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17. Two additional measures were taken to gauge the comparability of the spinal cords among groups. For each cord, the outline of the gray matter in every section examined was traced using a microprojector and the average gray matter area calculated; the rostral-caudal length of the SNB was also determined in each animal. No differences were observed in either measure [Fs(5, 19) < 1.6, P > 0.2], indicating that cords did not shrink after castration or show overall growth due to androgen treatment.
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18 November 1985; accepted 7 February 1986

Amplification and Rearrangement of Hu-*ets*-1 in Leukemia and Lymphoma with Involvement of 11q23

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The Hu-*ets*-1 oncogene was found to be rearranged and amplified 30-fold in one case of acute myelomonocytic leukemia in which a homogeneously staining region occurred on 11q23; the oncogene was rearranged and amplified approximately tenfold in a case of small lymphocytic cell lymphoma with an inverted insertion that also involved band 11q23. This work suggests that Hu-*ets*-1 is an unusual oncogene that can help explain the common involvement of chromosome band 11q23 in various subtypes of hematopoietic malignancies.

THE PRESENCE OF *ETS* AND *MYB* IN the acute leukemia virus E26 produces at least two differences in the pathogenicity of this virus as compared to the myeloblastosis virus AMV, which contains only *myb*: (i) leukemias with a myeloid as well as an erythroid phenotype are induced in birds infected by E26 and (ii) myeloblasts transformed by E26 appear to be completely blocked in differentiation and are not inducible by tumor promoters (*1*). While two different c-*ets* loci have been detected in the human, mouse, and cat genomes (*2*), a single colinear c-*ets* homologue may be present in the chicken genome. In humans, these loci were called Hu-*ets*-1 and Hu-*ets*-2 (*2*) and were mapped to chromosome bands 11q23-24 (*3*) and 21q22.3, respectively (*2*). Specific chromosomal abnormalities involving 11q23—such as reciprocal translocations, deletions, inverted insertions (inv ins), and homogeneously staining regions (HSR's) (*4–6*)—have been associated with acute myelomonocytic leukemia (AMMoL) (*4*), acute lymphocytic leukemia (ALL) and small lymphocytic cell lymphoma (SLCL) (*5*), and myeloproliferative syndromes (*6*). Because of these observations, we decided to investigate whether Hu-*ets*-1 is altered in leukemic and lymphomatous cells with 11q23 chromosomal abnormalities.

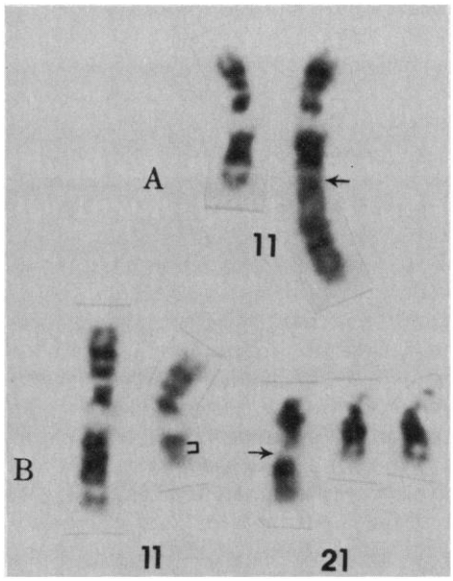

Fig. 1. Pairs of G-banded chromosomes 11 in patient 1 with acute myelomonocytic leukemia (AMMoL) and an HSR 11q23 (A); and chromosomes 11 and 21 in patient 2 with small lymphocytic cell lymphoma (SLCL) with inv ins (21;11)(q22.3; q23q14.2), +21 (B).

The G-banding pattern on chromosome 11 from patient 1 with AMMoL (M4) and an HSR 11q23 as the only chromosomal defect is shown in Fig. 1A; Fig. 1B displays an inv ins(21;11)(q22.3; q23q14.2), +21 in an SLCL patient (patient 2). In patient 2, the segment 11q14.2q23 from one chromosome 11 is inverted before being inserted into band 21q22 so that band 11q23 [where Hu-*ets*-1 maps (*2*)] and band 21q22 [where Hu-*ets*-2 maps (*2*)] become contiguous in the abnormally long chromosome 21.

The Hu-*ets*-1 probe, pRD6K (Fig. 2A), is a 5.7-kilobase (kb) fragment of human DNA (*2*). Southern blot hybridization (*7*) revealed amplification of Hu-*ets*-1 in Bam HI–digested DNA from patients 1 and 2 (Fig. 2B). For comparison, a normal blood donor DNA sample obtained during chemotherapy-induced remission from patients 1 and 2 are shown.

In the case of the DNA from patient 1, an additional band of approximately 9.0 kb was present after Bam HI digestion. The signal intensity from the rearranged fragment was slightly less than the high signal intensity of the other amplified band which migrated with the same mobility as the 14-kb band present in normal controls.

Additional evidence for the amplification as well as rearrangement of Hu-*ets*-1 was obtained by digesting DNA from patients 1 and 2 with the restriction enzyme Xba I and by hybridizing the blots with the pRD6K probe. Both patients showed evidence of amplification and rearrangement (Fig. 2C, lanes 4 and 5).

Rearrangement was indicated by the pres-

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ence of bands of intermediate sizes (approximately 2.1 and 2.3 kb for patients 1 and 2, respectively). In additional experiments, we used the filter previously hybridized to the pRD6K probe, and an 830–base pair (bp) probe (pRD700) that has been mapped 3′ to pRD6K and is adjacent to the discontinuity between the loci of Hu-*ets*-1 and Hu-*ets*-2 (Fig. 2A). The region recognized by the Hu-*ets*-1 probe pRD700 was amplified but not rearranged in DNA's from these patients (Fig. 2C, lanes 8 and 9). These data suggest that the entire region identified by the pRD6K and pRD700 probes is amplified in both patients, but that the DNA sequence alteration is inside fragments detected by pRD6K and is therefore 5′ to pRD700. The lower degree of amplification of some bands recognized by the pRD6K probe in the DNA sample from patient 2 can be explained by the particular structure of the amplified-rearranged region; a similar degree of amplification was detected by the probe pRD700.

Since patient 2 also had a breakpoint at band 21q22 where Hu-*ets*-2 has been mapped, the probe pH33 [which is specific for the Hu-*ets*-2 locus (Fig. 2A)] was also hybridized to the washed filter that had previously been hybridized with pRD6K and pRD700. DNA from patient 1 showed no amplification or rearrangement and was comparable to a normal control (Fig. 2C, lanes 10 and 12). DNA from patient 2, however, had an extra 1.3-kb band and lacked the 0.9-kb band (Fig. 2C, lane 11) as compared to the normal blood donor and patient 1 DNA's (Fig. 2C, lanes 10 and 12). These changes could have resulted from a submicroscopic deletion(s) or other mechanisms causing loss of heterozygosity as has been shown for the K-*ras*, *Rb*-1, and *WAGR* loci that are deleted in neoplastic cells (*8*). The possibility of a polymorphic variation with these enzymes was ruled out because a remission sample from patient 2, as well as blood samples of 27 normal individuals, showed no polymorphic variation. The results of the combined use of specific probes for Hu-*ets*-1 and Hu-*ets*-2 suggest that Hu-*ets*-1 is amplified and rearranged in both patients and that Hu-*ets*-2 is altered in patient 2.

Amplification of Hu-*ets*-1 was quantified by densitometric analysis of several Southern blots from patient 1 and 2 DNA's that had been digested with Bam HI and Hind III. As an internal control, we washed the Hu-*ets*-1 probe from the filter and rehybridized it with the probe specific for the human heavy-chain immunoglobulin Cμ gene, as described (*9*). The hybridization intensity indicated that a similar amount of DNA was present on each lane of the gel; this was also observed when the same samples were digested with Xba I and rehybridized with the Hu-*ets*-2 probe (Fig. 2C, lanes 10–12). X-ray films of the same filter were exposed for different amounts of time and films in which the intensity of the signal was proportional to exposure time were analyzed (*10*). From these and other experiments, we estimate that the Hu-*ets*-1 sequence was amplified 30- to 40-fold in DNA from patient 1 and approximately 10-fold in DNA from patient 2.

In situ hybridization studies (*11*) were carried out to confirm the chromosomal localization of Hu-*ets*-1 in 25 prometaphases of leukemic blasts from patient 1 (Fig. 3). Roughly half of all grains were on chromosome 11; 49 of 113 chromosomal grains were scattered over all chromosomes and the rest were preferentially localized on 11q23, with 13 grains on the normal chromosome and 51 on the HSR 11q23 (fourfold increase). We did not have fresh chromosome preparations from leukemic blasts of patient 2 for in situ hybridization studies.

To determine whether abnormal patterns for the Hu-*ets*-1 locus are associated with other abnormalities involving chromosome 11q23, we analyzed fibroblastic or lymphocytic cultures from patients with birth defects and different chromosomal translocations—t(11;22), t(9;11), t(6;11), and

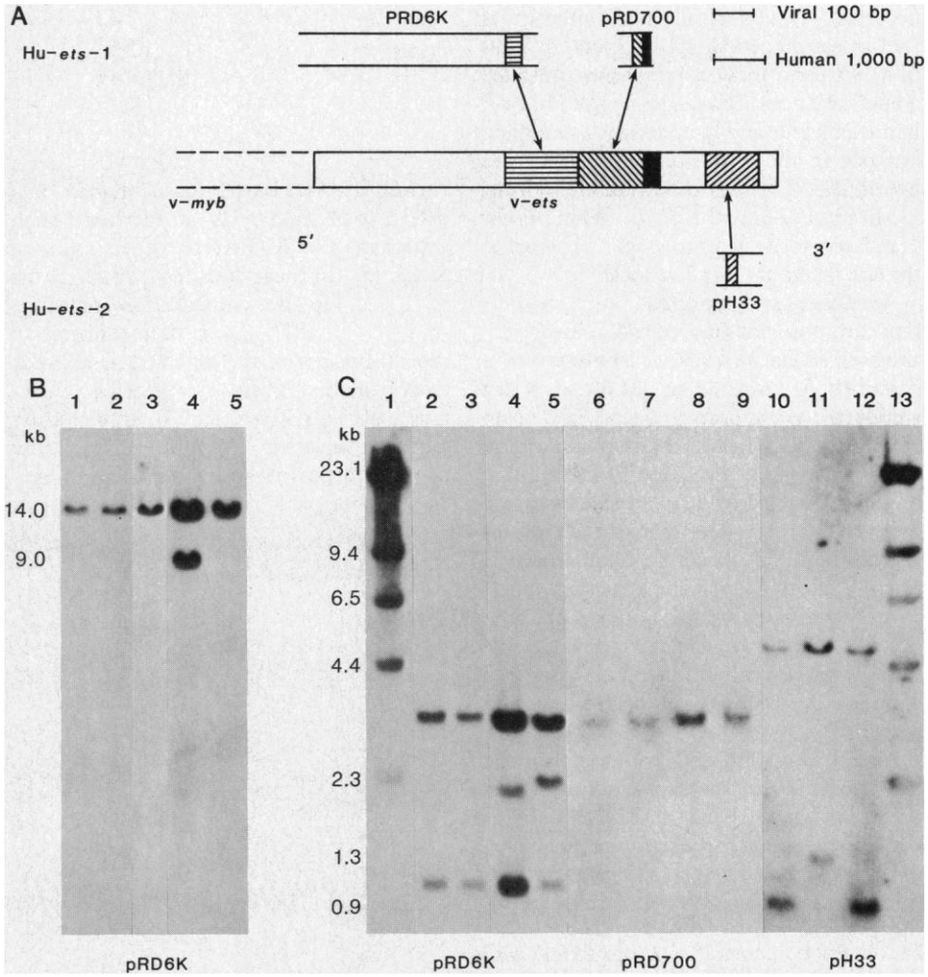
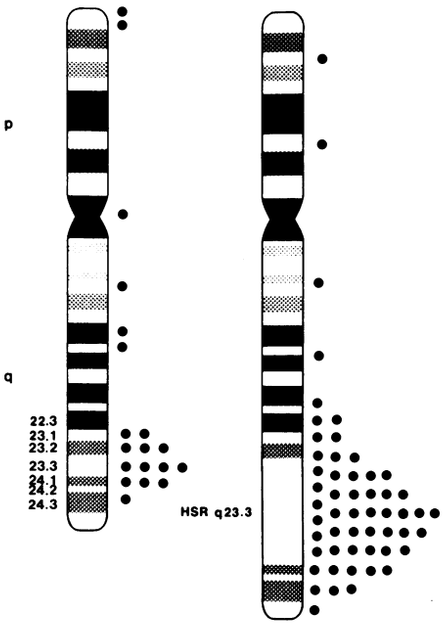


Fig. 2. (A) Hu-*ets*-1–specific probes pRD6K and pRD700 and Hu-*ets*-2–specific probe pH33 (*2*). For comparison, v-*ets* from the virus E26 is also shown. Solid boxes represent the Hu-*ets*-1/Hu-*ets*-2 common region; boxes with horizontal or diagonal lines show regions of homology between v-*ets* and Hu-*ets*-1 or Hu-*ets*-2. (B) DNA hybridization analysis of Hu-*ets*-1 abnormalities with DNA (10 μg) that had been digested with Bam HI and hybridized with the 5.7-kb insert of pRD6K that is specific for Hu-*ets*-1. (Lane 1) Normal blood donor; (lanes 2–4) marrow samples from patient 1 while in remission, patient 2 while in remission, and patient 1 during the leukemic phase, respectively; (lane 5) lymphomatous DNA from patient 2. (C) Hybridization analysis with Hu-*ets*-1 and Hu-*ets*-2 specific probes. DNA (10 μg) was digested with the enzyme Xba I and the same filter was sequentially hybridized with the pRD6K (lanes 1–5), pRD700 (lanes 6–9), and pH33 (lanes 10–13). Molecular weight markers of Hind III–digested DNA were also added to lanes 1 and 13 [nick-translated ^{32}P-labeled DNA (approximately 10^4 cpm/μg)]. Lanes 4, 8, and 10 correspond to patient 1 DNA; lanes 5, 9, and 11 to patient 2 DNA; lanes 2, 6, and 12 are normal blood donor controls; and lanes 3 and 7 are samples from patient 1 while in remission.

Fig. 3. In situ hybridization of Hu-*ets*-1–specific probe RD6K to chromosome 11 of leukemic blasts of patient 1. Grain distribution in 25 prometaphases is represented in the normal chromosome 11 and in the chromosome 11 with an HSR.



t(X;11)—involving band 11q23 (*12*). Hybridization analysis of DNA from these patients showed no amplification or rearrangement for the Hu-*ets*-1 locus (Fig. 4, lanes 1–5). Additional negative controls included one ANLL and one non-Hodgkin's lymphoma case with no abnormalities of 11q23 (Fig. 4, lanes 6 and 7).

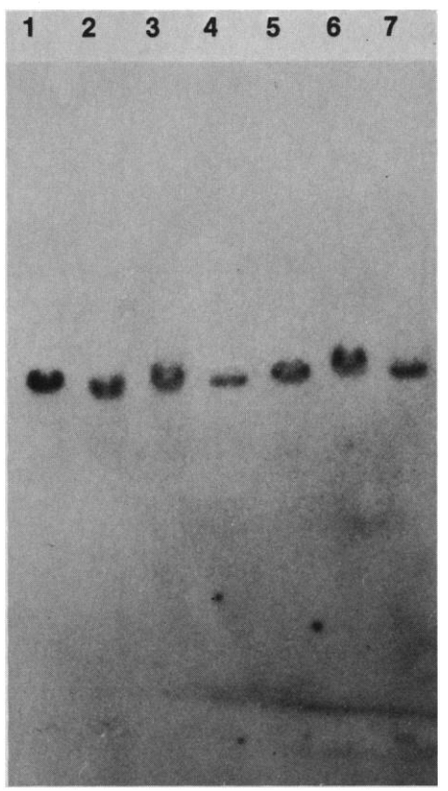
Thus, Hu-*ets*-1 is altered in two cases of hemapoietic malignancies in which different abnormalities of chromosomal band 11q23 occurred. An abnormally high intensity as well as rearranged signal in Southern blots of DNA from these two patients indicated amplification and rearrangement of Hu-*ets*-1 in the leukemic and lymphomatous samples, but not in the remission samples. In situ hybridization studies confirmed the normal localization of Hu-*ets*-1 on 11q23 in patient 1 and also identified the amplified signal at the site where the HSR is located.

Amplified genes are often located on aberrant chromosomal sites (*13, 14*). However, a situation similar to what we found has been described for a clone of HL60 in which amplified c-*myc* appears to be localized on an HSR at 8q24, which is the normal location of c-*myc* (*15*). The elevation in the number of grains detected on 11q23 HSR by in situ hybridization was less than the degree of amplification calculated by Southern blotting. While the explanation for this difference is not clear, a similar situation has been described for the human carcinoma cell line A431 in which rearranged and unrearranged bands of the *erb*B proto-oncogene are amplified (*14*).

Alteration of Hu-*ets*-1 could explain the high frequency of karyotypic abnormalities involving band 11q23 in ANLL patients with myelomonocytic phenotype, in which the translocation t(9;11)(p22;q23) appears to be the most common abnormality (*4, 5*).

Fig. 4. Hybridization analysis of pRD6K with Bam HI–digested DNA (10 μg) from four patients with congenital nonhematological disorders and 11q23 abnormalities. (Lane 1) Normal cell DNA; patient DNA's from cell lines GM 0084 (lane 2) with t(11;22)(q23;q11), GM 0364 (lane 3) with t(9;11)(p24;q23), GM 1605 (lane 4) with t(6;11)(p23;q23), GM 3552 (lane 5) with t(X;11)(q26;q23). Negative controls from an ANLL patient with t(15;17)(q22;q11.2) and an NHL patient with t(14;18)(q32;q21) and no involvement of 11q23 are shown on lanes 6 and 7, respectively. Hybridization of the same DNA samples after digestion with Xba I also showed a pattern of hybridization undistinguishable from normal controls.

An inv ins(21;11)(q22.3; q23q14.2) or a del(11)(q14.2q23),+21 is associated with approximately 20 percent of all cases of SLCL (*5*). In these disorders, however, the finding of Hu-*ets*-1 amplification and/or rearrangement may appear more difficult to explain because of the different (lymphoid) nature of this disease. Nevertheless, leukemias with a t(4;11)(q21;q23), until recently classified as acute lymphocytic, are now believed to possess an undifferentiated phenotype with both myelomonocytic and lymphoid character (*16*). These leukemias have also been shown to contain rearrangements of the immunoglobulin heavy-chain Cμ genes, a marker considered characteristic of lymphoid differentiation (*9, 17*).



Preliminary data indicate that similar alterations in the Hu-*ets*-1 locus may be present in the leukemic cells from one AMMoL case with a translocation t(6;11)(q27;q23), from two AMMoL cases with t(9;11)(p22;q23) and from one case of myelodysplastic syndrome or preleukemia with t(2;11)(p13;q23). Band 11q23 is involved in several translocations, deletions, insertions and HSR's in leukemias (*4*), lymphomas (*5*) and myeloproliferative syndromes (*6*). Thus, Hu-*ets*-1 may be an unusual oncogene that, depending on other chromosomal sites involved, helps determine different phenotypes or is altered according to differentiation-specific pathways.

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18. We thank J. Silver and M. Israel for helpful discussion. Partially supported by NCI grants CA-31024 and CA-33314.

9 October 1985; accepted 8 January 1986