Expression of c-abl in Philadelphia-Positive Acute Myelogenous Leukemia

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The identical cytogenetic marker, t(9;22)(q34;q11) (Philadelphia [Ph] translocation), is found in approximately 90%, 20%, and 2% of adult patients with chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), and acute myelogenous leukemia (AML), respectively. In CML, the molecular events resulting from the Ph translocation include a break within the bcr locus on chromosome 22, transfer of the c-abl protooncogene from chromosome 9 to 22, and formation of an aberrant 210-kD bcr-abl fusion protein (p210c-abl). Recently, the absence of bcr rearrangement and expression of a distinct aberrant 190-kD abl protein (p190c-abl) has been described in Ph-positive ALL, with the suggestion that the two abl variants may be pathogenetically associated with myeloid v lymphoid leukemia.

One of the most striking examples of the association between a specific cytogenetic aberration and activation of a cellular protooncogene occurs in chronic myelogenous leukemia (CML). In 1960, Nowell and Hungerford1 made the seminal discovery of a consistent chromosomal aberration—the Philadelphia (Ph) chromosome—in CML. The Ph chromosome is a shortened chromosome 22 resulting from a reciprocal translocation, t(9;22)(q34;q11).2 Molecular analysis has revealed that the 22q11 breakpoint occurs within a breakpoint cluster region (bcr) spanning about 5.8 kilobase (kb).3 Importantly, the c-abl protooncogene is transferred from its normal residence on chromosome 9 to chromosome 22 in juxtaposition to the 5′ bcr sequences that remain on the latter chromosome. The resulting fused bcr-abl gene expresses a novel 210-kD protein; the normal c-abl counterpart is a 145-kD protein.6 The aberrant 210-kD bcr-abl fusion protein (p210c-abl) is active as a tyrosine phosphokinase,4 a property of potential fundamental importance to CML because this enzymatic activity has been shown to exert its effects. Rearrangement of bcr and expression of a bcr-abl message occur in virtually all patients with CML but not in patients with other hematologic disorders, strongly implicating these molecular events in the pathogenesis of CML.

Approximately 20% of adult patients with acute lymphoblastic leukemia (ALL) also carry the t(9;22)(q34;q11) anomaly.5 Several investigators have shown molecular heterogeneity of chromosome 22 breakpoints in Ph-positive ALL.6,7 At least one half of reported patients do not have rearrangement within the 5.8-kb bcr locus. The break at 22q11 may occur between the bcr and C5 region of the immunoglobulin light-chain cluster gene.10 Recently, our laboratory12 and others13,14 have demonstrated a novel aberrant 190-kD c-abl protein (p190c-abl) in bcr rearrangement-negative, Ph-positive ALL; p210c-abl was not found. Presumably, p190c-abl results from recombination of c-abl with a DNA segment upstream of the 5.8 kb bcr locus. Based on these results, it appeared that subtle alterations in genomic configuration relative to the bcr region may correlate with distinct disease phenotypes. A question that arose is whether the generation of p190c-abl rather than p210c-abl is associated with the development of a lymphoid as opposed to myeloid phenotype or, alternatively, of an acute rather than a chronic leukemic process. One approach to dissecting these possibilities was to study samples from patients with a rare leukemia subtype—Ph-positive acute myelogenous leukemia (AML). In this paper, we demonstrate absence of bcr rearrangement in a Ph-positive AML patient and expression of a 190-kD c-abl protein indistinguishable from that found in bcr rearrangement-negative, Ph-positive ALL patients. Our results indicate that p190c-abl is not specific for the lymphoid phenotype but instead may be important in the manifestation of an acute rather than chronic Ph-positive leukemic process.

PATIENT AND METHODS

Case report. In May 1986, patient VI presented with Ph-positive AML. Her past medical history included a diagnosis of carcinoma of the right breast in 1965. She underwent mastectomy and postoperative chest wall irradiation (3,000 rad). In 1970, a left breast carcinoma was found and subsequently removed by mastectomy. She returned to M.D. Anderson Hospital and Tumor Institute every 4 to 6 months for follow-up evaluation. As part of each visit, a CBC was done. In January 1986, the blood counts were normal: WBC count, 6 × 10³/µL with a normal differential count; hemoglobin, 12.7 g/dL; platelet count, 226 × 10³/µL. At the time of her routine return appointment in May 1986, she felt well. There was no evidence of recurrent breast cancer. Surprisingly, laboratory tests revealed a grossly elevated WBC count of 179 × 10³/µL with 94% blasts. Her hemoglobin content was 8.1 g/dL; platelet count, 28 × 10³/µL; and leukocyte alkaline phosphatase score, 5% (normal.
range, 40% to 225%). A bone marrow aspirate and biopsy specimen revealed a cellularity of 95% with 84% blasts. There was no peripheral blood or bone marrow basophilia. The majority of the bone marrow cells stained positive for myeloperoxidase, consistent with a diagnosis of AML. Reactivity with monoclonal antibody against the differentiation marker My4 was about 50% positive. Cell surface markers for OKTI I (T cell surface marker) and surface immunoglobulin (B cell marker) were negative. These results as well as morphological analysis of the bone marrow by light and electron microscopy indicated a diagnosis of M4 (myelomonocytic) subtype of AML (French-American-British classification). Karyotype analysis (Giemsa-stained chromosomes) revealed a hyperdiploid (51 to 87 chromosomes) Ph-positive clone in 25 of 25 metaphases. The patient was treated with high-dose cytosine arabinoside and achieved complete hematologic remission after one course. However, the aberrant cytogenetic clone persisted in 10% of the cells. The patient remains in hematologic remission at 6 months. Blood for molecular studies was obtained immediately before initiating chemotherapy and stored at −70°C until analysis. Informed consent, in accordance with institutional guidelines, was obtained for chemotherapeutic procedures and for drawing blood.

Antisera. Rabbit antisera were made against the following peptides: the predicted hydrophilic v-abl sequence DEV-EKELGKRGTRGC-C (abl389-403); the predicted high-term potential domain of c-abl (Chou-Fasman predictive model for protein secondary structure, KENLLAGPSENPDN-C (abl3-3); the region DDESGPLGYGLNVC-C (bcr3) within bcr exon 3; and the NH2-terminal peptide sequence 1 to 17 derived from the Heisterkamp et al5 bcr sequence (bcr1). The methodology has been previously reported in detail by Kloetzer et al.

DNA analysis. DNA was prepared by cell lysis, proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation. DNA was analyzed by restriction enzyme digestion, Southern blotting, and hybridization of filters to nick-translated probes. The methodology has been previously described by our laboratory.2 Rearrangement in AML patient VI was distinct from the 190,000-mol wt band of anti-bcr3 serum directed against the 1585 kb bcr probe.15 As shown in Fig 2A, lanes 1 and 3. To demonstrate that p210 is bcr-abl specific, we preincubated each of the antisera with the peptide against which they were made (Fig 2A, lanes 2 and 4). This procedure results in blocking of antisera-specific bands but not nonspecific background phosphorylation. To date, we have studied fresh samples from 16 Ph-positive CML blast crisis patients (myeloid blast crisis, ten patients; lymphoid, five patients; undifferentiated, one patient); all patients produced p210 (Fig 2A, lanes 5 and 6; data not shown).19 We have recently demonstrated that a Ph-positive ALL patient without bcr rearrangement expresses a distinct aberrant p210 rather than p210 abl.12 The mRNA encoding p190 abl lacks at least some bcr sequences and is smaller (7.4 kb) than the 8.5-kb CML-derived bcr-abl transcript.15 As shown in Fig 2A (lanes 7 and 9), bcr rearrangement—negative AML patients VI does not produce p210 abl despite the presence of the Ph translocation; however, a 190-kD abl-related protein is detected by two anti-abl sera (Fig 2A, lane 7, and Fig 2B, lane 1). Figure 2B (lanes 1 and 3) demonstrates that the abl-related product of AML patient V1 comigrates with the p190 abl of a Ph-positive bcr rearrangement—negative ALL patient.12 Similar to the situation in ALL,12 AML-derived p190 abl is not detected by anti-bcr3 serum directed against bcr exon 3 (Fig 2A, lanes 9 and 10) or by anti-bcr1 serum directed against the most 5′ portion of the Heisterkamp bcr sequences15 (data not shown).

A 190,000–molecular weight (mol wt) band is also observed in K562 cells (Fig 2A, lanes 1 and 3). This band may represent a second translational product of bcr-abl or a proteolytic fragment of p210 abl. The p190 abl of AML patient V1 is distinct from the 190,000–mol wt band of Ph-positive CML cells because anti-bcr serum clearly recog-
The end stage of CML, termed blast crisis, strongly resembles Ph-positive acute leukemia: the leukemic cells lose their capacity for terminal differentiation, and similar clinical characteristics and laboratory parameters are observed. Further, occasional chemotherapy-induced transition of Ph-positive ALL and AML to CML is well documented. Based on these observations, it has been postulated that the disease of some patients presenting with Ph-positive acute leukemia actually represents the blast transformation stage of CML, which escaped diagnosis during its early, often asymptomatic chronic phase. Even so, it seems likely that at least some ALL and AML patients with t(9;22) have a bona fide acute presentation. Because Ph-positive acute leukemia and CML are similar but retain some distinct features, it may be that related but not identical molecular events are involved in their pathogenesis.

Interestingly, some ALL patients carrying a t(9;22), which is cytogenetically indistinguishable from that found in CML, do not have bcr rearrangement. Our work[2] as well as that of other groups[10,11] has revealed a novel c-abl protein—p190bcr-abl—in Ph-positive, bcr rearrangement-negative ALL patients; p210bcr-abl is not expressed. In the current report, we demonstrate that the Ph tranlocation in AML can also be associated with a 22q11 break outside of the bcr locus and production of p190bcr-abl without an accompanying p210bcr-abl. Our data suggest that p190bcr-abl differs from p210bcr-abl by virtue of the absence of bcr sequences in the N-terminal of the former protein. Similar to the situation in ALL[12] AML-derived p190bcr-abl is enzymatically active as a protein kinase, an observation consistent with a direct role for this aberrant protein in the malignant process. A literature review (Table 1) revealed that about 95% of Ph-positive CML patients (including at least 20 blast crisis patients) but fewer than 50% of Ph-positive acute leukemia patients have bcr rearrangement. Further, p210bcr-abl appears to be expressed in all chronic[19] and acute leukemia patients[12] with bcr rearrangement. Although the two molecular subgroups of Ph-positive acute leukemia have similar clinical and morphological features,[10,11] their distinct genomic configurations may have biologic relevance. The presence of bcr rearrangement makes Ph-positive acute leukemia virtually indistinguishable from CML blast crisis[26] and suggests a single disease state, with the acute leukemia representing the blast transformation phase of subclinical CML. Conversely, Ph-positive bcr rearrangement-negative ALL and AML may reflect true de novo acute leukemia as evidenced by the following: (a) bcr rearrangement negativity is almost never found in CML and (b) p190bcr-abl without p210bcr-abl appears to be associated exclusively with the acute leukemia phenotype. This concept is supported by our current observations because a preceding undetected CML state can be ruled out in our Ph-positive, bcr rearrangement-negative AML patient because of the fortuitous availability of normal blood counts obtained 4 months before diagnosis. Analysis of additional patients will be needed to strengthen the generality of these concepts. However, our data suggest that the presence or absence of proximal bcr sequences attached to c-abl may modulate this gene's tumorigenic potential for chronic v acute leukemogenesis.

Despite the presence of p190bcr-abl instead p210bcr-abl, the subgroup of bcr rearrangement-negative, Ph-positive acute leukemia patients bears a close clinical resemblance to bcr rearrangement-positive, Ph-positive acute leukemia patients[10,11] and as mentioned earlier, both subgroups resemble CML blast crisis. The molecular events leading to the inevitable malignant evolution of CML from a chronic to an acute leukemia phenotype remain largely unexplored; however, because bcr rearrangement is found in the early benign
events are required. If expression of p190 without and Giemsa staining. Nature 243:290, 1973

chronic myelogenous leukemia. Science 32: 1497, 1960

production of pl90c in addition to p210 occurs during chromosome in chronic myelocytic leukemia. Nature 300:765, 1985


are clustered within a limited region, phase, it seems likely that superimposed secondary genetic alterations occur during the course of CML and plays a role in blast transformation? Further analysis of c-abl expression in the nosological spectrum of Ph-positive hematologic malignancies is in progress and should help elucidate the role of molecular variants of this gene on leukemogenesis.

REFERENCES


Table 1. Summary of Studies Analyzing Ph-Positive Leukemia Patients for bcr Rearrangement

<table>
<thead>
<tr>
<th>Type of Leukemia</th>
<th>Source</th>
<th>No. of Patients Analyzed</th>
<th>No. of Patients with bcr Rearrangement (%)</th>
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<tr>
<td>CML</td>
<td>Groffen et al$^3$</td>
<td>17</td>
<td>17</td>
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<tr>
<td></td>
<td>Poponeo et al$^8$</td>
<td>14</td>
<td>10</td>
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<td></td>
<td>Collins$^7$</td>
<td>20</td>
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<tr>
<td></td>
<td>Shalrid et al$^{13}$</td>
<td>72</td>
<td>70</td>
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<tr>
<td>Totals</td>
<td>Rodenhuis et al$^6$</td>
<td>123</td>
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<tr>
<td>ALL</td>
<td>Erikson et al$^{10}$</td>
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<td>0</td>
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<tr>
<td></td>
<td>de Klein et al$^{11}$</td>
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<td>9</td>
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<td></td>
<td>Kurzrock et al$^{12,24}$</td>
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