THE BCL-2 GENE: A REGULATOR OF PROGRAMMED CELL DEATH

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INTRODUCTION

The bcl-2 gene was cloned as a novel gene located at a chromosomal translocation breakpoint in follicular B cell lymphomas by three groups in 1985. Follicular lymphomas are indolent tumors, difficult to eradicate but slow to progress, and are believed to originate from centrocytic B cells in germinal centers. 85% or more of follicular lymphomas carry a t(14;18) (q32;q21) leading to the expectation that a new cellular oncogene would be activated by this translocation. The bcl-2 gene is found on chromosome 18 at q21 and juxtaposed in a tail to head fashion to elements of the immunoglobulin heavy chain locus on chromosome 14 in the 14;18 translocation (Figure 1). As expected, the bcl-2-Ig fusion mRNA is overproduced in t(14;18) cells, leading to

Figure 1. Structure of the t(14;18) translocation. Involvement of immunoglobulin recombinase is recognized by loss of internal D-J chromosome 14 sequences from der 14 and der 18 breakpoints as normally occurs during V(D)J recombination.
elevated levels of the 26 kd bcl-2 protein and changes in cellular phenotype 7,8.

The breakpoints in the immunoglobulin heavy chain gene occur at the 5' end of a joining (J) region and 3' to a diversity (D) region, with loss of intervening DNA and addition of extra nucleotides characteristic of "N" segments 9. This is notable because it dates the t(14;18) as a pre-B cell event, occurring during immunoglobulin VDJ recombination, and indicates significant normal differentiation occurs following the translocation to achieve the mature B cell phenotype of the malignant cell. Additional findings indicate the low probability of further steps culminating in full malignant transformation following an initial t(14;18). Detection of t(14;18) breakpoints by PCR analysis of DNA from tonsil samples containing reactive hyperplasia demonstrated a 54% prevalence in otherwise healthy individuals 10. This remarkable finding has been recently confirmed 11. In addition to other genetic events, extracellular signals may be important. Most follicular lymphomas have undergone an immunoglobulin heavy chain constant region switch recombination to the IgG isotype, implying subsequent exposure to cognate antigen may be a limiting step for progression of t(14;18)-bearing clones.

The breakpoints on chromosome 18 are also remarkably focused, suggesting sites of increased chromosomal breakage or cryptic recombination signals. The most frequent location lies in the 3' untranslated sequence of the bcl-2 gene, with 60% of breakpoints occurring within a 150bp sequence (mbr, major breakpoint region). Additional sites occur 3' to the bcl-2 gene (mcr, minor chromosomal rearrangement) and 5' to the bcl-2 gene 12,13,14. Immunoglobulin recombination signal sequences (heptamer-space-nonamer) are not found near chromosome 18 breakpoint sequences. A putative recombination motif homologous to prokaryotic chi sequences has been found adjacent to several chromosomal breakpoints, including the mbs and mcr bcl-2 regions and within the immunoglobulin heavy chain locus 15. Recently, a 45-Kd nuclear protein which binds chi-type sequences in the bcl-2 mbr and mcr breakpoints has been discovered 16. Early B cell lines were found to possess a specific endonuclease activity targeting the mbr region. A plausible model involves homologous recombination between breakpoint sequences on chromosomes 14 and 18, mediated by a chi-specific recombinase and V(D)J-recombinase, with possible involvement of the 45-Kd binding protein.

RESULTS AND DISCUSSION

The initial description of the cellular function of the bcl-2 gene in 1988 examined the effect of bcl-2 overexpression on cellular viability following withdrawal of interleukin-3, a growth and survival factor from FDC.P1 promyeloid cells 17. BcI-2 increased viability in this context, but did not cause FDC.P1 cells to lose their dependence on IL-3 for proliferation or provide an additional proliferative stimulus. The cell death induced by IL-3 withdrawal can be shown to be typical of apoptosis or programmed cell death 18. Characteristic internucleosomal DNA fragmentation and cellular morphological changes are prevented by bcl-2, implying that bcl-2 acts at an early step in the apoptotic pathway. These results have been confirmed in a variety of models of cellular survival factor withdrawal, although important exceptions have been noted. These cell deaths associated with IL-2, IL-6, and ciliary neurotrophic factor (CNTF) withdrawal otherwise resemble apoptosis, and the lack of bcl-2 effect suggests that multiple intracellular apoptotic pathways exist and include bcl-2-resistant and sensitive varieties 19,20.

Evidence supporting an in vivo role for bcl-2 in regulating cell death has been less easy to come by, in part due to the greater difficulty in quantitating cell survival in vivo and in part due to the need to understand the function of physiologic levels of bcl-2 within normal cells. These problems not withstanding, existing data suggests that bcl-2 functions as one, or perhaps the, important physiologic regulator of cell survival in cell lineages in which apoptosis determines cell turnover or developmental cell loss. The most compelling case can be made for B lymphocytes on which most studies to date have focussed as the cell of origin for t(14;18) bearing tumors. Transgenic mice bearing a bcl-2-Ig transgene overexpress bcl-2 in B lymphocytes and their precursors and develop polyclonal B lymphocyte hyperplasia 21,22. B lymphocytes are normally short-lived and the transgenic B cells are not proliferating, implying enhanced survival for their expansion. These cells have a dramatically prolonged survival in vitro, with a significant fraction surviving up to