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BCL2

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Synonyms

- B-cell **leukemia**/lymphoma-2 gene (*Bcl2*)
- Bcl2
- Apoptosis regulator Bcl2
- B-cell CLL/lymphoma 2
- B-cell lymphoma protein 2

Definition

The Bcl2 family of proteins belong to a peculiar class of proteins regulating **apoptosis**, **cell cycle**, **differentiation**, and **autophagy**; in oncology, the genes coding for these proteins could not be defined neither as dominant transforming **oncogenes** (such as **myc**), nor **tumor suppressor genes** (such as **p53**). They could be best defined as apoptosis-related genes, a definition that stresses the importance of apoptosis (and of its dysregulation) in the genesis and development of cancer in humans and other species. Dysregulation of apoptosis is involved also in the development of diseases other than cancer, such as autoimmune diseases, AIDS and various degenerative pathologies.

Characteristics

The Bcl2 family encompasses several members divided in anti-apoptotic and pro-apoptotic proteins; among the anti-apoptotic are Bcl2 and BclXL, whereas among the pro-apoptotic are Bax, Bak, **Bid** and Bad. These proteins contain conserved Bcl2 homology (BH) domains (termed BH1, BH2, BH3 and BH4), together with a transmembrane domain, all being identified as crucial for the regulation of apoptosis. In addition, based on functional studies and the conservation of BH domains, the Bcl2 family can be further divided into three subgroups. The Bcl2 subgroup includes all anti-apoptotic proteins, such as Bcl2 and BclXL. The Bax subgroup consists of pro-apoptotic members, such as Bax, Bak, and Bad. Both groups contain more than one BH domain. The third subgroup contains BH3-only proteins, such as Bid and Bim, which can interact with either anti-apoptotic proteins or pro-apoptosis members. The observation that inhibitors and inducers of cell death interact with each other by forming homodimers or heterodimers suggests that apoptosis is regulated, at least in part, by protein-protein interaction. By means of two alternative transcripts (a and b), Bcl2 codes for a protein of 205 amino acids (Bcl2b), or 239 amino acids (Bcl2a); both proteins contain BH domains for homo/heterodimerization with members of the Bcl2 family of proteins. The BH4 domain is required for anti-apoptotic activity and also for interaction with the serine-threonine kinase encoded by **Raf-1**, an oncogene homologous to **protein kinase C**, that is the target of several tumor promoters. The hydrophobic carboxyl terminus of the protein determines association with cellular membranes; also this tail seems necessary for the anti-apoptotic function. Proteins of the Bcl2 gene family are evolutionarily conserved from the Sponges to man.

Functions

The main biological function of Bcl2 is to inhibit apoptosis or, conversely, to promote cell survival. Other related biological functions concern the control of cell cycle. In fact, Bcl2, as well as the anti-apoptotic members of this family of proteins, are anti-proliferative by facilitating G0, thus suggesting that cell survival is maintained at the expense of proliferation. In hematopoietic cell lines, these functions are crucial for differentiation, and Bcl2 might also have a direct role in cell fate decision beyond strict cell survival. In addition, Bcl2 family members are involved in the control of autophagy. As far as cell survival is concerned, it appears that the cell fate is dependent on the amount of intracellular Bcl2 protein; an increase (over-expression) of Bcl2 protein is associated with prolonged survival and apoptotic protection, whereas its decrease is associated with apoptosis or enhanced sensitivity to apoptosis-inducing agents. For example, HIV-specific CD8+ T cells show a significantly reduced expression of Bcl2, potentially priming them to apoptosis. Conversely, in the development of cancer, Bcl2 over-expression inhibits the apoptosis of cancer cells bearing mutations, thus being a key determinant of neoplastic cell expansion and resistance to anticancer treatments. As a consequence, cancer cell death is delayed, and cancer cell accumulation occurs.

At the molecular level, inhibition of apoptosis as well as control of cell cycle, differentiation and autophagy, occur through a complex process of protein-protein interaction. In the inhibition of apoptosis this process involves heterodimerization, especially with the pro-apoptotic member of the Bcl2 family. In addition to homo/heterodimerization within the Bcl2 family members, the anti-apoptotic members of the Bcl2 family also interact with other proteins regulating apoptosis, such as **caspsases** and **APAF1**. Formation of complexes with these proteins involved in the actuation of apoptosis prevent them to initiate the protease cascade eventually leading to cell death.

The multiple independent functions of Bcl2 proteins are mediated by the BH domains and the hydrophobic helices. These functions can be grouped in two main categories: 1. A function as membrane channels for ions and proteins. 2. A function as membrane adaptor/docking proteins. The first hint about Bcl2 function came from studies on the three-dimensional structure of the Bcl2 analogue, anti-apoptotic Bcl-XL. It showed a surprising similarity to the pore-forming domains of some bacterial toxins that cause the formation of channels for ions, proteins or both. It was observed that Bcl2 and its homologues are localized to intracellular membranes, in particular, the outer **mitochondrial membrane**, the endoplasmic reticulum and the intracellular membrane of the nuclear envelope. In these areas they have a membrane transport function for calcium ions and proteins. The channels created by Bcl2 insertion into membranes resemble the pores formed by certain bacterial toxins. Thus, the two long hydrophobic helices of the protein core insert deeply through the phospholipid bilayer, perpendicular to the membrane surface, and the rest of the protein undergoes conformational changes resembling the opening of an umbrella with the five surrounding amphipathic helices resting on the top of the membrane. The ability to form channels, by insertion of the two hydrophobic helices, is essential for Bcl2 anti-apoptotic function. However, by analogy with other channel-forming proteins, the Bcl2 channels are formed by two or more proteins of the Bcl2 family. Thus, there is the possibility that anti- and pro-apoptotic members of the Bcl2 family form homo- or heterodimers. In fact, the pro-apoptotic members of the family also have channel forming activity, although the channels formed by these proteins might have different transport selectivity or subcellular localization. Heterodimerization of anti- and pro-apoptotic Bcl2 family proteins might lead to the formation of different channels or, alternatively, the heterodimers might be unable to form channels at all. Schematically, the channels formed by Bcl2 and the other anti-apoptotic members prevent apoptosis, possibly transporting back, and thus antagonizing, the pro-apoptotic factors that outflow through the channels formed by the pro-apoptotic members of the Bcl2 family. For example, Fas ligand, a well characterized inducer of apoptosis, activates a member of the caspase family (caspase 8) that cleaves pro-apoptotic Bid. Once truncated, Bid translocates to mitochondria where it might function as a channel protein to release cytochrome c, thus activating cytosolic caspases that are the terminal effectors of apoptosis. Bcl2 inhibits the release of cytochrome c either by plugging the channels opened by Bid, or by transporting cytochrome c back to the mitochondria. Also in this case, the level of expression and the ratio between anti-apoptotic and pro-apoptotic Bcl2 family proteins is critical in deciding cell death or survival.

In addition to the channel forming properties, Bcl2 family proteins interact with a number of signal transducing proteins involved in apoptosis and other crucial cellular processes. These include the protein kinase C homologue Raf-1, the **G-proteins H-Ras** and R-Ras, the p53-binding protein p53-BP2, the pro-apoptotic protein CED-4, (homologue to APAF1), and the protein phosphatase calcineurin. These interactions are mediated by specific BH domains; for example, the BH4 domain has been reported to bind with calcineurin, Raf-1, and CED-4. The association between Bcl2 and these proteins might be responsible for their translocation to intracellular membranes where Bcl2 is anchored. This may lead to changes of their activity, such that they might be sequestered and inactivated, or targeted for interaction with other membrane-associated proteins. For example, Raf-1 is a serine/threonine kinase that transduces mitogenic signals from membrane receptors to the nucleus. Association between Raf-1 and Bcl2 causes translocation of the protein kinase to the mitochondrial membrane where Bcl2 is located. Once there, Raf-1 phosphorylates and inactivates Bad, one of the pro-apoptotic members of the Bcl2 family. Phosphorylated Bad is sequestered in the cytosol, engaged by an adaptor protein termed 14-3-3, and thus unable induce apoptosis. In the absence of growth/survival factors (such as in IL-3 deprivation of IL-

3-dependent hematopoietic cell lines), Raf-1 is not activated and the unphosphorylated Bad is able to induce apoptosis. Protein-protein interaction is also responsible for Bcl2 biological functions other than control of apoptosis. In fact, interaction between the catalytic domain of Raf-1 and the BH4 domain of Bcl2 in multipotent hematopoietic progenitor cells is critical in determining the erythroid/myeloid fate of differentiating cells. Another protein originally isolated as a Bcl2-interacting protein, is Beclin 1, the first identified mammalian autophagy gene product. Bcl2 negatively regulates Beclin 1-dependent autophagy and Beclin 1-dependent autophagic cell death, thus raising the possibility that Bcl2 family members also regulate autophagy.

Regulation

The first association between Bcl2 and human cancer was observed in follicular lymphomas bearing the t(14;18) chromosomal translocation by which the gene was cloned. This translocation brings the Bcl2 gene to chromosomal location 18q21 into juxtaposition with the immunoglobulin heavy-chain locus at 14q32, resulting in transcriptional de-regulation of the Bcl2 gene. This event does not involve alterations of the coding regions of the gene. Subsequently, Bcl2 over-expression was recognized as a general feature of various types of hematological and solid malignancies. Thus, many members of the Bcl2 family have been found to be differentially expressed in various malignancies, and some are useful prognostic cancer biomarkers. Whether through its function as a channel protein or as an adaptor/docking protein, the final result on cell fate, however, depends upon the level of expression of Bcl2. Therefore, the control of Bcl2 expression has been the object of numerous studies of transcriptional, translational and post-translational regulation. Over-expression of Bcl2 has been associated with hypomethylation in the promoter region. In normal cells, once apoptosis is initiated, Bcl2 is proteolytically cleaved by caspases. Interestingly, the cleaved protein, lacking the BH4 domain, has pro-apoptotic activity, and causes the release of cytochrome c into the cytosol thus promoting further caspase activity. Bcl2 family proteins are also regulated by phosphorylation that affects their activity and conformation. The structural analysis of anti-apoptotic members of Bcl2 family led to the discovery of an unstructured "loop region" near the N-terminus exposed to the cytoplasm. The anti-apoptotic members of Bcl2 family such as Bcl2 and Bcl-XL are phosphorylated on specific serine/threonine residues within this unstructured loop in response to diverse stimuli including treatment with chemotherapeutic or chemopreventive agents. In most instances, such phosphorylation has been associated with the loss of their biological (anti-apoptotic) function. The chemoresistant tumors often overexpress Bcl2/Bcl-XL. In these instances, the apoptosis yielding effect due to phosphorylation of anti-apoptotic Bcl2 family members is quite interesting because phosphorylation-dephosphorylation pathway of these anti-apoptotic proteins could be an ideal molecular target for therapy of subpopulation of cancer in which these cell death repressors are essential prognostic markers. Thus, further gaining the knowledge on the mechanism of inactivation of Bcl2/Bcl-XL by phosphorylation might be of significant importance to therapy for human malignancies in which over-expression of these anti-apoptotic proteins is recognized.

Bioactivity

Oncogenes and tumor suppressor genes modulate Bcl2 expression with profound results on death or survival of cancer cells. The tumor suppressor gene p53 can induce apoptotic cell death by down-regulation of Bcl2 and up-regulation of Bax. The p53-dependent negative response element on Bcl2 has the features of a transcriptional silencer, mediating inhibition of transcription in an orientation-dependent manner. In a variety of tumors, p53 expression is associated with apoptosis and with sensitivity to DNA damaging agents (anticancer drugs and **ionizing radiations**), by enhancing the transcription of a gene that favours apoptosis (Bax), at the same time blocking the transcription of a gene that would protect cancer cells from apoptosis (*i.e* Bcl2). Bcl2 over-expression is able to hinder p53-induced apoptosis, but it is ineffective against p53-dependent growth arrest. However, when Bcl2 is expressed together with the proto-oncogene c-myc, both p53-induced growth arrest and apoptosis are counteracted. In recent years, however, the role of mutations of single genes in the genesis of cancer has been questioned, and it was proposed instead that cancer is a chromosomal disease. According to this hypothesis, carcinogens initiate chromosomal evolutions via unspecific aneuploidies. By unbalancing thousands of genes, **aneuploidy** corrupts teams of proteins that segregate, synthesize and repair chromosomes. Aneuploidy is thus considered a steady source of karyotypic-phenotypic variations from which selection of further cancer-specific aneuploidies encourages the evolution and subsequent malignant progression of cancer cells. The rates of these variations are proportional to the degrees of aneuploidy, and can exceed conventional mutation by 4-7 orders of magnitude. In this scenario, the role of anti-apoptotic genes, such as Bcl2, is even more paramount as they provide the opportunity for cancer cells to survive despite gross aneuploidy and to accumulate complex, malignant phenotypes.

References

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