

Transcriptional Deregulation of Mutated BCL6 Alleles by Loss of Negative Autoregulation in Diffuse Large B Cell Lymphoma

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The BCL6 proto-oncogene encodes a POZ/zinc finger transcriptional repressor expressed in germinal center (GC) B cells and required for GC formation. In ~35% of diffuse large B cell lymphomas (DLBCL) and 5–14% of follicular lymphomas (FL), the BCL6 locus is altered by chromosomal translocations that deregulate its expression by a mechanism known as promoter substitution. In addition, the BCL6 5' non-coding sequences are targeted by multiple somatic mutations that cluster within ~1.5 kb from the transcription initiation site and are found in 30–40% of normal GC B cells (frequency: $0.14 \times 10^{-2}/\text{bp}$) as well as in a fraction of all GC-derived B cell lymphomas.^{1,2} The structural features, distribution, and specificity for GC B cells strongly suggest that BCL6 mutations are introduced by the same mechanism that targets immunoglobulin V genes in the GC. Nonetheless, the biological role of these mutations in normal and malignant B cell development remains unclear.

The present study was aimed at investigating the effect of BCL6 mutations on gene transcriptional regulation. To this end, the BCL6 5' noncoding region (~5 kb) from 24 tumor cases [6 DLBCL, 5 Burkitt lymphoma (BL), 4 FL, and 5 chronic lymphocytic leukemia (CLL), corresponding to 35 mutated alleles] or sorted GC B cells ($N = 20$ mutated alleles) was linked to the luciferase gene, and the activity of the corresponding constructs was evaluated in transient transfection/reporter gene assays in two BCL6 permissive lymphoma lines (Ly1 and MUTUI-BL59). We found that, while mutant alleles derived from normal GC cells, or from BL, FL, and CLL cases, displayed comparable activity to that of a wild-type construct, four (33%) of the 12 DLBCL-associated alleles were significantly overexpressed (4–18-fold) in both transfected lines. Among multiple mutations scattered throughout the BCL6 5' non-coding sequences of these four alleles, single nucleotide substitutions, all located within the first noncoding exon of the gene, were found to be responsible for the observed overexpression.

These mutations affect two adjacent BCL6 binding sites and were found to prevent BCL6 from binding to its own promoter, both *in vitro* in electrophoretic mobility shift

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assays and *in vivo* in chromatin immunoprecipitation assays. Since these sites are normally used by BCL6 to negatively autoregulate its expression levels, our results suggest that DLBCL-associated mutations disrupt the negative feedback loop and can contribute to lymphomagenesis by allowing deregulated transcription of BCL6. Indeed, deregulated expression of the endogenous BCL6 gene could be documented in native lymphoma cells carrying a nucleotide substitution in one of the two exon 1 binding sites.

An expanded survey of DLBCL cases revealed that alterations in these sequences can be found in ~13% of the samples ($N = 4/32$); it is noteworthy that these samples all lack 3q27 chromosomal translocations, suggesting that exon 1 mutations and BCL6 rearrangements are mutually exclusive. Our data identify a novel mechanism for BCL6 deregulation and point to a broader involvement of this proto-oncogene in DLBCL pathogenesis, by indicating that deregulated BCL6 expression can be achieved not only by chromosomal translocations (~35% of cases) but also by mutations affecting the BCL6 exon 1 binding sites (~13% of cases).

REFERENCES

1. MIGLIAZZA, A. *et al.* 1995. Frequent somatic hypermutation of the 5' noncoding region of the *BCL6* gene in B-cell lymphoma. *Proc. Natl. Acad. Sci. USA* **92**: 12520–12524.
2. PASQUALUCCI, L. *et al.* 1998. BCL-6 mutations in normal germinal center B cells: evidence of somatic hypermutation acting outside Ig loci. *Proc. Natl. Acad. Sci. USA* **95**: 11816–11821.