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

Tomoki Naoe, Kunihiko Takeyama, Toshiya Yokozawa, Hitoshi Kiyoi, Masao Seto, Naokuni Uike, Teruo Ino, Atae Utsunomiya, Atsuo Maruta, Itsuro Jin-nai, Nanao Kamada, Naokuni Uike, Teruo Ino, Atae Utsunomiya, Chihiro Shimazaki, Shigeo Horiike, Yoshihisa Kodera, Hidehiko Saito, Ryuzo Ueda, Joseph Wiemels, and Ryuzo Ohno

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).

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.

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.

Received 2/22/00; revised 7/5/00; accepted 7/7/00.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by a Grant-in-Aid from the Japanese Ministry of Health and Welfare (9-3, 5-9).
2 To whom requests for reprints should be addressed, at Department of Infectious Diseases, Nagoya University School of Medicine, Nagoya 466-8560, Japan. Phone: 81-52-744-2955; Fax: 81-52-744-2801.

1 The abbreviations used are: TRL/MDS, therapy-related leukemia and myelodysplastic leukemia; NAD(P)H:NQO1, 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). Alkylating 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 Koseisho Leukemia Study Group conducted a surveillance study of TRL/MDS in Japan and obtained 256 patients with these 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 cytotoxic 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, and eight in M0 to M5, respectively. Two were chronic myelogenous 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 of blasts (REAB), and two were RAEB in transformation (RAEB-T).

Patients with de novo AML who were registered to AML-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 protocol (24, 25) conducted by the Japan Leukemia Study Group. In 1995, the Koseisho Leukemia Study Group provided 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’-AGTGGCATCTG-CATTCTGGT-3’ and 5’-GATGACTGCGCCAGTGTG-3’. The amplification was carried out in a thermocycler (Model 8700; 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, 56°C for 1 min, and 72°C for 2 min. An additional cycle was performed for 72°C for 10 min. The amplified fragments were digested with HinfI endonuclease (Biolabs, Inc., Beverly, MA) and analyzed on agarose gel electrophoresis. The paired primers for GST-M1 and -T1 were 5’-GAAGTCCTC-CCTGAAAAGCTAAAGCA-3’ and 5’-GTTGGGGCTCAATACCGTG-3’ (19), and 5’-TTCTCTACTGCTTCTCATACTC-3’ and 5’-TCACGGATCAAGGCAAGCA-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’-ACAAAGCTGTCCTGCCTAG-3’ and 5’-CCTACGTCACGTTCAAGC-3’. The amplification of CYP3A4 promoter region was performed using the following primers: 5’-GTAAGATCTTGAGTGGT-3’ and 5’-AGTCCATTATGTATGAG-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 on a DNA sequencer (373A; Applied Biosystems, Foster City, CA). Representative data of polymorphism is shown in Fig. 1.

**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 = 0.29, P = 0.033).
Clinical Cancer Research 4093

Table 1  Association of NQO1 genotype with TRL/MDS and de novo AML

<table>
<thead>
<tr>
<th>Genotype of NQO1</th>
<th>TRL/MDS (n = 58)</th>
<th>OR(^d) (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>Pro/Pro</td>
<td>17</td>
<td>1.0</td>
<td>186</td>
<td>1.0</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>(29.3%)</td>
<td>(Ref.(^b))</td>
<td>(45.3%)</td>
<td>(Ref.)</td>
<td>(34.0%)</td>
</tr>
<tr>
<td>Pro/Ser</td>
<td>27</td>
<td>0.98</td>
<td>161</td>
<td>0.53</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>(46.6%)</td>
<td>(0.28–1.68)</td>
<td>(39.2%)</td>
<td>(0.32–0.74)</td>
<td>(55.3%)</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>14</td>
<td>2.62</td>
<td>64</td>
<td>1.10</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>(24.1%)</td>
<td>(2.16–3.08)</td>
<td>(15.6%)</td>
<td>(0.48–1.72)</td>
<td>(10.6%)</td>
</tr>
</tbody>
</table>

\(^d\) OR, odds ratio.
\(^b\) CI, confidence interval.
\(^a\) Ref., reference.
\(^d\) Expected according to each allelic frequency and the Hardy-Weinberg law.

Table 2  Association between NQO1 genotype and clinical features of TRL/MDS

<table>
<thead>
<tr>
<th>FAB classification: M0/M1</th>
<th>Pro/Pro</th>
<th>Pro/Ser</th>
<th>Ser/Ser</th>
<th>Pro/Pro</th>
<th>Pro/Ser</th>
<th>Ser/Ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0/M1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>M2</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>M3</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>M4</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>M5</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>MDS</td>
<td>12</td>
<td>5</td>
<td>5</td>
<td>12</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>History of ETP therapy</td>
<td>Yes</td>
<td>10</td>
<td>4</td>
<td>Yes</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>History of radiation</td>
<td>No</td>
<td>11</td>
<td>17</td>
<td>No</td>
<td>11</td>
<td>17</td>
</tr>
<tr>
<td>therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of MDS</td>
<td>Yes</td>
<td>8</td>
<td>11</td>
<td>Yes</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Chromosome abnormalities</td>
<td>No</td>
<td>9</td>
<td>16</td>
<td>9</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>del(5q)/5–</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>del(7q)/7–</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>del(5q)/5– and del(7q)/7–</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Abnormal 11q</td>
<td>1</td>
<td>4(^b)</td>
<td>1</td>
<td>4(^b)</td>
<td>1</td>
<td>4(^b)</td>
</tr>
<tr>
<td>t(15;17)</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>inv(16)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Normal</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>UE or ND</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>MLL rearrangement</td>
<td>Present</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\) Chromosomal abnormalities were classified according to the primary change observed. UE, unrevaluated; ND, not done.
\(^b\) One of the four patients also had del(7q)/7– abnormality.

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 −298, indicating a very low frequency or a lack of variant type of CYP3A4 in Japanese persons. The above one case had a heterozygous mutation of G to A at position −230 (data not shown).

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/Ser 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
fail 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 with the de novo type, the product of which catalyzes ETP at the first oxidization step, we believe that the relevant role of CYP3A4 polymorphism should be analyzed in combination with the NQO1 polymorphism.

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/Ser 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 reported to be 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. The association of the NQO1 polymorphism with clinical features, including patients’ ages, karyotypes, and responses to therapy, is also an important issue for future studies.

Molecular epidemiological studies have unmasked the relationship between cancer and genetic factors as well as environmental factors. The majority are retrospective or cohort case-control studies, which have provided suggestive, but sometimes confusing, data. To elucidate the mechanism of gene polymorphism affecting the risk of leukemia, experimental studies of transformation in vitro and in vivo will be necessary in conjunction with epidemiological studies.

ACKNOWLEDGMENTS

We are grateful to our colleagues in the Koseisho Leukemia Study Group and in the Japan Adult Leukemia Study Group for sending us patient samples. We thank Yoko Tagawa for technical assistance.

REFERENCES

18. Hengstler, J. G., Arand, M., Herrero, M. E., and Oesch, F. Poly-

...
Clinical Cancer Research

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

Tomoki Naoe, Kunihiko Takeyama, Toshiya Yokozawa, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/10/4091

Cited articles
This article cites 32 articles, 14 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/10/4091.full#ref-list-1

Citing articles
This article has been cited by 21 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/6/10/4091.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/6/10/4091.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.