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.

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 Qiagen 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).

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 B-CLL200) with aggressive disease (Rai stage III/IV) for which there was no information regarding LOH on chromosome 11 or ATM protein expression.
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 presence of a somatic ATM mutation in a B-CLL case with LOH at 11q22–23 suggests that, in B-CLL, the ATM gene may be the target of allelic loss in the region.

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 higher 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 D11S2179 showed LOH at 13q14 (28), the site of a putative tumor suppressor gene involved in B-CLL, indicating that at least two distinct genetic/biochemical pathways can lead to B-CLL. As is the case in sporadic T-PLL/T-CLL (9–12), sporadic B-CLL shows a relative increase of missense over premature termination mutations characteristic of AT families. However, there is no clustering of mutations along the protein that would suggest an association between particular domain function inactivation and B-CLL leukemogenesis. Nonetheless, the above mentioned correlation of both TP53 inactivation (17) and chromosome 11 LOH (13–16) with poor survival in B-CLL, coupled with the known interaction among TP53 and ATM (29, 30), argues in favor of the involvement of particular aspects of ATM function in B-CLL progression. Furthermore, the increased incidence of malignancy in AT with 10% of patients developing tumors, 80% of which are of lymphoid origin (2), the phenotype of ATM-deficient mice (31, 32), and the finding of ATM mutations in both B and T-cell sporadic tumors, clearly indicate that ATM inactivation not only interferes with normal lymphoid function and differentiation but also represents an important step in the pathogenesis of these tumors.

The pattern of ATM inactivation in B-CLL and in T-cell tumors, loss of one allele and mutation of the remaining allele, is indicative of a classical tumor suppressor inactivation mechanism. However, this mechanism of inactivation does not eliminate the possibility that allelic loss at 11q uncovers a mutator phenotype that initiates or accelerates leukemic progression. Therefore, functional studies will be necessary to determine whether ATM functions as a tumor suppressor, mutator, or both.

Acknowledgments

We thank Terry Hyslop for performing the one-sided exact binomial test. R. Cusick for expert help with PCR amplification and purification of ATM exons, and T. Manshour for dedicated and expert technical help. We also thank G. Russo and M. Negri for valuable discussions and critical reading of the manuscript.

References


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