To the editor:

Recurrent ATM mutations in T-PLL on diverse haplotypes: no support for their germline origin

The ATM gene has been found to be mutated or deleted in the majority of cases of T cell prolymphocytic leukemia (T-PLL), and missense mutations were shown to cluster in the highly conserved gene region encoding the ATM kinase domain. Recently, Vanasse et al reported that as many as half of the individuals without ataxia-telangiectasia (A-T) who develop sporadic T-PLL are heterozygous carriers of mutations in the ATM gene. In a response, Stoppa-Lyonnet et al argued that there was no evidence in the literature for this assertion and provided a valuable set of data supporting a somatic origin of ATM mutations in this sporadic leukemia. Because nontumor DNA in T-PLL cases was largely unavailable in previous studies, it is unknown to what extent, if any, A-T heterozygosity is associated with a detectable risk of sporadic T-PLL. In addition, the majority of identified ATM mutations in T-PLL were different from those observed in A-T families.

If the ATM mutation in sporadic T-PLL is identical to a previously detected A-T patient allele, haplotype analysis of tumor DNA may be a useful way, in the absence of germline material, to distinguish between a somatic and germline origin of the mutation. Evidence of the same ancestral chromosome shared by a T-PLL and an A-T family would support a germline alteration, whereas an identical mutation in a different haplotype background argues for a recurrent mutation. Our previous analysis of 37 cases with T-PLL, largely from the United Kingdom, identified 3 tumors carrying the same mutations as previously found in the germline of A-T patients. Single strand polymorphism (SSCP) analysis and nucleotide sequencing identified only a single mutation in each of the 3 tumors and failed to detect the presence of a normal allele. The first tumor (1d5) carried a 9 base pair (bp) deletion (7636del9), the most common A-T allele reported so far, found in 15 apparently unrelated families. But this is the most common allele for DI15S2179 (36% in normal chromosomes not carrying an ATM mutation in the United Kingdom population), and in the majority of the remaining marker loci, the observed alleles differed. Although tumor 1c10 had allele 6 at DI15S2179 identical to 6 A-T patients in 3 families with the same 7271T>G mutation, this was the second most common allele for this locus in United Kingdom families (26%). But alleles at and distal to DI15S1778 and at and proximal of the DI15S1819 locus were different in T-PLL DNA compared with the founder haplotype in the A-T patients carrying the 7271T>G mutation. Finally, tumor BJ01, which was found to contain a truncating mutation 9139C>T, also showed a haplotype distinct from the 2 different haplotypes observed in 2 A-T families carrying the same 9139C>T mutation (Figure 1).

Although a marker locus mutation can explain occasional variant alleles in short tandem repeats (in particular in those differing by a single repeat unit), this mechanism is unlikely to explain the multiple allelic diversity observed between the A-T haplotypes and haplotypes of T-PLL tumors. In A-T patients founder haplotypes are conserved over the same range of markers as used here to analyze sporadic T-PLL tumors. It is unlikely, therefore, that the distance over which the markers are spread will allow changes from germline resulting in such allelic diversity. We conclude that the haplotypes in the region analyzed around the ATM locus are not the same as those carrying the same mutation in the germline. Therefore, our results are not consistent with the

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Figure 1. Haplotype analysis of T-PLL tumors and A-T patients carrying the same ATM mutation. haplotype associated with mutation 7636del9 in 8 United Kingdom families. haplotype associated with mutation 7271T>G in 3 United Kingdom families. Not known which haplotype is associated with 9139C>T mutation in this family. haplotype associated with mutation 9139C>T in this family.
germline origin of reported changes in T-PLL tumors, although they ultimately cannot exclude it. In their response to the letter of Stoppa-Lyonnet et al., Vanasse and colleagues argue that recurrence of ATM mutations previously found in T-PLL does not fit well with their strictly somatic origin. Our present data illustrate that recurrent mutations in tumor DNA are compatible with their somatic origin. At present, we believe that there are no data to support a tacit acceptance of the hypothesis of the germline origin of ATM mutations in sporadic T-PLL. This, of course, can now be tested prospectively using matched normal and tumor DNA from a larger number of T-PLL cases with ATM mutations.

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References


To the editor:

Locus-specific regulation of HLA-A and HLA-B expression is not determined by nucleotide variation in the X2 box promoter element

HLA class I expression is controlled by several regulatory pathways. The X2 box is a crucial element of the SXY regulatory module, which controls the transactivation of HLA class I and β2-microglobulin and of HLA class II and their accessory genes. Critical in this class II transactivator (CIITA)–induced transactivation is the cooperative binding of a multiprotein complex consisting of RFX, ATF/CREB, and NFY on the SXY regulatory module.

Recently, Girdlestone presented evidence to suggest that nucleotide variation in the X2 box would be responsible for a differential regulation between HLA-A and HLA-B. It was concluded that the X2 box of HLA-A did not bind ATF/CREB factors, leading to a lack of or weaker induction of this locus by CIITA and that an interaction and synergy between CIITA and RelA would compensate for the lack of ATF/CREB binding. Here we present data that, similar to HLA-B, the X2 box of HLA-A binds ATF/CREB and mediates, as part of the SXY regulatory module, CIITA-induced transactivation. This demonstrates that the X2 box is not the basis for locus-specific regulation of HLA-A and HLA-B genes. Our methods were as follows:

**Plasmids:** Luciferase reporter plasmids used contained a 228 bp BgIII-AhAl HLA-A*0201 promoter fragment (pGL3-B250), a 140 bp PpaMI-AhAl HLA-A*0201 promoter fragment (pGL3-A140), or a 269 bp AspI-AhAl HLA-B*0702 promoter fragment (pGL3-B250) cloned into pGL3-Basic (Promega, Madison, WI), as described. The X2 box mutant constructs (pGL3-AmX2 and pGL3-BmX2) contained a 4 bp mutation in the X2 box region (HLA-A2: TACAACGCG; HLA-B7: TCGTGACGCG). These were generated by overlap extension polymerase chain reaction (PCR), as described previously. The expression vectors pRSV-RelA (p65) and pREP4-CIITA were described previously.

**Transient transfection:** Cell lines and the calcium phosphate precipitation method of transfection were described previously. In each of 4 wells of a 6-well plate, 0.2 × 10⁶ Tera-2 cells were transfected with a DNA precipitate containing 1 μg firefly luciferase pGL3 reporter plasmid, 0.5 μg pREP4-CIITA, and/or 1 μg pRSV-RelA (p65) and 0.2 μg Renilla luciferase control plasmid (pRL-SV40 or pRL-RSV). Cells were harvested 3 days after transfection. Luciferase activity was determined using the dual luciferase reporter assay system (Promega) and a luminometer (Tropix, Bedford, MA).

**Electrophoretic mobility gel shift assay (EMSA):** Preparation of nuclear extracts and EMSA was performed as described previously. The nucleotide sequence of the X2 probe of HLA-A*0201 was determined.
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