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Absence of structural mutations of the BAK gene in B-cell lymphomas

Bak is a Bcl-2 family member with pro-apoptotic activity. Mutations in the coding region of Bak have been described in human gastrointestinal cancers. We examined the status of the Bak gene in ninety-two B cell lymphomas. We found that structural mutations in the BAK gene are not involved in B cell lymphomagenesis.

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Evading apoptosis is a key feature in the malignant transformation of normal B-cells to lymphoma cells. BAK, a Bcl-2 family member, is the principal antagonist of the anti-apoptotic protein BCL-XL and interacts with BAX to form membrane pores leading to mitochondrial dysfunction and release of cytochrome c.¹⁻³ Mutations in the coding region of Bak have recently been described in human gastric and colorectal cancers.⁴ Therefore, we examined the status of the Bak gene in 92 paraffin-embedded B-cell lymphomas, peripheral blood samples from 20 healthy donors and 10 paraffin-embedded samples of non-neoplastic oral tonsils for mutations in the five coding exons (exon 2-6). Genomic DNA extraction, SSCP-PCR analysis and direct sequencing of samples that showed mobility-shifted bands on the gel were performed as previously described.⁵ Primers located within the intron sequences were used to amplify the five coding exons of BAK.⁴ Bak protein expression was evaluated using immunohistochemical analysis with polyclonal anti-human Bak antibody (DAKO, Copenhagen, Denmark) in all samples, as previously described.⁵ SSCP analysis of exons 3, 5 and 6 did not show any abnormal mobility indicative of sequence alteration in normal peripheral blood, non-neoplastic tonsils or in B-cell lymphomas. An abnormal mobility was, however, frequently observed in exon 2 of samples from peripheral blood, non-neoplastic tonsils, and lymphomas of germinal and post-germinal center origin (i.e. follicular lymphomas, diffuse large B-cell lymphomas and extra-nodal marginal zone lymphomas, MALT-type), but not in lymphomas of pre-germinal center origin (mantle cell and small lymphocytic lymphomas) (Figure 1A and Table 1). This alteration, monoallelic or biallelic, consists of a C to T transition in codon 14 (TGC to TGT), without translation into an amino acid substitution. In 6 patients demonstrating the exon 2 sequence

Table 1. Exon 2 Bak mutational analysis in B-cell non-Hodgkin's lymphomas and normal lymphoid tissues.

	Number	Normal of cases	Monoallelic	Biallelic mutation	Mutated (%) mutation
Mantle cell lymphoma	7	7	-	-	0
Small lymphocytic lymphoma	7	7	-	-	0
Follicular lymphoma	32	20	10	2	37.5
Diffuse large B-cell lymphoma	27	20	7	-	26
Marginal zone lymphoma	17	9	7	1	47
Lymphoplasmacytoid lymphoma	2	1	1	-	50
Peripheral blood	20	11	8	1	45
Non-neoplastic tonsil	10	6	4	--	40

alteration, epithelial tissue was available as a non-lymphoid control. In contrast to the lymphoma samples, epithelial tissues did not show exon 2 mutations (Figure 1B). In the SSCP analysis of exon 4 we found a different migration pattern of the PCR fragments in one case with follicular lymphoma, consisting in a biallelic T to C transition in the non-coding region of the intron sequence flanking exon 4 (data not shown). Immunohistochemical analysis did not show any different expression of Bak protein between exon 2 mutated and exon 2 non-mutated samples. Our study shows that structural mutations in the BAK gene are not involved in B-lymphomagenesis. The distribution of BAK exon 2 mutation in different lymphoma types suggests a segregation of this mutation with a germinal/post-germinal center origin. We observed this mutation with comparable frequency in normal peripheral blood and non-neoplastic tonsils (Table 1), which contain predominantly B-cells of post-germinal center origin. In contrast we found no mutation in normal epithelial tissues of patients with B-cell lymphoma containing the exon 2 mutation. Taken together these data indicate the germinal center origin of the exon 2 mutation. The germinal center is the site of somatic hypermutation, contributing to the diversity of the variable region of the immunoglobulin (IgV) genes.⁶ Recently, other genetic regions have been identified as targets for the somatic hypermutation machinery.^{7,8} These include the 5' region of the Bcl-6 gene. The Bak mutation, as Bcl-6 and IgV mutations, is located within 2 Kb from the transcriptional initiation site. The distribution and frequency of Bak and Bcl-6 mutations appear to be similar. However, the Bak mutations occur at one hotspot in the coding region and can be found in a heterozygous or homozygous state, whereas Bcl-6 mutations are distributed within a 740 bp region of the 5' non-coding sequence and were all found in heterozygosity.⁷ The Bcl-6 mutations show a preferential targeting for the characteristic hotspot of the IgV hypermutation, the RGYW motif (R= purine, Y= pyrimidine and W= A or T).^{7,8} This motif is not present in the mutation site of Bak. It is, therefore, unlikely that Bak and Bcl-6 mutations in the germinal center are caused by the same mechanism. As the Bak mutation is a silent base change in the coding region there is no evident functional significance, but in combination with other gene mutations it may serve as an indicator for the germinal center transition of both normal and malignant B-cells. In conclusion, the absence of mutations in BAX and BAK and their ubiquitous expression in B-cell lymphomas suggest that cell survival in this type of neoplasia does not depend on the alteration of apoptotic agonists, but is most probably due to alterations of the apoptotic antagonists. However, further studies are needed

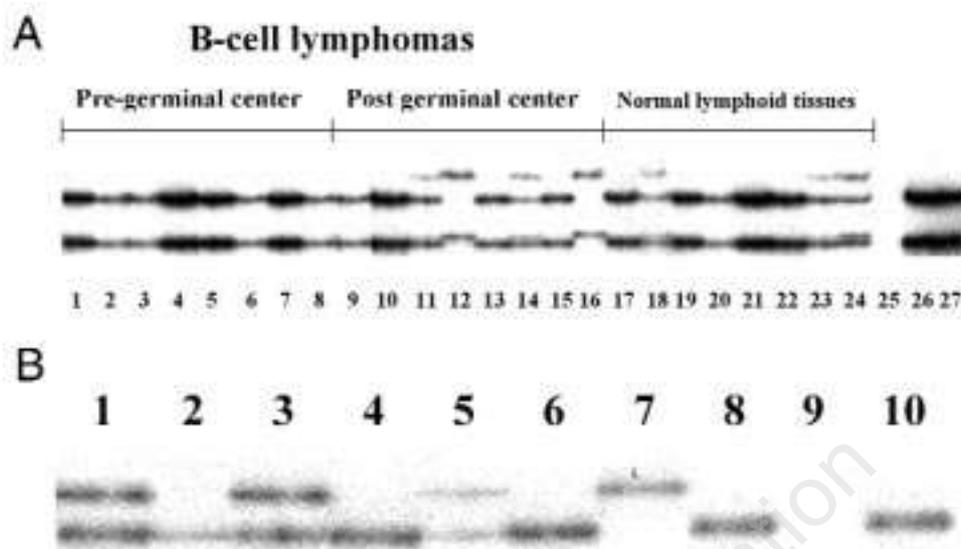


Figure 1. A: PCR-SSCP analysis of point mutations for a 165 base pair region encompassing Bak exon 2. The figure shows a PCR-SSCP analysis, in non-denaturing conditions, using genomic DNA from lymphomas of pre-germinal center origin: small lymphocytic lymphoma (samples 1-4) and mantle cell lymphoma (samples 5-8); from lymphomas of germinal and post-germinal center origin: follicular lymphoma (samples 9-12) and large B-cell lymphoma (samples 13-16); and from normal lymphoid tissue of non-neoplastic tonsils (samples 17-20) and peripheral blood (samples 21-24). Sample 25: negative control using water instead DNA. Samples 26 and 27: PCR-SSCP analysis using DNA from skin of normal donors. **B:** PCR-SSCP analysis of Bak exon 2, in denaturing conditions, using MDE gel. The figure shows a migration pattern of 4 follicular lymphomas in lanes 1, 3, 5, and 7, with their relative normal non-lymphoid controls in lane 2 (skin tissue), 4 (gastric tissue), 6 (pericardial tissue) and 8 (skin tissue). Lane 9: negative control using water instead DNA. Lane 10: skin of normal donor.

to arrive at a more precise definition of the alterations in the apoptotic pathways in malignant B-cells that program them for cell survival.

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