RAPID COMMUNICATION

All Patients With the T(11;16)(q23;p13.3) That Involves MLL and CBP Have Treatment-Related Hematologic Disorders

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The involvement of 11q23-balanced translocations in acute leukemia after treatment with drugs that inhibit the function of DNA topoisomerase II (topo II) is being recognized with increasing frequency. We and others have shown that the gene at 11q23 that is involved in all of these treatment-related leukemias is MLL (also called ALL1, Htrx, and HRX).

In general, the translocations in these leukemias are the same as those occurring in de novo leukemia [eg, t(9;11), t(11;19), and t(4;11)], with the treatment-related leukemias accounting for no more than 5% to 10% of any particular translocation type. We have cloned the t(11;16)(q23;p13.3) and have shown that it involves MLL and CBP (CREB binding protein). The CBP gene was recently identified as a partner gene in the t(8;16) that occurs in acute myelomonocytic leukemia (AML-M4) de novo and rarely in treatment-related acute myeloid leukemia. We have studied eight t(11;16) patients, all of whom had prior therapy with drugs targeting topo II with fluorescence in situ hybridization (FISH) using a probe for MLL and a cosmid contig covering the CBP gene. Both probes were split in all eight patients and the two derivative (der) chromosomes were each labeled with both probes. Use of an approximately 100-kb PAC located at the breakpoint of chromosome 16 from one patient revealed some variability in the breakpoint because it was on the der(16) in three patients, on the der(11) in another, and split in four others. We assume that the critical fusion gene is 5′ MLL/3′ CBP. Our series of patients is unusual because three of them presented with a myelodysplastic syndrome (MDS) most similar to chronic myelomonocytic leukemia (CMMoL) and one other had dyserythropoiesis; MDS is rarely seen in 11q23 translocations either de novo or with t-AML. Using FISH and these same probes to analyze the lineage of bone marrow cells from one patient with CMMoL, we showed that all the mature monocytes contained the fusion genes as did some of the granulocytes and erythroblasts; none of the lymphocytes contained the fusion gene. The function of MLL is not well understood, but many domains could target the MLL protein to particular chromatin complexes. CBP is an adapter protein that is involved in regulating transcription. It is also involved in histone acetylation, which is thought to contribute to an increased level of gene expression. The fusion gene could alter the CBP protein such that it is constitutively active; alternatively, it could modify the chromatin-association functions of MLL.

MATERIALS AND METHODS

Patient samples were obtained with informed consent at the University of Chicago (patient no. 1),* Tufts Medical Center (patient...
A Consequences of t(11;16)

5' centromere  BCR  3' telomere

MLL

MLL-CBP

CBP

CBP-MLL

B

CBP genomic breakpoints in t(11;16)

5' centromere  3' telomere

+ with CBP aa 1-346

+ with CBP aa 722-1120

no. 2), the University of Texas Southwestern Medical Center (patients no. 3 and 4), the University of Kiel (patient no. 5), the Medical University of South Carolina Medical Center (patients no. 6 and 7), and the St Jude Children’s Research Hospital (patient no. 8). The bone marrow specimens were processed for cytogenetic analysis using standard procedures. In some cases, material was also frozen for subsequent use for DNA and/or RNA.

FISH was performed as previously reported. Initially, we used several YACs to try to map the breakpoint. YAC 615F4 from NAD labeled 16p13 very weakly, with additional stronger signals on 17q23 and 12 centromere. One other YAC from JB also was not useful; YAC 541E10 from the CEPH library gave a weak signal on chromosome 16, band p13, and signals on the satellites of chromosomes 13 and 22. A genomic probe cloned from the der(16) chromosome breakpoint junction from patient no. 1 mapped near the CBP gene (Fig 1). FISH analysis was pursued using a series of cosmid clones from JB that span the CBP locus (Tes2, 541E10-C23, Tes5, 541E10-C53, and 376E2-C1) and cosmids from NAD (C365F4, C444A4, C443G8, C388H4, C304A10, C312B2, C379G3, C330H2, C58E12, and C307E6). Rearrangements of the MLL gene were identified using the MLL probe (Oncor, Inc, Gaithersburg, MD).

Studies correlating FISH results with morphology were undertaken using methods previously published. Briefly, freshly made bone marrow aspirate smears were stained with Wright’s stain and coverslipped with Pro Tex (Lerner Labs, Pittsburgh, PA), and images of the Wright-stained cells were videotaped. After the coverslips were removed, the slides were soaked in Xylene for 5 minutes, air-dried, and processed for FISH. The results of the FISH analysis were correlated with morphology by comparing the stored images of the Wright-stained cells and the FISH results. The cells were scored as showing one of three patterns: (1) der(11)/der(16) (1 red, 1 green, and 2 fusion signals); (2) normal pattern (2 red signals and 2 green signals); and (3) indeterminate.

RESULTS

Clinical features. All eight of the patients had been treated previously for a variety of malignancies, including...
leukemia, lymphoma, and other solid tumors (summarized in Table 1). Every patient’s treatment included a drug that targeted topo II, usually etoposide or teniposide, but also doxorubicin; the doses ranged from 2,200 mg/m2 to 8,100 mg/m2. In addition, six of the patients had received alkylating agents. All of the patients achieved a complete remission as a result of their initial therapy but developed a secondary hematologic disorder within 6 to 60 months. Every patient had a t(11;16) often accompanied by other abnormalities (6 patients). The secondary hematologic disease was variable with four patients having t-AML and three having t-MDS. One other patient (no. 7) had dyserythropoiesis that resolved without treatment concomitant with normalization of his karyotype. MDS appeared to be most compatible with chronic myelomonocytic leukemia (CMMoL). Seven of eight patients received treatment for their secondary disease, including bone marrow transplantation in four children or young adults. Four patients are undergoing treatment or are in remission and three are dead.

**FISH.** We performed FISH using the MLL probe on all eight patients. Patients no. 1, 2, and 5 had previously been shown to have an MLL rearrangement. Cells from all patients showed a split MLL probe that labeled both the der(11) and der(16) chromosomes. We used some or all of the cosmids probes for the CBP gene and showed that these probes labeled the normal chromosome 16 as well as both the der(16) and der(11) chromosomes, indicating that the CBP gene was split by the translocation (Figs 1 and 2, upper row, A and B). Our FISH analysis with the PAC genomic probe, which is about 100 kb and contains CBP coding sequences, determined some variability in the breakpoint in CBP because the genomic probe remained on the der(16) in three patients, was translocated to the der(11) in one patient, and was split in four others (Fig 2, middle row, C, D, and E). In the latter patients, more of the PAC remained on the der(11) than was translocated to the der(11). The fusion gene on the der(11) chromosome, which we believe contains the critical junction consists of 5’ MLL and 3’ CBP, and the der(16) would contain 5’ CBP and 3’ MLL.

Using RT-PCR, we cloned the MLL-CBP junction from patients no. 1 and 6; the CBP breakpoint in patient no. 1 is at the same amino acid as the MOL-CBP fusion, whereas in patient no. 6 it is more telomeric, occurring just before the bromodomains of CBP.

The lineage analysis in patient no. 6 using FISH showed that the t(11;16) (MLL/CBP) was present in the expanded monocytic series and that it was also present in a subpopulation of the mature granulocytic and erythroid elements. Virtually all of the monocytes showed the der(11) der(16) pattern, whereas only a subset of granulocytes (20%) and erythroid precursors (10%) were involved. None of the small mature-appearing lymphocytes evaluated showed the fusion (Table 2 and Fig 2F, G, H, and I, upper and lower panels).

## DISCUSSION

In addition to the eight patients whom we have studied, we are aware of three other patients with a t(11;16), patients no. 9 through 11 in Table 1. The t(11;16) appears to be unusual, because all of the cases of leukemia or myelodysplasia contain the critical junction consists of 5’ MLL and 3’ CBP.

### Table 1. Summary of t(11;16)(q23;p13) in Patients With Treatment-Related Acute Leukemia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age at diagnosis of first malignancy</th>
<th>Type of Leukemia</th>
<th>Karyotype</th>
<th>Present Status</th>
<th>Treatment for secondary disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10/M</td>
<td>AML-M2; t(8;21)</td>
<td>46,XX,t(11;16)(q23;p13)[18]/46,i(17)(q10)[2]/46,XX</td>
<td>Alive in remission</td>
<td>Targeted topo II, usually etoposide or teniposide, but also doxorubicin.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>64/F</td>
<td>Breast cancer</td>
<td>46,XX,t(11;16)(q23;p13)[23]/45,i(17)(q10)[2]/46,XX</td>
<td>Alive in remission</td>
<td>Targeted topo II, usually etoposide or teniposide, but also doxorubicin.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>36/F</td>
<td>t-B-ALL</td>
<td>46,XX,t(11;16)(q23;p13)[13]/46,XY,i(17)(q10)[2]/46,XX</td>
<td>Alive in remission</td>
<td>Targeted topo II, usually etoposide or teniposide, but also doxorubicin.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>26/F</td>
<td>Chronic myelomonocytic leukemia</td>
<td>46,XX,t(11;16)(q23;p13.3)[12]/46,XX,i(17)(q10)[2]/46,XX</td>
<td>Alive in remission</td>
<td>Targeted topo II, usually etoposide or teniposide, but also doxorubicin.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>73/F</td>
<td>Mantle cell</td>
<td>46,XX,t(11;16)(q23;p13)[10]/46,XX,i(17)(q10)[2]/46,XX</td>
<td>Alive in remission</td>
<td>Targeted topo II, usually etoposide or teniposide, but also doxorubicin.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>16/F</td>
<td>Ewing's sarcoma</td>
<td>46,XX,t(11;16)(q23;p13)[13]/46,XX,i(17)(q10)[2]/46,XX</td>
<td>Alive in remission</td>
<td>Targeted topo II, usually etoposide or teniposide, but also doxorubicin.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>19/M</td>
<td>t-B-ALL</td>
<td>46,XX,t(11;16)(q23;p13)[13]/46,XX,i(17)(q10)[2]/46,XX</td>
<td>Alive in remission</td>
<td>Targeted topo II, usually etoposide or teniposide, but also doxorubicin.</td>
<td></td>
</tr>
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presence of MLL/CBP translocation on the der(11) and der(16) chromosomes, whereas the lymphocyte shows the pattern of a normal (nonclonal) tMDS. The monocyte, granulocyte, and erythroid precursor show the pattern of one red, one green, and two fusion signals, indicating the precursor, and mature lymphocyte (F, G, H, and I, respectively) and the corresponding FISH results below from patient no. 6 with CMMoL-like no. 1 showing the PAC on the normal 16 and on the der(11). Lineage analysis in patient no. 6. Lower panel. Monocyte, granulocyte, erythroid precursor, and mature lymphocyte (F, G, H, and I, respectively) and the corresponding FISH results below from patient no. 6 with CMMoL-like tMDS. The monocyte, granulocyte, and erythroid precursor show the pattern of one red, one green, and two fusion signals, indicating the presence of MLL/CBP translocation on the der(11) and der(16) chromosomes, whereas the lymphocyte shows the pattern of a normal (nonclonal) cell with two red and two green signals.

Table 2. Lineage Analysis in Patient No. 6: FISH Analysis: Number of Cells (%) With Each FISH Pattern

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>RGFF</th>
<th>RRGG</th>
<th>Indeterminate</th>
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<tbody>
<tr>
<td>Monocytes</td>
<td>67 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>6 (20.7)</td>
<td>15 (51.7)</td>
<td>8 (27.5)</td>
</tr>
<tr>
<td>Erythroid precursors</td>
<td>4 (10.5)</td>
<td>25 (65.8)</td>
<td>9 (23.7)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0 (0)</td>
<td>77 (96.3)</td>
<td>3 (3.7)</td>
</tr>
</tbody>
</table>

Abbreviations: RGFF, red, green, fusion, fusion; RRGG, red, red, green, green.
AT-rich DNA, the coadaptor and chromatin modification functions of CBP could be redirected to inappropriate genomic regions.

All 26 patients with t(8;16) reviewed by Hanslip et al. had AML, usually M4 or M5 with erythrophagocytosis; the incidence is estimated as 4 per 1,000 patients with AML. Three of these cases with t-AML are included in a review by Quesnel et al., which reports on a total of six cases with t-AML and t(8;16). Four of the six had received doxorubicin often in combination with alkylating agents. The interval for onset of treatment to leukemia was 7 to 23 months (7, 8, 9, 23, and 23 months). The subtype of leukemia was mainly M4, with one M5b; only one patient had a preceding MDS phase. Thus, for the t(8;16) as for most of the translocations with MLL, those with t-AML are a small fraction of the total number of patients.

In our comparison of the location of the genomic breakpoint in MLL in various translocations, we noted that 75% of patients with acute leukemia de novo had breaks in the centromeric half of the 8.3-kb breakpoint cluster region, whereas 75% of the treatment-related acute leukemia patients had breaks in the telomeric half of the MLL breakpoint cluster region. This telomeric portion contains a scaffold attachment region (SAR) and six of seven topo II cleavage sites. We postulated that this chromatin structure made this region more vulnerable to translocations in the presence of drugs that target topo II. In contrast, we know nothing about the location of SARs or putative topo II cleavage sites in CBP, because the genomic region of the CBP breakpoints has not been sequenced. However, as noted by others, CBP appears to be genomically very unstable.

A critical practical concern is whether patients develop treatment-related leukemia because of chance (bad luck) or because of an unusual sensitivity to the topo II inhibitors. One way to resolve the question is to save material on all such patients so that the translocation breakpoints can be cloned and sequenced. This information would provide data regarding the presence of unusual polymorphisms that could lead to genomic instability in a manner analogous to the triplet repeats in the fragile X syndrome. It has been noted by Winick et al. that 7 of 15 of their patients with t-AML were of Mexican origin. Whether this is a coincidence or evidence for a polymorphism that would cause no phenotype in the absence of unusual stress such as exposure to epipodophyllotoxins will only be clarified by further studies.

ACKNOWLEDGMENT

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REFERENCES


CD34+ cells purified from normal bone marrow using an antibody column. Cytocentrifuge preparation. (Courtesy of Alberto A. Marmont, MD, and Giovanna Piaggio, MD, Ospedale San Martino, Centro Trapianti di Midollo Osseo, Divisione Ematologia 2, 16132 Genova, Italy.)
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