CONCISE REPORT

Rearrangement of the Breakpoint Cluster Region and Expression of P210 BCR-ABL in a “Masked” Philadelphia Chromosome-Positive Acute Myeloid Leukemia

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The Philadelphia (Ph) translocation t(9;22)(q34;q11) occurs frequently in chronic myeloid leukemia (CML) but is less common in acute lymphoblastic leukemia (ALL) and rare in acute myeloid leukemia (AML). In most cases of CML and some cases of Ph+ ALL the protooncogene ABL from 9q34 is translocated to the breakpoint cluster region (bcr) of the BCR gene at 22q11 to form a chimeric gene encoding a novel 210-kd protein (P210 BCR-ABL) with enhanced tyrosine kinase activity. In other patients with Ph+ ALL and Ph+ AML, the breakpoint probably occurs in the first intron of the BCR gene; this results in a smaller chimeric gene which encodes a P190 BCR-ABL. We studied a patient with AML (FAB M6) arising de novo who had a “masked” Ph chromosome in association with extensive karyotypic changes. The leukemic cells initially showed rearrangement of the bcr, presence of a hybrid mRNA, and expression of the P210 BCR-ABL. These changes were absent in remission. These results support the concept that the BCR-ABL chimeric gene plays a crucial role in leukemogenesis but suggest that factors other than the position of the breakpoint in the BCR gene determine the lineage of the target cell for malignant transformation.

THE PHILADELPHIA chromosome (Ph), the product of the reciprocal translocation t(9;22)(q34;q11), is present in 90% to 95% of cases of chronic myeloid leukemia (CML). It also occurs to a lesser extent in acute lymphoblastic leukemia (ALL) but is rare in acute myeloid leukemia (AML). Variant translocations occur in 5% to 10% of Ph+ leukemias.1 These include “masked” Ph chromosomes in which the deleted chromosome 22 is not clearly recognizable because it is involved in additional rearrangements with one or more other chromosomes.

Molecular studies have shown that in Ph+ CML the breakpoint cluster region (bcr) located within the BCR gene at 22q11 and the protooncogene ABL located at 9q34 are juxtaposed on chromosome 22 to form a new chimeric BCR-ABL gene.2 This gene encodes a larger than normal protein P210 BCR-ABL with protein tyrosine kinase (PTK) activity.3 In contrast, Ph+ ALL falls into two molecular subgroups. One group has rearrangement within bcr (bcr+) and expression of P210 BCR-ABL; in the other, the breakpoint occurs within the first intron of the BCR gene (bcr−) and leads to expression of a smaller ABL-related protein P190 BCR-ABL.4 Ph+ AML may also be heterogeneous at the molecular level: Kurzrock et al5 described a patient with Ph+, bcr- AML whose leukemic cells expressed the P190, and Erikson et al5 reported a case of Ph+ AML with rearrangement of the bcr (bcr+).

We report a cytogenetic and molecular investigation of a de novo case of AML. The karyotype was complex and included a masked Philadelphia chromosome. The leukemic cells showed rearrangement of bcr and expressed P210 BCR-ABL.

MATERIALS AND METHODS

Case report. A 41-year-old woman presented with a short history of fever, cough, and symptoms of anemia. There was no preceding history of hematologic illness. Examination revealed marked hepatosplenomegaly. On her admission to the hospital, her blood count had a hemoglobin of 8.8 g/dL and platelets 51 × 10^9/L. Total WBC count was 26.5 × 10^9/L, with 66% neutrophils, 10% lymphocytes, 2% monocytes, 2% metamyelocytes, and 20% blasts. The marrow showed 85% infiltration by blasts, with deeply basophilic cytoplasm containing up to four nucleioli. Occasional blasts exhibited vacuolation. Intermediate and late normoblasts were absent. Cytocentrifuge staining with periodic acid-Schiff (PAS) and Sudan Black were negative, but 100% of the blast cells reacted with a monoclonal antibody for glycophorin A. The blood and bone marrow findings were therefore consistent with AML, French-American-British (FAB) type M6 (erythroleukemia).

Treatment was started with CCNU (lomustine) 200 mg/m^2 on day 1, daunorubicin 50 mg/m^2 on days 1 to 3, and cytosine arabinoside 100 mg/m^2 on days 1 through 7 (inclusive). The blood and bone marrow on day 42 after chemotherapy was started showed complete remission.

Cytogenetic analysis. Chromosome preparations were made with standard techniques from bone marrow and peripheral blood at presentation and during remission. Metaphases were Giemstained banded and karyotyped according to the International System (ISCN).

In situ hybridization.121-I-Labeled probes were hybridized in situ to metaphases obtained from direct preparations of bone marrow using the methods described previously.7 The probes used were a 1.4-kilobase (kb) (cDNA) ABL (from Dr O. N. Witte) and a 1.3-kb SIS probe (from Dr M. Waterfield).

PTK assay. Blast cells were separated from peripheral blood on discontinuous Percoll gradients, lysed, and assayed for in vitro ABL-protein tyrosine kinase activity as described previously.8

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Table 1. Distribution of Silver Grains on Chromosomes Involved in t(1;4;9;922) Following In Situ Hybridization With Probes for ABL and SIS

<table>
<thead>
<tr>
<th>Probe</th>
<th>Total No. of Metaphases</th>
<th>Total No. of Grains</th>
<th>No. of Grains on Chromosome (% of Total Grains)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1p−</td>
</tr>
<tr>
<td>ABL</td>
<td>50</td>
<td>97</td>
<td>2(2)</td>
</tr>
<tr>
<td>SIS</td>
<td>50</td>
<td>98</td>
<td>5(5)</td>
</tr>
</tbody>
</table>

*Masked Ph chromosomes.

**DNA analysis.** High mol-wt DNA from nucleated cells was isolated from the intermediate fraction of Percoll-separated cells and digested with restriction enzymes Bgl II, XbaI, and EcoRI. The DNA was size-fractionated by electrophoresis through 0.7% agarose gels, transferred to nitrocellulose, and hybridized to a 4.8-kb DNA probe spanning the bcr region (provided by Dr Kees Stam, Oncogene Sciences, Manhasset, NY).

**RNA analysis.** Total RNA was isolated from mononuclear cells using the lithium chloride/urea protocol. In brief, five oligonucleotide primers were made using an Applied Biosystems DNA synthesizer. The sequences of the oligomers are:

- Oligo A: 5' GAAGAAGTGTTTCAGAAGCTTCCC 3' (b1b2 sense strand).
- Oligo B: 5' GGTTACCAATTCAGCAGGTGAGCATCTGACTT 3' (a2 sense strand—the first ten nucleotides are an artificial restriction enzyme site incorporated to aid cloning if necessary).
- Oligo C: 5' GTGTGATACCCCTAAGGCCGGAG 3' (a3 antisense strand).
- Oligo D: 5' TGGATTTAAGCAGAGTTCCAAGGGCCTTCCAGGCCCAGTA 3' (b3a2 sense strand).
- Oligo E: GCTGACCATCAATAAGGAAGAAGCUCACAGCGGCCAGTA 3' (b2a2 sense strand).

The polymerase chain reaction (PCR) was performed as described. A combination of two primer pairs A/C and B/C were used. If a message coding for P210 is present in the cells the combination of primers A/C bracket a region of 319 or 394 base pairs (bp) depending on the position of the junction point within the bcr of the BCR gene. A fragment of 218 bp will be amplified by the primer pair B/C from any normal or hybrid ABL transcript present. Size estimations of the amplified material were made following electrophoresis through a 2% agarose gel. In addition, the size-fractionated DNA was transferred to Gene Screen Plus (New England Nuclear, Boston) and hybridized to oligomers representing specific sequences of the b3a2 junction (oligo D), b2a2 junction (oligo E) or to a universal ABL sequence probe (oligo B) which detects any of the amplified ABL products.

**RESULTS**

**Cytogenetic and in situ analysis.** At diagnosis, the patient showed a highly variable hyperdiploid karyotype (48,XX to 96,XX) with a predominant clone 48,XX. No normal metaphases were present. Trisomy 11 and a complex rearrangement involving chromosomes 1, 4, 22, and both homologues of chromosome 9, with or without additional abnormalities, were found in all (30 of 30) of the cells analyzed. Study of the metaphase chromosomes by in situ hybridization showed that the ABL probe was localized to the 9p− chromosome at 9q34 and to the derived 22q+ (masked Ph) chromosomes (Table 1), whereas SIS was found on the normal homologue at 22q13 and also on the distal region of 4q (Table 1). The karyotype of the predominant clone was therefore interpreted as 48,XX, t(1;4;9;9;22)(q24;q31;q34;p13;q11)+11,+der(22), the duplication of the derived 22q+(der(22)) resulting in a double masked Ph chromosome (Fig 1). After remission induction therapy, cytogenetic analysis of bone marrow cells at 2 and 5 months showed a normal karyotype (46,XX).
DNA, RNA, and protein analysis. Southern analysis of DNA demonstrated a rearranged band in addition to the germline band in the XbaI digest (Fig 2A, lane 2). This finding is consistent with a rearrangement within the bcr region. The presence of an active BCR-ABL chimeric gene was demonstrated by the results of the PTK assay which detected a 210-kd protein (Fig 2B, lanes 1 and 2). To confirm the presence of the hybrid gene coding for the 210-kd protein with ABL epitopes, RNA was isolated and subjected to specific amplification of the putative junction region by polymerase chain reaction. An amplified fragment of 394 bp bounded by the primers A/C from BCR and ABL gene sequences was observed (Fig 3A), suggesting the presence of a junction between the third exon (b3) of the bcr of the BCR gene and the common exon (a2) of the ABL gene. The predicted band of 218 bp was observed in the reaction with primer pair B/C (Fig 3A). Both these bands hybridize to the ABL primer (oligo B) (data not shown). In addition, the 394-bp band but not the 218-bp band hybridized to the primer (oligo D) spanning the predicted b3a2 junction sequence (Fig 3A), which was further confirmation of the hybrid identity of the amplified region. No hybridization was observed with the primer (oligo E) that spans the junction sequence of b2a2 (data not shown). Figure 3B is a schematic of the hybrid mRNA present in the leukemic cells showing position of oligomers used in this study. Sizes of the amplified units for primer pairs B/C (ABL) and A/C (b3a2) are also indicated. Hatched region represents the ABL sequences; open region represents BCR sequences. Boundaries of the three exons of the BCR gene refer to exons b1, b2, and b3 present within the bcr.

DISCUSSION

The consistency of the rearrangement of the bcr and formation of the functional chimeric gene BCR-ABL encoding P210 BCR-ABL in Ph+ and some patients with Ph− CML provides strong evidence for the importance of this molecular event in CML. Conversely, the smaller BCR-ABL
fusion gene encoding the P190 BCR-ABL has been suggested to be specifically associated with the Ph+ acute leukemias that arise de novo. Several recent studies, however, have shown that some cases of Ph+ ALL show bcr rearrangement and express P210; some of the cases showed no evidence of a preceding CML in chronic phase. Our data show that the same molecular changes, bcr rearrangement and expression of P210, are also present in a patient with masked Ph+ AML. One case of Ph+ AML patient with bcr rearrangement was reported previously, but detailed hematologic and protein data were not provided. It must be argued that the patient described in this report may not actually be a Ph+ AML arising de novo, but rather may have CML in erythroid transformation. However, the absence of features in the peripheral blood and marrow (eg, eosinophilia, basophilia, and/or thrombocytosis) that might have suggested an occult preceding CML, the morphologic abnormalities in the erythroid series and, most important, the ease with which both hematologic and karyotypic remission was obtained are consistent with de novo AML.

Therefore, the association of P210 BCR-ABL with CML and P190 BCR-ABL with the acute leukemias are probably loose associations and further exceptions will probably be recognized. Thus, other secondary events must be responsible for determining the predominant malignant lineage. Molecular rearrangements underlying the t(9;22) are usually conserved in masked Ph translocations despite extensive karyotypic changes. In many of these cases, the clinical features are identical to those of patients with CML with the simple Ph translocation. One or other additional chromosomal changes might activate another gene (or protooncogene) involved in specifying the lineage for malignant transformation, however.

Although no specific chromosomal translocations occur regularly in erythroleukemia, both CML in erythroid transformation and AML M6 may be associated with gross disruption of the karyotype with bizarre chromosomal rearrangements. Several clonal abnormalities were noted in the leukemic cells of our patient, only one of which (trisomy 11) could be associated with a known protooncogene H-RAS located at 11p15. Other clonal chromosomal rearrangements involving chromosome 1, which has a nonrandom involvement in human neoplasia, and chromosome 4 with a breakpoint at fragile site 4q31 (fragile sites have been implicated in predisposition to chromosomal arrangements associated with malignancy) were also present. Thus, we speculate that one or other of the additional cytogenetic changes that occurred in this patient’s leukemic cells might have led to activation or deletion of other oncogenes—events which dictated to some extent the phenotypic characteristics of this leukemia.

We conclude that the results of this study provide further evidence for the central role of the BCR-ABL fusion gene in the pathogenesis of leukemia but suggest that factors other than the precise position of the breakpoint within the BCR gene must be important in specifying the target cell for the malignant transformation.

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REFERENCES

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