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Advances in Brief

An Altered 11-Kilobase Transcript in Leukemic Cell Lines with the t(4;11)(q21;q23) Chromosome Translocation[1]

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Abstract

The 11q23 chromosome band is frequently associated with chromosomal aberrations in human leukemias. We have previously cloned a DNA fragment derived from chromosome 11 which could be used as a probe to detect rearrangements in DNAs from the leukemic cells of patients with the t(4;11), t(9;11), and t(11;19) translocations. In this study we now show that the same probe detects DNA rearrangements in malignant cells from patients with the t(1;11), t(6;11), t(10;11), and del (11q23) chromosomal abnormalities. A second probe obtained from a region located centrometric to the breakpoint cluster detects major and minor transcripts of 12.5 and 11.5 kilobases, respectively, in all cell lines examined. The same probe identifies an altered 11-kilobase RNA in all three independent cell lines with the t(4;11)(q21;q23) chromosome translocation.

Introduction

11q23 chromosome abnormalities have been reported in ALL,[3] and in AML, most commonly of the M4 and M5a subtypes (1). The t(4;11)(q21;q23), t(11;19)(q23;p23), and t(1;11)(p32;q23) chromosome translocations are found in 10%, 2%, and <1% of ALL, respectively. Reciprocal translocations between chromosome region 11q23 and chromosomal regions 9p22, 6q27, 1p21, 2p21, 10p11, 17p25, and 19p13 are found in 5–6% of AML. In addition, interstitial deletions at 11q23 have been detected in both ALL and AML. Clinically, rearrangements of 11q23, in particular the t(4;11), have some distinct features: the patients are often quite young (less than 1 year of age); have very high blast count; and show poor prognosis. In many patients the leukemic cells express both B-cell and myeloid markers (2), and the disease is consequently considered "biphenotypic."

Using either somatic cell hybrids (3–5) or the fluorescent *in situ* hybridization technique (6), it was possible to position the breakpoints on chromosome 11 to a region between the *CD3* and *PBGD* genes. Rowley *et al.* (6) used a *CD3* probe to clone a 350-kilobase human DNA fragment from a YAC library. This YAC spanned the t(4;11), t(9;11), t(11;19), and t(6;11) breakpoints as indicated by fluorescent *in situ* hybridization analysis. Using probes derived from both sides of the breakpoint cluster region these investigators identified a 12.5-kilobase RNA in cells with or without 11q23 abnormalities. Furthermore, a probe located telomeric to the cluster region detected two additional transcripts of 11.5 and 11 kilobases in the RS 4;11 cell line, as well as in all hematopoietic and nonhematopoeitic cells tested (7). The gene coding for the three RNAs was termed *MLL* (7). We have cloned a *CD3*-containing YAC from the human DNA YAC library of Washington University and prepared a cosmid library from the insert (8). A probe isolated from one of the cosmids detected rearrangements in the great majority of DNAs from the leukemic cells of patients with the t(4;11), t(9;11), and t(11;19) chromosome translocations. The breakpoints clustered within a small DNA region of <5.8 kilobases, termed the ALL-1 locus (8). In addition, we found that a pool of probes derived from two cosmids detected RNAs of 0.4, 5, and 11–12 kilobases in several cell lines. Studies at this region have now been extended.

Materials and Methods

Cells. The cell lines PC3 and LNCaPFGC (prostate adenocarcinomas) as well as RS 4;11 [ALL with t(4;11)] were obtained from the American Type Culture Collection. Lines GM607 and GM1500 (Epstein-Barr virus-immortalized B-lymphocytes) were obtained from the National Institute of General Medical Sciences. The B1 cell line [ALL with t(4;11)] was a gift from T. Grunberger and A. Cohen at the Hospital for Sick Children in Toronto (9). Other cell lines used included MV4;11 [ALL with t(4;11)] (10), SKDHL (B-cell lymphoma) (11), Jurkat (T-cell lymphoma) (12), 697 (pre-B leukemia) (13), ALL-1 (B-cell leukemia) (14), K562 and KCl-22 (erythroid and myeloid acute phase of chronic myeloid leukemia) (15, 16), and T98G (glioblastoma) (17).

Probes. The 0.7-kilobase *Dde*I fragment used to detect 11q23 rearrangements (8) was further purified from human repetitive sequences by digestion with the *Alu*I enzyme and electrophoretic removal of an internal 240-base pair fragment. The resulting terminal fragments were ligated together and subcloned into a plasmid vector. A 1-kilobase fragment isolated by *Eco*RI digestion of cosmid 20 (8) was used to detect transcripts. The 1-kilobase fragment was localized to the left terminus of cosmid 20 (8) ~10 kilobases centromeric of the breakpoint cluster. Its position relative to the breakpoint cluster was confirmed by showing that it was present in a DNA from a somatic cell hybrid containing the der 11 but was absent from a hybrid containing the der 4 (hybrids were a gift from Dr. Carol Jones from the Eleanor Roosevelt Cancer Institution at Denver).

DNA and RNA Analysis. DNA and RNA were extracted and analyzed using conventional procedures. Aliquots of 5–10 μg of polyadenylated RNA were electrophoresed on 1.1% agarose gels containing formaldehyde as described (18). The size of the transcripts was estimated on the basis of the electrophoretic mobilities of ribosomal RNAs and of an RNA ladder purchased from BRL.

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[3] The abbreviations used are: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; YAC, yeast artificial chromosome.

Results

The finding of tight clustering of the breakpoints on chromosome 11 in the three most common 11q23 abnormalities (8) raised the possibility that the same region is rearranged in other chromosomal aberrations involving 11q23. To test this hypothesis we digested tumor DNAs from the leukemic cells of patients with t(6;11)(q27;q23), t(1;11)(p34;q23), t(10;11)(p11–15;q23), and del (11)(q23) with *Bam*HI, *Xba*I, *Eco*RV, and *Hind*III enzymes and subjected them to Southern analysis using the modified 0.5-kilobase *Dde*I fragment as a probe. Some of the results are shown in Fig. 1. Rearranged fragments were found in the DNAs of patients with t(6;11), t(1;11), and t(10;11) (Fig. 1 *Lanes a*, *d*, and *e*, respectively) and in two patients (Fig. 1 *lanes f* and *g*) of five with interstitial deletion in 11q23 [the three negative patients had del (11)(q21q23)]. The patients with t(6;11) and t(10;11) as well as one of those with del(11)(q23) showing rearrangement had AML; the rest of the patients tested had ALL.

To further analyze transcription of the genomic DNA adjacent to the breakpoint cluster region, segments of cosmid 20 found fully or partially free of repetitive sequences were examined as probes to polyadenylated RNAs obtained from a variety of hematopoietic and nonhematopoeitic cell lines. Three ALL cell lines, MV 4;11, RS 4;11, and B1 containing the t(4;11) chromosomal translocation, were included in the analysis. These three cell lines had rearrangements at the breakpoint cluster region as shown previously (8) or here (Fig. 1, *Lanes b* and *c*). A 1-kilobase *Eco*RI DNA segment used as a probe and located centromeric to the breakpoint cluster region (see "Materials and Methods") identified a 12.5-kilobase RNA in all cell lines (Fig. 2). A minor species of 11.5 kilobases was detected in most of the samples without involvement of 11q23, but because of technical reasons it was not possible to determine if this RNA was present in the cells with the t(4;11) translocation. A transcript of 11 kilobases was detected in the three cell lines with the t(4;11) chromosome translocation (Fig. 2, *Lanes c*, *f*, and *k*). The width of this band on the autoradiogram suggests that it corresponds to two comigrating RNA species. The 11-kilobase RNA was not detected in any of the cell lines lacking 11q23 aberrations (Fig. 2).

Discussion

The results shown here, in combination with those reported previously (8), indicate that the same breakpoint cluster region

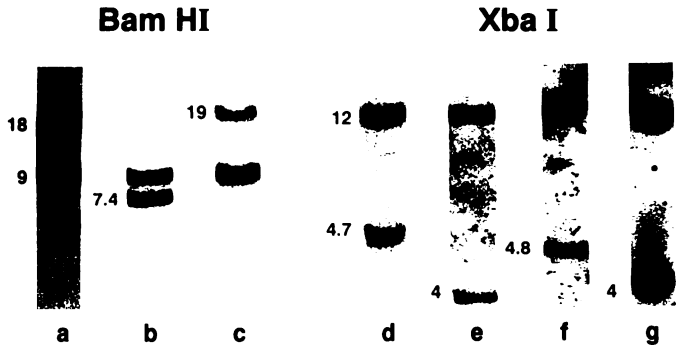

Fig. 1. Southern blot analysis of DNAs from primary tumors and cell lines with 11q23 abnormalities. *Lane a*, patient CH with t(6;11); *Lane b*, the B1 cell line with t(4;11); *Lane c*, the RS 4;11 cell line with t(4;11); *Lane d*, patient JB with t(10;11); *Lane e*, patient ML with t(1;11); *Lane f*, patient SO with del(11)(q23); *Lane g*, patient RE with del(11)(q23). *Numbers*, kilobases. The germline *Bam*HI and *Xba*I fragments are of 9 and 12 kilobases, respectively.

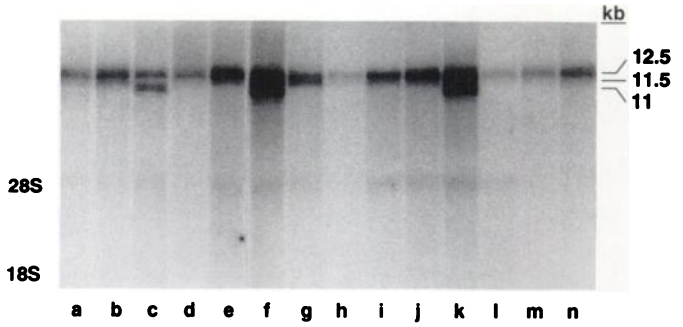

Fig. 2. Northern blot analysis of RNAs from cell lines. Lines included SK DHL (*Lane a*), KCL22 (*Lane b*), MV 4;11 (*Lane c*), T98G (*Lane d*), ALL-1 (*Lane e*), B1 (*Lane f*), K562 (*Lane g*), Jurkat (*Lane h*), GM607 (*Lane i*), 697 (*Lane j*), RS4;11 (*Lane k*), GM1500 (*Lane l*), LNCaPFGC (*Lane m*), PC3 (*Lane n*). *28S* and *18S*, migration of ribosomal RNA.

is rearranged in at least seven different 11q23 abnormalities, including six types of translocations as well as interstitial deletions. Three samples with 11(q21q23) deletions, one sample with t(11;15)(q23;q22), and one sample with t(11;X)(q23;q26) did not show rearrangements within the locus (not shown). In addition, in 1 of 12, 1 of 9, and 2 of 9 patients with t(4;11), t(9;11), and t(11;19) chromosome translocations, respectively, we were not able to demonstrate rearrangements using the *Dde*I probe. Finally, the breakpoint in the RC-K8 cell line containing the t(11;14)(q23;q32) is apparently telomeric to the locus discussed here (19). In all of these cases other unidentified loci on chromosome 11 might be involved. Alternatively, the ALL-1 locus might be affected in these patients too, but this may occur at a different site.

Using a new probe we identified three polyadenylated transcripts; two of them (12.5- and 11.5-kilobase species) are expressed in most or all cell lines, but the third (11-kilobase RNA) was detected solely in cell lines with the t(4;11) abnormality. RNA species of similar size were recently reported by others (7). However, while our probe, which is located centromeric to the breakpoints, detects all three RNAs, Ziemin-van der Poel *et al.* (7) reported that their probe (no. 1), which is derived from the same general location, detects predominantly the 12.5-kilobase species. While we detect the 11-kilobase transcript solely in leukemic cells with the t(4;11) chromosome translocation, the other study identifies an 11-kilobase mRNA in the RS4;11 cell line as well as (in small amounts) in all cells tested (7). These differences between the two studies should be resolved once more informative probes (complementary DNAs) are made available. Our results show, however, a clear qualitative alteration in the expression of a region adjacent to the breakpoint cluster region on chromosome 11 in cells with the t(4;11) chromosome translocation. It is still to be determined whether the 11-kilobase aberrant RNA originates by a mechanism involving gene truncation, gene fusion, or modified splicing.

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