Orotate Phosphoribosyltransferase Gene Polymorphism Predicts Toxicity in Patients Treated with Bolus 5-Fluorouracil Regimen

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Abstract

Purpose: We investigated whether the determination of orotate phosphoribosyltransferase (OPRT) and thymidylate synthase (TYMS) polymorphisms could predict the toxicity of 5-fluorouracil (5-FU) in colorectal cancer patients.

Experimental Design: The determination of OPRT and TYMS genotypes were done in genomic DNA extracted from blood by PCR amplification in 69 patients treated with bolus 5-FU as adjuvant chemotherapy. Associations between these polymorphisms and toxicity were evaluated retrospectively.

Results: The Ala allele in OPRT Gly212Ala polymorphism and the two tandem repeats (2R) in TYMS promoter polymorphisms were associated with grade 3 to 4 neutropenia and diarrhea. The multivariable logistic regression models revealed that only TYMS promoter polymorphism had an independent value to predict grade 3 to 4 neutropenia [odds ratio, 19.2 for patients with the 2R allele compared with patients with homozygous with the three repeat (3R) alleles], whereas both OPRT and TYMS promoter polymorphisms were independent predictive factors for grade 3 to 4 diarrhea (odds ratio, 13.3 for patients with the Ala allele compared with patients in the Gly/Gly genotype and 11.1 for patients with the 2R allele compared with patients in the 3R/3R genotype). A significant difference was observed in the time to onset of severe toxicity, defined as grade 4 neutropenia and/or grade 3 to 4 gastrointestinal toxicities according to OPRT and TYMS promoter polymorphisms.

Conclusion: OPRT Gly212Ala polymorphism seems to be a useful marker for predicting toxicity to bolus 5-FU chemotherapy. Prospective translational treatment trials including larger number of patients are needed to confirm our results.

Despite the recent development of various new drugs, 5-fluorouracil (5-FU) still plays a major role in chemotherapy for colorectal cancer. Although the drug has been in clinical use for almost 50 years, there is still no clear methodology to identify patients who are likely to benefit most from the treatment. Even with different doses and schedules, the response rate of metastatic colorectal cancer is only 21% when treated with 5-FU and leucovorin (1), with the incidence rates of grade 3 to 4 hematologic toxicity being 31% with 5-FU bolus and 4% with continuous infusion regimens, respectively, and grade 3 to 4 nonhematologic toxicity of 13% to 14% in both regimens (2). Therefore, there is a clear need to identify markers to predict the treatment effect and toxicity to provide a rational basis for treatment selection.

The main mode of action of 5-FU is thought to be the inhibition of thymidylate synthase (TS), an essential DNA synthetic enzyme that catalyses the methylation of dUMP to dTMP, through the binding of fluorodeoxyuridine monophosphate (FdUMP) to TS protein (3). A polymorphic 28-bp tandem repeat in the promoter enhancer lesion (TSER), which usually presents as a double-tandem repeat (2R) or a triple-tandem repeat (3R), has been described in the 5′-untranslated region of the TS gene (TYMS; ref. 4). This polymorphism may affect the translational efficacy of the gene and predict not only the response (5–8) but also the toxicity (7, 9) when treated by fluoropyrimidine-based chemotherapy. More than 80% of 5-FU given is inactivated by dihydropyrimidine dehydrogenase (DPD) in the liver (10). In patients suffering from severe 5-FU-associated toxicity, several mutations in the DPD gene (DPYD) have been identified (11).

Although the importance of the value of TS and DPD in the cytotoxicity of 5-FU is recognized (12), the contribution of phosphorylation is necessary to activate 5-FU into its nucleotides (13). The preferential use of the pathway directly to FUMP by orotate phosphoribosyltransferase (OPRT) was revealed to correlate with the cytotoxicity of 5-FU (13–15). FUMP is then phosphorylated to fluorouridine diphosphate, which can be either converted to FdUMP or phosphorylated to the active metabolite fluorouridine triphosphate (3). Fluorouridine triphosphate is extensively incorporated into RNA (F-RNA), disrupting normal RNA processing and function (3). In a mouse model, 5-FU-induced gastrointestinal toxicity has been reported...
to be due to F-RNA, regardless of FdUMP levels (16). Additionally, potassium oxonate, an inhibitor of OPRT, decreased the levels of FUMP followed by a decrease in F-RNA by ~70% in the small intestine, and there was a reduction in gastrointestinal toxicity (16, 17). Taken together with these results, an association between OPRT expression in normal tissues and 5-FU-induced gastrointestinal toxicity should be considered.

Uridine monophosphate (UMP) synthase is a bifunctional enzyme catalyzing the last two steps of de novo pyrimidine biosynthesis, OPRT and orotidine-5'-monophosphate decarboxylase (18). Loss of enzymatic activity results in hereditary orotic aciduria, a rare autosomal recessive disorder (19). Molecular investigation of UMP synthase deficiency in Japanese orotic aciduria patients revealed three rare disease-related single nucleotide polymorphisms of R96G (A-to-G transition; nucleotide 286), G429R (G-to-C transition; nucleotide 1283), and V109G (T-to-G transition; nucleotide 326), which compromise OPRT function. In addition, two common non–disease-related polymorphisms, Gly213Ala polymorphism (G-to-C transition; nucleotide 638) within exon 3 and 440Gpoly within exon 6, were identified. Although 440Gpoly is a silent polymorphism, constructs encoding the Gly213Ala substitution within exon 6, were identified. Although 440Gpoly is a silent polymorphism, constructs encoding the Gly213Ala substitution seemed to confer a significant OPRT activity increase in S21 insect cells using a baculovirus expression system (>150% normal; ref. 19). These data indicate the possibility of predicting of 5-FU-induced toxicity based on OPRT Gly213Ala polymorphism in normal tissues.

The aim of this study is to determine whether OPRT Gly213Ala polymorphism and TYMS promoter polymorphisms are predictive of toxicity in patients receiving 5-FU adjuvant chemotherapy. We also evaluated OPRT mRNA expression and OPRT activity in normal colon mucosa in relation to OPRT Gly213Ala polymorphism.

Materials and Methods

Patients. From January 2000 to August 2002, all 69 consecutive patients, who underwent radical surgery and received bolus 5-FU therapy combined with leucovorin as adjuvant chemotherapy in the Department of Digestive and General Surgery of Saitama Medical School, were included. Eligible patients had histologically proven adenocarcinoma of the colon or rectum. Patients were eligible if they had Duke's B2 with evidence of obstruction, perforation, or invasion of adjacent organs, or Duke's C tumors, an Eastern Cooperative Oncology Group performance status of ≤1, and adequate bone marrow/renal/hepatic functions. Excluding criteria were any prior or concurrent radiation therapy or chemotherapy. Written informed consent was obtained from all patients to use their blood and tissue samples for research purposes, with the approval of the institute’s ethical committee.

This study cohort consisted of 43 men and 26 women, with a median age of 65 years ranging from 36 to 78 years. The performance status was 0 and 1 in 55 and 14 patients, respectively. The primary tumor was located in the colon in 56 cases and in the rectum in 13 cases. The depth of invasion according to the tumor-node-metastasis classification system were 15 T1, or T2 tumors, 33 T3 tumors, and 21 T4 tumors. There were 6 Duke's B2 and 63 Duke's C tumors.

All eligible patients were treated between 14 and 35 days after surgery with the Roswell Park regimen (500 mg/m² 5-FU i.v. bolus weekly for 6 weeks, given 1 hour after t-leucovorin infusion, combined with 250 mg/m² t-leucovorin by 2-hour infusion; four treatment cycles are given, each consisting of six weekly treatments followed by a 2-week rest period). Toxicity was recorded by grade according to the National Cancer Institute Common Toxicity Criteria version 2.0. Physical examination, a full blood count, and serum chemistry were done before every administration of drugs. Before the start of every injection, the drug dose was reevaluated according to toxicity. If the absolute neutrophil count was <1,500/μL, and if the platelet count was under 75,000/μL, then treatment was delayed until the recovery of bone marrow function. If grade 4 neutropenia or thrombocytopenia, or grade 3 to 4 gastrointestinal toxicities (i.e., diarrhea, stomatitis, nausea, and vomiting) were observed after the previous injection, the 5-FU dose was reduced to 400 mg/m² in subsequent courses. Severe toxicity was defined as grade 4 neutropenia or grade 3 to 4 gastrointestinal toxicities of both.

Blood and Tissue samples. Blood sampling was done preoperatively in all 69 patients. DNA from peripheral blood mononuclear cells was isolated using a modified QiAamp DNA Blood Maxi kit protocol (Qiagen, Santa Clarita, CA) and was quantified using a PicoGreen dsDNA Quantitation kit (Molecular Probes, Eugene, OR).

In 37 patients who were enrolled initially in this study, we made archival fresh frozen samples of normal colonic mucosa, located apart from the primary tumor, at the time of surgery. Immediately after resection, the normal mucosa was divided into two equal portions of at least 500 mg each. Both portions were fresh frozen in liquid nitrogen until the time of RNA extraction and measurement of OPRT activity.

Genotyping. Samples were genotyped for OPRT Gly213Ala polymorphism by Assay-by-Design by Applied Biosystems (ABI, Foster City, CA), as previously described (20). Reactions were done with the following protocol on a GeneAmp PCR 9700 or 7700 ABI Sequence Detection System: 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. A post-PCR plate read on the 7700 was used to determine genotype. Taqman Single Nucleotide Polymorphism Genotyping Assay (Applied Biosystems), prevalidated assay including the specific primer and probes, was used for genotyping (assay ID C_1901447-10, rs1801019).

The genotyping of TYMS promoter polymorphisms was carried out by using PCR protocol as described previously (7). Products of 210 bp (2R/2R), 238 bp (3R/3R), or both of products (2R/3R) were observed.

Analysis of OPRT activity and mRNA expression. OPRT activities in frozen samples was measured by the paper disc method (21, 22). Briefly, the tissue samples were homogenized in a 2-fold volume of 50 mmol/L Tris-HCl buffer (pH 7.5) containing 1.5 mmol/L MgCl₂ and 2 mmol/L DTT. After centrifuging (105,000 × g, 1 hour, 4°C), 200 μL of supernatant were collected and incubated at 37°C with 1.6 μmol/L [³H]5-FU (2.5 nCi), 2 μmol 5-phosphoribosyl 1-pyrophosphate, 6 μmol β-glycerophosphate, and 240 nmol α₅-methylene adenosine 5'-diphosphate in a total volume of 200 μL. Aliquots of the reaction mixture were removed after 5, 10, and 15 minutes of incubation, and the reaction was stopped immediately by placing them in a boiling water bath. After centrifugation, 20 μL of the supernatant were spotted onto anion exchange filter paper disc made from DEAE-cellulose, and the disc was repeatedly washed to remove unreacted [³H]5-FU. The filter paper disc was placed in a scintillation vial followed by the additional 8 mL of Scintisole EX-H (Wako, Tokyo, Japan), and the radioactivity of [³H]FUMP was measured. The OPRT activity (pmol/min per mg protein) was calculated based on the amount of FUMP produced, which was proportional to the radioactivity, and the protein concentration in the enzyme solution was measured by the method of Lowry et al. (23).

Total RNA for each sample was isolated using the RNeasy mini kit (Qiagen, Inc., Chatsworth, CA) followed by cDNA synthesis as previously described (15). Quantitation of cDNA of the OPRT gene and an internal reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was done by a fluorescence based real-time detection method (Taqman), as described previously. The PCR reaction mixture consisted of 600 mmol/L of each primer; 200 nmol/L probe: 2.5 units AmpliTaq Gold Polymerase; 200 μmol/L each dATP, dCTP, and dGTP, 400 μmol/L dTTP, 5.5 mmol/L MgCl₂, and 1× Taqman Buffer A containing a reference dye, to a final volume of 25 μL (all reagents from Applied Biosystems).
Results

Distributions of OPRT Gly213 Ala polymorphism and TYMS promoter polymorphism. OPRT Gly213 Ala polymorphism was successfully assessed for all 69 patients. The genotype TYMS promoter polymorphisms were obtained for 65 patients because of missing four samples. The distribution of the Gly213 Ala genotype was 8.7% Ala/Ala [6 patients; 95% CI: 4.0-14.9%], 37.7% Ala/Gly (26 patients; 95% CI, 26.2-49.1%), and 53.6% Gly/Gly (37 patients; 95% CI, 41.9-65.4%). The frequency of the rare allele (Ala) was 27.5%. The distribution is in close agreement with that predicted by the Hardy-Weinberg equilibrium. The distribution of the TYMS promoter polymorphism was 4.6% 2R/2R (3 patients; 95% CI, 0.0-19.5%), 16.9% 2R/3R (11 patients; 95% CI, 13.3-20.6%), and 78.5% 3R/3R (51 patients; 95% CI, 74.8-82.1%). The frequencies of the 2R and 3R allele were 13.1% and 86.9%, respectively.

There was no statistical association of OPRT Gly213 Ala and TYMS promoter polymorphisms with the clinicopathologic features, such as age, gender, performance status, location of primary tumor (colon, rectum), tumor depth of invasion, and Dukes’ classification (data not shown).

Analysis of toxicity in terms of genotypes. The most prevalent toxicities reported were neutropenia seen in 10.1% (6 patients with grade 3 and 1 patient with grade 4) and diarrhea in 15.9% (10 patients with grade 3 and 1 patient with grade 4) of all patients. Severe toxicity, defined as grade 4 neutropenia or grade 3 to 4 gastrointestinal toxicities or both, was observed in 11 of all 69 patients: one patient experienced grade 4 neutropenia and diarrhea, and two patients experienced grade 3 diarrhea and nausea or vomiting. No toxic deaths were observed in this study.

Grade 3 to 4 neutropenia was observed in 2.7% (1 of 37) of patients in the Gly/Gly group compared with 15.4% (4 of 26) in the Ala/Gly group and 33.3% (2 of 6) in the Ala/Ala group (Kruskal-Wallis test, \( P = 0.0393 \); Table 1). There was grade 3 to 4 neutropenia in 66.7% (2 of 3) of patients with the 2R/2R genotype compared with 27.2% (3 of 11) in the 2R/3R group and 3.9% (2 of 51) in the 3R/3R group (Kruskal-Wallis test, \( P = 0.0005 \); Table 1). There was grade 3 to 4 diarrhea in all six patients with the Ala/Ala genotype compared with 15.4% (4 of 26) in the Ala/Gly group and 2.7% (1 of 37) in the Gly/Gly group (Kruskal-Wallis test, \( P < 0.0001 \); Table 1). Grade 3 to 4 diarrhea was observed in 66.7% (2 of 3) of patients in the 2R/2R group compared with 36.4% (4 of 11) in the 2R/3R group and 9.8% (5 of 51) in the 3R/3R group (\( P = 0.0070 \), Kruskal-Wallis test; Table 1).

All six patients in the Ala/Ala genotype experienced severe toxicity, whereas 4 of 26 patients in the Ala/Gly genotype and 1 of 36 patients in the Gly/Gly genotype did (\( P < 0.0001 \), Kruskal-Wallis test; Table 1). There was severe toxicity observed in 2 of 3 patients with 2R/2R genotype compared with 4 of 11 patients in the 2R/3R group and 5 of 51 patients in the 3R/3R group (\( P = 0.0005 \), Kruskal-Wallis test; Table 1). An OR of 16.4 (95% CI, 2.0-136.7) was observed for patients with the Ala allele compared with the group of patients homozygous for the Gly allele, whereas an OR of 4.9 (95% CI, 1.7-28.1) was observed for patients with the 2R allele compared with the group of patients homozygous for the 3R allele.

Using multivariate analysis, only TYMS promoter polymorphism (2R/2R < 2R/3R, 3R/3R) was found to be a statistically significant risk factor for grade 3 to 4 neutropenia, with an OR of 19.2 (95% CI, 2.2-334.4; \( P = 0.016 \); Table 2). Both OPRT Gly213 Ala polymorphism (OR, 13.3; 95% CI, 1.9-280.9; \( P = 0.026 \)) and TYMS promoter polymorphism (OR, 11.1; 95% CI, 1.6-117.0; \( P = 0.022 \)) were independent variables associated with grade 3 to 4 diarrhea.

In addition, a significant difference was observed in the time to onset of severe toxicity according to OPRT Gly213 Ala and TYMS promoter polymorphisms (\( P < 0.0001 \) and \( P = 0.0009 \), respectively, log-rank test; Fig. 1). The median time to onset of severe toxicity was 3 weeks (range, 1-5 weeks) in six patients with the Ala/Ala genotype and 6 weeks (range, 3-13 weeks) in 26 patients with the Ala/Gly genotype. Only one patient with the Gly/Gly genotype experienced severe toxicity, at 8 weeks from the start of chemotherapy.

Among 11 patients with severe toxicity, one patient with the Ala/Ala OPRT polymorphism and the 2R/2R TYMS polymorphism needed to be hospitalized due to coincidentally occurring grade 4 neutropenia and diarrhea, and the treatment was stopped. 5-FU-based chemotherapy was successfully following by the treatment postponement and the dose reduction in four patients (three patients with the Ala/Gly genotype and one patient with the Gly/Gly genotype), whereas treatment was halted in the remaining six patients (three patients with the Ala/Ala genotype and three patients with Ala/Gly genotype) because of severe toxicity, although we attempted dose modification.

OPRT activity and mRNA expression in relation to Gly213 Ala variants. In 37 patients who were enrolled initially in this
study, both OPRT mRNA expression and OPRT activity were measured in normal colon mucosa. The distribution of the Gly213Ala genotype in these 37 patients was 8.1% in Ala/Ala (3 patients; 95% CI, 0.0-16.9%), 29.7% in Ala/Gly (11 patients; 95% CI, 15.0-44.5%), and 62.2% in Gly/Gly (23 patients; 95% CI, 46.5-77.8%), which is similar in all 69 patients. We examined the relationship between OPRT mRNA expression and the OPRT Gly213Ala polymorphism. The median gene expressions in patients in the Ala/Ala group and the Ala/Gly group were 1.62 and 0.87, respectively, whereas the median gene expression in patients in the Gly/Gly group was 0.53 (Kruskal-Wallis test, \( P < 0.0001 \); Fig. 2).

Analysis of the Gly213Ala polymorphism with respect to OPRT activity revealed a significant association between the genotype and OPRT activity in normal colon mucosa. The median OPRT activity in patients with the Ala/Ala genotype and the Ala/Gly genotype were 0.26 and 0.13 pmol/min per mg protein, respectively, whereas the median OPRT activity in patients with

<table>
<thead>
<tr>
<th>Table 1. Association between OPRT Gly213Ala and TYMS promoter genotypes and the occurrence of toxicities</th>
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</thead>
<tbody>
<tr>
<td><strong>OPRT Gly213Ala genotypes</strong></td>
</tr>
<tr>
<td>Ala/Ala</td>
</tr>
<tr>
<td>No. patients (%)</td>
</tr>
<tr>
<td>Neutropenia (no. patients)</td>
</tr>
<tr>
<td>Grade 0-2</td>
</tr>
<tr>
<td>Grade 3</td>
</tr>
<tr>
<td>Grade 4</td>
</tr>
<tr>
<td>Diarrhea (no. patients)</td>
</tr>
<tr>
<td>Grade 0-2</td>
</tr>
<tr>
<td>Grade 3</td>
</tr>
<tr>
<td>Grade 4</td>
</tr>
<tr>
<td>Severe toxicity( ^1 )</td>
</tr>
<tr>
<td>Not experienced</td>
</tr>
<tr>
<td>Experienced</td>
</tr>
</tbody>
</table>

**NOTE:** Toxicities were graded according to version 2.0 of the National Cancer Institute Common Toxicity Criteria. \( P \) as calculated with the nonparametric Kruskal-Wallis test. \( ^* \) \( P \) with regard to toxicity comparing grade 0 to 2 versus grade 3 to 4. \( ^1 \) Grade 4 neutropenia and/or Grade 3 to 4 gastrointestinal toxicities.

Table 2. Association between patient characteristics and polymorphisms in OPRT Gly213Ala and TYMS promoter and grade 3 to 4 neutropenia and diarrhea by logistics analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. patients</th>
<th>Grade 3 to 4 neutropenia</th>
<th>Grade 3 to 4 diarrhea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OR (95% CI)</td>
<td>( P )</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt;70)</td>
<td>37</td>
<td>1</td>
<td>0.990</td>
</tr>
<tr>
<td>(\geq70)</td>
<td>28</td>
<td>1.0 (0.2, 4.9)</td>
<td>1.5 (0.2, 16.1)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>1</td>
<td>0.336</td>
</tr>
<tr>
<td>Female</td>
<td>26</td>
<td>2.2 (0.4, 12.0)</td>
<td>0.6 (0.1, 4.8)</td>
</tr>
<tr>
<td>Performance status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>52</td>
<td>1</td>
<td>0.127</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>3.6 (0.7, 19.0)</td>
<td>4.5 (0.6, 50.2)</td>
</tr>
<tr>
<td>OPRT Gly213Ala</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly/Gly</td>
<td>32</td>
<td>1</td>
<td>0.072</td>
</tr>
<tr>
<td>Ala/Gly, Ala/Ala</td>
<td>33</td>
<td>7.4 (1.2, 144.1)</td>
<td>4.5 (0.5, 111.9)</td>
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<tr>
<td>TYMS promoter</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3R/3R</td>
<td>51</td>
<td>1</td>
<td>0.003</td>
</tr>
<tr>
<td>2R/3R, 2R/2R</td>
<td>14</td>
<td>13.6 (2.5, 106.1)</td>
<td>19.2 (2.2, 334.4)</td>
</tr>
</tbody>
</table>

**NOTE:** Multivariate analysis was done by using the patient characteristics (age, gender, and performance status), OPRT Gly213Ala genotype, and TYMS promoter genotype.
the Gly/Gly genotype was 0.07 (Kruskal-Wallis test, \( P = 0.0002 \); Fig. 2). The Ala allele showed a significant association with higher OPRT activity (median, 0.13 and 0.07 pmol/min per mg protein for patients with the Ala allele and those with the Gly/Gly genotype, respectively; Mann-Whitney \( U \) test, \( P < 0.0001 \)).

There was a positive correlation between OPRT mRNA level and OPRT activity in normal colon mucosa (Spearman rank correlation coefficient 0.71, \( P < 0.0001 \)).

**Discussion**

In this study, we showed that OPRT Gly\(^{213}\)Ala polymorphism could help in predicting toxicity of grade 3 or 4 neutropenia and diarrhea among colorectal cancer patients who received 5-FU adjuvant chemotherapy. Especially, OPRT Gly\(^{213}\)Ala and TYMS promoter polymorphisms were independent factors to predict grade 3 to 4 diarrhea, whereas only TYMS promoter polymorphism had the independently predictive valuable for grade 3 to 4 neutropenia.

There is ample evidence that a deficiency of DPD is associated with severe toxicity after the administration of 5-FU. In patients who are deficient for DPD, 5-FU clearance is dramatically reduced and standard doses of 5-FU cause excessive toxicity in these patients (10, 26). DPD deficiency is caused at least in part by DPYD polymorphisms that result in complete or partial loss of DPD activity (11, 27, 28). To date, the most common consistent data are for allele DPYD\(^*2\), which is a splice site mutation (G to A) that causes skipping of exon 14 (IVS14+1G>A), resulting in the production of a truncated mRNA and the formation of a defective protein (27). This defective protein degrades rapidly followed by decreased detectable DPD activity (28). This mutation was commonly
detected in 24% to 28% of all patients suffering from severe 5-FU toxicity, whereas the frequency of the mutant allele is actually 0% in the Japanese population (11, 29, 30). These results show that this mutation might be justified for screening for DPD deficiency, not predicting toxicity before 5-FU-based chemotherapy. Thus, we did not perform the genotyping of DPYD*2 in this study.

Unlike in DPYD, the significant inverse association between the number of the 28-bp tandem repeats in TSER and severe toxicity to 5-FU is relevant. Patients with homozygous 2R/2R genotype experienced severe toxicity (grade 3) with an incidence of 63% compared with 32% in the group 2R/3R and 27% in the group 3R/3R, when treated with 5-FU-based chemotherapy for metastatic colorectal cancer (7). Subsequently, an OR of 20 has been reported for patients with the homozygous genotype carrying two 2R alleles compared with the reference group of patients homozygous for the 3R alleles in TSER, when treated with adjuvant or palliative 5-FU-based chemotherapy (9). We confirmed the association between TYMS polymorphism and severe toxicity to 5-FU, even in the Japanese population. The frequency of the 2R allele has been reported to be 19% in the Japanese population, which is lower than the 40% among Caucasian population (31).

There is little knowledge about the relation between the toxicity and polymorphism in genes responsible for 5-FU activation. We showed for the first time that OPRT Gly113Ala polymorphism could help in predicting toxicity, especially grade 3 to 4 diarrhea, among colorectal cancer patients who received 5-FU chemotherapy. Patients with the Ala allele were 16 times more likely to have severe toxicity compared with those with the Gly/Gly genotype. Moreover, a conspicuous finding was that the onset of severe toxicity occurred earlier from the start of chemotherapy in patients with the Ala/Ala genotype than those with other genotypes. To the best of our knowledge, the positive association between genotype and the onset of toxicity has not been previously reported. OPRT Gly113Ala polymorphism might predict not only the risk of toxicity but also the time of the occurrence of severe toxicity.

In our population with colorectal cancer, the frequency of the Ala allele of OPRT Gly113Ala polymorphism was 27.5%, quite similar to the 26% previously reported in the Japanese healthy population (19). Of note, albeit based on a small number of patients, OPRT Gly113Ala polymorphism statistically correlates with OPRT activity in normal colon mucosa. This result is concordant with the in vitro study (19). The Ala allele was also associated with increased OPRT mRNA level in normal tissue, leading to the hypothesis that this polymorphism could play a role in mRNA stability and translation. The mechanism of induction of gastrointestinal toxicity was reported to be due to the incorporation of F-RNA, not the inhibition of the biosynthesis of dTMP through conversion of 5-FU to dFdUMP (16). Hence, higher OPRT activity, leading to elevate the level of F-RNA in small intestine after the administration of 5-FU, could be reasonably expected to increase the likelihood of severe diarrhea in patients with the Ala allele.

In conclusion, OPRT Gly113Ala polymorphism seems to be a significant predictor of 5-FU-induced toxicity in patients treated with bolus 5-FU adjuvant therapy. OPRT Gly113Ala polymorphism had an independent value only for prediction of severe diarrhea, whereas TYMS promoter polymorphism was an independent factor to predict both severe neutropenia and diarrhea. These findings could generate new questions concerning the correlation of OPRT Gly113Ala and TYMS polymorphisms and the predictive value of combining the information from two polymorphisms. At present, we cannot answer the questions because the current study was a limited retrospective one involving a relatively small number of patients. Prospective translational treatment trials, including larger number of patients, are needed to corroborate our results and resolve these questions.

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References


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