Loss of DCC Gene Expression Is of Prognostic Importance in Acute Myelogenous Leukemia

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ABSTRACT

Purpose: Expression of the deleted in colorectal carcinoma (DCC) gene has been found to be lost in some patients with acute myelogenous leukemia (AML). Although this finding is critical to leukemogenesis, its prognostic significance remains uncertain. To evaluate this, loss of DCC gene expression in AML patients and their prognostic significance were investigated.

Experimental Design: A group of 170 patients with AML was analyzed. DCC gene expression in AML cells was determined by a semiquantitative reverse transcriptase-PCR. Simultaneous mutation analyses of the p53, N-ras, and FLT3 genes were performed in all of the AML cells by single-strand conformation polymorphism and sequencing subsequent to PCR. The importance of loss of DCC expression was evaluated by Cox proportional analysis and the Kaplan-Meier method.

Results: Loss of DCC expression was detected in 47 patients (27.6%). The p53, N-ras, and FLT3 mutations were detected in 20 (11.7%), 42 (24.7%), and 26 (15.2%) patients, respectively. The durations of overall survival (OS) and complete remission (CR) of the 47 DCC-negative AML patients were significantly shorter than that of the 123 DCC-positive patients (P < 0.0045 and <0.0060, respectively). Univariate and multivariate analyses showed that loss of DCC expression was an unfavorable prognostic factor for both OS (P < 0.0053 and <0.0084, respectively) and CR duration (P < 0.0146 and <0.0371, respectively). The 64 DCC-positive patients with wild p53, N-ras, and FLT3 had statistically better CR attainment compared with the other 106 patients (P < 0.0001).

Conclusions: Loss of DCC gene expression was shown to be an independent prognostic factor in AML patients. Thus, loss of DCC gene expression might serve as an important molecular marker for predicting the CR duration and OS of patients with AML.

INTRODUCTION

Carcinogenesis is a multistep process in which accumulation of additional genomic alterations drives tumor progression (1, 2). Nonrandom chromosomal loss or deletion suggests that oncogenes are also involved in AML (23, 24). Mutations of the p53 gene have been observed in AML, although the incidence of these events is lower than in solid tumors (5). Mutations of the N-ras gene are also associated with the pathogenesis of AML. N-ras gene mutations are found in 20–30% of AML patients (6, 7). An internal TD of exons 11 and 12 and mutation of aspartic acid at codon 835 (Asp835) in the FLT3 gene are sometimes involved (in ~20% of AML patients; Ref. 8). The mutant N-ras and FLT3 are ligand independently phosphorylated and play an important role in the proliferation of leukemia cells (9).

The DCC gene, which is deleted in colorectal cancer, has been identified as a possible tumor suppressor gene (10). DCC gene expression has been found to be lost in some patients with acute leukemias or myelodysplastic syndromes (11, 12). Although these findings suggest that a loss of DCC expression is critical to leukemogenesis, the DCC function that may mediate tumor suppression remains unclear (13). Recently, the DCC protein was found to transduce signals, resulting in activation of caspases (14) and inhibition of cdk1 (15). These observation suggest that DCC protein product may suppress carcinogenesis.

To evaluate the role of the DCC gene in leukemias, we studied the expression of the DCC gene in AML using the RT-PCR and investigated its PS in relation to the duration of CR or the survival time. Because p53 mutations, as well as N-ras and FLT3 activation, have been detected in many AML patients and may influence the CR duration and survival time (9, 16, 17), simultaneous mutation analyses of the p53, N-ras, and FLT3 genes, as well as cytogenetic analysis, were performed for all of the AML patients. The present study is the first report about simultaneous analyses of these genes.

PATIENTS AND METHODS

Patients. After obtaining informed consent, 170 patients with AML, who were classified by the FAB criteria into types M0-M7 (M0: n = 15; M1: n = 39; M2: n = 58; M3: n = 18; M4: n = 23; M5: n = 12; M6: n = 3; M7: n = 2), were
evaluated. All these patients were untreated previously. None of them had secondary AML for another neoplasm. The 170 patients comprised 112 men and 58 women. The median age was 57 years (range, 15–79). The patients were treated at the Hospital of Nippon Medical School from 1986 through 2000. The induction regimens for AML patients comprised the same conventional chemotherapy (BHAC-DMP regimen), consisting of 170 mg/m² behenoyl cytarabine, 70 mg/m² 6-mercaptopurine, and 20 mg/m² prednisolone daily for 15 days and 25 mg/m² daunorubicin on days 1, 2, 5, 6, and 9. Fifteen cases of M0 AML were treated with the same BHAC-DMP regimen with 1.4 mg/m² vincristine on days 1 and 8 of the therapy (18). Cases aged ≥70 years were given a 70% dose of the same chemotherapy regimen. Eighteen M3 patients were treated with 45 mg/m² all-trans retinoic acid (19). After achieving CR, two courses of consolidation chemotherapy and six courses of intensification chemotherapy were administered. The duration of follow-up was 0.1–118 months (mean 20.6 months).

CR was defined as <5% blasts in normo-cellular bone marrow with normal levels of peripheral neutrophil and platelet counts. OS was calculated from the 1st day of therapy to death. CR duration for patients who achieved CR was measured from the date of CR to relapse or death. Nine patients who underwent BMT were censored at the date of BMT.

Analysis of DCC Expression by Semiquantitative RT-PCR. After obtaining informed consent, we prepared total RNA from the mononuclear cells of the bone marrow according to our standard protocols (11). DCC expression was analyzed by our standard RT-PCR protocol (11) and a semiquantitative RT-PCR protocol, as described previously (20).

Briefly, mononuclear cells of the bone marrow, which contains ≥90% leukemic cells, were obtained by Ficol-Hypaque centrifugation (Lymphoprep, Neegard, Norway). The total RNA of mononuclear cells was extracted with an RNAzol kit (Biotex Laboratories, Inc., Houston, TX) or by the CsCl method. The concentration of the RNA was determined by spectrophotometry, and semiquantitative RT-PCR was performed with slight modification. RNA (500 ng) was reverse transcribed using random 9-mer primer. Then the synthesized cDNA was subjected to PCR analysis. PCR was performed with 10 μl of cDNA reaction mixture by using 0.0125 A260 nm units of each oligonucleotide primer DCC1 and DCC2. These oligonucleotide primers are the same ones used by Fearon et al. (11). PCRs of 35 cycles for DCC and 25 cycles for β–actin were performed, consisting of 30 s at 94°C, 30 s at 55°C, and 1 min at 75°C. The RT-PCR reactions for DCC and β–actin were done in a multiplex fashion. The PCR products (10 μl) were extracted with phenol, precipitated with ethanol, and electrophoresed. The PCR products were visualized directly in ethidium bromide-stained gels and photographed (Fig. 1). The quality of the RNA and the validity of the PCR amplifications were determined by RT-PCR of β–actin. Semiquantification of the photogenic signals was performed using an MCID image analysis system (Imaging Research, Inc., St. Catherines, Ontario, Canada; Ref. 20). The entire width of the lane was analyzed with appropriate background subtraction. All bands in one photograph were analyzed together. The relative intensity was defined as the ratio of the DCC signal (233 bp) to that of β–actin (530 bp). As Fig. 1 shows, OIH-1 cells, (21) which show normal expression of the DCC gene, and KML-1 cells, (22) in which DCC expression is lost, were used to semiquantify the DCC mRNA.

A relative intensity of ≤10% for the DCC products compared with that of β–actin was defined as negative DCC (i.e., loss of DCC expression). A relative intensity of ≥11% for the DCC products compared with that of β–actin was defined as positive DCC (i.e., normal DCC expression). The semiquantitative RT-PCR was performed twice or three times for each patient.

Analysis of N-ras Gene Mutations. DNA from mononuclear cells of the bone marrow was prepared according to our standard protocols (23). Mutations of the N-ras gene were detected by our standard protocol as described previously (23, 24).

Analysis of p53 Gene Mutations. Mutations of the p53 genes were detected by our standard protocol as described previously (23).

Analysis of FLT3 Gene Mutations. The PCR reaction was performed according to the procedure of Kiyoi et al. (25) Genomic PCR for TD mutation was performed using the primers 11F (5′-GCAATTTAGTATGAAAGGCCAGC-3′) and 12R (5′-CTTTGACGATTTGACGGAACC-3′). Genomic PCR for Asp835 mutation was performed using the primers 17F (5′-CCGCCAGGAACGTGCTTG-3′) and 17R (5′-TCAGCCTCACATTTGCCC-3′; Ref. 26). The PCR mixture containing 500 ng of genomic DNA was incubated with 0.5 unit of Takara

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**Fig. 1** A, quantitation of DCC mRNA by RT-PCR assay. The total RNA of OIH-1 cells was diluted with KML-1 RNA to the concentrations indicated. The lanes represent KML-1 in OIH-1 at dilutions of KML-1 alone (1), 50:1 (2), 20:1 (3), 10:1 (4), 5:1 (5), 2:1 (6), and OIH-1 alone (7). Lane M, molecular markers; B, RT-PCR assay of AML samples. Lanes 1, OIH-1 cells; Lane 2, KML-1 cells; Lanes 3, 6, and 9, DCC-positive samples; Lanes 4, 5, and 7, DCC-negative samples.
Taq DNA polymerase (Takara, Shiga, Japan). Denaturing, annealing, and extension steps were performed at 94°C for 30 s, 56°C for 1 min (for TD), or 59°C for 1 min (for Asp835) and 72°C for 2 min, respectively, for 35 cycles, including an initial 3-min denaturation step at 94°C and a final extension step at 72°C for 10 min. The PCR products were visualized directly in ethidium bromide-stained agarose gels and photographed.

The sequences of abnormal-sized FLT3-TD products were determined according to our original protocols (27). The PCR products of abnormal size were cut out from an agarose gel and purified. The fragments were subcloned into the Eco RV site of pGEM-5Zf(+) cloning vector. The PCR products were sequenced using an ABI sequencer with dye terminators (Perkin-Elmer, Warrington, United Kingdom). All sequences were confirmed by sequencing in both orientations.

The PCR products of FLT-Asp835 were digested with Eco RV and subjected to electrophoresis on an agarose gel. If the PCR products showed undigested band, the PCR products directly sequenced on a DNA sequencer (310; Applied Biosystems).

Statistical Methods. OS was calculated from the 1st day of therapy until death. Patients who underwent BMT were excluded from the study on the date of the transplant.

The following patient characteristics before treatment were analyzed: age, sex, FAB classification, peripheral WBC count, cytogenetic findings, N-ras mutation, p53 mutation, FLT3 mutations, and DCC expression. Analysis of frequencies was performed using Fisher’s exact test for 2 x 2 tables or Pearson’s c² test for larger tables. Survival probabilities were estimated by the Kaplan-Meier method, and differences in the survival distributions between the DCC-positive and DCC-negative groups were tested with the Log-rank statistic (28). The PS of the study variables was assessed using the Cox proportional hazards model. These statistic analyses were performed with SAS ver6.12 software (SAS Institute, Inc., Cary, NC). For all analyses, the Ps were two tailed, and a P < 0.05 was considered statistically significant.

RESULTS

Loss of DCC Expression. We identified normal DCC expression (DCC positive) in 123 patients (M0: n = 10; M1: n = 27; M2: n = 41; M3: n = 13; M4: n = 20; M5: n = 8; M6: n = 2; M7: n = 2) and loss of DCC expression (DCC negative) in 47 patients (M0: n = 5; M1: n = 13; M2: n = 17; M3: n = 5; M4: n = 3; M5: n = 4; M6: n = 1; M7: n = 0). The DCC-negative group comprised 16 women and 31 men. The survival time and CR duration of the DCC-negative AML patients were shorter than those of the DCC-positive patients, and these differences were significant (P < 0.0045 and <0.0060, respectively; Log-rank test), as shown in Fig. 2, A and B.

Analysis of N-ras, p53, and FLT3 Mutations. N-ras gene mutation was detected in 42 (M0: n = 1; M1: n = 12; M2: n = 10; M3: n = 2; M4: n = 9; M5: n = 5; M6: n = 1; M7: n = 1) of the 170 patients (24.7%). Mutations at codons 12, 13, and 61 were observed in 28, 13, and 5 patients, respectively. Nine of the 42 patients had multiple mutations at codon 12, and 1 had a multiple mutation at codon 13. Of the total N-ras gene point mutations found in the 42 patients, a G to A transition was the most frequent (32 of 51). A G to T transition was found less often (8 of 39). We could not discriminate, here, whether one leukemia sample contained multiple subclones of cells, each with a different N-ras mutation, or whether >1 bp in the codon was mutated in every cell of the malignant clone. No correlation was found between N-ras mutation and the survival time (P < 0.488; Fig. 3A) or between N-ras mutation and the CR duration (P < 0.314; Fig. 4A).
correlation was found between FLT3 mutation and the survival time (P < 0.223; Fig. 3D). However, the correlation was found between FLT3 mutation and the CR duration (P < 0.0116; Fig. 4D).

The AML Subgroup with no Mutations Correlated More Closely with CR Duration and OS. In the total of 170 patients, 28 patients had loss of DCC expression with wild-type N-ras, p53, and FLT3 genes (negative DCC/wild N-ras, p53, and FLT3), 64 patients had normal DCC expression with wild-type N-ras, p53, and FLT3 genes (positive DCC/wild N-ras, p53, and FLT3), 59 patients had normal DCC expression with one or more of mutant-type N-ras, p53, and FLT3 genes (positive DCC/mutant N-ras, p53, FLT3), and 19 patients had loss of DCC expression with one or more of mutant-type N-ras, p53, and FLT3 genes (negative DCC/mutant N-ras, p53, and FLT3). The clinical characteristics of these four groups were compared (Table 1). The 64 positive DCC/wild N-ras, p53, and FLT3 group had statistically better CR attainment compared with the other 106 patients (P < 0.0001). Especially, the positive DCC/wild N-ras, p53, and FLT3 group had statistically better CR attainment compared with the negative DCC/wild N-ras, p53, and FLT3 and the positive DCC/mutant N-ras, p53, and FLT3 groups (P < 0.0005 and <0.0001, respectively). The OS and CR duration were further analyzed for these four groups.

The OS of the positive DCC/wild N-ras, p53, and FLT3 group was significantly different from the other groups (P < 0.0006, <0.0001, and <0.0004, respectively; Fig. 5A). The CR duration of the positive DCC/wild N-ras, p53, and FLT3 group was significantly different from the other groups (P < 0.0008, <0.0001, and <0.0003, respectively; Fig. 5B).

Unfavorable Prognostic Factors for OS or CR Duration. The prognosis of AML patients depends on factors such as the age, initial WBC counts, FAB type classification, karyotype, and immune phenotype (Tables 2 and 3). Among these factors, cytogenetic data are thought to be the most important prognostic factor. On the basis of the cytogenetic findings, the 170 patients were segregated into three groups: a good risk group (n = 33) was defined by a karyotype of t(8;21), t(15;17), or inv(16); a poor risk group (n = 26) by t(9;22), 11q23 alterations, del(5), or del(7); and a standard risk group (n = 111) by normal or other altered karyotypes. Univariate and multivariate analyses for the OS or CR duration were performed (Tables 2 and 3).

Univariate analysis showed that unfavorable prognostic factors for the OS included the following: male gender (P < 0.0163), FAB type other than M2 and M3 (P < 0.0089), age of ≥60 years (P < 0.0033), poor risk karyotype compared with good risk karyotype (P < 0.0001), and mutant p53 (P < 0.0001), loss of DCC expression (P < 0.0053), and FLT3-Asp835 (P < 0.0232; Table 1). Multivariate analysis for OS showed that the poor risk karyotype compared with the good risk karyotype was the strongest unfavorable factor (relative risk, 0.149; P < 0.0001), followed by mutant p53 (P < 0.0002), mutant FLT3-TD (P < 0.0072), and loss of DCC expression (P < 0.0084). Leukocytosis, FAB type, mutant N-ras, mutant FLT3-Asp835, and male gender were less important.

Univariate analysis showed that unfavorable prognostic factors for the CR duration were the following: male gender (P < 0.0162), FAB type classification other than M2 and M3 (P < 0.0041), age of ≥60 years (P < 0.0167), poor risk...
Loss of DCC as a Prognostic Factor in AML

Karyotype was the strongest unfavorable factor (P < 0.0001; multivariate analysis for CR duration showed that poor risk karyotype was significantly associated with CR duration and OS. In our previous analysis of acute leukemia, expression of the DCC gene was absent in 20–30% of AML patients, in some of whom allelic loss in the DCC gene was observed (11, 12). In the 37 DCC-negative patients, allelic loss in the DCC gene was observed in only 2 AML patients with loss of DCC expression (data not shown; Ref. 11). Another 35 AML patients had no abnormality at the Southern level. Cytogenetic analysis also revealed 3 patients had monosomy 18 abnormality, and 2 AML patients had 18q21 abnormality. Thus, no abnormality of chromosome 18 was observed by G banding in 32 AML patients showing intragenic loss of heterozygosity in the DCC gene (29). The excellent correlation between loss of DCC and intragenic DCC loss of heterozygosity suggests that this may be an important

Table 1  Clinical characteristics of 170 patients with AML

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 170)</th>
<th>Positive DCC/wild N-ras, p53, and FLT3 (n = 64)</th>
<th>Negative DCC/wild N-ras, p53, and FLT3 (n = 28)</th>
<th>Positive DCC/mutant N-ras, p53, and FLT3 (n = 59)</th>
<th>Negative DCC/mutant N-ras, p53, and FLT3 (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>112/58</td>
<td>39/25</td>
<td>15/13</td>
<td>45/14</td>
<td>16/3</td>
</tr>
<tr>
<td>Age</td>
<td>55.4 (15–79)</td>
<td>56.2 (15–79)</td>
<td>57.9 (15–76)</td>
<td>55.5 (17–78)</td>
<td>49.1 (23–74)</td>
</tr>
<tr>
<td>WBC (10^9/liter)</td>
<td>26.2 (0.6–483)</td>
<td>26.4 (0.7–124)</td>
<td>15.9 (0.7–121)</td>
<td>51.0 (0.6–333)</td>
<td>69.9 (4.7–483)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>121</td>
<td>57</td>
<td>16 (P &lt; 0.0005)</td>
<td>33 (P &lt; 0.0001)</td>
<td>15</td>
</tr>
<tr>
<td>Failure</td>
<td>49</td>
<td>7</td>
<td>12</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor risk</td>
<td>26</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Standard risk</td>
<td>111</td>
<td>45</td>
<td>10</td>
<td>41</td>
<td>15</td>
</tr>
<tr>
<td>Good risk</td>
<td>33</td>
<td>11</td>
<td>12</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5 Kaplan-Meier analysis of OS (A) and CR duration (B) of the four types of AML patient groups with wild- or mutant-type N-ras, p53, and FLT3 according to DCC expression (Table 1). DCC−, DCC positive; DCC−, DCC negative; W, wild; M, mutant. The FLT3 implies TD and Asp835 mutations of the FLT3 gene.

Table 2  Unfavorable prognostic factors for OS in 170 patients

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0.0163</td>
<td>0.3504</td>
</tr>
<tr>
<td>Age</td>
<td>0.0033</td>
<td>0.0204</td>
</tr>
<tr>
<td>FAB types other than M2 and M3</td>
<td>0.0089</td>
<td>0.6631</td>
</tr>
<tr>
<td>WBC count</td>
<td>0.0111</td>
<td>0.6376</td>
</tr>
<tr>
<td>Cytogeneticsb</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Loss of DCC expression</td>
<td>0.0053</td>
<td>0.0084</td>
</tr>
<tr>
<td>Mutant p53</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mutant N-ras</td>
<td>0.4918</td>
<td>0.2473</td>
</tr>
<tr>
<td>Mutant FLT3-TD</td>
<td>0.1000</td>
<td>0.0072</td>
</tr>
<tr>
<td>Mutant FLT3-Asp835</td>
<td>0.0232</td>
<td>0.4800</td>
</tr>
</tbody>
</table>

a  Mean (range) values are indicated for age and WBCs. The numbers of cases are shown for FAB type, cytogenetics, and outcome.

b  P < 0.0375 and 0.029 compared with the negative DCC/wild N-ras, p53, and FLT3 group by Mann-Whitney’s U test, respectively.

Karyotypes were segregated into three groups.

b  Comparison between the good risk and poor risk karyotype groups.

DISCUSSION

In the present study, we showed that loss of DCC expression is significantly associated with CR duration and OS. In our previous analysis of acute leukemia, expression of the DCC gene was absent in 20–30% of AML patients, in some of whom allelic loss in the DCC gene was observed (11, 12). In the 37 DCC-negative patients, allelic loss in the DCC gene was observed in only 2 AML patients with loss of DCC expression (data not shown; Ref. 11). Another 35 AML patients had no abnormality at the Southern level. Cytogenetic analysis also revealed 3 patients had monosomy 18 abnormality, and 2 AML patients had 18q21 abnormality. Thus, no abnormality of chromosome 18 was observed by G banding in 32 AML patients with loss of DCC. In colorectal tumorigenesis, loss of DCC expression was present exclusively in colorectal tumors harboring intragenic loss of heterozygosity in the DCC gene (29). The excellent correlation between loss of DCC and intragenic DCC loss of heterozygosity suggests that this may be an important
Table 3  Unfavorable prognostic factors for CR duration in 170 patients

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>Sex</td>
<td>0.0162</td>
<td>0.3598</td>
</tr>
<tr>
<td>Age</td>
<td>0.0167</td>
<td>0.0392</td>
</tr>
<tr>
<td>FAB types other than M2 and M3</td>
<td>0.0041</td>
<td>0.4861</td>
</tr>
<tr>
<td>WBC count</td>
<td>0.1038</td>
<td>0.7637</td>
</tr>
<tr>
<td>Cytogenetics**</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Loss of DCC expression</td>
<td>0.0146</td>
<td>0.0371</td>
</tr>
<tr>
<td>Mutant p53</td>
<td>0.0001</td>
<td>0.0003</td>
</tr>
<tr>
<td>Mutant N-ras</td>
<td>0.3485</td>
<td>0.3067</td>
</tr>
<tr>
<td>Mutant FLT3-TD</td>
<td>0.2849</td>
<td>0.0235</td>
</tr>
<tr>
<td>Mutant FLT3-Asp835</td>
<td>0.0268</td>
<td>0.0616</td>
</tr>
</tbody>
</table>

* Karyotypes were segregated into three groups.
** Comparison between the good risk and poor risk karyotype groups.

Although we could not entirely rule out the possibility that a weak adverse interaction exists between mutant N-ras, p53, FLT3, and loss of DCC expression. Because gene alterations are associated with aberrant signal transduction, these mutations may be additively or synergistically associated with leukemia progression. Intriguingly, the groups with one or more mutations had significantly less CR attainment and a poorer prognosis than the normal DCC/wild N-ras, p53, and FLT3 group. These observations suggest that the DCC gene may functionally act as a tumor suppressor gene in leukemogenesis. However, the PS of the p53 mutation is the most important factor in AML leukemogenesis, along with the poor risk karyotype. The present data, as well as those in previous reports (32), may indicate that the p53 plays a more important role in leukemogenesis than the other genes.

Earlier reports indicate that FLT3-TD mutation is associated with OS. In the present study, unfortunately. FLT3-TD mutation did not show a correlation with a worse OS or short CR duration by Kaplan-Meier curves and the Log-rank test. However, FLT3-TD mutation was associated with the OS and the CR duration by multivariate analysis. The reasons for the discrepancy in these statistical analyses may be the sample size. FLT3 mutation may be meaningful as a PS factor. The predictive value of N-ras mutations is still controversial from the present data and from other recent reports (8). The present study failed to find any correlation between N-ras mutation and CR duration or survival time, although mutations of N-ras may well be involved in the pathogenesis of AML.

For the DCC gene, there is no report as to its PS in leukemias. Our present study revealed that loss of DCC expression is a useful and potent prognostic marker in AML patients. One recent study in patients with colorectal cancer dealt with the PS of the DCC protein in neoplasms (33). Although the function of the DCC gene as a tumor suppressor gene remains obscure, the present findings are of particular interest and are added to the current understanding of its role in cell differentiation and leukemogenesis (12).

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