ATM Mutations in B-Cell Chronic Lymphocytic Leukemia

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Abstract

Mutations in the ATM gene located on the long arm of chromosome 11 at 11q22–23 cause ataxia-telangiectasia, an autosomal recessive disorder that is associated with increased incidence of malignancy and, particularly, lymphoid tumors. A role for ATM in the development of sporadic T-cell chronic leukemias is supported by the finding of loss of heterozygosity at 11q22–23 and ATM mutations in leukemias carrying TCL-1 rearrangements. Approximately 14% of B-cell chronic lymphocytic leukemia (B-CLL), the most common adult leukemia, carry deletions of the long arm of chromosome 11 at 11q22–23. Loss of heterozygosity at 11q22–23 and, more recently, absence of ATM protein, have been associated with poor prognosis in B-CLL. To determine whether the ATM gene is altered in B-CLL, we have sequenced individual ATM exons in six B-CLL cases. We show that the ATM gene is mutated in a fraction of B-CLLs and that mutations can be present in the germ line of patients, suggesting that ATM heterozygotes may be predisposed to B-CLL.

Introduction

AT2 is an autosomal recessive disorder that is characterized by, among other symptoms, predisposition to malignancy (1, 2). The responsible gene, ATM (mutated in AT), located at 11q22–23, was identified by positional cloning (3, 4). Adult ATM homozygotes are predisposed to T-cell prolymphocytic leukemia/T-CLL (5). In homozygous ATM mutants, T-CLL develops in a T-cell clone carrying a TCL1 or, rarely, MTCPI rearrangement and additional genetic changes (6–8). Similarly, in sporadic T-CLL, in which TCL1 translocations are common, LOH at 11q22–23, with mutation of the remaining ATM allele, has been described (9–12). LOH on chromosome 11 in a region including the ATM gene has also been described in B-CLL (13–16), the most common human leukemia in the Western hemisphere; the molecular pathogenesis of B-CLL remains largely unknown (17). In a minority of B-CLLs, the BCL1/CyclinD1 and BCL2 oncoproteins are activated by translocations (17). The TP53 tumor suppressor gene is mutated in ~10% of cases, and unknown genes are thought to be targeted by chromosome 13 deletions, chromosome 12 abnormalities, and chromosome 11 deletions found in more than 40, 30, and 10% of B-CLL cases, respectively (13–17). Poor outcome in B-CLL has been associated with chromosome 12 abnormalities as well as with TP53 inactivation, 11q deletions (13–17), and absence of ATM protein (18). To determine whether ATM mutations play a role in B-CLL pathogenesis, we examined the ATM gene in five paired normal/tumor B-CLL cases exhibiting LOH at the D11S2179 marker located within the ATM locus (3), no detectable ATM protein, and shorter survival times than patients expressing ATM protein (18). In addition, we analyzed a sporadic B-CLL case (CLL200) with aggressive disease (Rai stage III/IV) for which there was no information regarding LOH on chromosome 11 or ATM protein expression.

Materials and Methods

Patient Samples. Five patients were treated at the M. D. Anderson Cancer Center and were previously found to have LOH at 11q22–23 and to lack ATM protein expression (18); one patient was seen at the University of California San Diego and was selected randomly from available B-CLL cases. All patients had immunophenotypic and morphological features of B-CLL with leukemic cells that were positive for both CD-19 and CD-5. Normal samples consisted of bone marrow obtained at clinical remission and were negative for B-CLL as determined by morphological, flow cytometric (CD5+, CD19+, <5%), and molecular studies (no immunoglobulin heavy or light chain rearrangement).

Sequence Analysis. DNA was extracted from patient material as described elsewhere (18). DNA from the San Diego patient was isolated by conventional methods, and RNA was isolated with RNAzol as recommended by the manufacturer (Tel-Test Inc., Friendswood, TX). Amplified products were purified with the Qiagroup PCR purification kit (Qiagen, Valencia, CA) and sequenced with the corresponding forward and reverse primers using the dideoxy terminator reaction chemistry in an automated ABI 377 sequencing system (with stretch configurations; Applied Biosystems-Prism-Perkin Elmer, Foster City, CA). Sequences from tumor cases were aligned and compared to the normal ATM sequence using the Sequencher program (Genecodes, Ann Arbor, MI). When changes were identified, they were confirmed by repeating the PCR and sequencing the new amplification product. To determine whether the identified change was present in the germ line or represented an acquired mutation, the same exon in which a mutation had been identified in the tumor was amplified on two separate PCRs from the corresponding normal DNA (except for CLL200, for which normal DNA was not available), and the products were sequenced.

Results and Discussion

As described previously (19), we proceeded to sequence individual exons of the ATM gene (20) in the six B-CLL cases described above. In addition to previously described common and rare polymorphisms (19, 21), we identified four missense mutations, two of which had previously been reported in AT families from the United Kingdom (22, 23). Because of limiting amounts of DNA, not all 64 coding exons were sequenced in all cases. Therefore, it is possible that, in the two cases in which we did not detect any alterations, the mutations are in nonsequenced exons (3 exons in one case and 11 exons in the other).

One of the four missense mutations results in a splicing error (in CLL200), and this is the only one for which there is no information regarding the patient’s germ line. Tumor DNA from this patient showed a 7865C→T transition resulting in the creation of a new
splice site within exon 55 (Fig. 1a). Amplification and sequencing of the ATM mRNA from this tumor revealed the absence of normal ATM mRNA and production of an aberrant ATM mRNA species with a deletion of the last 63 nucleotides of exon 55, disruption of the open reading frame, and creation of a premature stop codon at residue 2623 within exon 56 (Fig. 1b). This mutation, therefore, could result in a truncated protein product lacking the COOH-terminal phosphatidylinositol 3'-kinase domain, the most highly conserved region, and one that ATM shares with a family of proteins from different species, including cell cycle regulators as well as DNA damage and telomere length control proteins (24).

One leukemia with LOH at D11S2179 showed a previously unreported missense mutation at nucleotide 995 and absence of the normal allele. This A→G transition replaces the tyrosine at 332, which is conserved in mouse, with a cysteine. Alternatively, the A→G transition could result in the synthesis of an aberrant mRNA because it creates a new splice donor site at nucleotide 995 within exon 10. Sequencing of normal tissue DNA from this case revealed that the patient was homozygous for the wild-type allele, indicating that the A→G transition present in the leukemic cells represents an acquired mutation of the ATM gene (Fig. 2). The previous description of four (including the 5071A→C) germ-line mutations in 88 cancer families, giving a carrier rate of 4.5% in this group (21), and our finding of 2 carriers among 34

Finally, two other patients with LOH at D11S2179, both Caucasians, showed missense mutations in the tumors with residual amplification of the normal allele due to the presence of a few normal cells in the tumor sample. Analysis of granulocyte DNA from both cases showed that they were heterozygous and carried the mutant allele in their germ line. Fig. 3 shows the sequence of the normal and tumor tissues of one of these patients. One patient showed a 5071A→C nucleotide transversion, resulting in a S1691R change and the other showed a 7271T→G transversion resulting in a V2424G change. Both mutations had previously been described in AT families (22, 23). The 5071A→C mutation was described in an AT patient (23) and in the germ line of a breast cancer patient with a family history of cancer (21). The T7271G mutation, a founder mutation in the British Isles, was described in both the homozygous and compound heterozygous states in AT families with a variant phenotype showing decreased cerebellar degeneration and slower progression (22, 23) as well as in a case of sporadic T-cell leukemia (9). Table 1 presents a summary of identified mutations.

The question of whether AT heterozygotes, which are thought to constitute between 0.2 and 1% of the Caucasian population (25, 26), have an increased risk of developing tumors, remains to be answered. However, the previous description of four (including the 5071A→C) germ-line mutations in 88 cancer families, giving a carrier rate of 4.5% in this group (21), and our finding of 2 carriers among 34
informative Caucasian patients with sporadic B-CLL (see Ref. 18 for LOH analysis of these patients), giving a carrier rate of 5.9%, strengthen the argument of increased susceptibility.

The hypothesis that the observed carrier frequency of 5.9% in B-CLL is significantly grater than the 1% estimate in the general population was tested using a one-sided exact binomial test that assures that a significant probability (P) is valid despite the small sample size (27). The test is significant with a P = 0.045, suggesting that the observed carrier rate is indeed higher than expected. In addition, because only 4 of 34 normal samples were analyzed (four germ-line DNAs from patients with LOH and mutations), the actual carrier rate in B-CLL is likely to be higher. However, the actual frequency of the two alleles in question in the Caucasian population in North America is unknown and may be significantly higher than the 1% assumed here. Furthermore, cancer predisposition is a complex trait with numerous environmental influences and much larger studies outside the scope of this work will be needed to answer this question.

This work represents the first report of ATM involvement in sporadic B-CLL. Our finding of both germ-line and somatic ATM mutations in B-CLL and the poor outcome of B-CLL with LOH at 11q22–23 (14–17) point to the important role of this gene in B-cell leukemogenesis. Interestingly, none of the cases showing LOH at 11q22–23 revealed no evidence for mutation. Cancer Res., 60: 6427–6431, 1997.


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Table 1 Summary of ATM mutations in B-CLL

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Residue</th>
<th>RNA</th>
<th>Germ line</th>
</tr>
</thead>
<tbody>
<tr>
<td>7865C→T</td>
<td>New splice site</td>
<td>Aberrant</td>
<td>ND*</td>
</tr>
<tr>
<td>995A→G</td>
<td>Y332C</td>
<td>ND</td>
<td>WT</td>
</tr>
<tr>
<td>5071A→C</td>
<td>S1691R</td>
<td>ND</td>
<td>Het</td>
</tr>
<tr>
<td>7271T→G</td>
<td>V2424G</td>
<td>ND</td>
<td>Het</td>
</tr>
</tbody>
</table>

* ND, not determined; WT, homozygous wild type; Het, heterozygous carrier.


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