Impact of Solute Carrier Family 29 Member 1 (SLC29A1) Single Nucleotide Polymorphisms on mRNA Expression in Peripheral Blood Mononuclear Cells

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Solute carrier family 29 member 1 (SLC29A1), which is broadly expressed in human tissues, is involved in the cellular uptake of antiviral and anticancer nucleoside analogs, ribavirin (RBV), cytarabine, and gemcitabine. Although several genetic variants have been identified in SLC29A1 gene, it is unclear whether or not the variants are responsible for SLC29A1 activity. Understanding the genetic contribution to transporter activity can lead to individual variation of drug response in antiviral and anticancer treatment including nucleoside analogs.

Several single nucleoside polymorphisms (SNP) of SLC29A1 gene, have been identified so far, though our interest was focused on what were associated with drug response with an allelic frequency of >0.1 in Japanese. Four SNP, rs177C>G, 647T>C, 1171G>A, and 1288G>A, provided amino acid mutations of SLC29A1 and none of them was known to provide change in transporter activity. Frequency of these mutations is less than 0.021 in Asian or Caucasian population. On the other hand, it has been reported that three SNPs, rs6932345, rs747199 and rs760370, existing on intron of SLC29A1 gene, were associated with drug response and mRNA expression with the frequency of >0.1 in Japanese.

Tsubota et al. recently reported that rs6932345 in SLC29A1, a major RBV transporter gene, was a significant genetic factor associated with the outcome of pegylated-interferon (IFN)/RBV combination therapy in patients with mono-infectected hepatitis C virus (HCV). They found that patients with the rs6932345 AA genotype (wild-type) had a higher rate of rapid and sustained virological response and a higher RBV trough concentration than did patients with the AC/CC genotypes (mutation carrier). Another research group also found that rs760370, an SNP highly linked with rs6932345, was associated with rapid virological response in IFN/RBV therapy for patients co-infected with human immunodeficiency virus and HCV. These observations are quite interesting to understand the impact of SLC29A1 activity on IFN/RBV combination therapy, in which SLC29A1 plays an important role in intracellular uptake and gastrointestinal absorption of RBV.

Although wild-type rs6932345 may provide higher activity of SLC29A1 than the mutant type, no data have been available on such an association, including data on mRNA expression.

We, therefore, examined the impacts of the rs6932345 genotype on SLC29A1 mRNA expression in peripheral blood mononuclear cells (PBMCs), one of the targets of HCV. The impact of another SNP rs747199 was also examined, in the upstream region of the translation initiation site of SLC29A1. Because the rs747199 located on the putative transcription factor binding site is known to be associated with SLC29A1 mRNA expression on PBMC. SNP rs760370 was not examined in the present study because it highly linked with rs6932345 at a frequency of 99%.

MATERIALS AND METHODS

Determination of mRNA Expression The study was approved by the ethical committee of University of Tsukuba Hospital, and written informed consent was obtained from all study participants. Whole blood samples (2.5 mL) were collected in PAXgene Blood RNA tubes (QIAGen GmbH, Hilden, Germany) from 46 healthy Japanese individuals (28 men and 18 women). Immediately after the room temperature incubation for two hours, the samples were subjected to PAXgene Blood RNA Kit (QIAGen) to isolate total RNA. It was confirmed that wild-type for rs6932345 and rs747199 showed higher SLC29A1 mRNA expression in PBMCs.

Key words single nucleotide polymorphism; solute carrier family 29A1; mRNA expression; ribavirin

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gene. Since these transcripts variants possess common coding sequence for SLC29A1 protein, we designed primers on NM_004955 (exons 8 to 10) to measure the mRNA expression. The primers for determining SLC29A1 mRNA were 5'-AGA CCA AGT TGG ACC TCTA TTAG GC-3' (forward) and 5'-CAC GGCT TGG AAA CAT CCC AAT-3' (reverse); and for determining ubiquitin C (UBC) mRNA were 5'-ATT TGG TTC-3' (forward) and 5'-GCA GTG GCA CAA ACA CCA-3' (reverse). The mRNA data were normalized to UBC as the reference gene using delta-delta Ct method.\textsuperscript{[13]}

**Genotyping for the SLC29A1** Genomic DNA isolated from PBMC using the QIAamp DNA Mini Kit (QIAGEN) was subjected to genotyping of SLC29A1 rs6932345A>C and rs747199G>C. PCR reaction was performed with the following primers: rs6932345, 5'-CGG AGC CTC ACA GCT GTA TCT C ATG GGT-3' (reverse) and 5'-TGG TGT GGG GAA TGT GTC GCG GTT CTT G-3' (forward) for rs747199, 5'-TGA CTG AGG TCA AAC CAG AGG-3' (forward) and 5'-GAG TGT GGG GAA TGT GTC AGT-3' (reverse). The mRNA data were normalized to UBC as the reference gene using delta-delta Ct method.\textsuperscript{[13]}

**RESULTS AND DISCUSSION**

The allele frequencies for rs6932345A>C and rs747199G>C were 0.163 and 0.152, respectively, which were similar with those in previous reports for East-Asian population\textsuperscript{[5]} and in HapMap project database.\textsuperscript{[8]} The level of SLC29A1 mRNA in the rs6932345 AA genotype (wild-type) was 1.71 times that in the AC/CC genotype (mutation carrier) (\(p<0.05\)) (Table 1). Similar results were observed for rs747199, in which the mRNA level in the wild-type genotype was 1.73 times that in the mutant type (\(p<0.02\)) (Table 1). rs747199 was linked with rs6932345 at a frequency of 84.8%. When rs6932345 and rs747199 were compared in combination in the wild-type and mutant genotypes, the mRNA level in the wild-type genotype was 1.85 times that in the mutant type (\(p<0.01\)) (Table 1).

The present results revealed that wild-type rs6932345 is associated with higher SLC29A1 mRNA expression in PBMC as compared with the mutant genotype. It is confirmed that SLC29A1 mRNA expression showed positive correlation with cellular uptake of RBV in hepatocyte cell lines.\textsuperscript{[4]} SLC29A1 mRNA knockdown reduced RBV uptake level in OR6 cells.\textsuperscript{[15]}

Thus, mRNA expression is responsible for the transport activity of SLC29A1. Since higher mRNA expression in PBMC may produce higher intracellular uptake and gastrointestinal absorption of RBV, Tsutoba’s findings that patients with wild-type rs6932345 possessed higher blood RBV and responded well to IFN/RBV therapy might be relevant.\textsuperscript{[5]} The present results showed that rs747199 is linked with rs6932345 and that it also participated in regulation of SLC29A1 mRNA expression. When compared with rs6932345 alone, genotyping of both rs747199 and rs6932345 showed a clear difference in mRNA expression between the wild-type and mutant genotypes (Table 1), suggesting that rs747199 is another SNP that can permit more sophisticated assessment of the pharmacodynamics of RBV.

In conclusion, we confirmed that wild-type rs6932345 and rs747199 showed higher SLC29A1 mRNA expression in PBMCs. These data support the recent report that major RBV transporter gene SLC29A1 polymorphism influences treatment response. The clinical impact of rs747199 on the outcome of IFN/RBV therapy should be confirmed in future studies.

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