Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4922–4926, May 1995
Medical Sciences

Involvement of the *ALL-1* gene in a solid tumor

(chromosomal translocations/self-fusion)

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Communicated by Hilary Koprowski, Thomas Jefferson University, Philadelphia, PA, February 21, 1995

ABSTRACT Translocations involving chromosome band 11q23, found in 5–10% of human acute leukemias, disrupt the *ALL-1* gene. This gene is fused by reciprocal translocation with a variety of other genes in acute lymphoblastic and myelogenous leukemias, and it undergoes self-fusion in acute myeloid leukemias with normal karyotype or trisomy 11. Here we report an alteration of the *ALL-1* gene in a gastric carcinoma cell line (Mgc80-3). Characterization of this rearrangement revealed a three-way complex translocation, involving chromosomes 1 and 11, resulting in a partial duplication of the *ALL-1* gene. Sequencing of reverse transcription–PCR products and Northern blot analysis showed that only the partially duplicated *ALL-1* gene was transcribed, producing an mRNA with exon 8 fused to exon 2. This report of *ALL-1* gene rearrangement in a solid tumor suggests that *ALL-1* plays a role in the pathogenesis of some solid malignancies. The absence of the normal transcript in this cell line, in association with the loss-of-heterozygosity studies on chromosome 11q23 seen in solid tumors, suggests that *ALL-1* is involved in tumorigenesis by a loss-of-function mechanism.

The *ALL-1* gene, located at human chromosome 11 band q23, is involved in chromosome translocations associated with *de novo* and secondary acute lymphoblastic leukemias (ALLs) and acute myeloid leukemias (AMLs) (1). Approximately 80% of infant ALL, 60% of infant AML, and 6% of ALL and AML in older children and adults show cytogenetic abnormalities at 11q23 (2, 3). In addition, >75% of secondary leukemias associated with therapy with inhibitors of topoisomerase II show alterations at 11q23 and *ALL-1* gene rearrangements (4).

In leukemias with translocations involving 11q23, the *ALL-1* gene fuses with one of many different genes. In fact, at least 20 different reciprocal translocations involving band 11q23 have been described. Some of the *ALL-1* partner genes have been cloned and characterized: *AF1p* from chromosome 1; *AF4* from chromosome 4; *AF6* from chromosome 6; *AF9* from chromosome 9; *AF17* from chromosome 17; *ENL* from chromosome 19; and *AFX* from human chromosome X (5–12). Among these different genes, only *AF9* and *ENL* share sequence homology (8, 9). The *ALL-1* gene was called HRX, MLL, or Htrx by others (7, 13, 14). Recently, by examining specimens from patients with AML without chromosome translocations we have observed that the *ALL-1* gene may undergo self-fusion resulting in a partially duplicated gene and a transcript with an in-frame fusion of either exon 6 or exon 8 with exon 2. This observation suggests that an alteration of the ALL-1 protein product is the critical event in the oncogenic conversion of *ALL-1* gene, whereas fusion partners might not play an important role in the oncogenic process (15, 16). Although it is evident that *ALL-1* is involved in leukemogenesis, it is still not clear whether the altered ALL-1 protein acts through a mechanism of gain or a loss of function.

The *ALL-1* gene, which spans ≈100 kbp and consists of at least 21 exons, encodes a very large protein of ≈431 kDa (6, 7). The ALL-1 protein has regions of homology with the *Drosophila* trithorax gene, which regulates the thoracic segmentation of the *Drosophila* through positive regulation of the genes of the Antennapedia and Bithorax complexes (6, 7, 14). The ALL-1 protein contains recognizable protein motifs and homologies: (*i*) at the N terminus it has several AT hook motifs similar to those present in high-mobility-group proteins, which bind (A + T)-rich DNA or cruciform DNA; (*ii*) it has two centrally located zinc-finger domains, which are also implicated in protein/DNA and/or protein/protein interactions; and (*iii*) it has a region of homology with DNA methyltransferases (17). Recently Zeleznik-Le *et al.* (18) have suggested that the ALL-1 protein may be a member of a family of bifunctional transcriptional factors because it contains both activation and repression domains. It is still not known, however, whether the ALL-1 protein functions as an activator or a repressor of transcription *in vivo*, or both.

Genetic studies have revealed that rearrangements resulting in loss of function, such as deletions, are the most frequent genetic alterations in solid tumors (19). Chromosome translocations leading to gene fusion and oncogene activation have, however, been described frequently in soft tissue sarcomas. For example, in Ewing sarcoma and in primitive neuroectodermal tumors, the *EWS* gene at band 22q12 fuses either with the *FLI-1* gene at band 11q24 or with the *ERG* gene at band 21q22, leading to the formation of a chimeric transforming gene. Other examples are as follows: the *SYT–SSX* gene fusion in synovial sarcomas with the t(X;18)(p11.2;q11.2) chromosome translocations; the *TLS–CHOP* fusion gene found in myxoid liposarcoma with t(12;16)(q13;p11), and the fusion of the *PAX-3* homeotic gene with the *FKHR* gene in t(2;13)(q35;q14) translocations involved in alveolar rhabdomyosarcoma (20–23).

The molecular characterization of the t(1;17) and t(17;22) translocations in neurofibromatosis type 1 has led to the cloning of the *NF1* gene (24, 25), encoding neurofibromin, a protein that down-regulates p21ras. Additional studies demonstrated that the *NF1* gene is a tumor-suppressor gene (26–28). In fact, mutations of the *NF1* gene that cause familial neurofibromatosis also occur in somatic cells and can lead to the development of tumors such as colon adenocarcinoma, myelodysplastic syndrome, and anaplastic astrocytoma (29, 30).

Because *ALL-1* is expressed in a large variety of tissues, we sought to determine whether this gene, which plays a major role in human acute leukemias, might also be involved in the pathogenesis of solid tumors.

Here we report an *ALL-1* gene rearrangement in a cell line derived from a gastric carcinoma lacking cytogenentic evidence of chromosome 11q23 translocations (Mgc80-3). The molecular analysis of this rearrangement shows a partially

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Abbreviations: LOH, loss of heterozygosity; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; RT, reverse transcription.

duplicated *ALL-1* gene, similar to the rearrangement observed in acute leukemias without cytogenetic 11q23 alterations.

MATERIALS AND METHODS

Cell Lines and Rodent–Human Hybrids. Gastric carcinoma cell lines Mgc80-3 and Kato III (31, 32) were from Si-Chun Ming (Temple University, Philadelphia). Other cell lines were AGS, RF-1, RF-48, Hs746T (stomach adenocarcinoma); SW48, LoVo, (colon adenocarcinoma); SW1463, SW837 (rectum adenocarcinoma); LNCaP, DU145, PC-3 (prostate adenocarcinoma); NCI-H460, A549 (lung cancer); Caov 3 (ovarian carcinoma); JAR (chorioncarcinoma); Hep G2 (hepatocarcinoma); 143B (osteogenic sarcoma); and RS 4;11 [ALL with t(4;11)] were obtained from the American Type Culture Collection. Colon adenocarcinoma cell line SW1222 was provided by E. Mercer (Thomas Jefferson University). Melanoma cell lines WM164 and WM793 have been described (33, 34). Melanoma cell lines SB-1, SB-3, and M21 were established in the laboratory of Soldano Ferrone (New York Medical College, Valhalla).

Rodent–human hybrid cell lines have been described (35–38) or were from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Coriell Institute, Camden, NJ). Hybrids retaining partial chromosomes have also been described (37, 38).

Southern Blot Analysis. Approximately 8 μg of genomic DNA was digested with several restriction enzymes (*Bam*HI, *Hin*dIII, *Eco*RI, *Bgl* II, *Kpn* I, and *Xba* I). The digestion products were separated on 0.8% agarose gel, blotted onto positively charged nylon membranes, and hybridized as described (39) with the B859 probe (an 859-bp *Bam*HI cDNA fragment that spans the *ALL-1* breakpoint cluster region between exon 5 and exon 11). The filters that showed rearranged bands with the B859 probe were stripped and probed with SAS1 (a 289-bp DNA probe from intron 1 of the *ALL-1* gene) (15).

RNA Blot Analysis. RNA was extracted by the guanidinium thiocyanate method (40). Aliquots of 10 μg were electrophoresed in 1.1% agarose gel containing formaldehyde, blotted onto nylon membranes, and hybridized with the B859 probe.

Reverse Transcription (RT)–PCR. Total cDNA from the Mgc80-3 cell line was prepared with 2 μg of RNA using the Superscript preamplification system for first-strand cDNA Synthesis (GIBCO/BRL). PCR was done by using *Taq* DNA polymerase (Perkin–Elmer/Cetus). Oligonucleotide primers are 3.1c (5′-AGGAGAGAGTTTACCTGCTC-3′) from exon 3, 5.3 (5′-GGAAGTCAAGCAAGCAGGTC-3′) from exon 5, 6.1 (5′-GTCCAGAGCAGAGCAAACAG-3′) from exon 6, and 3.2c (5′-ACACAGATGGATCTGAGAGG-3′) from exon 3. The first PCR on the total cDNA was done with primers 3.1c and 5.3. Nested PCR on 0.5 μl of the first PCR product was done with primers 3.2c and 6.1. PCR products were analyzed by agarose gel electrophoresis, subcloned into the TA cloning vector (Invitrogen), and sequenced.

Molecular Cloning. Two bacteriophage libraries were made from size-fractionated *Eco*RI (ZAP II) and *Bgl* II (EMBL3) digests of the Mgc80-3 genomic DNA. Recombinants were identified by filter hybridization in the ZAP II library with the B859 probe and in the EMBL3 library with the SAS1 probe. Construction of the libraries, screening, labeling, hybridization, and restriction enzyme mapping were done by standard techniques (41). Positive clones in the ZAP II library were isolated and subcloned into the Bluescript vector by the *in vivo* excision protocol (Stratagene). Subclones from the EMBL3 library were constructed in the pBluescript II plasmid vector. All clones were characterized by Southern blot hybridization and were subsequently mapped and sequenced. PCR amplification using *Taq* polymerase and *Taq* extender PCR additive (Stratagene) was done in Mgc80-3 genomic DNA by using oligonucleotide primers (B1T3-F1, 5′-GCTCTCAGGTCCTTCCTTCTTC-3′; and 0.9K-F1, 5′-TTCCCATCGCTCTTTCCCAAG-3′). The PCR product (MCH1) was subcloned in the TA vector and sequenced. All the sequences were determined by cycle sequencing with an Applied Biosystems 373A DNA sequencer. Programs from the Genetics Computer Group were used for data analysis.

RESULTS

To test the hypothesis that the *ALL-1* gene could be altered in solid tumors, we have examined 26 cell lines derived from 10 different types of solid neoplasms. A cDNA probe (B859) that

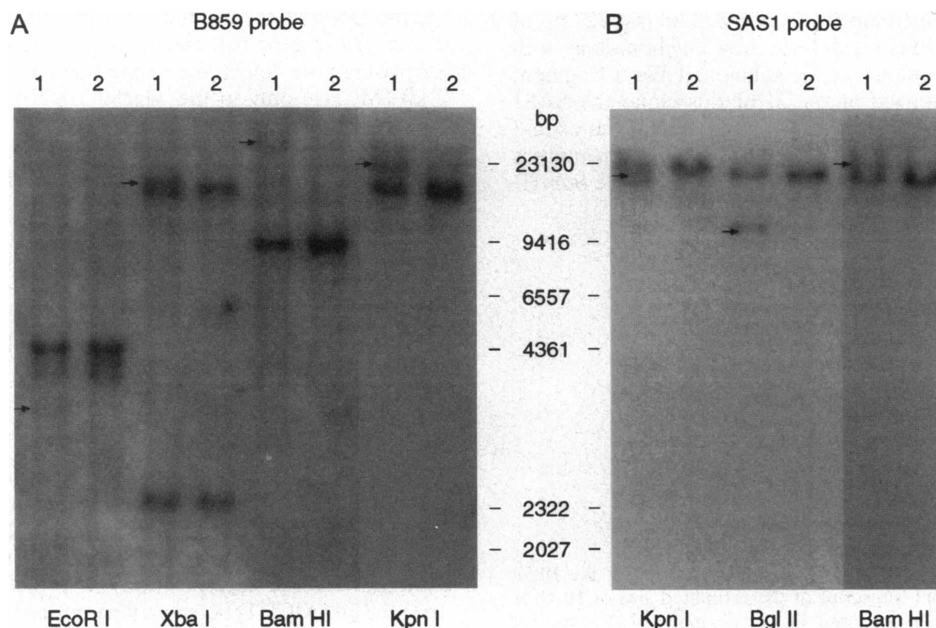

FIG. 1. Southern blot analysis of *ALL-1* showing rearrangements in the Mgc80-3 DNA. Lanes: 1, Mgc80-3; 2, human placenta. *A* was hybridized with probe B859; *B* was hybridized with probe SAS1. The restriction enzymes used to digest genomic DNAs are indicated at bottom.

spans the *ALL-1* breakpoint cluster region involved in acute leukemias (6, 8) was used to detect rearrangements of the *ALL-1* gene by Southern blot in these cell lines. As shown in Fig. 1*A*, a gastric carcinoma cell line (Mgc80-3) showed rearranged bands after DNA digestion with several enzymes. The presence of an *ALL-1* gene rearrangement in the Mgc80-3 cell line was surprising because we had previously reported the lack of cytogenetic evidence of chromosome 11 abnormalities in this cell line (42). However, in recent studies we have described the *ALL-1* self-fusion and partial duplication in acute myeloid leukemias with a normal karyotype or trisomy 11 (15, 16). We next examined the genomic DNA of the Mgc80-3 cell line with a probe (SAS1) that detects rearrangements associated with *ALL-1* partial duplication. The SAS1 probe showed rearranged bands on Southern blot, but in contrast with the AMLs mentioned above, these bands did not comigrate with the rearranged bands detected by the B859 probe, except for the *Kpn* I rearranged band (Fig. 1*B*).

We have also examined *ALL-1* expression in the Mgc80-3 cell line, using B859 as a probe, by RNA blot analysis (Fig. 2). We used as control the RNA of a gastric carcinoma cell line with no evidence of *ALL-1* alterations (Kato III) and the RS 4:11 cell line that contains a t(4;11) chromosomal translocation and shows two transcripts of the approximate size of 15 kb and 12.7 kb (43). We detected only an ≈18-kb altered transcript, indicating expression of an altered *ALL-1* gene and absence of the normal *ALL-1* transcript in the gastric carcinoma cell line.

To determine whether the altered RNA was derived from a partially duplicated *ALL-1* gene, we performed a RT–PCR on Mgc80-3 total RNA using oligonucleotide primers from exon 5 in forward orientation (primer 5.1) and from exon 3 in reverse orientation (primer 3.1c) specific for the *ALL-1* duplication (15). An amplified band of the predicted size was detected (Fig. 3*A*). Sequence analysis of the nested PCR product showed an in-frame fusion of exon 8 with exon 2 (Fig. 3*B*).

To define the mechanism of the partial duplication of the *ALL-1* gene, we cloned the two rearranged bands detected by probes B859 and SAS1: the 3.8-kb B859 *Eco*RI fragment (E3-15) and the 12.5-kb SAS1 *Bgl* II fragment (B4M). The two genomic DNA clones were isolated and analyzed by restriction enzyme analysis and DNA sequencing (Fig. 4*A*). The sequence of the whole E3-15 clone showed that the breakpoint was in intron 8, 876 bp downstream from exon 8. The last 727 bp of the 3′ end of the E3-15 clone did not show any homology with known genes. The sequence of the subcloned *Xba* I fragment of the B4M clone showed at the 3′ of this clone the SAS1 sequence followed by exon 2–intron 2 and part of the exon 3 of the normal *ALL-1* gene. Nevertheless, by restriction analysis of the B4M lambda clone we were unable to detect the *Bam*HI restriction site normally located 5.8 kb upstream of exon 2. The sequence of the first 2.5 kb of the B4M clone located 6.8 kb upstream of exon 2 did not show homology to known loci. This result was not informative because the sequence of intron 1 of the *ALL-1* gene has not yet been determined. However, as shown in Fig. 4*D*, two oligonucleotide primers (B1-T3F; 0.9K-F1) were designed in an attempt to close the gap between the E3-15 and B4M clones by PCR amplification. The PCR analysis was done by using as templates the Mgc80-3 genomic DNA, the DNA of the hybrid 7300 containing a chromosome 11, the DNA of the cosmid 20 containing the entire intron 1 of the *ALL-1* gene (6), and normal human DNA as a control. Surprisingly we detected a strong band of the estimated size af 2 kb (MCH1) only in the Mgc80-3 DNA and in the normal human DNA.

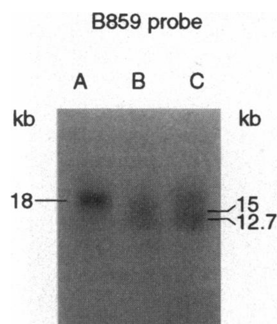

FIG. 2. RNA blot analysis of ALL-1 transcripts using the B859 probe. Only an abnormal transcript of the estimated size of 18 kb is present in the Mgc80-3 cell line (lane A). The normal ALL-1 transcript of 15 kb is detectable in Kato III (lane B) and RS 4:11 (lane C) cell lines. An additional transcript of 12.7 kb, which derives from *ALL-1/AF4* fused genes, is indicated in the RS 4:11 cell line (lane C) (43).

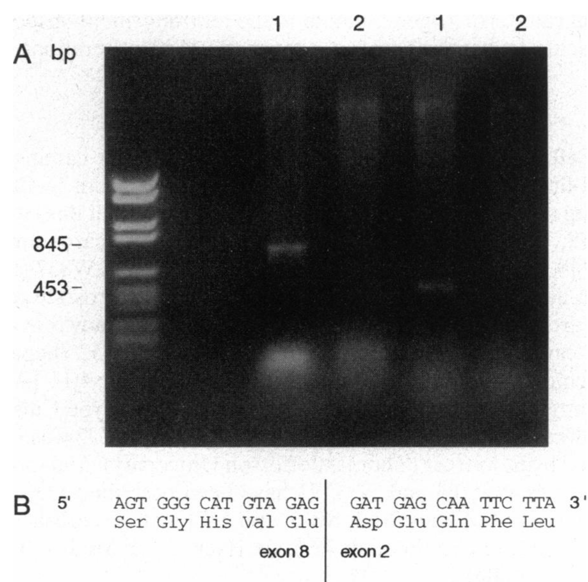

FIG. 3. (*A*) Ethidium bromide-stained agarose gel showing PCR amplification and nested amplification of the Mgc80-3 RT product (lane 1) and of the normal control RT product (lane 2). A major band of 845 bp has been obtained by using primers from exon 5 in forward orientation (5.3) and from exon 3 in reverse orientation (3.1c). A minor band of 453 bp was the product of a nested amplification on the RT–PCR product using primers from exon 6 in forward orientation (6.1) and from exon 3 in reverse orientation (3.2c). (*B*) Sequence analysis of the nested PCR product shows in-frame fusion of exon 8 with exon 2 of the *ALL-1* gene. Amino acid translation is shown beneath the DNA sequence.

To identify the chromosomal location of the MCH1 fragment we performed a further PCR analysis with the same set of primers (B1-T3F; 0.9K-F1) on a panel of somatic hybrids covering all human chromosomes. The result revealed that the fragment interposed between intron 8 and intron 1 of the *ALL-1* gene derived from chromosome 1p31.2-32.3. The MCH1 fragment was cloned and sequenced. A FASTA database search against GenBank did not reveal any homology with known DNA sequences.

DISCUSSION

This study demonstrates *ALL-1* gene alteration and self-fusion in malignant cells derived from a solid tumor. In this gastric adenocarcinoma-derived cell line, the *ALL-1* gene underwent a partial duplication of the region involving exon 2 through exon 8 with the interposition of a piece of chromosome 1p31.3-32.1 (MCH1) between intron 8 and the duplicated exon 2. The sequence of the cloned cDNA showed a fusion of exon 8 to exon 2, indicating that the interposed MCH1 fragment of chromosome 1 was spliced out. Thus, the role of the chromo-

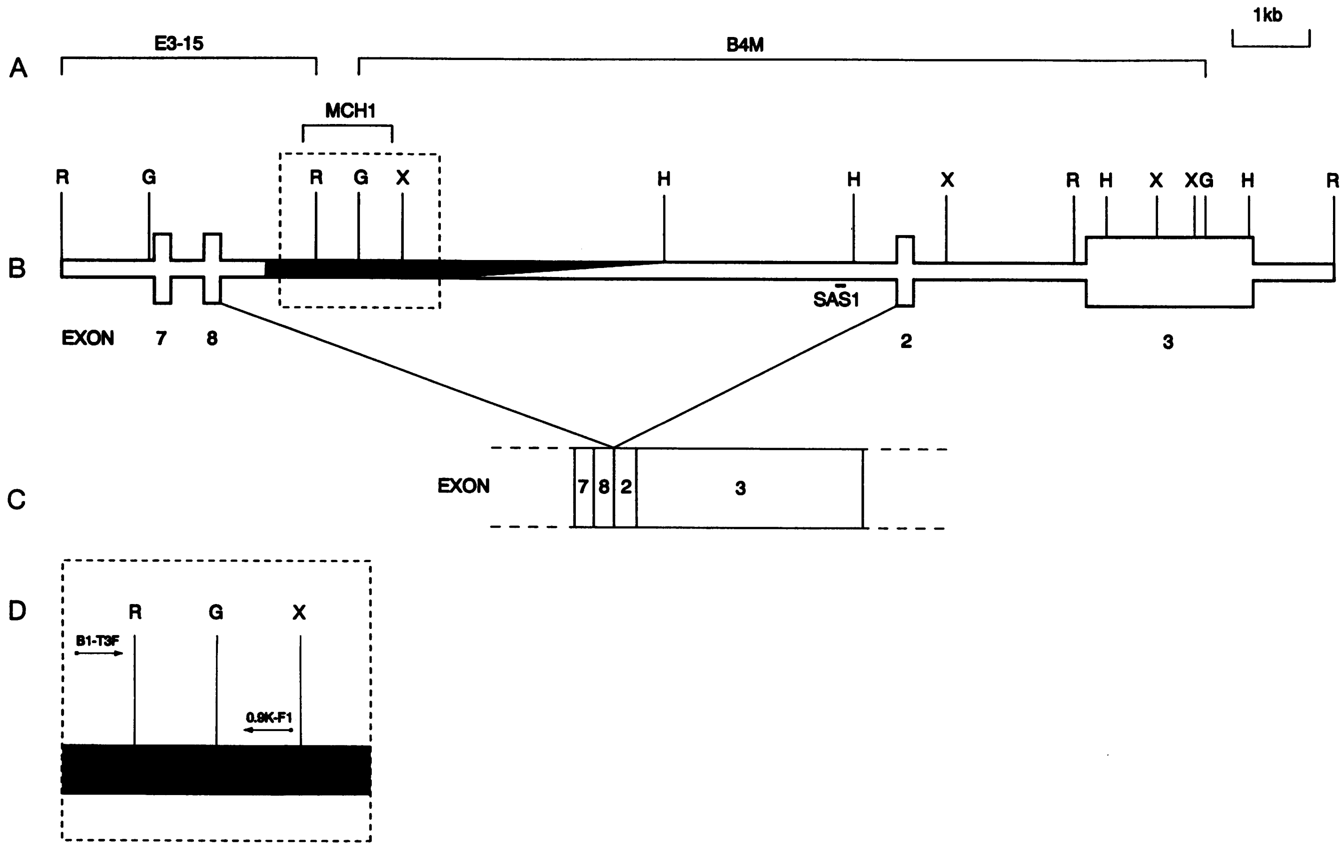

FIG. 4. Schematic representation of the three-way complex translocation in Mgc80-3 cells. (*A*) Lambda phage and plasmid clones covering the *ALL-1* genomic rearrangement. E3-15 is a 3.8-kb *Eco*RI lambda ZAP II clone. B4M is a 12.5-kb *Bgl* II lambda EMBL3 clone. MCH1 is a 2-kb PCR product cloned in the TA cloning vector. (*B*) Physical map of genomic rearrangement. Exons are indicated by enlarged boxes. Black area represents chromosome 1-derived fragment. The 3′ junction between chromosome 1 and chromosome 11 remains undetermined, as indicated. Some restriction enzyme sites are also shown: R, *Eco*RI; G, *Bgl* II; X, *Xba* I; H, *Hin*dIII. (*C*) ALL-1 cDNA structure. Exon 8 is fused with exon 2, and the intervening chromosome 1 fragment is spliced out. (*D*) An enlargement of the scheme in *B* shows position of the primers used to amplify the MCH1 fragment. The B1-T3F oligonucleotide primer was 721 bp upstream of the *Eco*RI site at the 3′ end of the E3-15 lambda clone. The 0.9K-F1 oligonucleotide primer was 62 bp upstream of the *Xba* I site at the 5′ end of the B4M lambda clone. The amplified product is ≈2 kb.

some 1-derived sequence remains unclear. It may be a residual DNA fragment resulting from an intermediate step in this three-way translocation.

Because *ALL-1* can fuse with many different genes, and even with itself, to cause acute leukemias, it seems possible that the mechanism of malignant transformation involves loss rather than gain of function, where the altered ALL-1 protein in which the N terminus is disconnected from the rest of the protein may bind to the normal *ALL-1* product, leading to its functional inactivation. The normal bands detected by Southern blot analysis may also derive from the partially duplicated gene, and the lack of the normal transcript suggests that the wild-type *ALL-1* gene is either absent or transcriptionally silent in this cell line. The absence of the wild-type allele might be explained by a possible involvement of both *ALL-1* alleles in the complex translocation. Although Southern blot analysis of the Mgc80-3 genomic DNA cannot determine whether the wild-type *ALL-1* gene is intact, the existence of two apparently normal chromosomes 11 in this cell line does not allow discrimination between the following possibilities: (*i*) a partially duplicated gene present together with the normal gene or (*ii*) a partially duplicated gene resulting from the reduplication of the chromosome carrying the translocation. Because in leukemias the wild-type *ALL-1* is generally transcribed together with the partially duplicated *ALL-1*, we had previously speculated about the different mechanisms that might block normal ALL-1 function (11).

Because we do not observe the expression of the normal *ALL-1* gene and presence of only the partially duplicated *ALL-1* gene in the gastric carcinoma cells, it seems possible that malignancy involves the loss of the normal *ALL-1* in this gastric tumor.

Although chromosomal translocations have been studied mostly in hematological neoplasms and soft tissue sarcomas, there are few reports regarding the role of chromosomal translocations in carcinomas, the most common human tumors (44).

Although chromosome translocations are usually associated with activation of oncogenes, the *NF1* gene on chromosome 17q11.2 represents an example of a tumor-suppressor gene that can be knocked out by chromosomal translocations involving band 17q11.2 (45). Additional studies, however, have demonstrated that point mutations are the most frequent genetic changes involving the *NF1* gene, leading to loss of NF1 function (29, 30). It has also been demonstrated that the disruption of the *RB1* locus by chromosomal translocation at 13q14.2 represents a predisposing mutation in some retinoblastoma cases (46).

We hypothesize that the *ALL-1* gene may play an important role in malignant transformation of cells of solid tissues, such as carcinomas.

Loss of heterozygosity (LOH) studies have revealed that region 11q22-q24 is frequently deleted in colon, cervical, breast and ovary carcinomas, and melanoma (47–52). In some cases the narrowed region of LOH does not include the *ALL-1* locus (48, 49); however, these results do not rule out the possibility that a region including the *ALL-1* gene might also be a target of LOH, independent of the region *D11S35–D11S29* recently identified. In fact, we have shown that marker

D11S528, which is telomeric to *D11S29* where *ALL-1* maps, has a frequency of LOH higher than *D11S29* (49). Based on the fact that the *ALL-1* gene is rearranged in a solid tumor, investigations of LOH at region 11q23-q24 and point mutation analysis of the *ALL-1* gene should be performed in carcinomas to assess the role of *ALL-1* in these tumors.

Mutations that modify the structure of the normal ALL-1 protein could play a role in the tumorigenesis of both hematological and solid neoplasms.

We thank Drs. Soldano Ferrone, Si-Chun Ming, and Ed Mercer for providing cell lines; Tatsuya Nakamura for providing cosmid 20; and Teresa Druck for help with the mapping study. We are grateful to A. Ar-Rushdi for helpful discussion and to Janet Finan for her support in the cytogenetic analysis. This work was supported by the Falk Medical Research Trust and by Grants CA39860 and CA50507 from the National Cancer Institute and DHP71028 from the American Cancer Society.

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