2 and BCL2-IGH fusion mRNAs demonstrate the same 2.5-hour short half-life (Clearly et al, 1986, Tsujimoto et al1986, Seta et al1988)

Protein

Description
The BCL2 gene encodes a 26 kd protein consisting of 239 amino acids with a single highly hydrophobic domain at its C-terminus, which enables it to localize mainly in the mitochondrial outer membrane, and to a lesser extent in the nuclear envelope and the membrane of the endoplasmic reticulum (Chen-Levy 1989). The main role of BCL2 protein is to maintain the integrity of the mitochondrial membrane, preventing cytochrome c release and its subsequent binding to APAF1 (apoptosis activating factor-1). The protein contains all four BCL2 homology (BH) domains (BH1 to BH4). BH1, BH2 and BH3 constitute the hydrophobic cleft through which the protein interacts and forms homo- and heterodimers with the pro-apoptotic members of the BCL2 family of proteins (Thomadaki 2006, Reed 2006). BCL2 increases the survival kinetics of the cell specifically by blocking apoptosis. Thus it prevents the cell from going into suicidal activities that usually require ATP, new RNA, and protein synthesis, and inducing a variety of cellular ultrastructural changes such as cell shrinkage, nuclear fragmentation, and DNA degradation.

Expression
BCL2 expression is widespread in immature tissues prenatally whereas its expression becomes highly restricted with maturation. BCL2 is extensively expressed in immature B-cells and memory B cells but is temporally down-regulated in germinatal center B-cells, partially because of repression by BCL6. High levels of BCL2 have been detected in thymus throughout the medulla, in spleen and in lymph nodes as well as early in the embryonic kidney. Decrease in its expression levels is observed in motor-neurons as well as in pre-B cells being prepared to differentiate (Thomadaki 2006).

Function
BCL2 Protein Family and Apoptosis
The BCL2 family is a prototype of a large family of evolutionarily related proteins that share a high degree of homology although they exert different functions. This family consists of 25 pro-apoptotic and anti-apoptotic members which interact to maintain a balance between newly forming cells and old dying cells. These proteins are localized to the membrane of mitochondria and endoplasmic reticulum, operating as guardians of these organelles (Thomadaki 2006, Reed 2008, Aki et al 2014)).
BCL2 family proteins play central roles in cell death regulation and can regulate diverse cell death mechanisms including apoptosis, necrosis and autophagy. They are the key regulators of the mitochondrial pathway of apoptosis. This pathway is required for normal embryonic development and for preventing cancer. These proteins control the permeabilization of the mitochondrial outer membrane (MOM) that releases cytochrome c and other apoptotic factors into the cytosol. BCL2 family proteins share up to four BCL2 homology domains (BH1, BH2, BH3, & BH4) and are generally divided into two categories, anti-apoptotic and pro-apoptotic proteins based on their intracellular function and sequence homology. The anti-apoptotic proteins, BCL2, BCL2L1 (BCL-XL), BCL2L2 (BCL-W), MCL1, BCL2A1 (A1/BFL-1), share homology within four domains (BH1-4). These proteins form a characteristic helical bundle fold, which is critical for their ability to bind to the pro-apoptotic BCL2 family members and thereby exert their antiapoptotic function. The pro-apoptotic proteins such as BAX, BAK1, and BOK share BH1-3 domains whereas other pro-apoptotic proteins, such as BCL2L11 (BIM), BAD, and BID, contain only the BH3 domain and are known as BH3-only proteins (Figure 3). In normal situations, the BH3-only proteins are inactive or exist at low levels in the cell. However, in the presence of apoptotic stimuli the BH-only proteins become activated by post-translational modifications or their expression are increased. The stimulation of BH3-only proteins induces BAX-BAK oligomerization. After their oligomerization, BAX and BAK directly cause MOM permeabilization, a critical step in apoptosis (Aki et al 2014). The role of anti-apoptotic BCL2 proteins is to neutralize pro-apoptotic BH3-only proteins and thus inhibit their effect on BAX-BAK activity and MOM permeabilization. The balance between pro-survival and pro-death BCL-2 proteins is a major factor in determining if cells undergo apoptosis in response to cell stress.

**Control of Proliferation by BCL2**

Although BCL2-family proteins are key players in the control of mitochondria-based apoptosis, they can also control cell proliferation. High levels of BCL2 protein were reported to be associated with a lower proliferative capacity of human lymphoma, suggesting a negative control on proliferation. The anti-proliferative effect of BCL2 acts mainly at the level of the G0/G1 phase of the cell cycle. Deletions and point mutations in the BCL2 gene show that in some cases the anti-proliferative activity of BCL2 can be dissociated from its anti-apoptotic function (Bonnefoy et al, 2004). This indicates that the effect of BCL2 on cell cycle progression can be a direct effect and not only a consequence of its anti-apoptotic activity. BCL2 appears to mediate its anti-proliferative effect by acting on both signal transduction pathways (NFAT, ERK) and on specific cell cycle regulators. In addition, BCL2 cooperated with MYC to promote proliferation of B-cell precursors.

**Figure 3** BCL-family proteins have 1-4 domains (BH1, BH2, BH3 or BH4) and a transmembrane domain (TM). Anti-apoptotic BCL2-family members contain all four BH domains. Proapoptotic BCL2-family members are either multi-domain or BH3-only proteins.

**Implicated in**

Apoptosis is essential for normal embryonic development, maintenance of tissue homeostasis, and development and function of the immune system. In contrast, processes that interfere with normal apoptosis promote cell survival and,
potentially, oncogenesis. Therefore, dysregulation of
BCL2 family has a major role in tumor formation.
The BCL2 family is also involved in other diseases,
such as autoimmune, infectious and neurodegenerative disorders. The autoimmune
disease such as type 1 diabetes can be caused by
defective apoptosis, and schizophrenia may result from
an abnormal ratio of pro- and anti-apoptotic
factors (Strasser et al, 2011). On the other hand,
there is increasing evidence that BCL2 family
proteins also have additional functions in other
cellular processes, such as mitochondrial
morphology and metabolism, which remain largely
unexplored. Apoptosis is regulated by a balance of
pro-apoptotic factors and anti-apoptotic factors.

**t(14;18)(q32;q21)**
The **t(14;18) Breakpoints in Lymphoma**
The t(14;18)(q32;q21) constitutes the most common
chromosomal translocation in human lymphomas,
being present in over 85% of follicular lymphoma
(FL) and in up to 30% of diffuse large B-cell
lymphoma (DLBCL). BCL2 is normally located on
chromosome 18q21.33 in a telomere to centromere
orientation. The molecular consequence of the
**t(14;18)** juxtaposes of the BCL2 gene next to IGH locus on the der(14) chromosome, in the same
transcriptional orientation as the IGH gene. The
breakpoints on chromosome 18 are clustered with
50-60% fall within a 2.8 kb major breakpoint region
(MBR), located in the untranslated 3’ UTR of the
BCL2 gene, and another 25% falls in the minor
cluster region (MCR) (Bakhshi 1987, Willis and
Dyer 2000). Others cluster within a third, intermediate cluster region midway between the
MBR and MCR while other breakpoints have been
described scattered through this region. In rare
variant translocations involving the IG light chain
loci, t(2;18)(p12;q21) and (18;22)(q21;q11.2), the
breakpoints are located in the 5’ noncoding region of
the BCL2 gene. The breakpoints on chromosome 14 occur most commonly just 5’ of IGH JH segments
within sequences that typically show evidence of
exonucleolytic “nibbling”, N-hp additions, and D
segment addition, events that occur normally during
attempted V(D)J recombination. Less commonly,
IGH breakpoints may occur at sites 3’ of the JH segments, or rarely even in IG switch regions. BCL2
mRNA expression is up-regulated in the translocated allele through the action of IGH E[1] enhancer
sequences, which are highly active in germinal
center B cells. In the cases of rearrangements falling
in the MBR, a BCL2/IGH fusion mRNA transcript
is produced whereas rearrangements in the MCR
lead to increased levels of a normal BCL2 mRNA
(Aster and longtine 2002). The t(14;18) translocation
or its variants does not interrupt the protein-encoding
region of BCL2 gene so that the normal and the
translocated alleles produce the same-sized, 26-kd
protein, a member of a family of proteins involved in
the regulation of apoptosis. The somatic
hypermutation mechanism associated with the IGH
gene often induces mutations in the BCL2 gene
which may further dysregulate its expression and can
also lead to point mutations in the coding regions of
Therefore, a subset of t(14;18) positive lymphomas
do not express intact BCL2 protein due to somatic
mutations of the gene.

The majority of follicular lymphomas in adults
depend on BCL2 overexpression which is almost
always the result of t(14;18) translocation or its
variants. BCL2 overexpression sustains cell survival
but is not sufficient for FL development, thus other
genetic lesions or epigenetic events are required.
This is supported by the observation that BCL2
transgenic mice develop polyclonal hyperplasia of
mature, long lived non-dividing B cells. With time,
a fraction of BCL2 transgenic mice develops
generative disorders. The autoimmune
region of BCL2 gene so that the normal and the
translocated allele produces the same-sized, 26-kd
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The **Cytogenetics**

**Diagnostic use of BCL2**

Fluorescence in situ hybridization (FISH) is widely
used in clinical laboratories to detect BCL2 gene
rearrangement in fresh tissue and paraffin embedded
tissue sections using dual color translocation DNA
probes (Figure 4). BCL2 protein expression by
immuno-histochemistry represents a rapid and
inexpensive method to identify BCL2
overexpression. In normal tissue, BCL2 antibodies
react with B-cells in the mantle zone, as well as some
T-cells. Because BCL2 expression is down-
regulated in normal germinal centers, the presence
of BCL2 protein can help to distinguish follicular
lymphomas from reactive follicular hyperplasia.
However, positive cells increase considerably in
follicular lymphoma, as well as many other forms of
cancer. In some cases, the presence or absence of
BCL2 staining in biopsies may be significant for the
patient’s prognosis or likelihood of relapse.
Figure 4  Karyotype from follicular lymphoma showing t(14;18)(q32;q21) [arrow]; Insert: FISH with IGH/BCL2 DNA probes showing a double fusion signals confirming t(14;18) [red & green fused signals]

BCL2 Expression in Cancer

BCL2 is upregulated in almost 50% of all human cancers, consistent with its role as an apoptotic regulator (Cory et al, 2003, Yip and Reed, 2008, Reed 2008). The majority of small cell lymphoma such as chronic lymphocytic leukemia (CLL), marginal cell lymphoma, and mantle cell lymphoma, over-express BCL2, although less than 5% of those patients have detectable BCL2 gene rearrangement (Hanada et al, 1993). Increased expression of BCL2 is also found in nearly all patients with acute lymphocytic leukemia and frequently in acute myeloid leukemia (Yip and Reed 2008). Most of adult FL cases have overexpression of BCL2 protein however, the pediatric type FL is negative for BCL2 expression. Approximately 30% of DLBCL patients are categorized as having relatively high BCL2 expression (Monni et al, 1997). BCL2 may also play a role in non-hematologic tumors, and inappropriate expression has been observed in solid tumors such as prostate, breast, and small cell and non-small cell lung cancers. In small cell lung cancer, high BCL2 expression in >90% of patients have been reported (Hellemans et al 1995, Jiang et al 1995, Anagnostou et al 2010, Henriksen et al 1995). Ovarian, neuroblastoma, bladder, colorectal, and some head and neck cancers have all exhibited high expression of BCL2.

Mechanisms of BCL2 Activation

Besides chromosomal translocations as a key mechanism for activation of the BCL2 gene in lymphoma, overexpression as a result BCL2 amplification has been demonstrated in non-Hodgkin's lymphomas and small cell lung cancers (Monni, O, 1997). Other contributing mechanisms are deletion of endogenous microRNAs (miRs) such as MIR195, MIR24-2 and MIR-365B that normally repress BCL2 gene expression (Cimmino et al., 2005). This mechanism has been documented in CLL, where the genes encoding MIR15 and miR16 become deleted or inactivated by mutations in >70%

Expression of BCL2 and Prognosis

Multiple studies have shown that high levels of BCL2 gene expression is a negative risk factor correlated with severity of malignancy. Elevated expression of BCL2 in AML was shown to be associated with poor clinical response to chemotherapy (Campos et al, 2005). BCL2 expression correlates negatively with overall survival within a specific subgroup of DLBCL (Iqbal et al, 2006). Additionally, several studies have demonstrated a correlation between elevated BCL2 expression and poor prognosis in melanoma, breast, prostate, small cell lung, colorectal and bladder cancers (Anagnostou et al, 2010). Further studies have proven that higher BCL2 expression leads to resistance to chemotherapy and radiation (Reed 2008, Review).

BCL2 for Targeted Therapy

Some cancers, particularly non-Hodgkin lymphoma, are dependent on BCL2 for survival. BCL2 is also involved in the development of resistance to chemotherapeutic agents, further stressing the importance of targeting the BCL2 gene in cancer therapeutics. Numerous approaches have been developed to block or modulate the production of BCL2 at the RNA level, at the protein level, or at the DNA level. From a clinical perspective, treatment with novel, potent BCL2 inhibitors either alone or in combination with conventional therapies hold significant promise for providing beneficial clinical outcomes. The BCL2 targeted drug has the potential
to enhance cell killing when used alone or in combination with traditional cytotoxic agents, which may lead to greater efficacy and reduce toxicity of chemotherapy (Ebrahim et al 2016, Delbridge et al, 2016).

Antisense Oligonucleotides (ASOs)
Antisense technology involves the use of a sequence that is complementary to a specific mRNA which inhibits its expression and subsequently induces a blockade in the transfer of genetic information from DNA to protein. Oblimersen sodium, an example of ASO agent, is an 18-base antisense phosphorothioate oligonucleotide compound designed to specifically target the first six codons of the human BCL2 mRNA sequence, resulting in degradation of BCL2 mRNA and a subsequent decrease in BCL2 protein translation and intracellular concentration (Herbst et al, 2004). Oblimersen has been evaluated for suitability of the treatment of a number of cancers, including small cell lung cancer, prostate cancer, renal cell carcinoma, as well as non-Hodgkin lymphomas. This compound is already well advanced in clinical trials for the treatment of refractory CLL, multiple myeloma and melanoma. Studies showed that treatment with Oblimersen alone resulted in some long-term disease-free survival, while combination therapy with cyclophosphamide resulted in long-term disease-free survival with no histological or molecular evidence of lymphoma. Of note, treatment with antisense oligonucleotides lowers the concentration of other chemotherapies required for treatment, decreasing side effects and toxicity.

Small-Molecule Protein Inhibitors (SMIs)
Small-molecule inhibitors (SMIs) are a group of drugs designed to mimic BH3-only proteins to inhibit the action of anti-apoptotic BCL2 proteins. They compete with pro-apoptotic BCL2 to occupy BH3 docking grooves on the surfaces of anti-apoptotic family members, thus functioning as valuable anti-neoplastic drugs. One such drug is Obatoclax (GX15-070) D, a new experimental pan inhibitor of BCL2-family proteins, particularly to MCL1, as many hematologic malignancies depend on this protein for survival. Obatoclax can induce oligomerization of BAK in the mitochondria, interrupting its function, and activate caspases leading to cell death and cell cycle arrest. Obatoclax was shown to overcome drug resistance and potentiate the efficacy of traditional chemotherapeutic agents.

DNA interference (DNAi)
DNAi therapeutic drugs, such as PNT2258, are a class of nucleic acid-based therapy that contain sequences designed against noncoding, non-transcribed regions of genomic DNA upstream of gene transcription initiation sites, thus effectively blocking its transcription. DNAi can interact with genomic DNA leading to an apoptotic cell death cascade by gene silencing. This approach is aimed at blocking BCL2 gene transcription. Evaluations of this technology in preclinical and early clinical studies are very encouraging and strongly support further development of DNAi as cancer therapeutics.

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