Hu-*ets*-2 is Translocated to Chromosome 8 in the t(8;21) in Acute Myelogenous Leukemia

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ABSTRACT: *The human genome contains two distinct loci with homology to the viral ets gene, the transforming sequence of the E26 avian erythroblastosis virus; these loci, Hu-ets-1, and Hu-ets-2, have been mapped to 11q23 and 21q22, respectively. Using in situ chromosomal hybridization, we have demonstrated that Hu-ets-2 is translocated to chromosome #8, the chromosome containing the critical or conserved junction, as a result of the t(8;21)(q22;q22) in acute myelogenous leukemia. Another protooncogene, c-mos, is also retained at the conserved junction, suggesting that one or both of these genes may play a role in the pathogenesis of acute myelogenous leukemia.*

INTRODUCTION

The avian erythroblastosis virus, E26, induces both myeloblastosis and erythroblastosis in vivo and transforms both erythroid and myeloid progenitor cells in vitro [1]. The transforming gene of E26 has a tripartite origin, being composed of viral gag sequences as well as sequences from the chicken myb and ets genes [1]. In humans, two distinct genes with homology to the viral *ets* oncogene have been identified [2]. These genes are structurally distinct, transcriptionally active, and are located on two human chromosomes [2, 3]. Thus, the human Hu-*ets*-1 locus has been mapped to chromosome #11 at band q23 [2–4], and Hu-*ets*-2 has been localized to chromosome #21 at band q22 [3, 5; Le Beau et al., unpublished results]. These chromosomal regions contain the breakpoints in several of the recurring abnormalities associated with acute leukemia. Specifically, band 11q23 is involved in the t(9;11) of acute monocytic leukemia (AMoL) [6], and acute myelomonocytic leukemia (AMMoL), as well as in the t(4;11) in acute lymphoblastic leukemia (ALL) [7, 8], and 21q22 is the breakpoint of the t(8;21) in acute myelogenous leukemia (AML) with maturation [9].

Molecular analysis of each of these rearrangements have suggested that the Hu-*ets*-1 and Hu-*ets*-2 sequences may play a role in the pathogenesis of the corresponding leukemia. With respect to the t(9;11), the Hu-*ets*-1 gene is translocated to the short arm of chromosome #9 in proximity to the α-interferon sequences [10]. Similarly, the analysis of somatic cell hybrids prepared by the fusion of Chinese ham-

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ster cells with leukemia cells with a t(4;11) has revealed that Hu-*ets*-1 is translocated to 4q [11]. Southern blot analysis of leukemia cells with the t(9;11) or t(4;11) and of the cell hybrids did not reveal any rearrangement of the Hu-ets-1 sequences [10, 11].

With respect to the t(8;21), analysis of somatic cell hybrids prepared by fusing Chinese hamster cells with leukemia cells with a t(8;21) indicated that the Hu-*ets*-2 gene was present in the cell hybrid which contained the 8q− chromosome, but not in the hybrid containing the 21q+ chromosome [11]. The Hu-*ets*-2–specific probe used in these experiments was derived from the 3′ region of the gene. Rearrangements of this locus have not been identified by Southern blot analysis of DNA from the hybrids or from leukemia cells with a t(8;21) using the same probe. The relocation of Hu-*ets*-2 to 8q is interesting since the 8q− chromosome appears to contain the critical or conserved junction. We have previously shown that the oncogene c-*mos* remains on the 8q− chromosome in metaphase cells with the t(8;21) [12]. By using in situ chromosomal hybridization we have also determined that the Hu-*ets*-2 gene is translocated from chromosome #21 to the conserved junction on chromosome #8.

METHODS AND RESULTS

To determine if the Hu-*ets*-2 gene is involved in the breakpoint of the t(8;21) we performed in situ chromosomal hybridization using a Hu-*ets*-2–specific genomic probe, H33 [2], to metaphase cells prepared from leukemic bone marrow cells of three AML-M2 patients with a t(8;21) [13]. The results of this hybridization are given in Table 1 and are illustrated in Figure 1. In hybridizations using the Hu-*ets*-2 probe, we observed specific labeling on the normal chromosome #21, as well as on the rearranged chromosome #8 (8q−). These results indicate that this gene is translocated to #8 as a result of this chromosomal rearrangement.

The distribution of labeled sites on the normal homologs 8 and 21 and on the rearranged chromosomes, 8q− and 21q+, observed in metaphase cells from each patient is illustrated in Figure 1. Of 100 metaphase cells examined from patient 1 [46,XY,t(8;21)(q22;q22)(100%)], 12 (12%) were labeled on the normal chromosome #21 (Fig. 1a). The labeled sites on this chromosome were clustered at band q22; this cluster of grains represented 8.8% of all labeled sites (11 of 125; $p < 0.0005$). Three labeled sites were noted on the 21q+ chromosome; none were located at the translocation breakpoint junction. In 15 metaphase cells, labeled sites were noted on the 8q− chromsome; they were clustered at the translocation breakpoint junction at band 8q22 and at band 21q22 (11 of 125, 8.8%; $p < 0.0005$). The normal #8 homolog had only five labeled sites distributed on both the short and long arms (4.0%; $p > 0.2$).

Analysis of metaphase cells from two other patients with this rearrangement gave similar results (Table 1; Figure 1). Of 100 metaphase cells examined from patient 2 [46,XX,t(8;21)(q22;q22)(15%)/47,XX,+4,t(8;21)(q22;q22)(85%)] that were hybridized with Hu-*ets*-2, nine were labeled on band q22 of the normal #21, and ten were labeled on 8q22 or 21q22 of the 8q− chromosome (Fig. 1b). These clusters of sites represented 7.5% (nine of 120) and 8.3% (ten of 120) of all labeled sites, respectively. Both the normal #21 and the 8q− were significantly labeled ($p < 0.0005$). In contrast, neither the normal #8 nor the 21q+ chromosome showed significant labeling. Analysis of metaphase cells from this patient in a second hybridization experiment gave identical results. For patient 3 [46,XX(40%)/45,X,−X,t(8;21)(q22;q22)(60%)], only 44 metaphase cells were available for analysis. Of these, six were labeled on band q22 of the normal #21, and seven were labeled on 8q22 of the 8q− chromosome (Fig. 1c). A total of 49 grains were ob-

Table 1 In situ hybridization of the Hu-*ets*-2 probe to metaphase cells with a t(8;21)

Case	Total number of labeled sites (number of metaphase cells analyzed)	Number of labeled sites (%)							
		Normal #8		8q−		Normal 21		21q+	
		Total	q22	Total	q22 Junction	Total	q22	Total	q22 Junction
1	125(100)	5(4)	0	15(12)[a]	11(8.8)	12(9.6)[a]	11(8.8)	3(2.4)	0
2	120(100)	4(3.3)	0	16(13.3)[a]	10(8.3)	13(10.8)[a]	9(7.5)	0	0
3	49(44)	2(4.1)	0	7(14.3)[a]	6(12.2)	7(14.3)[a]	6(12.2)	0	0

[a] χ^2 value corresponds to a $p < 0.0005$.

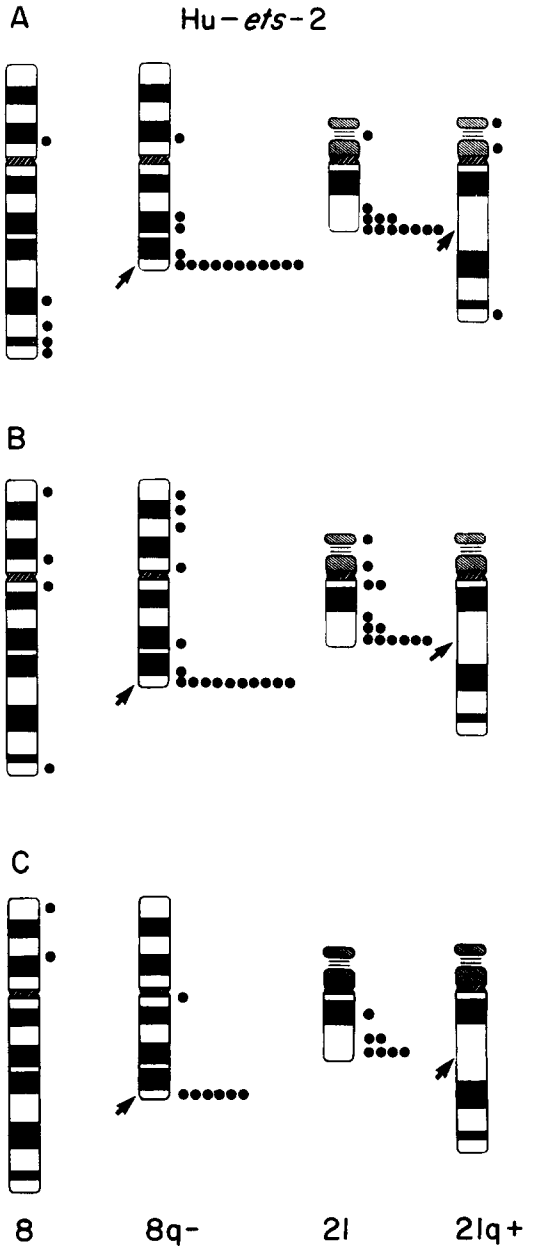

Figure 1 Distribution of labeled sites on chromosomes 8 and 21 and on the translocation derivatives 8q− and 21q+ after hybridization of the Hu-*ets*-2 probe to metaphase cells from three AML patients with a t(8;21)(q22;q22). Clusters of grains can be observed at band q22 on the normal chromosome #21, and at the breakpoint junction on the 8q− chromosome. The arrows identify the breakpoint junctions. Metaphase chromosomes were hybridized with the H33 probe which contains a 1.02 kb insert of the human genomic Hu-*ets*-2 sequences cloned in pBR322. Radiolabeled probe was prepared by nick-translation of the plasmid DNA with tritiated deoxynucleoside-triphosphates to a specific activity of 2×10^8 dpm/μg. In situ hybridization was performed as described previously [13]. Metaphase cells were hybridized at 2 or 4 ng probe/ml of hybridization mixture. The results of these hybridizations indicate that the Hu-*ets*-2 gene is relocated as a result of this translocation to the long arm of chromosome #8.

served; thus, these clusters of grains represented 12.2% and 14.3% of all labeled sites, respectively. The normal #8 (two grains) and the 21q+ chromosome (zero grains) were not significantly labeled.

DISCUSSION

Our results are consistent with those obtained from the analysis of somatic cell hybrids [11], indicating the Hu-*ets*-2 is distal to the translocation breakpoint on chromosome #21, and that this gene is relocated to the 8q− chromosome as a result of the t(8;21) in AML-M2. Southern blot analysis of leukemia cells from eight patients with a t(8;21) using a probe containing the 3′ region of the Hu-*ets*-2 gene did not reveal any rearrangement of the Hu-*ets*-2 gene [11]; thus, the proximity of this gene and its relationship to the translocation breakpoint is unknown. The results of Southern blot analysis and in situ chromosomal hybridizations using probes representing the 5′ region of this gene may clarify this issue. Nevertheless, preliminary evidence suggests that the t(8;21) is associated with an altered expression of the Hu-*ets*-2 gene [11].

Finally, the identification of two oncogenes, c-*mos* and Hu-*ets*-2, in proximity to the conserved breakpoint junction on the 8q− chromosome is intriguing; this phenomenon has not been identified in the other recurring chromosomal abnormalities analyzed to date. Caubet et al. using in situ chromosomal hybridization have suggested that the c-*mos* gene may be located at 8q11 rather than q22, and therefore may be irrelevant with respect to the t(8;21) [14]; however, our studies using this technique and a similar c-*mos* probe do not support this hypothesis [12]. Clarification of the precise role of c-*mos* and Hu-*ets*-2 in the pathogenesis of AML with the t(8;21) will await a detailed molecular characterization of the sequences located at the breakpoints, of the transcriptional activity of c-*mos* and Hu-*ets*-2, and of the proteins encoded by these genes.

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