Chronic Lymphocytic Leukemia

ATM mutations in B-cell chronic lymphocytic leukemia

Tumor DNA samples from 56 patients with sporadic B-CLL chronic lymphocytic leukemia (B-CLL) were analyzed for mutations in the *ATM* gene, using exon scanning single strand conformation polymorphism (SSCP). Fifteen patients (27%) showed a pattern compatible with the presence of a somatic mutation. In only five patients, however, was a mutation possibly associated with the disease status detected, suggesting that *ATM* mutations make only a minor contribution to the pathogenesis of B-CLL.

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Approximately 20% of patients with B-cell chronic lymphocytic leukemia (B-CLL) carry deletions in the chromosomal region 11q22.3-q23.[1] Furthermore, about one third of B-CLL patients show reduced or absent levels of *ATM* protein.[2] Both of these features are associated with poor prognosis.[1-2] Although previous studies have shown a high frequency of *ATM* mutations in patients with B-CLL,[3-7] only a small number of cases have been analyzed to date and recent studies have shown that *ATM* gene alterations are rare in familial B-CLL.[7-8] Moreover, various studies of B-CLL patients, including a selection of cases with loss of hybridization (LOH) at 11q and different methods for detecting alterations with variable gene coverage of the *ATM* gene, have not provided accurate estimates of the actual proportion of B-CLL cases carrying mutated/inactivated *ATM* genes.

Single strand conformation polymorphism (SSCP) analysis of the *ATM* gene was carried out on 56 unrelated B-CLL patients. Forty-one patients (73%) showed a wild type pattern. Fifteen (27%) showed a pattern compatible with the presence of a mutation in the malignant clone, which was confirmed by sequence analysis. Thus, the incidence of *ATM* alterations in our group of patients is comparable to that reported by Stankovic *et al.* (32%).[6] In total, nine different aberrant patterns were detected in 11 patients (Table 1). Six

Table 1. Summary of ATM nucleotide changes detected in B-CLL patients.

Patient	*Exon*	*Nucleotide sequence change*	*Predicted protein*	*Previously reported*
UK17	6	295A→G	S99G	no
UK31	19	2572T→C	F858L	polymorphism[a,e,i]
SW15	19	2572T→C	F858L	polymorphism[a,e,i]
UK20	24	3161C→G	P1054R	polymorphism[a,b,e,] B-CLL[f]
UK31	24	3161C→G	P1054R	polymorphism[a,b,e,] B-CLL[f]
SW15	24	3161C→G	P1054R	polymorphism[a,b,e,] B-CLL[f]
UK7	27	3666A→G	L1222L	no
SW24	36	5071A→C	S1691R	B-CLL[g], A-T[g]
SW24	39	5557G→A	D1853N	polymorphism[c,e,i]
SW25	39	5557G→A	D1853N	polymorphism[c,e,i]
SW19	47	IVS46-24-25delAG	not known	no
UK4	49	6919C→T	L2307F	B-CLL[i]
UK1	IVS62	IVS62+8A→C	not known	polymorphism[d]
UK23	IVS62	IVS62+8A→C	not known	polymorphism[d]
UK31	IVS62	IVS62+8A→C	not known	polymorphism[d]

[a]Vorechovsky, Rasio 1996, Cancer Res; [b]Vorechovsky, Luo 1996, Cancer Res; [c]Sandoval 1999, Hum Mol Genet; [d]Castellvi-Bel 1999, Hum Mut; [e]Thorstenson 2001, Am J Hum Genet; [f]Stankovic 1999, Lancet; [g]Bullrich 1999, Cancer Res; [g]Stankovic 1998, Am J Hum Genet; [i]Yuille 2002, Blood.

Table 2. Summary of B-CLL patients with ATM nucleotide changes.

Patient	*Nucleotide sequence change(s)*	*Sex*	*Age at diagnosis (years)*	*WBC count (×10⁹/L)*	*Years of follow-up*	*Indolent/ active disease*	*Chemotherapy*	*Other*
UK1	IVS62+8A→C	no data						
UK4	6919C→T	M	81	105	21	active	yes	hemolysis
UK7	3666A→G	no data						
UK17	295A→G	M	66	56	4	indolent	no	splenomegaly
UK20	3161C→G	M	66	17	13	indolent	no	
UK23	IVS62+8A→C	no data						
UK31	2572T→C 3161C→G IVS62+8A→C	F	74	113	15	active	yes	splenomegaly
SW15	2572T→C 3161C→G	F	56	20	5	indolent	no	
SW19	IVS46-24-25delAG	M	42	16	4	indolent	no	lymphadenopathy
SW24	5071A→C 5557G→A	M	68	30	3	indolent	no	
SW25	5557G→A	F	82	48	3	active	yes	hemolysis, splenomegaly

of these changes (in seven patients) have been observed previously, and three of them have been suggested as potential disease associated mutations rather than polymorphisms. A mutation possibly associated with the disease was detected in only five patients in our series (UK17, UK7, SW24, SW19 and UK4). All mutations, except for the intron change IVS46-24-25delAG, were amino acid substitutions. All of the mutations identified appeared in a heterozygous form, that is, no LOH was detected in the *ATM* locus.

At least 15 patients carried a mutation within the *ATM* gene. However, several of the alterations do not fulfil the criteria for a genuine, pathogenic mutation. The exon 24 transversion (3161C→G),[5] has been found at a frequency of 2.2% in the Northern European population, arguing against a causal relationship between this alteration and malignancy.[9] Three of our patients carried this mutation. One of them, UK20, showed mutations in over 3% of the VH gene B-CLL sequence. As *ATM* mutant B-CLLs have been suggested to lack VH mutations,[6] our findings support the notion that 3161C→G represents a polymorphism.

Another alteration, which was also present in the germline DNA of the patient, was located in exon 36, 5071A→C[3]. This change was previously described as a pathogenic mutation in an ataxia-telangectasia (A-T) patient[10] and has also been found in a breast cancer patient.[11] We, however, observed this change in 2 of 220 (0.91%) Swedish controls using SSCP. Since the overall frequency of this allele is in excess of the estimated population frequency of *ATM* heterozygotes in the Swedish population (0.52%),[12] it is not likely to represent a genuine A-T associated allele nor does it appear to predispose to the development of B-CLL.

The alteration in exon 49, 6919C→T (L2307F) has previously been found in a B-CLL patient and is a non-conservative change and might thus confer a disease risk. However, the absence of co-segregation of aminoacid changes in the B-CLL families studied previously, implies that the non-conservative amino acid changes only confer small genotypic risks.[7] The remaining three of the previously reported changes in exons 19 and 39 and in intron 62 are all known polymorphisms (Table 1). Three of the *ATM* changes have not been described previously. The change in exon 27, 3666A→G does not alter the amino acid sequence. The deletion IVS46-24-25delAG does not affect the coding region, but may theoretically influence splicing of the gene. The substitution located in exon 6, 295A→G (S99G), is not located in any of the known functional domains of the gene. The patient presenting this change, UK17, showed mutations in over 9% of the VH B-CLL gene sequence, indicating a non-pathogenetic role.[6]

The possible role of *ATM* mutations found in B-CLL is still unclear. Our study contained consecutive cases and patients were not selected for LOH at 11q. The indolent clinical picture of the patients with *ATM* alterations in our study (Table 2) did not match the aggressive disease found in previous studies, suggesting that we have a less biased estimate of the incidence of *ATM* mutation in B-CLL. Although previous studies have suggested that *ATM* mutations are associated with a substantially increased risk of developing B-CLL,[13] our study shows that *ATM* inactivation is associated with tumorigenesis[14] only in a small subset of B-CLL.

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