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BCL2 Gene: Current Relevance to Clinical Oncology

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INTRODUCTION

THE BCL2 gene, whose name derives from its association with B-cell lymphoma, is an unusual and fascinating candidate proto-oncogene. It has been identified by its proximity to a translocation site on chromosome 18q21 in t(14;18) translocation present in the majority of nodular low-grade lymphomas and in a proportion of high-grade lymphomas. In t(14;18) the BCL2 gene is placed adjacent to the immunoglobulin (Ig) heavy-chain gene on chromosome 14q32. The consequence is deregulation of expression of the BCL2 gene with increase in BCL2 protein which may be an important step in the pathogenesis of B-cell lymphomas. Increased understanding of the control mechanism regulating the production of BCL2 and the function of the BCL2 protein opens the door to the *in vivo* manipulation and possible treatment of low grade non-Hodgkin lymphoma.

BCL2 GENE AND ITS PRODUCT

The BCL2 gene on chromosome 18q21 consists of three exons separated by large introns [1]. The whole gene spans at least 230 kb [2]. The coding portions of the gene lie within exons 2 and 3 with large untranslated regions, both at the 5′ and 3′ ends.

The gene encodes two proteins—26 kDa BCL2 alpha and 21 kDa BCL2 beta, which differ only at the carboxy terminal [3]. At the time of initial sequencing the function of the BCL2 protein was not known. The only clue to its possible role in oncogenesis was limited to some sequence homology with a predicted Epstein–Barr virus (EBV) protein [4]. Antibody localisation, fractionation experiments and the hydrophobic nature of the carboxy terminal suggested association of the BCL2 protein with intracellular membrane [5, 6] and search was on for its role as a receptor mediating growth factor signals. The putative transmembrane localisation and possible transduction properties of the BCL2 [7] suggested growth factor receptor/-transducer function and the initial candidate was a G protein [8, 9]. However it seems unlikely that BCL2 belongs to a GTP-binding protein family [10] particularly as it has now been localised as an integral protein of the inner mitochondrial membrane [11] which is unique among proto-oncogenes. The main function of the inner mitochondrial membrane is in energy metabolism essential to cell survival. It includes functions such as oxidative phosphorylation, electron and metabolite transport and these would be novel functions for a putative oncogene.

The amount of BCL2 present in normal lymphocytes is small as it cannot be detected by immunohistochemical staining with BCL2 antibodies. However, the level fluctuates with stages of differentiation and proliferative activity. Transcription of the BCL2 transiently increases when the pre-B cell is developing into mature B cells and subsequently is downregulated [12]. BCL2 mRNA levels also increase in B and T cells in response to proliferation signals [13], although the protein remains undetectable by immunohistochemistry in normal active germinal centres. BCL2 is therefore a mitochondrial membrane protein which appears to be involved in growth regulation of B and T cells.

The translocation of BCL2 in t(14;18) results in deregulation of gene expression which leads to excessive levels of BCL2 protein in pre-B and mature cells. There is increased transcription rate of a fused BCL2/Ig chimaeric mRNA [14] but the final BCL2 protein is structurally normal. High levels of BCL2 confer a survival advantage on the B cell and this may be a factor in the development of malignancy in these cells (see below).

BCL2 gene in lymphoma

The BCL2 gene has been cloned from translocation breakpoint on chromosome 18. The translocation has been detected by karyotyping, Southern blotting with BCL2 probes and by polymerase chain reaction (PCR). It is found in the majority of follicular low-grade lymphomas, in 20–30% high grade lymphomas [15–18] and in up to 10% of cases of chronic lymphatic leukaemia [19] and 20–30% of Hodgkin's disease [20]. The lack of detection in some follicular non-Hodgkin lymphomas with commonly used probes to *mbr* and *mcr* breakpoint regions does not exclude the involvement of BCL2, as the BCL2 product may be detected by antibodies in tissue sections [21] or by probes outside of the two common breakpoint regions [16]. BCL2 translocation is not detected in small cleaved follicular centre cell lymphoma (centrocytic lymphoma on Kiel classification) [22]. It is also not detected in lymphoma of mucosa-associated lymphoid tissue (MALT) [23] although the BCL2 protein can be seen in these tumours by immunostaining [24]. These findings suggest that the increase of BCL2 protein is the common event in low grade lymphoma which in the majority of cases is due to t(14;18) and rarely due to other mechanisms.

BCL2 rearrangement has also been detected by PCR in Hodgkin's disease tissue [20] and it is speculated that follicular lymphoma and Hodgkin's disease may coexist or they may share common pathogenesis [25].

Role of BCL2 in oncogenesis

Transfection of BCL2 into NIH3T3 cells, although failing to induce transformation *in vitro* causes tumours when transfected cells are injected into mice [3]. However BCL2 transfected into normal haemopoietic cells does not immortalise them or induce proliferation but prolongs cell survival of growth factor deprived haemopoietic cell line [26]. Transfected BCL2 in association with activated (transgenic) c-*myc* gene prolongs cell survival

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and occasionally promotes proliferation which is growth factor independent [27]. High levels of BCL2 can also protect B and T lymphoblasts under stress, and transfection of BCL2 into Epstein–Barr virus infected human lymphoblastoid B cell line also confers growth advantage [28].

In vivo evidence of involvement of BCL2 in oncogenesis centres around experiments in transgenic mice with deregulated BCL2 gene. Mice initially develop follicular hyperplasia with extended B cell survival [29–30] which progresses to diffuse large cell lymphoma and in some cases this occurs with the rearrangement of c-*myc* gene [31]. In doubly transgenic mice with deregulated BCL2 and c-*myc* there is rapid development of primitive lymphoid tumours suggesting marked synergy [32]. Such synergy has also been observed in T cells transfected with BCL2 and MYC genes [33].

These findings are compatible with the clinical experience of the behaviour of low grade lymphoma. The long indolent phase of the disease characterised by t(14;18) with BCL2 deregulation may be due to a prolonged survival of B lymphocytes which will be susceptible to further genetic alteration. The progression to a more malignant phase is associated with new genetic events such as c-*myc* activation. The co-existence of BCL2 and c-*myc* translocation has been demonstrated in some high grade lymphomas [34, 35] and the appearance of c-*myc* translocation has been detected on transformation of a low-grade lymphoma bearing t(14;18) [36].

It suggests that the BCL2 protein may extend cell survival by blocking programmed cell death and this is compatible with the indolent phase of low grade lymphoma. Histological transformation to aggressive lymphoma is associated with other oncogenic events of which c-*myc* translocation is one.

The cause of BCL2 deregulation

The commonest cause of BCL2 deregulation is t(14;18) translocation and its full understanding brings us closer to the primary oncogenic event. The sequencing of the translocation site of BCL2 on 18q21 and Ig heavy chain J region on 14q32 revealed that translocation occurs primarily within two short regions of the BCL2 described as the major breakpoint region (*mbr*) and the minor cluster region (*mcr*). A number of possible mechanisms for translocation have been suggested. The favoured mechanism is the involvement of a common recombinase which is normally responsible for Ig gene and T-cell receptor (TCR) gene rearrangement. In this model the translocation occurs by recognition of heptamer-spacer-nonamer sequences on chromosome 18 which are the signals for the joining of Ig and TCR genes. Translocation is therefore considered as a mistake of normal Ig and TCR gene rearrangement. There are arguments against this mechanism fully discussed by Tycko and Sklar [37]. In addition the sequencing of derivative chromosome 18 shows the proximity of D sequences [38, 39] which suggest translocation in a more mature B cell where Ig gene rearrangement has already occurred. Alternative model of translocation suggests an illegitimate pairing of staggered double strand DNA breaks [40]. Although mutation in BCL2 gene has been found in cell lines and may play a role in translocation or deregulation, it has not been firmly demonstrated in follicular lymphomas [41]. The precise mechanism of translocation mechanism remains at present unclear.

CLINICAL APPLICATION

The excess of BCL2 protein seems critical in extending and maintaining survival of B cells. The therapeutic aim should be to reverse the BCL2 deregulation. At present this is difficult as the control mechanisms which govern the deregulation are not fully worked out. However a possibility remains to block the BCL2 production at the translation or transcription level. Early *in vitro* experiments show successful inhibition of BCL2 expression by specific antisense oligonucleotides in leukaemic cell culture leading to inhibition of cell growth and survival [42].

There has been a considerable spin-off from the molecular understanding of BCL2 translocation. The translocations although occurring within relatively short DNA segments of *mbr* or *mcr* are specific for each cell clone and can be used as clonal markers. Molecular studies with Southern blotting or PCR can distinguish clonal proliferation of non-Hodgkin lymphoma from benign proliferation. The absence of translocation is however not helpful as it does not exclude the diagnosis of lymphoma. The detection of BCL2 on immunostaining is also compatible with the diagnosis of low grade lymphoma and can be used to distinguish this from benign hyperplasia. The presence of translocation in 20–30% of high grade tumours means that it is not possible to distinguish low from high grade lymphoma on molecular probing or immunohistochemistry. BCL2 can be used as a clonal marker which confirms the persistence of an original clone of cells during histological transformation from low to high grade lymphoma.

Detection of minimal disease

With primers specific for BCL2 gene at the mbr or mcr loci and primers for the J_H region of Ig heavy chain, it is possible to differentially amplifying t(14;18) translocation sequences by PCR. With this technique it is possible to detect up to $1 : 10^5$ cells bearing chromosomal translocation. This has been applied to the detection of tumour DNA from lymph nodes, bone marrow and peripheral blood of patients who are apparently disease free [43, 44].

Prognosis

Although the presence of t(14;18) is of no clear prognostic significance in nodular lymphomas, prognostic information can be obtained from the detection of other chromosomal changes which may confer more aggressive biological behaviour. The BCL2 translocation in high-grade lymphoma may predict a relapsing course of disease. Detection of t(14;18) in high-grade non-Hodgkin lymphoma is associated with worse progression free survival, although it has no influence on overall survival [45]. In already relapsed high-grade lymphoma the detection of BCL2 rearrangement seems to confer worse prognosis [45, 46]. These findings although not entirely consistent suggest a persistence of BCL2 bearing clone with indolent behaviour causing clinical recurrences.

CONCLUSION

The BCL2 is a novel type of proto-oncogene which plays an important role in the pathogenesis of lymphoma. The increased understanding of the mechanism of deregulation may lead to possible therapeutic intervention. At present molecular knowledge has been used for diagnosis, detection of minimal disease and as a prognostic indicator but the potential exists for this to be exploited to manipulate the course particularly of low-grade lymphoma.

BCL2 may also be involved in growth regulation in non-lymphoid tissue, and the search is on for its role in other tumours.

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