Correlation Between Molecular and Clinical Events in the Evolution of Chronic Myelocytic Leukemia to Blast Crisis


A patient with typical Philadelphia chromosome (Ph')-positive chronic myelocytic leukemia (CML) was studied during sequential phases of disease: (1) initial chronic phase; (2) myeloid blast crisis; (3) second chronic phase; and (4) accelerated disease. A point mutation in the coding sequence of the p53 gene first appeared concomitantly with the blast crisis and then disappeared with the re-establishment of a second chronic phase. The chromosomal concomitant of the molecular alteration was a deletion of 17p. These observations suggest that abnormalities of the p53 anti-oncogene are temporally related to the clinical progression of some cases of CML and are probably responsible for the development of blast crisis in these cases.

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After a chronic phase of variable duration, chronic myelocytic leukemia (CML) usually evolves into a more aggressive clinical disease with poorly differentiated myeloid or lymphoid blast cells populating the hematopoietic tissues. The Ph' chromosomal anomaly involving translocation of the c-ABL proto-oncogene from chromosome 9 to the bcr region of chromosome 22 is the pathogenetic molecular lesion in the chronic phase of disease, but the lesions responsible for evolution to the blast crisis phase of disease have not been definitely identified. Mutations in the first exon of the N-RAS oncogene are found in some acute-phase cell populations and are probably responsible for CML evolution in a small minority of cases. Recently, we and others observed deletions, rearrangements, and mutations of the p53 gene in blast crisis cases, and cell lines. The p53 gene is a cancer suppressor gene or "anti-oncogene." The exact frequency of alterations of the p53 gene is unknown, but probably exceeds 30%, suggesting that abnormalities of this anti-oncogene may frequently be responsible for clonal evolution of CML. A role for p53 as the molecular culprit in blastic crisis could more convincingly be argued if changes in the gene and in clinical disease were concomitant events. Therefore, we obtained cells, over a period of 2 years, from a patient whose disease went from chronic phase to blast crisis and then returned to chronic phase after intensive chemotherapy. The p53 gene in these cells was analyzed by complete sequencing of the coding exons. The results demonstrate that development of blast crisis and p53 gene alterations are linked events in some cases of blast crisis.

MATERIALS AND METHODS

Peripheral blood leukocytes and/or bone marrow mononuclear cells were separated and stored frozen after obtaining informed consent from the patient. DNA was isolated and the p53 gene analyzed by Southern blotting and sequencing. The sequence of bases in the coding exons 2 through 11 and most of the intron/exon junctions of the p53 gene were determined after amplification by the polymerase chain reaction (PCR) using appropriate 5' and 3' primers under conditions previously described. Multiple PCR reactions were performed on each sample to exclude the possibility of amplification-induced artifacts in base sequences. Conventional methods were used for routine hematologic and karyotypic analysis.

CASE REPORT AND RESULTS

A 34-year-old man was admitted to the hospital in February 1984 with a 6-week history of fatigue and night sweats. A diagnosis of CML was made on the basis of splenomegaly, typical blood and bone marrow abnormalities, and the presence of the Ph' chromosome. The disease remained in chronic phase for almost 6 years, requiring initial treatment with busulfan and subsequently with hydroxyurea or phenylalanine mustard. In January 1990, fatigue, fever, massive splenomegaly, and a rising blast count were noted, and a diagnosis of blast crisis was made (Table 1). Morphology and analysis with a panel of monoclonal antibodies including MY7, MY4, LM9, and anti-Tdt were consistent with a myeloid phenotype of the blastic clone.

Treatment with cytosine arabinoside, tetrahydouridine, and carboplatin was associated with return to a second chronic phase in late February/early March 1990, and this phase persisted under treatment with mitoxantrone until May 1990, when disease began to accelerate again with rising white counts in the blood, and 14% and 19% blasts in the blood and marrow, respectively (Table 1). A clear-cut blast crisis did not evolve at this time, but treatment with mitoxantrone and high-dose cytosine arabinoside was associated with the establishment of a brief third chronic phase in June 1990. The patient died of Escherichia coli sepsis in late July 1990, with disease again in early blastic transformation.

A blood sample obtained in September 1988, during the chronic phase of disease, showed a bcr gene rearrangement, a 46 XY Ph' karyotype, a heterozygous state for the bgl II polymorphism of the p53 gene (Fig 1, lane A), and a normal nucleotide sequence of the coding exons of the p53 gene (Table 1, Fig 2, lane A). The relevant codons of exon 7 are shown in Fig 2A.

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Table 1. Clinical and Molecular Events in the Evolution of CML

<table>
<thead>
<tr>
<th>Date</th>
<th>Phase of Disease</th>
<th>WBC (× 10⁹/L)</th>
<th>Platelets (× 10⁹/L)</th>
<th>Blasts (%)</th>
<th>Cytogenetics*</th>
<th>p53 Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/84</td>
<td>Diagnosis</td>
<td>124</td>
<td>415</td>
<td>6</td>
<td>46,XY, t(9q;22q)[100]</td>
<td>Blood</td>
</tr>
<tr>
<td>9/88</td>
<td>Chronic no. 1</td>
<td>155</td>
<td>419</td>
<td>10</td>
<td>46,XY, t(9q;22q)</td>
<td>Marrow</td>
</tr>
<tr>
<td>1/90</td>
<td>Blast crisis</td>
<td>87</td>
<td>173</td>
<td>41</td>
<td>46,XY[6]</td>
<td>71 Marrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>46,XY, t(9q;22q)[33]</td>
<td>45,XY, -16,DER(7p),7q+,11p+,17p-,t(9q;22q)[60]</td>
</tr>
<tr>
<td>5/90</td>
<td>Accelerated</td>
<td>172</td>
<td>305</td>
<td>14</td>
<td>46,XY, t(9q;22q)[40]</td>
<td>Unsuccessful</td>
</tr>
<tr>
<td>6/90</td>
<td>Chronic no. 3</td>
<td>86</td>
<td>410</td>
<td>1</td>
<td>46,XY[5]</td>
<td>46,Y,t(9q;22q),t(Xp;7p)[55]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>46,XY, t(9q;22q)[75]</td>
<td>46,Y,t(9q;22q),t(Xp;7p)[20]</td>
</tr>
</tbody>
</table>

Abbreviations: WBC, white blood cells; ND, not done.

*pThe percentage of cells with the given karyotype is shown in brackets [*].

In January 1990, when the disease was in early myeloid blast crisis, karyotype analysis for the first time showed a new and complex profile including persistence of the original Ph⁺-positive clone in 33% of the metaphases and the appearance of a cellular population comprising about 60% of dividing cells with abnormalities of chromosomes X, 7, 11, 16, and a deletion of 17p as well as the Ph¹ chromosome. It should be noted that the p53 gene resides on 17p. Southern analysis showed a loss of heterozygosity for the p53 locus (Fig 1, lane B) consistent with the chromosomal findings. Two independent blood samples at this time showed that the normal AAC of codon 239 of the p53 gene had been replaced by an AGC (Fig 2B), resulting in a change of asparagine to serine in the p53 protein. The much higher intensity of the mutant G relative to the normal A bands (Fig 2B) suggests that the malignant blasts had a single mutant allele of the p53 gene and that the faint A band results from the persistence of antecedent chronic-phase cells. Although other interpretations are possible, the pattern clearly shows that the mutant p53 allele was the dominant species in the blood in this blastic phase.

A second chronic phase was induced with multiagent chemotherapy. At this time, when there were 19 × 10⁹ white blood cells/L and less than 5% blasts in the peripheral blood, the karyotype analysis showed a 46XY pattern with the classical t(9q;22q) of the Ph¹ chromosome in one half of the dividing cells and an additional translocation involving chromosomes 7 and X in the remaining dividing cells. The 17p deletion was no longer detectable. Analysis of the complete coding sequence of p53 once again showed a normal pattern, indicating that the predominant clone during this second chronic phase had a normally coding p53 gene. The relevant area of codon 7 is shown in Fig 2C and demonstrates disappearance of the mutant G and return of the normal A at codon 239.

In May 1990, when disease was once again accelerating, a blood sample was obtained that contained 14% blast cells. Detailed sequencing of all coding exons showed a normal p53 gene structure at this time and Fig 2D shows the relevant area of exon 7; however, it should be noted that any mutation occurring in less than 20% of the cells would have been below the limits of detectability by direct sequencing.⁸

Cells obtained at intervals between 1988 and 1990 always...
had a rearranged bcr gene whose gross structure was unchanged throughout this period. Similarly, cells from all phases of disease were studied for alterations of exon 1 of N-RAS, and no abnormalities were found, indicating that activation of this gene by the common codon 13 mutation seen in many leukemias was not associated with change in disease status.

DISCUSSION

A patient with CML whose disease went into myeloid blast crisis and then returned to chronic phase with therapy permitted us to analyze sequential molecular alterations during different phases of disease. The coding sequence of the p53 gene was normal during the initial chronic phase of disease, but a mutation in the phylogenetically conserved region of the gene developed concomitantly with the evolution to myeloid blast crisis. With reinduction of the chronic phase, only structurally normal p53 gene sequences were again observed. The patient’s disease accelerated again after a few months, but the p53 gene structure remained normal. However, the percentage of blasts during this phase was only 14%, which is below the sensitivity of the sequencing analysis for detecting a point mutation. Therefore, we cannot definitely say that the first blast crisis clone did not recur a second time.

The p53 gene is located on the short arm of chromosome 17. The protein product of this gene was first identified as a consequence of its ability to bind to the transforming proteins of certain DNA tumor viruses, and it was soon observed that expression of this gene was often altered in malignant cell lines. When we examined human tumors for changes in p53, we first observed a consistent abnormality of this gene in human osteosarcomas. Subsequently, abnormalities of p53 were found in a wide variety of human tumors including colon, lung and breast cancers, and other sarcomas.

The weight of current evidence suggests that the p53 gene is an anti-oncogene. When the gene is deleted, disrupted by a molecular rearrangement, or altered by a mutation in its coding sequence, the result is a loss of its normal tumor-suppressive function. Thus, changes in the gene have generally been associated with progression rather than initiation of tumors. In 1989 we reported that deletions and rearrangements of the p53 gene were frequent in the blast crisis of CML, but were rare during the chronic phase and suggested that acquired changes in p53 were responsible for leukemic progression. However, this thesis could not be proven without a detailed analysis of the timing of molecular alterations relative to clinical events. Consequently, we undertook the present study.

Our current observations on a carefully studied patient indicate that the transition from chronic phase to blast crisis and a return to chronic phase of CML was accompanied by first a gain and then a loss of a clone of cells containing a structural abnormality of the p53 gene. The chromosomal alterations that accompanied these changes in molecular structure and clinical status included a deletion of 17p. Because the p53 gene is located on the short arm of chromosome 17, we infer that a chromosomal event resulted in loss of 17p sequences with retention of a single mutant allele of p53 in a clone of cells already carrying the Ph chromosome. A mutation in p53 coding sequences could have preceded or followed the loss of the normal allele with the result that only mutant p53 protein was produced by the new clone of cells. This circumstance is known to be associated with cellular immortalization in other biologic systems.

The mutation of the p53 gene, which we observed in our case, was a mis-sense mutation in the single allele of the p53 gene, which would have altered the protein in a region known to be a “hot spot” for mutations. Mutated p53 proteins fail to function normally and may even bind the normal p53 protein when one normal allele is retained by a tumor. Almost all of the p53 mutations thus far have been of this mis-sense variety, although we did find one CML blast crisis cell line in which a mutation was not in the coding sequence but resulted in defective processing of mRNA.

We interpret our observations on this case, as well as previous studies, as indicating that p53 gene alterations accompany and are probably responsible for the evolution of many cases of myeloid blast crisis.
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