

Expression of Bcl-2 and Bcl-2-Ig Fusion Transcripts in Normal and Neoplastic Cells

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Abstract

We examined the expression of the Bcl-2 gene at chromosome segment 18q21, that is translocated into the Ig heavy chain gene locus in t(14;18) bearing lymphomas. Bcl-2, while B cell associated, is expressed in a variety of hematopoietic lineages including T cells. Bcl-2 mRNA levels are high during pre-B cell development, the time at which the t(14;18) translocation occurs, but are down regulated with maturation. Like certain other oncogenes, Bcl-2 is quiescent in resting B cells but up-regulated with B cell activation. Mature B cell lymphomas with a t(14;18) have log-folds more mRNA than matched counterparts without the translocation. A sensitive S1 protection assay revealed that all transcripts in t(14;18) B cells were Bcl-2-Ig fusion mRNAs and originated from the translocated allele. Thus, there is a marked deregulation of Bcl-2 when it is introduced into the Ig locus in t(14;18) lymphomas.

Introduction

Distinct interchromosomal translocations are highly associated with specific types of lymphoid neoplasms, and their breakpoints have been localized to sites of *c-onc* genes or putative transforming gene (1-3). A translocation can alter an oncogene product or alternatively deregulate its expression (4-5).

The most common translocation in human lymphoid neoplasms is a t(14;18) (q32;q21) found in > 80% of follicular B cell lymphomas (3). We and others have previously cloned and characterized the site of chromosomal juncture on the derivative (der) 14 chromosome and used this to identify a new gene (Bcl-2) on chromosome segment 18q21 (Fig. 1)

(6-11). We noted that Ig diversity (D_H) segments uniformly mediated the juncture with 18q21 at the der(18) breakpoint, while Ig joining (J_H) segments fused with 18q21 at the der(14) breakpoint (11). This indicates that the t(14;18) occurs at the time of attempted D_H to J_H rearrangement, the earliest stage of Ig gene joining in a pre-B cell. We defined a major breakpoint region (mbr) within the 3' untranslated (UT) region of Bcl-2 on 18q21 where ~ 70% of translocations cluster (11). Analysis of normal and translocated cDNA products have indicated that the translocation results in a Bcl-2-Ig fusion transcript in which the translational open reading frame is not interrupted, but in which Ig heavy chain sequences are substituted for the Bcl-2 3' UT region (Fig. 1) (10) (Seto, M., and S. J. Korsmeyer, unpublished observations).

These structural findings prompted this examination of Bcl-2 expression in normal and neoplastic cells representing various hematopoietic lineages and stages of B cell activation and differentiation. Since deregulation was likely to be the primary abnormality in the Bcl-2-Ig fusion gene we compared the expression of Bcl-2 in its native location versus its translocated configuration.

Methods

Northern blot analysis. Oligo dT column purified poly (A) + RNA was selected from the guanidine thiocyanate prepared total RNA of cell lines. 5 μ g was denatured in formamide, electrophoresed on agarose-formaldehyde gels, and transferred to nitrocellulose paper (12). Northern blots were hybridized with a Bcl-2 probe (Fig. 1) as well as a γ -actin probe that revealed equivalent amounts of hybridizable RNA present in each lane (13).

S1 nuclease protection assay. An α -[32 P]dCTP synthetically radio-labeled complementary copy ssDNA probe was prepared by primer extension of an M13 clone. The DNA was digested with an appropriate restriction endonuclease and the ss probe prepared from an alkaline agarose gel. 100,000 cpm of probe was hybridized with 10 μ g of total RNA at 45°C for 16 h and then digested with 200 U of S1 for 1 h at 37°C (14). Protected DNA fragments were size separated on a 6% sequencing gel, fixed, dried, and autoradiography performed. In addition to the Bcl-2 probe defined in Fig. 3 a, each RNA was also hybridized with a β -actin probe that revealed equivalent amounts of hybridizable RNA in each sample (13).

Resting and activated B cells and T cells. Purified B cells were obtained by sheep red blood cell (RBC) depleting T cells (< 1%) and plate adherence depletion of monocytes (< 2%) from tonsillar mononuclear cells. These "resting B cells" were > 95% positive for surface Ig by indirect immunofluorescence. B cells were activated with a predetermined concentration of anti- μ chain antibody and B cell growth factor (BCGF, Cellular Products) (15). "Resting" T cells were sheep RBC purified from peripheral blood mononuclear cells and aliquots

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1. **Abbreviations used in this paper:** BCGF, B cell growth factor; der, derivative; mbr, major breakpoint region; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; UT, untranslated.

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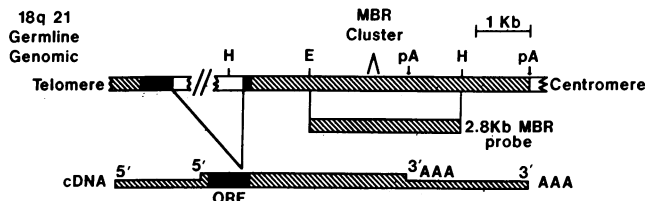


Figure 1. Schematic of the normal Bcl-2 genomic gene and cDNA products in a pre-B cell (Seto, M., and S. J. Korsmeyer unpublished observations). t(14;18) translocations cluster within a 2.8 kb EcoRI(E)-HindIII(H) mbr but leave the open reading frame (ORF) intact.

were activated with phytohemagglutinin (PHA) 1 μ g/ml and phorbol myristate acetate (PMA) 20 ng/ml. [3 H]Thymidine incorporation confirmed the induction of proliferation in the activated B and T cell preparations.

Results

Bcl-2 expression in hematopoietic lineages. Balm-4, a t(14;18) bearing B cell line (6), demonstrates many fold higher levels of a 6.5-kb mean-sized mRNA than non-B cell lines (Fig. 2 a). Early (HSB-2 and Jurkatt) (16) and more mature (Hut 102) (17) T cell lines demonstrate comparably low levels of Bcl-2. Bcl-2 transcripts are also found in the monoblastoid cell line, U-937 (16), while the erythroleukemia-like K562 (18) has only trace amounts.

Bcl-2 expression in translocated and nontranslocated B cells. The B-cell predominance of Bcl-2 expression prompted an analysis of serial stages of B cell differentiation. Comparable levels of mRNA were found in all B cells proven to carry the t(14;18) (6): SU-DHL-4, SU-DHL-6, Balm-4, and RL (Fig. 2 b). NU-DHL-1 is a 14q+ B cell lymphoma line in which the reciprocal partner is uncertain, but could be 18q21 (6). The major transcript displays considerable size microheterogeneity measuring from \sim 5.8 to 7.6 kb. Occasional smaller species including a 4.2-kb predominant mRNA in RL are noted. This variation results from alternative 5' and 3' ends (Seto, M., and S. J. Korsmeyer, unpublished observations). Burkitt lymphomas (Raji and LY67) (19) that lack the t(14;18) represent a pre-B to early B cell stage of maturation and have variable levels of Bcl-2. More mature B cell lines (BHM-23) and the Epstein Barr virus transformed secretory B cell lines (8392, SB, and CESS) (20) have markedly less Bcl-2. The most terminally differentiated cell (U-266), was derived from multiple myeloma (18) and displays no detectable mRNA on Northern analysis (S1 protection assay did demonstrate very low levels). In contrast the nontranslocated pre-B cell line (Nall-1) (21), displayed high levels of Bcl-2 mRNA (Fig. 2 b).

Bcl-2 expression in pre-B cells and comparable translocated and nontranslocated B cells. The high level expression of Bcl-2 in Nall-1 prompted an examination of additional B cell precursor cell lines (Nalm-6, Reh, and RS) (21), all of which displayed comparable levels to t(14;18) bearing mature B cells (Fig. 2 c). Mature B cell lines from diffuse large cell lymphomas that lacked a t(14;18) (SU-DHL-2, SU-DHL-9) and were phenotypically and histologically similar to those (SU-DHL-4, RL, SU-DHL-6) that possessed a t(14;18) were examined (22). Mature B cell lines with a t(14;18) had log-folds

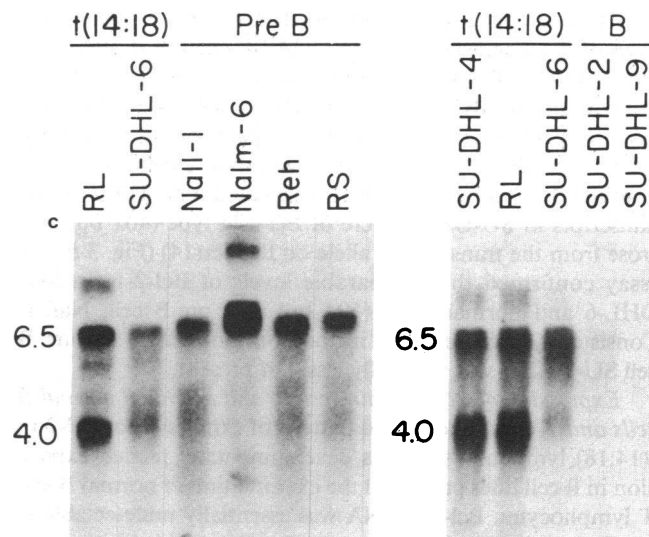
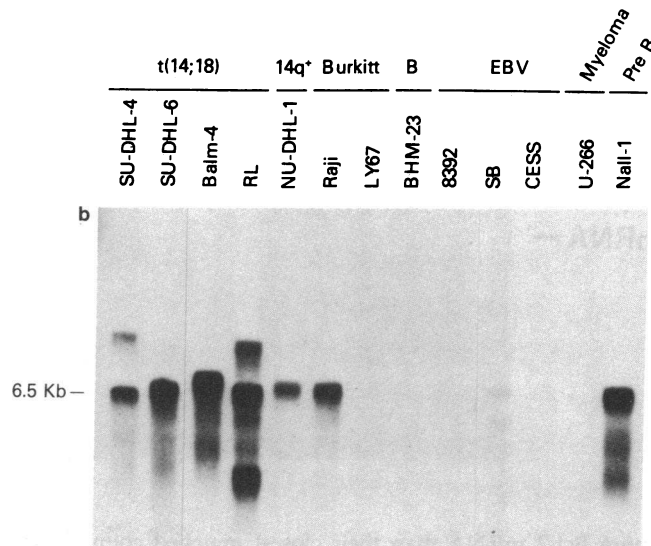
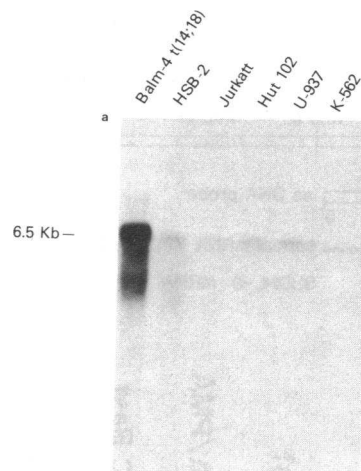


Figure 2. (a-c) 5 μ g pA RNA per lane Northern blots hybridized with the Bcl-2 2.8 kb mbr probe.

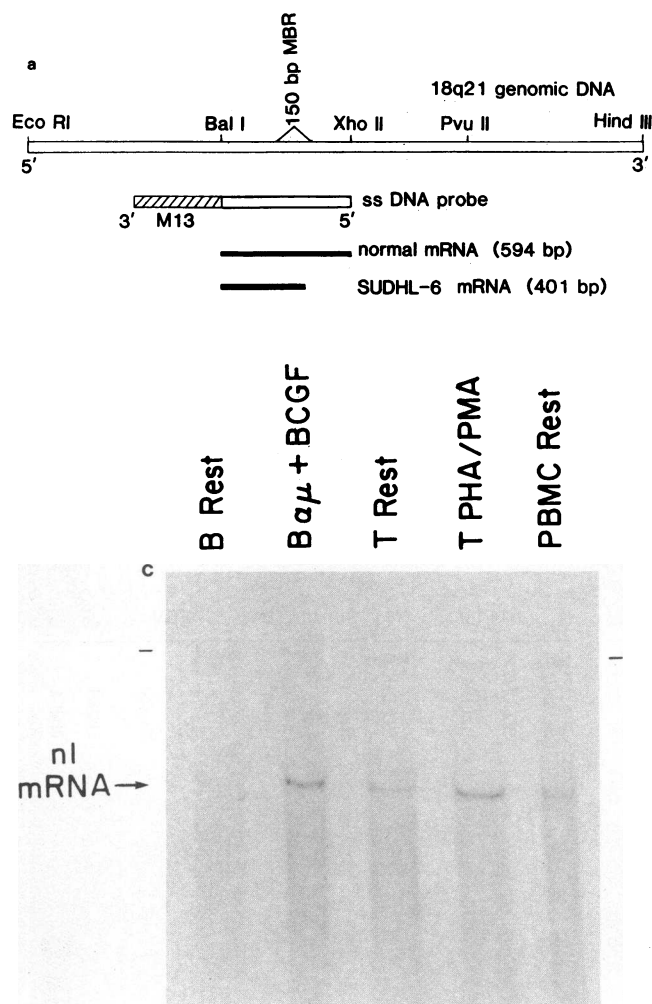


Figure 3. (a) Restriction map of 2.8 kb Eco RI-Hind III region of 3' UT region of Bcl-2 including 150 bp region of mbr where t(14;18) breakpoints cluster. A reverse complementary ssDNA probe possessed the mbr and additional M13 phage sequence. This probe was capable of discriminating any normal Bcl-2 mRNA (594 bp) from the SUDHL-6 Bcl-2-Ig fusion mRNA (401 bp as predicted by cDNA sequence). (b) S1 protection assay of 10 μ g total RNA in which DHL-6 Bcl-2-Ig fusion mRNA protects a 401-bp fragment while the normal Bcl-2 mRNA protects a 594-bp fragment. Dash marks denote residual native ssDNA probe. (c) S1 protection assay of 9 μ g total RNA in resting purified tonsillar B cells, B cells activated with anti- μ antibody and BCGF for 48 h, resting purified T cells, T cells activated with PHA/PMA for 48 h, and resting peripheral blood mononuclear cells.

more Bcl-2 mRNA than their closest matched counterparts lacking the translocation (Fig. 2 c).

All Bcl-2 transcripts in t(14;18) bearing cells arise from the translocated allele. We wished to determine if the elevated levels of Bcl-2 transcripts seen in t(14;18) bearing B cells were intact Bcl-2 mRNAs from the normal chromosome 18 or Bcl-2-Ig fusion mRNAs from the translocated allele. We created an S1 nuclease protection assay capable of discriminating the normal Bcl-2 transcript off a germline chromosome 18 from the Bcl-2-Ig fusion off the translocated gene (Fig. 3 a). All transcripts in SU-DHL-6 were of Bcl-2-Ig type (401 bp) and arose from the translocated allele on the der(14) (Fig. 3 b). S1 assay confirmed the comparable levels of Bcl-2-Ig in SU-DHL-6 and normal Bcl-2 (594 bp) in a pre-B cell, Nall-1. Consistently, Bcl-2 mRNA in the nontranslocated mature B cell SU-DHL-9 was markedly less, but present.

Expression of Bcl-2 within resting and activated normal B cells and T cells. The altered pattern of expression of Bcl-2 in t(14;18) lymphomas and its developmentally related expression in B cell lines prompted the examination of normal B and T lymphocytes. Bcl-2 mRNA was essentially undetectable in purified resting B cells. In contrast, B cells activated with anti- μ and BCGF demonstrated a marked elevation of Bcl-2 at a 48 h. time point shown in Fig. 3 c. Purified resting T cells demon-

strated more Bcl-2 mRNA than resting B cells and displayed an increment following activation with PHA and PMA. Resting peripheral blood mononuclear cells possessed Bcl-2 perhaps reflecting their predominantly T cell composition.

Discussion

The fact that Bcl-2 is translocated into the Ig locus in t(14;18) bearing B cell tumors but remains in its germline configuration in normal B cells suggests that the Bcl-2-Ig fusion is tumorigenic (6–11). This study revealed that the normal Bcl-2 steady state mRNA is high in pre-B cell lines but down regulated in mature B cell lines. Thus, Bcl-2 is highly expressed at a pre-B cell stage when it is also subject to translocation. While B cell associated, Bcl-2 is not lineage restricted as all other proliferating hematopoietic cell lines displayed at least minimal levels. Similar to the c-myc oncogene (23) this putative transforming gene was either nonexpressed or minimally expressed in resting B cells but was markedly augmented with the activation and proliferative signals of anti- μ plus BCGF. Despite the minimal levels of Bcl-2 mRNA in T cell lines, normal T cells displayed a surprising amount of Bcl-2 in resting as well as PHA/PMA activated T cells. This may indicate a difference in

the regulation of Bcl-2 within B versus T cell populations or might alternatively reflect unanticipated T cell activation during preparation.

A discriminatory S1 protection assay revealed that all transcripts of a t(14;18) bearing B cell were Bcl-2-Ig fusion mRNAs and arose entirely from the translocated gene on the der(14). This assay also confirmed the transcriptional direction of Bcl-2 and the head to tail orientation of the two fused genes. Furthermore, the absence of any normal Bcl-2 transcripts off of the germline chromosome 18 indicates a feedback suppression of the normal allele in t(14;18) cells.

This study reveals that the normal Bcl-2 gene is regulated in a nearly inverse fashion compared with Ig. Bcl-2 mRNA is high in pre-B cells but down regulated with differentiation, while Ig mRNA increases with terminal differentiation to a plasma cell. The introduction of Bcl-2 into the Ig locus results in a deregulation that manifests as inappropriately high levels of Bcl-2-Ig fusion transcripts at a mature B cell stage of development.

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