RAPID COMMUNICATION

Genomic Instability of Microsatellite Repeats and Its Association With the Evolution of Chronic Myelogenous Leukemia

By Chieki Wada, Shigeru Shionoya, Yumi Fujino, Hideo Tokuhiro, Tohru Akahoshi, Toyoaki Uchida, and Hideki Ohtani

Tumorigenesis has been shown to proceed through a series of genetic alterations involving protooncogenes and tumor-suppressor genes. Investigation of genomic instability of microsatellites has indicated a new mechanism for human carcinogenesis in hereditary nonpolyposis colorectal cancer and sporadic cancer and this instability has been shown to be related to inherited predisposition to cancer. This study was conducted to determine whether such microsatellite instability is associated with the evolution of chronic myelogenous leukemia (CML) to the blast crisis. Nineteen CML patients clinically progressing from the chronic phase to accelerated phase or blast crisis and 20 other patients in the CML chronic phase were studied. By polymerase chain reaction assay, DNAs for genomic instability in five separate microsatellites in chromosome arms 5q (Mfd27), 17p (Mfd41), 18q (DCC), 3p (C13-9), and 8p (LPL) were examined. Differences in unrelated microsatellites of chronic and blastic phase DNAs in 14 of 19 patients (73.7%) were demonstrated. Somatic instability in five microsatellites, Mfd27, Mfd41, DCC, C13-9, and LPL, was detected in 2 of 19 (10.5%), 8 of 19 (42.1%), 11 of 19 (57.9%), 4 of 17 (23.5%), and 4 of 17 (23.5%)

CHRONIC MYELOGENOUS leukemia (CML) is a clonal myeloproliferative disorder of primitive pluripotential stem cells and involves all hematopoietic lineages, the myeloid, erythroid, megakaryocyte, B-lymphoid, and sometimes T-lymphoid elements, but not skin fibroblasts or bone marrow stromal cells. CML almost always evolves from a chronic, relatively indolent disease to a more aggressive leukemia whose progressive stages are the accelerated phase and blast crisis characterized by increased cellular proliferation, maturation arrest, and karyotypic clonal evolution. CML cells show a characteristic cytogenetic abnormality, t(9;22)(q34;q11), which has been termed Philadelphia (PH) chromosome, and molecular alteration in the bcr-ab1 fusion gene. Although activation of bcr-ab1 tyrosine kinase may be essential to the pathogenesis of the chronic phase of CML, genetic alterations responsible for transition to the blast crisis are unknown. Other nonrandom cytogenetic changes as well as occasional activation of protooncogenes and alterations in tumor-suppressor genes such as the p53 gene in the evolution of some cases of blast crisis have been observed. Thus, it is possible that genomic instability and spontaneous error in DNA replication may significantly contribute to the evolution of CML to blast crisis. For confirmation of this point, examination was made of DNAs from 19 cases of blast crisis and the accelerated phase from the chronic phase for genomic instability in five separated microsatellites, Mfd27, Mfd41, DCC, C13-9, and LPL, was detected in 2 of 19 (10.5%), 8 of 19 (42.1%), 11 of 19 (57.9%), 4 of 17 (23.5%), and 4 of 17 (23.5%)

From the Departments of Clinical Pathology, Hematology, Internal Medicine and Urology, School of Medicine, Kitasato University, Sagamihara, Kanagawa, Japan. The diagnosis of CML was based on standard clinical and hematologic criteria. In the blast crisis or accelerated phase samples, markers such as myeloperoxidase, Sudan black, periodic acid Schiff reagent, nonspecific esterase, acid phosphatase, activity of terminal deoxynucleotidyltransferase, and lineage-specific monoclonal antibodies were analyzed. Buccal mucosa and peripheral blood samples of 20 other CML patients in lution of CML to the blast crisis. For confirmation of this point, examination was made of DNAs from 19 cases of blast crisis and the accelerated phase from the chronic phase for genomic instability in five separated microsatellites, Mfd27, Mfd41, DCC gene (deleted in colorectal carcinoma), C13-9, and LPL (lipoprotein lipase). Alterations in microsatellites and replication errors (RER ) consisted in frequent molecular abnormalities observed in the progress of CML.

MATERIALS AND METHODS

Clinical samples. Bone marrow or peripheral blood samples with CML were obtained from the Department of Hematology, Kita- sato University Hospital (Kanagawa, Japan). The diagnosis of CML was based on standard clinical and hematologic criteria. In the blast crisis or accelerated phase samples, markers such as myeloperoxidase, Sudan black, periodic acid Schiff reagent, nonspecific esterase, acid phosphatase, activity of terminal deoxynucleotidyltransferase, and lineage-specific monoclonal antibodies were analyzed. Buccal mucosa and peripheral blood samples of 20 other CML patients in


© 1994 by The American Society of Hematology.

0006-4971/94/8312-0045$3.00/0
the chronic phase were obtained after obtaining informed consent. Peripheral blood samples from five normal volunteers were also examined.

**Cyto genetic analysis.** Bone marrow or peripheral blood was cultured for 24 hours and processed for cytogenetic analysis using standard techniques.

**Isolation of DNA.** DNAs were isolated from Giemsa-stained slides. DNAs at the chronic phase and blast crisis as well as the accelerated phase in the same patient were prepared. Cells stripped off slides with disposable scalpels and cells from the buffy coat and EDTA). DNA samples were quantitated spectrophotometrically. Accelerated phase in the same patient were prepared. Cells stripped off slides with disposable scalpels and cells from the buffy coat and EDTA). DNA samples were quantitated spectrophotometrically.

**Oligonucleotide primers.** Primer pairs of five microsatellites, Mfd27, Mfd41, DCC, CI3-9, and LPL, were synthesized with an Applied Biosystems Model 392 DNA/RNA synthesizer (Applied Biosystems, Foster City, CA). Sequences of the primers are shown in Table 1. Chromosomal loci of the genes were arms 5q, 17p, 18q, 3p, and 8p, respectively.‡ Primers amplify the repeat sequence in an intron downstream of exon P in the DCC gene.

**Table 1. Oligonucleotide Primers of Microsatellites**

| Marker | Chromosome Location | Length* of Amplified 
DNA (bp) | Repeat Sequence | Nucleotide Sequence of Primers |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mfd27</td>
<td>5q (D5S107)</td>
<td>145</td>
<td>(CA)₅(AA)(CA)₇(GT)₅</td>
<td>5'&gt;GATCCACCAACTAACCACAAATC&lt;3'</td>
</tr>
<tr>
<td>Mfd41</td>
<td>17p12-p11.1 (D17S281)</td>
<td>159</td>
<td>(AC)₁₇</td>
<td>5'&gt;GGCATCTGCTAACCACAT&lt;3'</td>
</tr>
<tr>
<td>DCC</td>
<td>18q21</td>
<td>215</td>
<td>(TA)₉(N)₂(AT)₉</td>
<td>5'&gt;TTCTGGAAACCTACTGTCTA&lt;3'</td>
</tr>
<tr>
<td>CI3-9</td>
<td>3p21.3 (D3S643)</td>
<td>113</td>
<td>(AC)₁₅</td>
<td>5'&gt;GACGAAACTCAGCAACACTCC&lt;3'</td>
</tr>
<tr>
<td>LPL</td>
<td>8p22</td>
<td>116</td>
<td>(CA)₅(GT)₅</td>
<td>5'&gt;TATGGTCCTAAAGGTAACAC&lt;3'</td>
</tr>
</tbody>
</table>

**Abbreviation:** Mfd, Marshfield.

§ Sizes of amplified DNA fragments corresponded to the predominant allele.

† Primers amplify the repeat sequence in an intron downstream of exon P in the DCC gene.

**RESULTS**

**CML samples.** Nineteen cases of CML in the chronic phase and blast crisis or accelerated phase were studied. Phase, phenotype, karyotype, and leukemic blast (as a percentage) are specified in Table 2. The 19 cases consisted of 11 myeloid, 4 lymphoid, and 1 mixed phenotype of blast crisis and 3 accelerated phases with the myeloid phenotype.

There were 17 Ph¹-positive CMLs and 2 cases were Ph¹ negative in cytogenetic analysis at the chronic phase. Additional chromosomal changes at the blastic phase were noted in 18 patients. Isochromosome 17q [i(17q)] was detected in four blastic phases and t(17;22)(q23;q13) was seen in case no. 10 with a myeloid blast crisis. No cytogenetic abnormality in chromosomes 5, 18, 3, or 8 could be detected in the acute phase. Case no. 11 presented an extramedullary blast crisis with myeloid phenotype and DNA was isolated from the tumor. Duration of the chronic phase and survival time after the blast crisis are also indicated in Table 2. Patient with histories of CML showed no evidence of previous colorectal or HNPCC-associated cancer. No patient had a family history of cancer syndrome.

**Microsatellite instability.** Using the PCR-based assay, differences between chronic and blast phase DNA banding patterns for Mfd27, Mfd41, DCC, CI3-9, and LPL were identified.

The results of PCR amplification with Mfd27 primers are given in Fig 1. Normal alleles in five volunteer DNAs (nos. 1 through 5) were represented by one or two major bands. Some blastic phases had band patterns differing from those of chronic phases. Bands smaller (>2 bp) than those of the chronic phase were found in case no. 13 with the lymphoid blast crisis. Bands with a few basepair deletions (2 bp, minor change) were present in case no. 15 (lymphoid blast crisis).

Alteration in the Mfd27 microsatellite was seen in only two cases with the lymphoid blast crisis. No Mfd27 alteration
Table 2. Phenotype and Karyotype of Blastic Phase

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Age/Sex</th>
<th>Phenotype</th>
<th>Blast (%)</th>
<th>Additional Chromosomal Abnormalities</th>
<th>Chronic Duration (mo)</th>
<th>Survival (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chronic Phase</td>
<td>Blastic Phase</td>
<td>Ph'</td>
<td>15</td>
</tr>
<tr>
<td>Blast crisis</td>
<td>1</td>
<td>41/M</td>
<td>Myeloid</td>
<td>6.3</td>
<td>46.8</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>31/M</td>
<td>Myeloid</td>
<td>11.6</td>
<td>31.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>38/F</td>
<td>Myeloid</td>
<td>1.4</td>
<td>38.4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>45/M</td>
<td>Myeloid</td>
<td>16.8</td>
<td>71.6</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>47/F</td>
<td>Myeloid</td>
<td>8.8</td>
<td>40.2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>53/M</td>
<td>Myeloid</td>
<td>0.0</td>
<td>26.4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>22/F</td>
<td>Myeloid</td>
<td>4.2</td>
<td>81.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>42/F</td>
<td>Myeloid</td>
<td>4.2</td>
<td>78.3</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>70/M</td>
<td>Myeloid</td>
<td>2.4</td>
<td>40.2</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>50/M</td>
<td>Myeloid</td>
<td>10.6</td>
<td>50.8</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>38/M</td>
<td>Myeloid</td>
<td>0.0</td>
<td>0.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>76/M</td>
<td>Lymphoid</td>
<td>3.4</td>
<td>77.1</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>76/M</td>
<td>Lymphoid</td>
<td>2.4</td>
<td>61.5</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>48/M</td>
<td>Lymphoid</td>
<td>4.0</td>
<td>82.4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>37/F</td>
<td>Lymphoid</td>
<td>0.0</td>
<td>84.0</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>42/M</td>
<td>Lymphoid</td>
<td>5.0</td>
<td>90.5</td>
<td>+</td>
</tr>
<tr>
<td>Accelerated phase</td>
<td>17</td>
<td>38/M</td>
<td>Myeloid</td>
<td>0.6</td>
<td>5.4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>54/M</td>
<td>Myeloid</td>
<td>2.0</td>
<td>20.4</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>35/M</td>
<td>Myeloid</td>
<td>3.6</td>
<td>9.4</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviation: NE, not examined.

* Blast percentage in DNA isolated slides.
† Survival months after diagnosis of blastic phase.

was detected in other blastic phases, such as myeloid or mixed phenotypes. All results for the Mfd27 microsatellite were entirely reproducible in replicate assays. Results for assessment of microsatellite instability at Mfd27 are summarized in Table 3.

Figure 2 shows PCR with Mfd41 primers at the chronic phase and blast crisis. Different band patterns were noted for the Mfd41 microsatellite in cases no. 2, 4, 5, 6, 7, 9, 10 (myeloid blast crisis), and 18 (accelerated). Patterns corresponding to the blast crisis were those of a few smaller nucleotides bands in most cases. Alterations were caused by deletions of a few basepairs in the main repeated sequences. In case no. 5, loss of a larger allele and faint expanded bands were seen in the myeloid blast crisis. Expanded bands showing ladder patterns of larger and smaller alleles were observed in case no. 7. In case no. 18, a few base expansions were noted in the accelerated phase. Microsatellites of chronic and blastic phases were the same in cases no. 1 (myeloid), 13, 14 (lymphoid), and 17 (accelerated), as shown in Fig 2. In the blast crisis in cases no. 3, 8, 11, 12, 15, 17, and 19, the Mfd41 microsatellite manifested no instability (data not shown).
Table 3. Microsatellite Instability (RER+) in the CML Evolution

<table>
<thead>
<tr>
<th>No.</th>
<th>Microsatellite Instability</th>
<th>Frequency With Instability</th>
<th>RER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mfd27</td>
<td>Mfd41</td>
<td>DCC</td>
</tr>
<tr>
<td>1M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2/19</td>
<td>8/19</td>
<td>11/19</td>
</tr>
</tbody>
</table>

(10.5%) (42.1%) (57.9%) (23.5%) (23.5%) (52.6%)

Fig 3 shows microsatellite instability of the DCC gene containing simple repeated sequences. Alterations in band size during evolution to the blast crisis and accelerated phase from the chronic phase were noted. Alterations in the microsatellite of the DCC gene showed considerable variation and unusual patterns of genetic instability. Various abnormal band patterns not explainable as caused by loss of heterozygosity (LOH), such as nucleotide deletions and insertions and differing from those in the chronic phase, were observed in the blastic phase in cases no. 2, 3, 4, 5, 6, 7, 11, 12, 15, 16, and 18. The most frequent difference in band mobility was the number of deletions, which was approximately 2 bp to 30 bp in the blastic phase in cases no. 2, 4, 5, 6, 11, 12, 15, and 18. In cases no. 2, 4, 12, 15, and 18, newly deleted bands appeared in the blast crisis. Deleted and faint expanded bands appeared in the blast crisis in case no. 15. Expanded and deleted alleles were observed in the accelerated phase of case no. 18. A new larger allele appeared in case no. 16. Faint deleted bands were noted in the blast crisis of cases no. 3 (data not shown) and 12. No change in banding patterns for chronic and blastic phase DNAs could be seen in cases no. 1, 8, 10, 14, 17, and 19 (data not shown).

In cases no. 7, 9, 11, and 13, there was loss of one major allele in the blast crisis, possibly indicating LOH in the...
region of the DCC microsatellite. LOH patterns could be clearly seen in cases no. 9 and 13. Alterations in the DCC microsatellite in cases no. 7 and 11 provided an indication as to the relationship between instability and LOH. In these cases, microsatellite instability and LOH in the DCC microsatellite were complicated in nature. In case no. 11, differences in mobility accompanied by a number of nucleotide deletions after loss of an allele were evident. Alterations of the blast crisis in case no. 7 were of particular interest in that LOH was observed in one smaller allele and expansion was observed in another.

Alterations in the CI3-9 microsatellite were noted in cases no. 4M, 10M, 13L, and 15L. Instability at the LPL locus was found in cases no. 2M, 4M, 12L, and 13L. Figure 4 shows the genomic instability at multiple microsatellites in cases no. 4M and 13L. These blast crises were evaluated as typical RER+ phenotypes.

Alterations in the microsatellites were completely reproducible in replication or triplicate assay. To rule out the possibility of contamination or sample switching, DNAs were isolated again from slides at identical periods of chronic and blastic phases initially studied, followed by another PCR assay. The results of the second PCR from newly isolated DNAs were compared with those of the first assay. Identical alterations of the microsatellites were reproducibly detected. The results for assessment of PCR-based instability of Mfd27, Mfd41, DCC, CI3-9, and LPL are summarized in Table 3. Based on these results, alterations in Mfd27, Mfd41, DCC, CI3-9, and LPL for the chronic and blastic phases were detected in 2 of 19 (10.5%), 8 of 19 (42.1%), 11 of 19 (57.9%), 4 of 17 (23.5%), and 4 of 17 (23.5%) cases. Blastic phases with genetic instability in one or two microsatellites were noted in 14 of 19 cases (73.7%). Ten of 19 blastic phases (52.6%) had instabilities in at least two of five microsatellites and were categorized as RER+. Four of 19 cases (21.1%) had instability at one locus and five blastic phases (26.3%) without microsatellite instability could be evaluated as RER-. There was no case of blastic phase with mutations in all five microsatellites. In the DCC microsatellite, alterations, including instability and LOH, were observed in 13 of 19 cases (68.4%): instability in 9 cases, instability plus LOH in 2 cases, and only LOH in 2 cases.

An attempt was made to determine the association of microsatellite alteration (RER+) with age, sex, phenotype, blasts (as a percentage) in DNA preparation samples in chronic and blastic phases, Ph' chromosome in the chronic phase, cytogenetic abnormalities in the blastic phase, time to proceed from the chronic phase to blast crisis or accelerated phase, and survival time after diagnostic confirmation of the blastic phase. Alterations in Mfd27 were restricted to the lymphoid blast crisis and Mfd41 in the myeloid blastic phase. All phenotypes of the blast crisis and accelerated
phase showed altered microsatellite instability in the DCC, C13-9, and LPL. Patients without Ph1 chromosome in the chronic phase or additional chromosomal abnormalities in the blastic phase had one or two altered microsatellites. In four of five blast crises in which isochromosome 17q or t(17;22) was present, Mfd41 instability was observed. In four of eight myeloid blast crises with Mfd41 instability, chromosome 17 was not abnormal according to cytogenetic analysis. No significant association of microsatellite alteration (RER+) with age, sex, patient survival, duration of the chronic phase, or blasts (as a percentage) in chronic or blastic phases could be detected.

In the DCC microsatellite assay (Fig 3), abnormal bands were observed in chronic phase DNAs in cases no. 4, 5, and 6. Banding patterns appeared to be similar to those of LOH in the chronic phase in cases no. 2, 4, 15, and 16. Alterations at the DCC microsatellite could still be seen in the chronic phase in CML. In these cases, chronic phase DNAs were examined several times during CML. The same major allele was detected throughout all chronic phases. One major allele may be thus caused by homozygosity but not by LOH. Normal DNAs of CML patients were not obtained and LOH could thus not be confirmed in the chronic phase. PCR assay of the five microsatellites was conducted on DNAs from buccal mucosa and peripheral blood of 20 other CML patients in the chronic phase.

To confirm that alteration occurs in the chronic or blastic phase, alterations in the five microsatellites in normal buccal mucosa and peripheral blood of 20 other CML patients were analyzed. No alteration of the DCC band patterns could be detected in any patient with CML chronic phase. In the patient, case no. 4, at the chronic phase (lanes 1 through 3) and the myeloid blast crisis (B), PCR products of Mfd41 were analyzed. Alteration in Mfd41 microsatellite were detected at the time of the blast crisis. Autoradiograms of sequence gels are shown.

DISCUSSION

The present study on PCR-based microsatellite instability indicates for the first time the frequent occurrence of alterations in microsatellites (RER+) in the evolution of CML. Unstable microsatellites and RER+ have been demonstrated in some cancers such as HNPCC and HNPCC-associated and sporadic cancers.8-13 RER+ may possibly be related to heritable predisposition to cancer in affected members of HNPCC families. The incidence of RER+ is high (about 80%) in HNPCC familial cancers, but is less in sporadic cancer.8-11 In sporadic cancer, a significantly high incidence of RER+ has been observed in pancreatic (66.7%) and gastric cancers (38.6%) and particularly so in poorly differentiated gastric carcinoma (64.0%).11 In other sporadic cancers, instability is less frequent (3% to 28%). The instability of microsatellites in a nonepithelial tumor has yet to be determined. The present data indicate the incidence of microsatellite instability (RER+) in CML evolution to be significantly higher (52.6%) than in sporadic cancer. Its frequent occurrence in CML evolution is indication that genetic instability (RER+) may be importantly involved in the transition from chronic to blast crisis or acute stage of CML. Microsatellite instability in CML patients suggests simple repeated sequences to possibly be stable in stem cells in the chronic phase and microsatellites alterations to possibly occur in aggressive leukemic cells in the blast crisis. In CML, altered microsatellite instability, but not familial predisposition, may be a late molecular event associated with the pathogenesis of transition to blast crisis. Genomic instability in other cancers studies shows familial predisposition to cancer and occurs before or during the early stage of tumorigenesis.8-11 The late molecular event of microsatellite instability in CML may thus
come about through a series of steps different from those of
epithelial neoplasm in leukemogenesis.

The recessive model of tumor-suppressor gene indicates
two genetic events to be essential for producing a tumor
phenotype.21-23 Allelic loss and point mutations of remaining
alleles in tumor suppressor genes have been frequently ob-
served in various cancers. The present data on alterations in
the DCC microsatellite are of particular interest. Two
independent mechanisms for alterations of the DCC micro-
satellite in CML evolution, followed by LOH and instability,
are shown here for the first time. These alterations caused
by instability and LOH are the most frequently noted in the
CML blast crisis. DCC is a candidate tumor-suppressor gene
located on chromosome band 18q21, and loss of one DCC
allele or decreased DCC expression has been reported in
approximately 88% of colorectal cancers.24,25 DCC encodes
a cell surface glycoprotein with homology to the neuronal
cell adhesion molecule (N-CAM) family. Inactivation of the
DCC gene in hematologic malignancy has been little stud-
ied.24 Microsatellite alteration at an intron of the DCC gene
with high frequency may lead to qualitative or quantitative
alteration in this gene and its products. DCC product alter-
ations may lessen the adherence of CML cells to the stromal
matrix and thus prevent hematopoietic cell interactions with
consequent arrest of differentiation in CML. Intercellular
adhesion directly influences cellular differentiation.21,22,25 In-
activation of the DCC gene with microsatellite alteration
may be involved in some way with the evolution of CML.

On the average, there is one microsatellite per 100,000 bp
throughout the human genome and length polymorphism is
useful for linkage analysis.26,27 From the present results on
frequent alterations in multiple microsatellites at different
chromosomal sites, a great many microsatellites may be al-
tered in CML evolution. Genomic instability may possibly
be caused by an error during replication or repair and may
be correlated with tumorigenesis in HNPCC and other can-
cers.8,13,28,29 The human MSH2 gene has recently been cloned
and was found to be located on the HNPCC locus in chromo-
some 2p22-21.30 Mutations of hMSH2 gene, a candidate
HNPCC gene, cause instability of dinucleotide repeats and
have been detected in HNPCC kindreds and sporadic colo-
rectal cancers with microsatellite instability.30 Although the
defect in the replication/repair machinery of leukemic cells
has yet to be confirmed, mutations of the hMSH2 gene or
other genes in an hMSH2-dependent mismatch repair path-
way may cause highly frequent alterations of microsatellites
in CML evolution. The p53 gene whose mutations have
been detected in some myeloid blast crises may function to
maintain inherent genome mutability and control DNA repair
and cell cycle.6,7,21-25 Loss of the molecular functions that
control DNA replication/repair may increase the frequency
of accumulation of spontaneous mutations, leading to chro-
mosome loss and rearrangement in cancer.6,13,28,30 Loss of
fidelity in replication/repair machinery as indirectly indi-
cated here by microsatellite instability (RER+) may involve
additional chromosomal abnormalities that almost always
occur in the blast crisis and accelerated phase. Mutator genes,
such as the hMSH2 gene, that control DNA replication/repair
in leukemogenesis should be studied in greater detail to clar-
ify the molecular mechanism of CML evolution.

Alterations in microsatellites at multiple loci (RER+) may
be concluded because of the loss of fidelity in DNA
replication/repair during evolution from the chronic phase
to blast crisis. The PCR-based microsatellite instability assay
offers improved sensitivity and speed for diagnosing the
blast crisis as compared with standard cytogenetic analysis
and Southern blot hybridization. PCR-microsatellite assay
will become automated in the near future using DNA se-
quencer and microsatellite analysis software. PCR assay may
be a useful molecular means for diagnosing the blast crisis
in CML and useful for clinically predicting the emergence
of the second lethal phase of CML as well as for indicating
the most appropriate time for therapeutic intervention in the
blast crisis.

ACKNOWLEDGMENT

We are indebted to Y. Ohnuki, Y. Toyo-oka, M. Taira, and Dr
X.W. Guo for their technical assistance.

REFERENCES

1. Sawyers CL, Denny CT, Witte ON: Leukemia and the disrup-

M: Chronic myelogenous leukemia: A concise update. Blood 82:691,
1993

3. Rowley JD: A new consistent chromosomal abnormality in
chronic myelogenous leukemia identified by quinacrine fluores-

319:990, 1988

5. Liu E, Hjelle B, Bishop JM: Transforming genes in chronic

6. Ahuja H, Bar-Eli M, Advani SH, Benchimol S, Cline MJ:
Alterations in the p53 gene and the clonal evolution of the blast
crisis of chronic myelocytic leukemia. Proc Natl Acad Sci USA
86:6783, 1989

J, Snyder D, Foti A, Cline M: The spectrum of molecular alterations
in the evolution of chronic myelocytic leukemia. J Clin Invest
87:2042, 1991

8. Aaltonen LA, Peltomaki P, Leach FS, Sistonen P, Pylkkanen
GM, Kinzler KW, Vogelstein B, de la Chapelle A: Clues to the

9. Thiibodeau SN, Bren G, Schaid D: Microsatellite instability in

10. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Peruchio M:
Ubiquitous somatic mutations in simple repeated sequences reveal a

Genetic instability in pancreatic cancer and poorly differentiated

12. Risinger JI, Berchuck A, Kohler MF, Watson P, Lynch HT,
Boyd J: Genetic instability of microsatellites in endometrial carci-

13. Gonzalez-Zulueta M, Ruppert JM, Tokino K, Tsai YC,
Spruck CJH III, Miyao N, Nichols PW, Hermann GG, Horn T, Steven
K, Summerhayes JC, Sidransky D, Jones PA: Microsatellite instabil-

merizations at the DSS107, DSS108, DSS111, DSS117 and DSS118
Genomic instability of microsatellite repeats and its association with the evolution of chronic myelogenous leukemia [see comments]

C Wada, S Shionoya, Y Fujino, H Tokuiro, T Akahoshi, T Uchida and H Ohtani