Analysis of Genetic Polymorphism in NQO1, GST-M1, GST-T1, and CYP3A4 in 469 Japanese Patients with Therapy-related Leukemia/Myelodysplastic Syndrome and de novo Acute Myeloid Leukemia


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ABSTRACT

Several genetic polymorphisms in metabolic activation or detoxification enzymes have been associated with susceptibility to therapy-related leukemia and myelodysplastic leukemia (TRL/MDS). We analyzed gene polymorphisms of NAD(P)H:quinone oxidoreductase (NQO1), glutathione S-transferase (GST)-M1 and -T1, and CYP3A4, the enzymes of which are capable of metabolizing anticancer drugs, in 58 patients with TRL/MDS and in 411 patients with de novo acute myeloid leukemia (AML). Homozygous Ser/Ser genotype of NQO1 at codon 187, causing loss of function, was more frequent in the patients with TRL/MDS (14 of 58, 24.1%; OR = 2.62) than in those with de novo AML (64 of 411, 15.6%), and control (16 of 150, 10.6%; P = 0.002). Allelic frequencies of NQO1 were different between TRL/MDS and de novo AML (P = 0.01). In GST-M1 and -T1, the incidence of homologous deletion was similar among the three groups. The polymorphism of the 5' promoter region of CYP3A4 was not found in persons of Japanese ethnicity. These results suggest that the NQO1 polymorphism is significantly associated with the genetic risk of TRL/MDS.

INTRODUCTION

TRL/MDS are important late complications in patients with malignancies who received chemotherapy (1–3). Recently, molecular epidemiological studies have provided a new insight into the genetic background of TRL/MDS (4, 5).

Thus far there are some relevant genes which reportedly influence the risk of TRL/MDS. The cytochrome P450 enzymes are involved in metabolic activation of many carcinogens, and several polymorphisms significantly influence the metabolism (6). A member of P450 enzyme family, CYP3A4, metabolizes ETP to epipodophyllotoxin catechol followed by the generation of quinone metabolites (7–9). The quinone metabolites potentially generate DNA adducts, which enhance chromosomal breakage and recombination (10–11). A polymorphism in the CYP3A4 promoter region is associated with decreased gene expression and reportedly lowers the risk of TRL/MDS (4). The carcinogenic quinone metabolites are reduced to catechol by NAD(P)H:quinone oxidoreductase (NQO1), which is a member of a supergene family capable of detoxifying a number of electrophilic metabolites by catalyzing their conjugation to glutathione (15). Two of the members of the GSTs, GST-M1 and -T1, are absent in a significant percentage of the population (16–18). The deficiency of GSTs was associated with an increased risk of certain epithelial cancers, acute lymphoblastic leukemia, and AML (19). The GSTs are important candidate genes for TRL/MDS, because they are involved in the metabolism of the carcinogenic quinone metabolites. For NQO1, the loss-of-function polymorphism (Ser/Ser at codon 187) was associated with the genetic risk of TRL/MDS (4, 5). The 5' promoter region of NQO1 is important late complications in patients with malignancies who received chemotherapy (1–3). Recently, molecular epidemiological studies have provided a new insight into the genetic background of TRL/MDS (4, 5).

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3 The abbreviations used are: TRL/MDS, therapy-related leukemia and myelodysplastic leukemia; NAD(P)H:quinone oxidoreductase; GST, glutathione S-transferase; AML, acute myeloid leukemia; ETP, etoposide; FAB classification, French-British-American classification; OR, odds ratio; MPO, myeloperoxidase.
leukemia in black children, and de novo MDS (19–21), although there is controversy about MDS (22). Alkylation agents including melphalan are known substrates for GSTs (15). However, there is no report on the relationship between GST genotype and the risk of TRL/MDS.

Here, we conducted a case-control study to analyze the genetic susceptibility to TRL/MDS in Japanese patients and investigated the gene polymorphism of the above four enzymes; CYP3A4, NQO1, GST-M1, and GST-T1.

MATERIALS AND METHODS

Patients. In 1995, the Koseiho Leukemia Study Group conducted a surveillance study of TRL/MDS in Japan and obtained 256 patients with theses diseases (23). For this study, TRL/MDS was defined as AML or MDS following chemotherapy and/or radiotherapy for primary malignancies occurring at least 2 months after the start of the initial cytoxic therapies, as determined previously (23). Patients with chronic leukemia, myeloproliferative disorders, MDS, or acute leukemia were excluded from cases of primary neoplasms. However, if the TRL phenotype was clearly distinguished from primary leukemia, the patients were included. In 58 of the 256 patients, leukemia cells or DNA were available to us. For the primary neoplasms, 22 of the 58 patients received both radiation therapy and chemotherapy, 5 received radiation therapy only, and 31 received chemotherapy only. According to the FAB classification, each number of patients was as follows: two, five, six, six, nine, and eight in M0 to M5, respectively. Two were chronic myelomonocytic leukemia, nine were refractory anemia, nine were refractory anemia with excess blasts (REAB), and two were chronic leukemia cells or DNA were preserved, were studied. According to the FAB classification, each number of patients was as follows: 7, 72, 87, 89, and 92 protocols (24–26) conducted by the Japan Adult Leukemia Study Group, and whose leukemia cells or DNA were preserved, were studied. According to the FAB classification, each number of patients was as follows: 7, 72, 119, 120, 66, 22, 4, 1 in M0 to M7, respectively. Samples of TRL/MDS and de novo AML were provided through a limited number of hospitals (TRL/MDS from 24, de novo AML from 39 hospitals) for the purpose of molecular study after informed consent.

For the normal controls, we collected peripheral blood from 150 individuals working at two companies, separated geographically, after obtaining informed consent. The ages ranged from 22 to 60 years old, and the ratio of female to male was approximately 1:2.

Detection of Polymorphism. DNA purification and detection of the gene polymorphism were performed according to the published PCR methods (14, 17, 19, 20). Briefly, for the amplification of NQO1 gene fragment, a pair of sense and antisense primers were as follows: 5′-AGTGGCATCTGCATTTCTGTG-3′ and 5′-GATGACCTGCGCCAAGTGTG-3′. The amplification was carried out in a thermocycler (Model 9600; Perkin-Elmer/Cetus) with an initial denaturation step (8 min, 95°C), followed by 35 cycles consisting of three steps: 94°C for 30 s, 65°C for 1 min, and 72°C for 2 min. An additional cycle was performed at 72°C for 10 min. The amplified fragments were digested with Hpa II endonuclease (Biolabs Inc., Beverly, MA) and analyzed on agarose gel electrophoresis. The paired primers for GST-M1 and -T1 were 5′-GAAGTCCTCTGGAAAGCTTACGCCTCAATACGGTG-3′ and 5′-GTGGGGCTCAATACGGTG-3′ (19), and 5′-CTCTTACGGCGTCTCTATATCAGCC-3′ and 5′-TCACCCGATCATGCGCCAGCA-3′ (17), respectively. The presence or absence of GST genes was determined by using a differential PCR in which β-globin gene was coamplified in the same reaction tube. The primers for β-globin were 5′-ACAAACTGTCATCTAGC-3′ and 5′-CAACTTCATCCACGTCCCAATC-3′. The amplification of CYP3A4 promoter region was performed using the following primers: 5′-GTAAGATCTGTAGGCTGG-3′ and 5′-SGTAGTTCAATTTCTATAGG-3′ (27). The 205 bp-sized fragments were amplified and subjected to single-strand conformation polymorphism assay (28). A portion of the amplified products were directly sequenced using a Dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). Representative data of polymorphism is shown in Fig. 1.

Southern blot analysis of the MLL gene was performed as described previously (23).

Statistical Analysis. An analysis of frequencies was performed using the χ² test for 2 × 2 tables or the Pearson’s χ² test for larger tables. The Ps were calculated with StatView software (Abacus Concepts Inc., Berkeley, CA).

RESULTS

The NQO1 polymorphism at codon 187 was studied in 58 patients with TRL/MDS, 411 patients with de novo AML, and 150 healthy individuals. As shown in Table 1, the Ser/Ser genotype had an increased risk of TRL/MDS (OR = 2.62, P = 0.002 by the χ² test). Unexpectedly, the Pro/Ser genotype in de novo AML was less frequent than in healthy individuals (OR =
MDS because of insufficient DNA sample.

0.53, P = 0.002; Table 1). Allelic frequencies of Pro versus Ser in TRL/MDS, de novo AML, and healthy individuals were 52.6% versus 47.4%, 64.8% versus 35.2%, and 61.7% versus 38.3%, respectively. The Pro allele in TRL/MDS was more frequent than that in de novo AML (P = 0.01 by the χ² test) or that in healthy individuals (P = 0.09), whereas the frequency was similar in de novo AML and healthy individuals.

Clinical characteristics of TRL/MDS were compared among the three genotypes of NQO1, Pro/Pro, Pro/Ser, and Ser/Ser (Table 2). The genotype of NQO1 was not related to FAB subtypes, karyotype abnormalities, history of ETP therapy, or history of radiation therapy. Southern blot analysis of the MLL gene was performed in 57 of the 58 patients with TRL/MDS, and MLL gene rearrangements were detected in 9 patients (14.8%). However, there was no correlation between MLL rearrangements and the NQO1 genotypes (Table 2). In de novo AML, it was also studied whether NQO1 genotype was associated with a particular FAB subtype or with specific chromosomal abnormalities. However, no associations were found.

We next examined the polymorphism of the CYP3A4 promoter region in 58 patients with TRL/MDS and in 150 healthy individuals. The amplified fragments from all individuals had the same mobility on single-strand conformation polymorphism analysis with one exceptional case (Fig. 1). However, all of the sequences were the wild type for nucleotide position +217. As to CYP3A4, it was recently reported that a heterozygous mutation of G to A at position +298, indicating a very low frequency or a lack of variant type of CYP3A4 in Japanese persons. As to CYP3A4, we used leukemia cells instead of normal cells as the materials for this study. Accordingly, it must be kept in mind that somatic gene deletion of GST-M1 and GST-T1 gene was performed in 57 patients with TRL/MDS because of insufficient DNA sample.

We further studied the homozygous loss of the GST-M1 gene and the GST-T1 gene. The frequency of a null genotype was similar among the three groups (Table 3). The double-null genotype of GST-M1 and GST-T1 was also observed at a similar frequency (data not shown).

### DISCUSSION

In this study, we analyzed the relationship between genetic polymorphisms of various enzymes and TRL/MDS, in comparison with de novo AML and healthy individuals in Japan. NQO1 genotypes influenced the risk of TRL/MDS and de novo AML in different ways. The Ser/Ser type was associated with the increased risk of TRL/MDS, whereas the Pro/Pro type was associated with decreased risk of de novo AML. However, the genetic deletion of GST-M1 and -T1 had no association with these leukemias, and polymorphism of CYP3A4 was not found in Japanese persons. As to CYP3A4, it was recently reported that there is no variant type in Japanese persons (29). We used leukemia cells instead of normal cells as the materials for this study. Accordingly, it must be kept in mind that somatic gene alterations, including the loss of allele(s) and point mutations in the leukemia clone, might have influenced the genotype data.

The Ser/Ser type of NQO1 lacks enzymatic activity and
fails to detoxify quinone metabolites into the reduced form (13, 30). It was thus hypothesized that individuals lacking NQO1 activity would be at high risk of malignancies because of the exposure to procarcinogens, which are oxidized to quinone metabolites. The epidemiological study on benzene-toxification in China presented the first evidence that individuals with the Ser/Ser genotype had increased risk of subsequent hematotoxicity (31). The subsequent case-control studies on pediatric leukemia and TRL reported similar results (5, 14), although the carcinogens remain unclear in the former. The latter study reported that the increased risk was observed in TRL after cytotoxic therapy using alkylating agents or topoisomerase II-inhibitors, or in patients with clonal abnormalities of chromosomes 5 and/or 7 (5). In this study, we confirmed the association between the Ser/Ser genotype and an increased risk of TRL/MDS, but neither ETP therapy nor specific chromosomal abnormalities involving the MLL gene rearrangement were related to TRL/MDS with the Ser/Ser genotype. Because Japanese people were ethnically homogeneous as to CYP3A4, the product of which catalyzes ETP at the first oxidation step, we believe that the relevant role of NQO1 polymorphism in TRL/MDS is further confirmed by its isolation from the effects of CYP3A4. However, this and other studies did not consider the possibility that the NQO1 polymorphism had influenced the incidence of primary malignancies and/or the rate of long-term survival. Prospective study with appropriate stratification is required to obtain more conclusive data on NQO1 and TRL/MDS.

An unexpected finding in this study is that the Pro/Ser type was associated with a decreased risk of de novo AML. We studied a total of 411 patients with de novo AML, the samples of which were collected during three independent studies. Furthermore, this low frequency was observed in all FAB subtypes (data not shown). However, we cannot rule out that this might be derived from skewed sampling. According to the allelic frequencies, the distribution of NQO1 genotype in de novo AML and healthy individuals was not significantly out of the Hardy-Weinberg law, but slightly skewed in opposite directions (Table 1). Alternatively, individuals carrying the Pro type may actually be at low risk for de novo AML. NQO1 is known to catalyze the activation of some environmental procarcinogens, such as nitroaromatic compounds and heterocyclic amines (32), and the Ser/Ser type is adversely associated with an increased risk of lung cancer among smokers (33). Thus the intermediate NQO1 activity may be in favor of reducing the risk of de novo AML. More importantly, the NQO1 activity is complemented by MPO, which is known in benzene metabolism (31, 34). The balance between NQO1 and MPO activities must be more associated with the susceptibility of leukemia than the sole polymorphism of NQO1. The promoter polymorphism of the MPO gene was reportedly associated with the genetic risk of acute promyelocytic leukemia as well as the expression level of its product (35). Accordingly the NQO1 polymorphism should be analyzed in combination with the MPO polymorphism.

Table 3 Association of GST genotypes with TRL/MDS and de novo AML

<table>
<thead>
<tr>
<th>Genotype of GST-M1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TRL/MDS (n = 58)</th>
<th>OR (95% CI)</th>
<th>de novo AML (n = 411)</th>
<th>OR (95% CI)</th>
<th>Control (n = 150)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>32 (55.2%)</td>
<td>1.0 (Ref.)</td>
<td>184 (44.8%) (Ref.)</td>
<td>1.0 (48.7%)</td>
<td>73</td>
</tr>
<tr>
<td>Deleted</td>
<td>26 (44.8%)</td>
<td>0.77 (0–1.66)</td>
<td>227 (55.2%) (0.80–1.54)</td>
<td>1.17 (51.3%)</td>
<td>77</td>
</tr>
<tr>
<td>Genotype of GST-T1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Present</td>
<td>31 (53.4%)</td>
<td>1.0 (Ref.)</td>
<td>214 (52.1%)</td>
<td>69</td>
</tr>
<tr>
<td>Deleted</td>
<td>27 (46.6%)</td>
<td>0.74 (0–1.63)</td>
<td>197 (47.9%) (Ref.)</td>
<td>0.78 (46.0%)</td>
<td>81</td>
</tr>
</tbody>
</table>

<sup>a</sup> p = 0.28.<br><sup>b</sup> p = 0.40.

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