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Interferon and c-*ets*-1 Genes in the Translocation (9;11)(p22;q23) in Human Acute Monocytic Leukemia

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Gene probes for interferons α and β_1 and v-*ets* were hybridized to metaphase chromosomes from three patients with acute monocytic leukemia who had a chromosomal translocation, t(9;11)(p22;q23). The break in the short arm of chromosome 9 split the interferon genes, and the interferon-β_1 gene was translocated to chromosome 11. The c-*ets*-1 gene was translocated from chromosome 11 to the short arm of chromosome 9 adjacent to the interferon genes. No DNA rearrangement was observed when these probes were hybridized to genomic DNA from leukemic cells of two of the patients. The results suggest that the juxtaposition of the interferon and c-*ets*-1 genes may be involved in the pathogenesis of human monocytic leukemia.

SPECIFIC CHROMOSOMAL ABNORMALITIES are associated with defined types of leukemia (*1*). In two of these abnormalities, the t(8;14)(q24;q32) in Burkitt's lymphoma (BL) and the t(9;22)(q34;q11) in chronic myelogenous leukemia (CML), cellular proto-oncogenes associated with one of the chromosomal breakpoints (*2, 3*) are linked to another cellular gene. The immunoglobulin (Ig) genes are expressed specifically in B cells, which are the neoplastic cells in BL; c-*myc* is under the control of regulatory mechanisms that are related to the Ig genes. In CML, the *bcr* and c-*abl* genes are fused in the Philadelphia chromosome, resulting in transcription of a chimeric messenger RNA (mRNA) under the control of the *bcr* promoter.

Abnormalities of chromosome 11 involving band q23 are observed frequently in acute monocytic leukemia (AMoL), particularly in children (*4*). A common rearrangement, noted in about 10 percent of patients with AMoL, is the translocation (9;11)(p22;q23); the precise breakpoint in 9p, however, is a subject of disagreement. Analysis of trypsin-Giemsa–banded metaphase cells of our patients has indicated that all of band 9p21 remains on chromosome 9, and thus we have designated the breakpoint as 9p22. Other abnormalities observed in patients with AMoL include the t(11;19)(q23;p13) and del(11)(q23). Rearrangements involving 11q23 have also been reported in other hematologic malignant diseases. In particular, the t(4;11)(q21;q23) has been observed in patients with acute lymphoblastic leukemia (*5*), and leukemic cells with this abnormality may have cell surface markers characteristic of the monocyte lineage (*6*). The breakpoint in the t(9;11) at band 9p22 is also involved in other structural rearrangements. For example, deletions and translocations of the short arm of chromosome 9 are associated with a distinct subset of acute lymphoblastic leukemia (*7*).

Two sets of cellular genes have been localized to the vicinity of the breakpoints in the t(9;11) by in situ chromosomal hybridization. First, the cellular proto-oncogene c-*ets*-1 has been assigned to bands 11q23-q24 (*8*). The v-*ets* oncogene contains sequences that are homologous to cellular DNA located on chromosomes 11 and 21 (*9*). Two oncogenes, v-*ets* and v-*myb*, are transduced by the avian retrovirus E26 (*10*). This retrovirus causes a monoblastic neoplastic proliferation (myeloblastosis) as well as erythroblastosis in chickens (*10*).

Second, a cluster of interferon (IFN) genes, which include the family of IFN-α genes and the IFN-β_1 gene, has been assigned to the short arm of chromosome 9 at bands 9p13-p24 (*11*). The IFN-α and IFN-β_1 genes belong to a group of eukaryotic genes that encode polypeptides with antiviral and anticellular properties. These genes are not spliced, and their expression can be induced by viral infection in a large variety of cells (*12*). The IFN-α genes are a family of at least 17 closely related genes (*13*) that are clustered on the short arm of chromosome 9 (*11, 14*). Subsets of up to three IFN-α genes arranged in tandem have been isolated on one or a few overlapping genomic clones (*13*). Some of the genes are more closely linked than others, with distances ranging from 3.8 to 18 kilobases (kb) (*13*). The average distance between these linked genes is 10 kb. A second family of IFN-α–like sequences is interspersed in tandem arrangement between the IFN-α genes (*15*). Judging from the linkage disequilibrium of five restriction fragment length polymor-

Table 1. In situ hybridization of IFN and v-*ets* probes to metaphase cells with a t(9;11). The probes were prepared by nick translation of the plasmid DNA's with tritiated deoxynucleoside triphosphates to specific activities of about 3×10^7 dpm/μg: pR-78 contains human IFN-α_1 complementary DNA (cDNA) (*24*), pHFβ cDNA contains human IFN-β_1 cDNA (*25*), and p-ets-BB contains v-*ets* sequences derived from the virus E26 (*21*). In situ hybridization was performed as described previously (*26*). Metaphase cells were hybridized at 20 or 40 ng of probe per milliliter of hybridization mixture.

Probe	Patient	Number of labeled sites (number of metaphase cells analyzed)	Number of labeled sites (%)			
			Normal 9	t(9p)	Normal 11	t(11q)
IFN-β_1	1	128 (100)	15 (11.7)*	1 (0.8)	3 (2.3)	10 (7.8)*
	2	74 (50)	6 (8)*	2 (2.7)	2 (2.7)	6 (8)*
IFN-α_1	2	267 (82)	22 (8.2)*	20 (7.5)*	8 (3)	8 (3)
	3	52 (20)	6 (11.5)*	8 (13.5)*	0	3 (5.8)
v-*ets*	2	96 (60)	4 (4.2)	8 (8.3)*	11 (11.5)*	2 (2.1)
	3	50 (20)	1 (2)	6 (12)*	7 (14)*	0

*χ^2 value corresponds to $P < 0.0005$. The χ^2 analysis tests the hypothesis that labeling is random over all chromosomes.

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phisms, the IFN-β_1 gene, which is present in genomic DNA in a single copy (*16*), seems to be closely linked to the IFN-α gene cluster (*17*).

To determine whether the IFN genes or the c-*ets*-1 gene are involved in the breakpoints of the t(9;11), we performed in situ chromosomal hybridization of v-*ets*, IFN-α, and IFN-β_1 probes to metaphase chromosomes prepared from leukemic bone marrow cells of three AMoL patients who had a t(9;11). In hybridizations with the IFN-β_1 probe, we observed specific labeling on the normal chromosome 9 as well as on the rearranged chromosome 11 [t(11q)]; the IFN-α probe hybridized to the normal and rearranged chromosome 9 [t(9p)], but not to the t(11q) chromosome (Table 1 and Fig. 1). Specific labeling of both the normal chromosome 11 and the rearranged chromosome 9 was found after hybridization with the v-*ets* probe, indicating that c-*ets*-1, the homologous gene, was translocated to the short arm of chromosome 9.

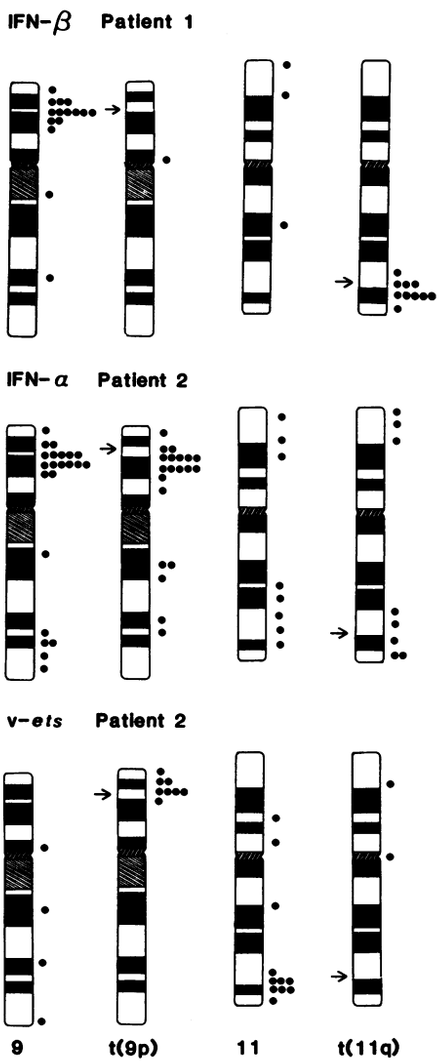

Fig. 1. Distribution of labeled sites on normal homologs 9 and 11 and on the translocation derivatives t(9p) and t(11q) that were hybridized to IFN-β_1, IFN-α, and v-*ets* probes. Metaphase cells were from two AMoL patients with a t(9;11)(p22;q23). The arrows identify the breakpoint junctions on the rearranged homologs.

In the hybridization of the IFN-β_1 probe to metaphase cells from patient 1, a cluster of grains was observed at bands p21 to p24 of the normal chromosome 9 (Fig. 1). The grains in this cluster represented 10.2 percent of all the grains observed over chromosomes (13/128; $P < 0.0005$ by χ^2 testing relative to the number that would be expected if there were a random distribution of grains over all chromosomes). A single grain was noted on the t(9p) chromosome. Labeled sites were found on the t(11q) chromosome in 10 metaphase cells; the grains were clustered at the translocation breakpoint junction at band 11q23, and at bands 9p22 to 9p24 (10/128; $P < 0.0005$). The normal chromosome 11 homolog had only three labeled sites, which were distributed over both the short and long arms (3/128; $P > 0.8$). Analysis of metaphase cells from patient 2 gave similar results (Table 1). Thus, the IFN-β_1 gene, normally located at 9p22 to p23, was translocated to chromosome 11 as a result of the t(9;11).

However, IFN-α genes remained on the t(9p) chromosome and, thus, are proximal to the t(9;11) breakpoint (Fig. 1 and Table 1). In the hybridization with the IFN-α probe to metaphase cells of patient 2, grains were clustered at bands p21 to 22 of the normal chromosome 9 (16/267; $P < 0.0005$) and the t(9p) chromosome (15/267; $P < 0.0005$). However, in the t(9p) chromosome, the cluster of grains extended over the material translocated from chromosome 11 (band 11q23) (Fig. 1). Eight labeled sites were noted on the normal chromosome 11, and eight on the t(11q) chromosome ($P > 0.3$). Although a small number of grains were distributed on the t(11q) chromosome along the region of the breakpoint junction, this labeling was not statistically significant. Moreover, an equivalent amount of label was noted on the distal long arm of the normal chromosome 11. Therefore, we found no evidence that any of the IFN-α genes were translocated to the t(11q) chromosome. Similar results were obtained in hybridizations of metaphase cells from patient 3; however, only a small number of metaphase cells were available for analysis from that patient (Table 1).

Hybridization of the v-*ets* probe to metaphase cells from patients 2 and 3 revealed specific labeling on two chromosome regions, the normal chromosome 11 at bands q23 to q25 and the t(9p) chromosome on the segment translocated from chromosome 11 at bands 11q23 to q25 (Fig. 1 and Table 1). For patient 2 (Fig. 1), the cluster of grains at 11q23 to q25 represented 8.3 percent of all labeled sites (8/96; $P < 0.0005$). Only two grains were noted on the t(11q) chromosome; neither was located on the long arm. The t(9p) chromosome contained eight labeled sites (8/96; $P < 0.0005$), which were clustered at the junction of the translocation breakpoints. The normal chromosome 9 homolog was not specifically labeled (4/96). The results obtained from the analysis of metaphase cells from patient 3 were consistent with these findings (Table 1).

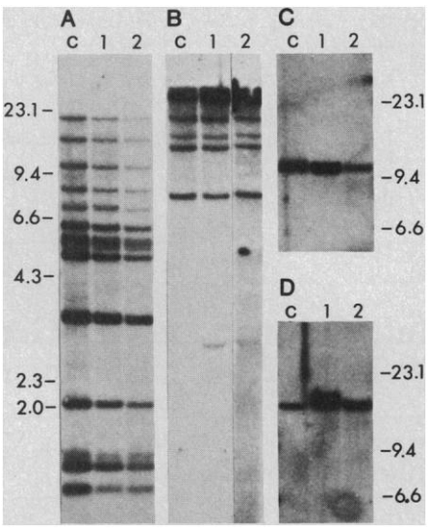

Fig. 2. Southern blots of DNA isolated from bone marrow cells of patients 1 and 2 (*27, 28*). Human placental DNA was used as a control (c). After restriction endonuclease digestion, (Hind III in A and C; Bam HI in B and D), the DNA fragments were separated on agarose gels and transferred to nylon filters. The filters were hybridized to an IFN-α probe (A and B) or to an IFN-β_1 probe (C and D). The sizes of the marker bands are given in kilobases. The labeled band at about 3 kb in the lanes from patient 2 is an artifact introduced by plasmid DNA that contaminated the human DNA preparation.

We analyzed the genomic DNA from two AMoL patients who had the t(9;11) by means of Southern blot hybridization with the IFN and v-*ets* probes. The IFN-α and IFN-β_1 probes only hybridized to germline-sized bands of Bam HI– and Hind III–digested patient DNA (Fig. 2). The v-*ets* probe hybridized to bands of 12.5, 6.5, and 3.6 kb in Bam HI–digested control human placenta DNA and in the DNA of the AMoL patients. After digestion of DNA with Hind III, the v-*ets* probe hybridized to bands of 14 and 2.6 kb, in control and patient DNA. Thus, the t(9;11) did not result in rearrangement of Bam HI or Hind III sites around the IFN genes analyzed. This implies that the transcriptional regulatory sequences associated with the IFN-β_1

promoter were also relocated to the t(11q) chromosome.

The 9p22 breakpoint of the t(9;11) splits the IFN gene cluster, thereby separating the IFN-β_1 gene from all or most of the IFN-α genes. The IFN-β_1 gene had previously been assigned to bands 9p21 to p24 by in situ hybridization (*11*). This gene was further sublocalized to 9p21 by Southern blot analysis of DNA from human cells which had a deletion of chromosome 9 at band p22 (*18*). Our results suggest that the IFN-β_1 gene is actually in 9p22 rather than in 9p21; also, they demonstrate that the IFN-β_1 gene is distal to the IFN-α genes. In addition, our findings define further the localization of the IFN-α genes to 9p13 to p21.

In the mouse as well as in man, the IFN-α genes and the IFN-β_1 gene are located on the same chromosome. In the mouse, the genes are located on chromosome 4 and are clustered together (*19, 20*). If a *cis* regulatory mechanism exists that affects transcription over the entire IFN gene cluster, then this mechanism may affect the function of a gene that is translocated to a position close to the IFN cluster.

In our investigations, the v-*ets* probe hybridized to the short arm of the t(9p) chromosome, indicating that the breakpoint on chromosome 11 is proximal to this gene. However, we could not determine the precise location of c-*ets*-1 relative to the breakpoint, nor its proximity to the IFN-α genes on the rearranged chromosome 9. If the c-*ets*-1 gene is close to the breakpoint at 11q23, its function may be affected by the IFN-α regulatory sequences. The fact that the sequences hybridizing to the v-*ets* probe in the Southern blots were of germline size does not invalidate this possibility, as the v-*ets* probe we used came from an avian virus and is truncated at its 5′ end. If the breakpoint is adjacent to or within the 5′ end of the c-*ets*-1 gene, a rearrangement might not be detected by Southern analysis when the v-*ets* probe is used.

Interferons have an antiproliferative effect on many types of cells, including monocytes (*21, 22*). It has been proposed that the secretion of IFN by monocytes that is induced after treatment with colony-stimulating factor 1 (CSF-1) is involved in a feedback control mechanism that regulates monocyte proliferation (*21*). This CSF-1–induced IFN secretion appears to be important in monocyte differentiation (*23*). It is unlikely, however, that the monoblastic proliferation associated with t(9;11) is a result of a defect in the IFN system. Even if the rearrangement of an IFN gene could disrupt its expression, it would not result in the complete inactivation of all IFN genes in the cluster. Nevertheless, if an oncogene such as c-*ets*-1 is relocated near an IFN gene and falls under the IFN regulatory mechanisms, the agents that normally induce and regulate expression of IFN genes may also induce the transcription of c-*ets*-1 and activate an alternative proliferative pathway.

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28. High molecular weight DNA was prepared by a modification of the method of Blin and Stafford (*27*). Portions (10 μg) of this DNA solution were digested with restriction enzymes (New England Biolabs) in the presence of 4.0 m*M* spermidine. The DNA digests were fractionated by electrophoresis on 0.8 percent agarose gel slabs and transferred onto "Gene Screen Plus" nylon membrane filters (New England Nuclear). The ^{32}P-labeled probes were prepared by nick translation of the plasmid DNA to a specific activity of 2×10^8 dpm/μg. The hybridization was performed in 50 percent formamide, 10 percent dextran sulfate, 1 percent sodium dodecyl sulfate (SDS), 5× Denhardt's solution, 5× SSC (SSC is 0.15*M* NaCl, 0.015*M* Na citrate, *p*H 7.0), 20 m*M* phosphate buffer (*p*H 6.8), denatured salmon sperm DNA (100 μg/ml), and ^{32}P-labeled probe (10^6 dpm/ml), at 42°C for 18 hours. The filters were washed in 0.1× SSC, 1 percent SDS, at 65°C for 2 hours, except for those hybridized to the v-*ets* probe, which were washed at 55°C for 2 hours.

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