Molecular Characterization of 16p Deletions Associated With Inversion 16 Defines the Critical Fusion for Leukemogenesis

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THE PERICENTRIC inversion of chromosome 16 [inv(16)(p13q22)] and the related translocation (16;16) [t(16;16)(p13;q22)] are well-established associations of the M4Eo subtype of acute myeloid leukemia (AML). Recently yeast artificial chromosomes (YACs) containing the p-arm breakpoints were isolated, and subsequently the genes disrupted by these chromosomal abnormalities at the 16p- and 16q-arm breakpoints were identified. The juxtaposition of downstream sequences of the p-arm myosin heavy chain gene (MYH11) with upstream sequences of the core binding factor beta gene (CBFB) from the q-arm results in an abnormal fusion gene on the p-arm (5'-CBFB/MYH11-3').

The product of this fusion gene has been identified as the critical product in some patients with inv(16) or t(16;16) AML. The putative alternative chimeric transcript generated on the q-arm (5'-MYH11/CBFB-3') has not been identified in three of these patients studied by reverse transcription-polymerase chain reaction (RT-PCR), and whether it may play a role in leukemogenesis has not yet been definitively determined.

Fluorescence in situ hybridization (FISH) analysis of inv(16) patient material provided rapid information on the mapping of cloned probes in relation to the breakpoints. Using probes proximal to the p-arm inversion breakpoint cluster region (p-ibc), these FISH experiments unexpectedly identified a deletion occurring in association with the inversion in rare patients. The presence of such deletions has recently been confirmed by two other groups. This finding suggests that 5'-MYH11 coding sequences might be deleted in some patients. If so, that would support the critical role of the 5'-CBFB/MYH11-3' transcript in the disease rather than the reciprocal transcript.

In this study we have conducted a FISH analysis on a large series of inv(16) or t(16;16) patients using p-arm cosmids and YAC probes to determine the frequency and the extent of the deletion centromeric to the p-ibc. The use of single-copy probes from both sides of the p-ibc in Southern hybridization analyses refined the limits of the deletion. Sequence analysis of portions of these probes has identified additional MYH11 exons to those already known. Further, identification of exons in the region proximal to the p-ibc, which was shown to contain sequences eliminated by the deletion, resolved the issue of the chimeric transcript critical in leukemogenesis, associated with these chromosome 16 inversions and translocations.

MATERIALS AND METHODS

Patients, samples, and cell lines. Forty-two patients with either inv(16) (38 patients) or t(16;16) (four patients) identified by routine cytogenetic analysis were included in this study. Additional cytogenetic abnormalities were present in some cases. The diagnosis was AML in 33 patients, RAEB-T in one patient, and not available in eight patients. Twenty-nine patients were studied at diagnosis; three patients were studied at both diagnosis and relapse; one patient at relapse only; status was unknown for eight patient samples; and one sample was cell line ME-1 from a relapsed AML patient with inv(16). Patient numbers were assigned in previous studies and, therefore, are not consecutive in this report. Peripheral blood specimens, obtained directly or by apheresis, and bone marrow specimens...
were cultured fresh or after cryopreservation and thawing. Cells were harvested for metaphase cells using standard protocols. Patient cells had been cryopreserved for up to 10 years, and cell suspensions or spread slides remaining after routine cytogenetics at diagnosis had been stored for up to 6 years. Not all samples yielded metaphase cells, and in these cases, interphase preparations were examined where feasible. A minimum of 200 nuclei were examined in interphase preparations, and the result was declared interpretable if at least 90% of nuclei displayed a consistent result.

**FISH probes.** Development and characterization of somatic cell hybrid 41XP91-3-20 (SCH-3-30), and YAC clones y757D7 and y854E2 have been described, as have the inter-Alu-PCR methods for isolating and amplifying human genomic contents from such clones for use as FISH probes. Identification of cosmids 35B11 and its contig, 16C3, LA2-2, and LA4-1 has been described. DNA was isolated using standard protocols. Probes were labeled with either biotin or digoxigenin-11-dUTP by nick translation.

**FISH.** FISH was performed using standard methodology. Slides were RNase treated, washed in 2× standard sodium citrate (SSC), dehydrated in an alcohol series, denatured in 70% formamide/2× SSC for 3 to 5 minutes at 70°C, and dehydrated in an ice-cold alcohol series. Probe was hybridized with Cot 1 DNA and placed in hybridization solution (50% formamide, 2× SSC, 10% dextran sulfate) to a final DNA concentration of 15 to 20 ng/μL. After denaturation at 70°C for 5 to 10 minutes, probe was quenched on ice for 10 minutes, reannealed at 37°C for 30 minutes, applied to denatured dehydrated slides, and incubated at 37°C overnight. Slides were washed three times in 50% formamide/2× SSC for 3 to 5 minutes at 42°C, twice in 2× SSC for 5 minutes at 42°C, and in either 1× or 0.2× SSC for 2 minutes at 37°C once and room temperature once. Signal detection and amplification was performed for biotin-labeled probes using fluorescein isothiocyanate (FITC)-avidin and antiantiavidin antibody from Oncor (Gaithersburg, MD). Digoxigenin-labeled probes were detected with rhodamine-tagged antidigoxigenin antibody (Oncor) and rabbit anti-sheep antibody as required for amplification as per manufacturer’s instructions. Slides were counterstained with propidium iodide/antifade or DAPI/antifade and examined with a Nikon fluorescent microscope (Nikon Inc, Melville, NY) equipped with a multiple-pass filter.

Pulsed field gel electrophoresis (PFGE), Southern blotting, filter hybridization, and RT-PCR were performed as previously described. Sequence analysis was performed on an Applied Biosystems Inc automated sequencer (Foster City, CA). Analysis was performed with PCGENE (Intelligenetics, Mountain View, CA).

**RESULTS**

**FISH identification of the p-arm deletion using cosmid, cosmid contig, and YAC probes.** FISH experiments on inv(16) patient metaphase cells were performed to localize cosmids probes with respect to the p-ibc. Cosmid 35B11 was shown by pulsed field mapping to contain sequences within 240 kb of the p-ibc. In the FISH experiments using 35B11 as probe, this cosmids mapped centromeric to the p-ibc, because the signal it generated moved to the q-arm on the inversion chromosome. As additional patients were studied, one was identified (patient 3) in whom a signal was generated on the normal chromosome 16 but not on the inv(16) chromosome. This finding indicated that the region on the inversion chromosome identified by this cosmid was deleted in the cells from this patient.

Cosmid 35B11 was contained within a cosmids contig spanning 120 kb. Four cosmids (57B2, 61B1, 43F6, 35B11) were chosen to provide a FISH probe spanning the entire contig. This larger probe not only provide a clear signal on each chromosome 16 in the metaphase cells of inv(16) patients, but also identified two clear spots in interphase cells (Fig. 1a). Consistent with the result in patient 3 using 35B11 alone, only one chromosome 16 from marrow metaphase cells had sequences that hybridized, and only one signal was detectable in the interphase nuclei (Fig 1b). These data indicated the deletion was at least 120 kb in size.

To determine the frequency of the deletion, cell samples from 42 patients with inv(16) or t(16;16) were studied by metaphase or interphase FISH with the 35B11 cosmids contig. A single signal indicated that the deletion was present in metaphase and/or interphase cell preparations from six patients (3, 5, 31, 35, 45, and 48) of 38 patients with inv(16) and from none of four patients with t(16;16). Thus, 15.8% of inv(16) patients and 14.3% overall were identified as having the sequences detected by the 35B11 contig deleted. The M4E0 cell line, ME-1, was one of the patient samples that did not show the deletion.

To establish that this deletion of p-arm sequences from one homologue of chromosome 16 is not present in the normal cells of the inv(16)/del patients identified, patient 5 was studied in remission. FISH analysis using the 35B11 contig showed two clear signals in metaphase and interphase cells. Furthermore, this patient and inv(16)/del patient 45 were restudied in relapse, and the leukemic clone was again characterized by the presence of inv(16) with the p-arm deletion establishing the relapse as from the original clone. Similarly, patient 10 who had inv(16) without the deletion at diagnosis retained the same molecular cytogenetic phenotype in subsequent relapse.

The position of the deletion with respect to the p-ibc was refined by metaphase FISH analysis of inv(16)/del patients using a cosmids probe, 16C3, which spans the chromosome 16 p-ibc region. This cosmids is shown in Fig 2A in relation to the 35B11 cosmids contig and in Fig 2B with the various patient breakpoints identified (see Southern data below). When used on metaphase cells from 14 inv(16) patients, the FISH signal generated by 16C3 was split in the inversion chromosome. The split was most readily detected in the rare patients with more centromeric breakpoints, because a larger p-arm fragment is retained (Figs 1c and 2B). When used as probe in the three inv(16)/del patients studied, 16C3 generated one clear signal on the normal chromosome (Fig 1e) with a weaker signal retained on the inversion chromosome (reflecting the more telomeric breakpoint in these patients), which was not identifiable in all metaphases (Fig 1d). This indicates that the deletion encompasses most of the unique sequences detected by this cosmids. Because 16C3 does not overlap the 35B11 contig (Fig 2A), the deletion must approach 160 kb in size (the sum of the contig and cosmids sizes).

The q-arm breakpoint region was studied for evidence of deletions. Cosmids LA2-2 and LA4-1 overlap and have been shown to span the q-arm breakpoint of the inversion. When used as a FISH probe, the signal was split between p- and q-arms as expected. This finding has been verified in nine inv(16) cases. Two of the inv(16)/del patients (patients 3
and 5) were studied with this probe. The signal was split in exactly the same manner as in the inv(16) patients without the deletion. Thus, no similar deletion event occurring in relation to the q-arm breakpoint has been identified.

The proximal limit of the deletion was narrowed by using large YACs that span the p-ibc (y854E2 and y757D7). These were found to span the deletion, as evidenced by splitting of the FISH signal between p- and q-arms in the inversion chromosome in all five inv(16)/del patients tested (patients 3, 5, 31, 35, and 45). The intensity of the remnant q-arm signal was consistently less in these cases as compared with the inv(16) patients without the deletion, providing further evidence of the loss of sequences in the former (Fig 1f and g). The smaller of the two YACS, y854E2, has a 550-kb human chromosome 16 DNA insert. The signal remaining in the deletion patients is substantial; therefore, the deletion must span considerably less than 550 kb. Conservatively, one can estimate from this visual evidence that at least 200 kb of signal remains, leaving 350 kb as the estimated upper size limit of the deletion.

Validation of FISH probes for detecting inv(16). Verification of the karyotypic abnormality of chromosome 16 was undertaken in all patients with available metaphase preparations. We also sought to confirm that the FISH probes used would detect the inversion in the presence of the deletion. Metaphase cells were analyzed by FISH using at least one, and in many cases all three, of the following probes: SCH-3-30, y854E2, and y757D7. Of the 42 patients analyzed by FISH for the presence of the deletion, metaphase preparations were available from 29, which included five of the six inv(16)/del cases (patients 3, 5, 31, 35, and 45). The cytogenetic abnormality involving chromosome 16 was confirmed by
DELETIONS IN INVERSION 16 LEUKEMIA

A

CENTROMERE

16p13

TELOMERE

16p13 - MYH11

BREAKPOINT CLUSTER REGION

5'

H

E

D

C

B

A

3'

NESA

16c3e

1 Kb

Fig 2. Physical organization of the p-ibc. (A) Summary of data derived from FISH probes. The 35B11 cosmid contig is within 240 kb of the p-ibc (pulsed field data) and spans 120 kb. Cosmid 16C3 spans the p-ibc and is 40 kb. Deletions eliminate signal from both these probes and, therefore, span a minimum of ~160 kb. YAC y854E2 not included in the figure has a 550-kb insert and spans the entire region, including the centromeric limit of the deletion. (B) Probes NESA and 16C3e used for Southern blotting are shown below the map. NESA is the most centromeric portion of the subcloned 5 kb EcoRI-Not I fragment. Genomic DNA sequenced in this study are represented as open bars. MYH11 exons 1-3 and 12 were identified from this. Exon 1 is the most 5' coding element identified to date but is not the initial exon. Exons 5 and 6 are postulated based on the cDNA fusion point for the common type A 5'-CBFB/MYH11-3' chimeric transcript (MYH11 base pair 1921) and the coding region identified in genomic sequence. The precise sizes of introns corresponding to breakpoints A, C, and D are uncertain. Exons 2-4 and 7-9 were identified previously. Exons 1 and 2 are MYH11 coding sequences immediately downstream of 5'-CBFB/MYH11-3' fusion points for transcript types D and C, respectively.

FISH in all patients tested, including the five inv(16)/del cases (Fig 1g). Thus, these probes readily detect the inv(16) even in the presence of the p-arm deletion.

Southern hybridization analysis. To confirm the FISH observation that sequences detected by cosmid 35B11 were deleted, an EcoRI repeat-free fragment of 35B11 known to detect rearranged macrorestriction fragments in inv(16) patient DNAs separated by PFGE was used as probe in the Southern hybridization analysis of inv(16)/del patient 5 (Fig 3). In the inv(16) patients without deletions, rearranged fragments were present in all four cases tested (patients 1, 2, 4, and 38) as expected. However, in patient 5, no rearrangement was detected, consistent with the interpretation that those sequences were deleted in the rearranged macrorestriction fragments.

To refine the localization of the deletions in relation to the p-ibc, two inv(16)/del patients (patients 3 and 5) from whom DNA was available were studied by conventional Southern analysis using probes from either side of the p-ibc (Fig 4). The results were compared with those from similar experiments conducted on DNA from inv(16) patients with
out deletions, other AML patients, and normal individuals. Probes 16C3e and NE5A identify fragments within 9 kb telomeric and 10 kb centromeric of the p-ibc, respectively (Fig 2B). Because the MYHII gene disrupted by the inversion is transcribed from centromere to telomere, 5' 16C3e is downstream and NE5A upstream with respect to the transcription direction of this gene. Both probes detect a common 17-kb HindIII fragment, which represents the normal unarranged fragment present in germline DNA. In DNA from inv(16) patients, we expect both probes to detect rearranged fragments because they are in close proximity to the p-ibc. The telomeric probe 16C3e should detect rearrangements resulting from fusion of p-arm 3'-MYHII with 5'-CBFB sequences from the q-arm as a consequence of the inversion. The centromeric probe NE5A should detect the reciprocal fusion on the q-arm consisting of 5'-MYHII and 3'-CBFB sequences. However, if sequences identified by the probe have been deleted, no rearranged fragment should be detected.

The telomeric probe 16C3e did, in fact, detect rearrangements in Kpn I or HindIII digests in all inv(16) cases tested (Fig 4A). This included inv(16)/del patients 3 and 5, and, therefore, we conclude that the deletion does not extend to sequences identified by 16C3e that are within 9 kb telomeric of the p-ibc. It should be noted that although a rearranged HindIII fragment was not detected in patient 5, one was clearly revealed using Kpn 1 (Fig 4A). This is most likely explained by the possibility that the rearranged fragment after HindIII digestion was similar in size to, and therefore could not be resolved from, the germline band. In contrast with these results, the centromeric probe NE5A did not detect rearranged bands in inv(16)/del patients 3 and 5 (Fig 4B and C). However, they were present as expected in all other inv(16) patients tested. The Southern and FISH results are summarized for these patients in Table 1, and they allow us to conclude that the deletion removes segments within 10 kb centromeric of, and presumably up to, the p-ibc.

**Sequence analysis.** Our interpretation of the above data indicated that the segments deleted from the abnormal chromosome 16 of inv(16)/del patients contain upstream MYHII sequences. To demonstrate this formally, portions of the subclone NE5 (from which probe NE5A was derived) and 16C3e were sequenced and compared with the MYHII sequence. Putative MYHII exons were located in both fragments, as shown in Fig 2B. This figure also includes previously published sequence information. Figure 5 outlines coding sequences identified in our subclones. Some ambiguities remain in the fine mapping of exon locations (Fig 2, legend). We conclude from these and the Southern hybridization data that the deletions described in the inv(16)/del cases contain upstream MYHII coding sequence.

**RT-PCR.** The chimeric transcript 5'-CBFB/MYHII-3' has been identified by an RT-PCR assay in a large number of inv(16) patients. 5 Because the deletion had been shown only to encompass segments—including MYHII sequences—centromeric of the p-ibc, we expect this chimeric transcript to remain intact in inv(16)/del patients. Inv(16)/

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**Table 1. Comparison of Inv(16)/Del and Inv(16) Molecular Studies**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Probe 35B11</th>
<th>Probe 16C3</th>
<th>Kpn I/NE5A</th>
<th>EcoRI/NE5A</th>
<th>HindIII/16C3e</th>
<th>Kpn I/16C3e</th>
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<tr>
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<td>R</td>
<td>R</td>
<td>R</td>
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<td>R</td>
<td>R</td>
<td>R</td>
<td>NR</td>
</tr>
<tr>
<td>3</td>
<td>Deleted</td>
<td>Deleted</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
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<td>Present</td>
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<td>R</td>
<td>R</td>
<td>NR</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>Present</td>
<td>Split</td>
<td>R</td>
<td>R</td>
<td>R</td>
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<tr>
<td>7</td>
<td>Present</td>
<td>Split</td>
<td>R</td>
<td>R</td>
<td>NR</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
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<td>Split</td>
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<td>R</td>
<td>NR</td>
<td>A</td>
</tr>
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<td>9</td>
<td>Present</td>
<td>Split</td>
<td>R</td>
<td>R</td>
<td>NR</td>
<td>A</td>
</tr>
</tbody>
</table>

 Patients 3 and 5 are inv(16)/del patients. Abbreviations: ND, not done; R, rearranged; NR, not rearranged.

* Transcript forms reflect different size RT-PCR products (see Results).

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**Fig 5. Genomic sequencing of MYHII p-ibc exons.** Exonic coding sequence and flanking sequences (upper and lower case, respectively) for exons 1-4 identified in NE5 clone and exon 12 in 16C3e clone. Exons 1 and 2 are not the first exons of the MYHII gene, but rather are the initial MYHII sequences of 5'-CBFB/MYHII-3' fusion transcripts types D and C, respectively. The CBFB/MYHII fusion point for B type transcript is shown as an arrow at position 1534 above exon 4. The Not I site at position 1575 on the transcript is internal to exon 4 and terminates the clone NE5. This Not I site is underlined.
del patients 3, 5, 31, and 35 were studied by RT-PCR. Primers used were from the 5’ region of CBFB and the 3’ region of MYH11 distal to the p-ibc. In all four patients the chimeric transcript was present. It was of the shortest form (type A), which is seen in the majority of inv(16) cases, in all four cases.6 Consistent with the Southern data, this result indicates that no coding sequences of MYH11 distal to the p-ibc have been deleted in these cases. It also verifies that the q-arm portion of the hybrid gene is intact at the cDNA level, as deduced from FISH data with a probe from the q-arm breakpoint.

RT-PCR findings in all patients studied by Southern hybridization are also included in Table 1. The genomic data generated by the Southern analyses correlated with RT-PCR findings. Rearrangements detected by 16C3e in KpnI digests are seen in patients with the smaller type A transcripts, which have transcripts of the longer 5’-CBFBMYH11-3’ region of CBFB and the MYH11 gene. Patients 2 and 6 rearranged with HindIII but not KpnI have transcripts of the longer varieties (types C and D), which include larger portions of MYH11 coding sequences in the fusion gene. Thus, upstream or centromeric breakpoints correlate with longer transcripts, and, conversely, downstream or telomeric breakpoints with shorter transcripts, indicating that variability in transcript length is generated by the amount of MYH11 included in the chimeric gene in most patients.6

Clinical data. It was of interest to establish whether any clinical differences were evident between the molecular cytogenetic subgroup of inv(16)/del patients and the other inv(16) patients. The clinical characteristics of the inv(16)/del patients are presented in Table 2. Time to treatment failure was compared for the six inv(16)/del patients and the 17 inv(16) patients without deletions on whom clinical data were obtainable. There was no difference observed. When stratified for patients receiving high-dose cytosine arabinoside (Ara-C)–based therapy, five inv(16)/del patients and 14 inv(16) patients were identified, and again no difference was observed.

### Table 2. Inv(16)/Del Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Karyotype*</th>
<th>FAB Classification</th>
<th>TTF (wks)</th>
<th>Status†</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 inv(16), +22(24)</td>
<td>M4Eo</td>
<td>144+</td>
<td>Alive</td>
<td></td>
</tr>
<tr>
<td>5 inv(16), +8(1)</td>
<td>M4Eo</td>
<td>67</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>31 inv(16)[12]</td>
<td>M4Eo</td>
<td>4</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>46.XX(11)</td>
<td>M4Eo</td>
<td>26</td>
<td>Dead</td>
<td></td>
</tr>
<tr>
<td>35 inv(16), +8(16)</td>
<td>M4Eo</td>
<td>52</td>
<td>Alive (3rd CR)</td>
<td></td>
</tr>
<tr>
<td>48 inv(16), +21(1)</td>
<td>M4Eo</td>
<td>77+</td>
<td>Alive</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FAB, French-American-British; TTF, time to treatment failure; CR, complete remission.
* Numbers in brackets indicate number of cells counted.
† Status as of June 1994.

### Discussion

The p-arm deletion associated with the inversion of chromosome 16 is a relatively infrequent event, identified by FISH analysis in only 6 (16%) of 38 inv(16) patients in this study. This result is not inconsistent with the frequency observed by other groups using similar techniques: 1 of 175 and 5 of 131 inv(16) cases. It could be argued that the large probes needed for FISH may not identify more subtle putative deletions, which could be more frequent. However, the incidence was unaltered by our Southern hybridization analysis of DNA from a subset of patients that did not show deletion by FISH. Because the latter analysis would have identified smaller deletions within 10 kb of the p-ibc, our FISH estimates of the deletion frequency may be correct. None of four t(16;16) cases demonstrated the deletion. However, if the incidence of deletions is similarly low among this group, the failure to detect any in the few patients studied does not exclude the possibility of deletions occurring in translocation patients.

The molecular characteristics of the deletions have been considerably refined. We have shown that a substantial amount of genetic material is deleted, with at least ~160 kb and at most an estimated 350 kb missing. The deletions were mapped to within 10 kb centromeric of the p-ibc. Identification of the 5'-CBFB/MYH11-3’ RT-PCR product in inv(16)/del patients identical in size to that in the majority of inv(16) patients without the deletion excludes deletion of MYH11 coding sequences telomeric to the p-ibc. Together, these mapping data are consistent with the interpretation that the deletions begin at the breakpoint and extend only proximally on the p-arm. Therefore, one may speculate, that both the inversion and the deletion occur simultaneously during the same complex molecular genetic event. It may be postulated that certain molecular characteristics of the p-ibc result in some degree of instability that predisposes to such inversion and deletion events. This instability in the genomic DNA may have contributed to the unstable nature of anonymous YAC clones containing portions of this region.7 The mechanisms involved in these events remain to be elucidated.

The molecular consequence of the rearrangement of chromosome 16 is the generation of two possible fusion genes: 5'-CBFB/MYH11-3’ on the p-arm and 5’-MYH11/CBFB-3’ on the q-arm. The presence of a p-arm deletion proximal to the p-ibc had been invoked to suggest that the important fusion gene for leukemogenesis is the p-arm 5'-CBFB/MYH11-3’ gene and not its counterpart.5 The demonstration here of upstream MYH11 sequences within the deleted region indicates that a substantial segment of the p-arm–derived MYH11 component of the postulated q-arm hybrid gene would be deleted, thus significantly truncating if not entirely abolishing the 5’ portion of the MYH11/CBFB hybrid gene. The clinical characteristics of inv(16) deletion and nondeletion patients are indistinguishable at diagnosis, providing further support for our conclusion that the 5’-CBFB/MYH11-3’ transcript is the operative factor in this leukemia. It could be argued that a possibility exists in the inv(16)/del patients for an additional fusion transcript to arise starting at the 5’ region of some unknown gene at the centromeric...
limit of the p-arm deletion and continuing into the 3′ region of CBFB on the q-arm. Although we have not excluded this possibility, it seems unlikely such a transcript would alter the leukemogenic process, as we are unable to discern phenotypic differences between deletion and nondeletion patients. Thus, future efforts will be focused on elucidating the role of the 5′-CBFB/MYH11-3′ transcript in leukemogenesis.

Other translocation events in acute leukemias have been associated with deletions. Chromosome band 11q23 is frequently involved in translocations occurring with several recipient chromosomes. In a recent study, 30% of samples tested showed evidence of the deletion of sequences of the MLL gene telomeric to the breakpoint. Translocation (8;21) associated with the M2-AML subtype has been reported to occur in association with the deletion of a short stretch of DNA from the AML1 gene region. In chronic myeloid leukemia, deletions within 3′BCR have been well characterized. The occurrence of deletions in association with several leukemia-related chromosomal rearrangements suggests common mechanisms may be involved.

In all of these translocations that generate two possible fusion genes, one appears to be critical. The transcript from the AML1-ETO fusion gene is the only product identified in the (8;21) translocation. Transcripts from both the PML-RARA and RARA-PML hybrid genes have been identified in t(15;17), however, whereas the former retains major functional domains of both genes and is considered the important molecule for leukemogenesis, the latter is of undetermined significance. Similarly, transcripts from both the BCR-ABL and ABL-BCR hybrids have been identified, with the former well established as critical for leukemogenesis. In these cases, the deletions have occurred in 3′ elements of the gene, which is the 5′ component of the critical fusion gene. Uniquely the inv(16)-associated deletion described here occurs in the 5′ portion of the MYH11 gene, which is the 3′ component of what we have deduced to be the critical fusion gene.

Recently it has been shown that in a subset of inv(16)/del patients the multidrug resistance protein (MRP) gene, which has been regionally mapped into the 16p segment containing the inversion breakpoint (16p13.12), was deleted. It was suggested, based on a comparison of five patients with MRP deleted and seven patients without deletion that the patients with the deletion had a better clinical outcome than those with two intact copies of this gene. We have not studied the MRP gene and cannot verify that the inv(16)/del patients in this report constitute a similar subset. However, under the likely assumption that MRP was deleted in our patients, a similar comparison of time to treatment failure between the six inv(16)/del patients and 17 inv(16) patients who did not have the deletion on whom sufficient clinical data were available demonstrated no difference. This result was unaltered when stratified for a uniform treatment approach. This finding is not surprising, as MRP has not been shown to confer resistance to Ara-C, which is the key chemotherapeutic agent in the therapy of inv(16) AML. However, it must be emphasized that much larger patient numbers need to be studied to draw definitive clinical conclusions regarding prognosis. Certainly, it is true that clinical subsets of inv(16) patients exist. For example, some are cured by chemotherapy whereas others relapse; some have leukemic organ infiltration whereas others do not; some have central nervous system involvement whereas others do not. Therefore, subset analysis at the molecular level would appear to be a worthwhile endeavor in an attempt to understand the biologic differences among similar leukemias. The molecular heterogeneity identified by the interstitial p-arm deletion in some patients may be a clue to further unraveling the inv(16) leukemias. Ultimately, finely tailored therapy may be possible.

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16p DELETIONS IN INVERSION 16 LEUKEMIA


Molecular characterization of 16p deletions associated with inversion 16 defines the critical fusion for leukemogenesis

P Marlton, DF Claxton, P Liu, EH Estey, M Beran, M LeBeau, JR Testa, FS Collins, JD Rowley and MJ Siciliano