TP53 Mutations Emerge at Early Phase of Myelodysplastic Syndrome and Are Associated With Complex Chromosomal Abnormalities

By Hiroto Kaneko, Shinichi Misawa, Shigeo Horike, Hiroyuki Nakai, and Kei Kashima

We examined TP53 mutation in 57 patients with myelodysplastic syndrome (MDS) at either the MDS phase or at the terminal leukemic phase using polymerase chain reaction-mediated single-strand conformation polymorphism (PCR-SSCP) analysis. TP53 mutations within exons 5 through 8 were found in seven patients. All these mutations were detected at the presentation of MDS whether these patients showed leukemic transformation or not. TP53 mutations were frequently found in patients with loss of the short arm of chromosome 17 (17p-) (three of seven patients with 17p-, 43%) and complex karyotypic abnormalities (five of 14, 38%). Among the seven patients with the TP53 mutation, four patients progressed to acute leukemia within 7 months from the diagnosis of MDS, and the remaining three died within 7 months without leukemic transformation. These findings suggest that mutations of the TP53 can be implicated in leukemic transformation and a poor prognosis in MDS.

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MATERIALS AND METHODS

Patients. Bone marrow samples were collected from 57 patients with MDS after obtaining informed consent. The diagnosis of MDS and its typing were made according to the French-American-British (FAB) morphologic criteria.14 Alterations of TP53 are observed in several classes of hematologic malignancies; acute myeloid leukemia,2 myelodysplastic syndrome (MDS),3 and chronic myelogenous leukemia (CML) at blast crisis,15 although the frequency is relatively low. In MDS, Jouveaux et al16 detected TP53 mutation in five of 151 patients, and Sugimoto et al17 found three cases among 44 patients in MDS phase, but none in six samples of leukemic phase. Thus, the alteration of the TP53 seems to be partly responsible for the development of MDS, but not for the leukemic transformation.

A correlation between TP53 mutation and abnormality of chromosome 17 was reported by several investigators.6,9,10,11 They showed that patients with loss of the short arm of chromosome 17 (17p-) had TP53 mutations more frequently than those with normal chromosome 17 homologue. However, it was also reported that those patients who showed 17p- as the sole abnormality seldom had a TP53 mutation.10,11 Furthermore, a complex chromosome abnormality is closely related to a genomic instability caused by TP53 inactivation.4

In this study, we examined exons 5 to 8 of the TP53 in 57 patients with MDS because the mutations have been reported to be accumulated in this region.18 The most recent samples available were first screened using polymerase chain reaction-mediated single-strand conformation polymorphism (PCR-SSCP) analysis,13 and the TP53 mutations were confirmed by direct sequencing. In cases where a TP53 mutation was detected, we examined earlier samples whenever possible. Furthermore, because we examined the TP53 mutation and the karyotypes in the same materials, we determined if there was a correlation between TP53 mutations and chromosomal findings.

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Table 1. Clinical Phase of MDS and TP53 Mutation

<table>
<thead>
<tr>
<th>Phase of MDS Examined</th>
<th>No. of Cases Examined</th>
<th>No. of Cases With TP53 Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS phase only</td>
<td>41</td>
<td>3</td>
</tr>
<tr>
<td>Leukemia and MDS phases</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Leukemia phase only</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>7</td>
</tr>
</tbody>
</table>

(12%) (Fig 1). Three patients had RAEB (cases 1 through 3), two of whom were examined at presentation (cases 1 and 2). Four patients had transformed to acute leukemia (cases 4 through 7), and the interval from the initial diagnosis of MDS to the leukemic change was 1.5, 2.5, 4, and 7 months, respectively (Table 2). When we retrospectively analyzed DNA samples obtained at diagnosis of MDS, aberrant bands were also seen at equal migration in each patient (Fig 1).

All of the abnormalities were identified by direct sequencing. All were missense point mutations. As we summarize in Table 2, a single bp substitution was detected in each patient. Five of these seven mutations were either A to G, G to A, or C to T transitions, and the remaining two were A to T or G to T transversions. In case 4, the point mutation at codon 136 (CAA → TAA) generates a stop codon that produces a 258 amino acids shorter TP53 protein. Direct sequencing analysis showed coexistence of normal sequence bands, especially in cases 2 and 3, which may represent a residual normal allele or contamination by normal cells (Fig 2).

**TP53 mutation and chromosome findings.** Thirty-six of 57 patients (63%) had an abnormal karyotype, including seven who showed a 17p- (Table 3). Among karyotypically abnormal cases, 14 had a complex karyotype containing three or more aberrations, and the remaining 22 showed a relatively simple abnormal karyotype with less than three aberrations. Mutations of TP53 were detected more frequently in patients with a complex karyotype (five of 14, 36%) than those with a simple abnormal karyotype or a normal karyotype (two of 43, 4.6%) (P = .009 by χ² test with Yates’ continuity correction). With respect to loss of a 17p, three of seven patients (43%) with a 17p- showed a TP53 mutation, in contrast to four of 50 patients (8%) with a normal pair of chromosome 17 (P = .044; Table 3). Furthermore, a TP53 mutation was not detected in the two patients showing a 17p- as a sole chromosome abnormality.

**DISCUSSION**

We detected TP53 mutations in seven of 57 patients with MDS (12%). This incidence is rather high when compared...
Table 2. TP53 Mutation, Clinical Outcome, and Karyotypes of Seven Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>FAB*</th>
<th>TP53 Mutation</th>
<th>Transformation†</th>
<th>Survival*</th>
<th>Karyotype at the Latest Examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55/M</td>
<td>RAEB</td>
<td>Codon 141 TGC → TAC (Cys) (Tyr)</td>
<td>–</td>
<td>7 mo</td>
<td>45,XY, –4,del(5)(q11q33), +del(7)(p14), +8, +10, –13, –16, –18,10/46,idem, +mar8</td>
</tr>
<tr>
<td>3</td>
<td>81/M</td>
<td>RAEB</td>
<td>Codon 205 TAT → TGT (Tyr) (Cys)</td>
<td>–</td>
<td>1.5 mo</td>
<td>47,XY,der(14)t(1;14)(p13;p12)[2]/44,idem,+mar[4]/44,idem,+r[8]/46,XY[1]</td>
</tr>
<tr>
<td>4</td>
<td>76/M</td>
<td>RAEB → M4</td>
<td>Codon 136 CAA → TAA (Glu) (Stop)</td>
<td>1.5 mo</td>
<td>14 mo+</td>
<td>44,XY,del(5)(q22q35), dic(7;12)(q11;11p11),add(11)(q11);p11;q22), –13, –15,add(17)(p13)[9]/46,XY[2]</td>
</tr>
<tr>
<td>5</td>
<td>31/F</td>
<td>RA → M6</td>
<td>Codon 277 TGT → TTT (Cys) (Phe)</td>
<td>2.5 mo</td>
<td>17 mo</td>
<td>45,XX,del(5)(q22;35), –13(6)[46,XX[3]</td>
</tr>
<tr>
<td>7</td>
<td>76/M</td>
<td>RAEB-T → M2</td>
<td>Codon 193 CAT → CTT (His) (Leu)</td>
<td>7 mo</td>
<td>7 mo+</td>
<td>45,XY,add(5)(q11), –18,add(19)(p13)[10]</td>
</tr>
</tbody>
</table>

* Type of MDS at diagnosis and type of leukemia in cases of transformation to acute leukemia.
† Interval between diagnosis of MDS and leukemic transformation.
* + indicates that the patient is alive.

with previous reports.5,7 This may reflect a high incidence of a 17p- in our series (7 of 57, 12%) because it was reported to be seen in approximately 7% of MDS patients11,19 and a TP53 mutation is correlated with abnormality of chromosome 17 as we and other investigators have described.5,9,20 Actually, a TP53 mutation can be detected more frequently in patients with a 17p- (43%) than in those with a normal pair of chromosome 17 (8%) in our series. Among seven TP53 mutations observed in our series, 5 (cases 1, 2, 4, 5, and 6) affected the nucleotide within the four hot-spot regions correlated with evolutionarily conserved domains that are considered to be functionally important.12 Moreover, all seven mutations affected the same codon of TP53 reported to alter in various human malignancies, including 5 (cases

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Fig 2. Sequencing analysis of 7 patients. The left half of each panel shows the normal sequence and the right half the sequence of a patient. A normal allele is simultaneously observed in cases 2 and 3. Nucleotide substitutions of all cases are summarized in Table 2.

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cases). Furthermore, among 3 patients who showed a CML.* In contrast to this hypothesis, our findings suggest a mutation in the MDS phase, the same mutations detected in the terminal stage had not been conducted.13

Other intriguing questions are when the TP53 is mutated during the course of MDS and how these mutations are involved in the leukemic change. A higher incidence of mutated TP53 in the transformed leukemia phase (four of 16, 25%) appears to reflect some role of TP53 inactivation in the progression to overt leukemia. However, the longitudinal analysis provided the important finding that, without exceptions, the same mutations detected in the terminal stage had already occurred at the presentation of MDS (four of four cases). Furthermore, among 3 patients who showed a TP53 mutation in the MDS phase, 2 patients were examined at the time of diagnosis of MDS. Alteration of the TP53 has been reported to be a late genetic event in the carcinogenesis model established in colorectal cancer21 or in blast crisis of CML.26 In contrast to this hypothesis, our findings suggest a different role of TP53 mutation in MDS. Alternatively, MDS patients who represent a TP53 mutation are already in an advanced stage of MDS, regardless of leukemic manifestation. Previous investigations on the TP53 mutation in MDS failed to demonstrate this fact because the longitudinal analysis had not been conducted.5,7

Our findings have a prognostic implication; in this study MDS patients with an altered TP53 frequently progressed to overt leukemia (4 of 7, 57%) within 7 months from the initial diagnosis of MDS. In the remaining, the three patients were deceased within 7 months. Totally, a median leukemia-free survival of patients with TP53 mutation was 4 months, and this was significantly shorter than that of patients without the mutation (median 72 months, \(P < .01\) by generalized Wilcoxon test). We speculate that this difference reflects the fact that some of the patients with TP53 mutation died of other causes before eventual leukemic transformation. Jonveaux et al6 described that 3 of 5 patients with a TP53 mutation progressed to acute leukemia and their prognosis was poor. Taken together, these findings suggest that when the TP53 mutation has emerged at the presentation of MDS, these patients represent a group with a poor prognosis and a high risk of leukemic change. In other words, leukemic transformation is partly predictable by analyzing the TP53 at the diagnosis of MDS. In those patients with a TP53 mutation, a careful follow-up seems to be necessary because of the risk of early leukemic change. More intensive treatment may have to be considered for these patients.

Consequently, TP53 alteration appears to play a certain role in the progression to overt leukemia and contributes to a poor prognosis in some patients with MDS. Lane4 describes that normal TP53 monitors the integrity of the genome and that tumor cells with inactive TP53 cannot carry out a cell-cycle arrest at G1 if DNA is damaged. He speculated that cells with TP53 mutations become genetically less stable and accumulate mutations and chromosomal rearrangements at an increasing rate. However, only 25% of patients (4 of 16) with leukemic transformation showed a TP53 mutation. Thus, a majority of MDS patients transform to acute leukemia without a mutation. As several genetic alterations have also been reported to contribute partly to the leukemic transformation,22-26 other mechanisms leading to the progression of MDS remain to be elucidated.

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