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Common single nucleotide polymorphisms of the *MDR1* gene have no influence on its mRNA expression level of normal kidney cortex and renal cell carcinoma in Japanese nephrectomized patients

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Abstract In this study, we have quantified the mRNA expression levels of multidrug resistance gene 1 (*MDR1*) in the normal kidney cortex and renal cell carcinoma (RCC) segments from 24 Japanese nephrectomized patients by real-time polymerase chain reaction (PCR). The mRNA expression level of *MDR1* in RCC segments was significantly decreased in comparison with each normal segment ($P=0.0042$, by Student's paired t -test). In addition, the ten common single nucleotide polymorphisms (SNPs) of the *MDR1* gene in the patients were assessed using the PCR-restriction enzyme fragment length polymorphism method to investigate the influence of these SNPs on its mRNA expression levels. The allele frequencies of these SNPs were comparable with our previous report in the Japanese recipients of living-donor liver transplantation (Goto et al., Pharmacogenetics 12:451–457; 2002). *MDR1* expression levels in the normal kidney cortex were independent on the five SNPs, which were polymorphic in the Japanese population. Furthermore, the effect of the SNPs on expression levels of *MDR1* mRNA in RCC segments was not recognized. These findings suggest that the common SNPs in the *MDR1* gene have no influence on the expression of its transcript in RCC segments as well as in the normal kidney cortex.

Keywords *MDR1* · SNP · Kidney · Renal cell carcinoma

Introduction

An ATP-driven efflux pump P-glycoprotein (Pgp), the multidrug resistance 1 (*MDR1* or *ABCB1*) gene product, is expressed in the plasma membrane of several tumor and normal cells and mediates extrusion of endogenous compounds, xenobiotics and drugs including anticancer drugs, cardiac glycosides, immunosuppressants, and anthracycline antibiotics (Ambudkar et al. 1999). Besides Pgp one of component molecules for the multidrug resistance of tumor cells, the transporter is now accepted as playing an important role for intestinal absorption, tissue distribution, and biliary or urinary excretion of drugs in normal tissues (Ambudkar et al. 1999).

Single nucleotide polymorphisms (SNPs) exist in the *MDR1* gene, and the relationship between SNPs and the expression/function of *MDR1* has been reported by several laboratories. First, Hoffmeyer et al. (2000) represented that the C3435T SNP in the *MDR1* gene influenced the expression level of Pgp in the duodenum and that the absorption rate of orally administered digoxin was dependent on the SNP in healthy Caucasians. However, following examinations comparing SNPs in the *MDR1* gene with drug absorption and/or the intestinal expression level of Pgp, *MDR1* have not been reproducible (Kim et al. 2001; Kurata et al. 2002; Goto et al. 2002; Gerloff et al. 2002). Recently, Siegmund et al. (2002) demonstrated that the allele T of cDNA 3435 in the *MDR1* gene reduced Pgp expression in the kidney. However, there are few reports illustrating in detail the renal *MDR1* expression with influences by its genetic variations.

Renal malignant tumors account for more than 2% of cancer incidence, and renal cell carcinoma (RCC) compose the majority in renal tumors. RCC shows resistance against chemotherapy (Hartmann and Boke-meyer 1999), and it is suggested that Pgp is involved in the chemoresistance of RCC (Fojo et al. 1987; Kakehi et al. 1988). Siegmund et al. (2002) also suggested that

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the C3435T SNP in the *MDR1* gene would be a risk factor for the development of RCC in Caucasians. In spite of suggesting the significance of Pgp and/or *MDR1* SNPs on the patients with RCC described above, the role of Pgp for chemoresistance of RCC has remained to be elucidated.

In the present study, we quantified the mRNA levels of *MDR1* in the normal kidney cortex and RCC segments from Japanese nephrectomized patients with renal tumors. In addition, the ten common SNPs of the *MDR1* gene in these patients were examined, and the association between genotypes and expression level was assessed.

Materials and methods

Patients

Twenty-four Japanese patients who were surgically nephrectomized with RCC at Kyoto University Hospital were enrolled in this study. The subjects consisted of 18 men and six women whose ages ranged from 39 to 74 (63.6 ± 8.5 , mean $\pm$ SD) years. The nephrectomized segments of the normal cortex and RCC were obtained after receiving written informed consent. The study was performed in accordance with the Declaration of Helsinki and its amendments, and was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine.

Isolation of total RNA and genomic DNA

Total RNA and genomic DNA from a renal homogenate in a guanidinium thiocyanate solution were isolated with a MagNA Pure LC RNA isolation kit II and DNA isolation kit (Roche Diagnostic GmbH, Mannheim, Germany), respectively, as previously described (Goto et al. 2002; Motohashi et al. 2002). The isolated total RNA was reverse-transcribed, and the single stranded DNA was used for quantification of *MDR1* mRNA levels. Genotyping of the *MDR1* gene was performed using genomic DNA.

Quantification of *MDR1* mRNA expression levels

MDR1 mRNA levels in the normal kidney cortex and RCC segments were measured by real-time polymerase chain reaction (PCR), as previously described (Motohashi et al. 2002). The primer/probe set for the specific amplification of *MDR1* was designed according to parameters incorporated in the Primer Express software (PE Biosystems, Foster City, CA, USA). The forward and reverse primers were GCTCAGACAGGATGTGAGTTGGT (position; 2812–2834, accession number M14758 in GenBank database) and CCTGGAACCTATAGCCCCTTAAAC (position; 2897–2920), respectively. The sequence of TaqMan probe was AAAAACACCACTGGAGCATTGACTACCAGG, corresponding to the position 2846–2875. Real-time PCR was performed in a total volume of 20 μ l containing 2 μ l of reverse-transcribed cDNA, 1 μ M forward and reverse primers, 0.2 μ M TaqMan probe, and 10 μ l TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The PCR condition was as follows: 50 cycles of 94°C for 15 s and 60°C for 60 s. The copy number of the target mRNA sequence in the starting materials was established by determining the fractional PCR threshold cycle number (Ct) at which a fluorescence signal generated during the replication process passed above a threshold value. The initial amount of target mRNA in each sample was estimated from the Ct value with a standard curve generated using known amounts of standard

plasmid DNA. Glyceraldehyde-3-phosphate dehydrogenase mRNA was also quantified as an internal control with glyceraldehyde-3-phosphate dehydrogenase control reagent (Applied Biosystems).

Genotyping of the *MDR1* gene

The genotype of the *MDR1* gene was investigated by PCR-restriction enzyme fragment length polymorphism methods. The specific primers and restriction enzymes used in this study were as previously described (Goto et al. 2002). The PCR conditions were as follows: after denaturing at 94°C for 3 min, the PCR was performed with 1 μ M of each primer and Taq DNA polymerase (Takara, Shiga, Japan), according to the following profile: 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, 35 cycles, following by a single additional 10-min extension at 72°C. The PCR products were digested with or without restriction enzymes and separated on 3.5% agarose gel.

Statistical analysis

The difference in the logarithmically transformed data of *MDR1* mRNA expressions between normal cortex and RCC was analyzed using Student's paired *t*-test. The correlation between the *MDR1* genotype and its mRNA expression was analyzed using the Mann-Whitney *U* test. *P* values < 0.05 were considered to be significant.

Results

Quantification of *MDR1* mRNA in normal kidney cortex and renal cell carcinoma

Figure 1 shows distribution histograms of logarithmically transformed mRNA levels of *MDR1* in the normal kidney cortex and RCC segments. The average mRNA expression levels of *MDR1* in the normal kidney cortex and RCC segments were 19.0 and 6.6 amol/ μ g total RNA, respectively. A statistical significant difference of the *MDR1* mRNA levels in normal kidney cortex and RCC segments of each patient was observed ($P = 0.0042$, Fig. 2).

Genotype frequency of ten common SNPs of the *MDR1* gene in nephrectomized patients

The genotype of the *MDR1* gene from the normal kidney cortex and RCC segments was assessed at ten common nucleotide positions in 24 Japanese patients. No differences in *MDR1* genotypes from both segments of each patient were found. Frequencies of the genetic variants are summarized in Table 1. In this study, variants at exon 2–1, cDNA 61, cDNA 307, cDNA 1199, and exon 12 + 44 were not observed. A variant leading to amino acid exchange was observed only at cDNA 2677. The allele frequency of the cDNA 2677 was 45.8% for allele T and 18.8% for allele A. At cDNA 1236 and 3435, which do not influence amino acid substitute, genetic variants were recognized. Allele frequencies were 66.7% for allele T at cDNA 1236 and 52.1% for allele T at cDNA 3435. A complete linkage was observed

Fig. 1A, B Distribution histograms of MDR1 mRNA expression levels in normal kidney cortex (A) and renal cell carcinoma (B). MDR1 mRNA levels were logarithmically transformed to improve normality. The arrows indicate the mean values of MDR1 mRNA expression levels

Fig. 2 Effect of malignant transformation on renal MDR1 mRNA levels in 24 patients with renal cell carcinoma

between cDNA 1236 C/T and an intronic variant of exon 6+139 C/T. Another intronic variant at exon 17–76 was determined with the frequency of 31.3% for allele A. These allele frequencies were comparable with our previous reports in Japanese recipients of living-donor liver transplantation.

Correlation between *MDR1* SNPs and MDR1 mRNA expression in normal kidney cortex and renal cell carcinoma

Next, we examined the association between the *MDR1* gene and its mRNA levels in the normal kidney cortex and RCC. As shown in Fig. 3, the significant effects of each SNP on MDR1 mRNA expression in the normal kidney cortex and RCC segments were not recognized. However, the MDR1 mRNA levels in the kidney were significantly reduced by transformation to RCC with C/T of cDNA 1236 and exon 6+139, T/A and T/T of exon 17–76, G/T of cDNA 2677, and C/T at cDNA 3435.

Discussion

In the present study, MDR1 mRNA levels in the human kidney cortex were quantified using the real-time PCR method. We previously examined its mRNA expression levels in the small intestine in Japanese recipients of living-donor liver transplantation. The MDR1 mRNA levels in the kidney cortex were about 40-fold higher than those in the intestinal mucosa (Goto et al. 2002; Hashida et al. 2001). Even if the mucosal samples derived from patients in end-stage liver failure were considered, the MDR1 mRNA contents in the kidney cortex were markedly higher compared to the intestine. To our knowledge, this is the first study of the quantification of MDR1 mRNA level in the human kidney cortex.

It is generally accepted that the majority of administered digoxin, a cardiac glycoside, is excreted into urine and that Pgp plays an important role for its tubular secretion (Tanigawara et al. 1992). In addition, concomitant administration with various drugs, including cyclosporin A, calcium channel blockers, macrolide antibiotics, and azole antifungal drugs elevates serum concentration of digoxin, at least in part, via renal tubular Pgp (Rodin and Johnson 1988; Wakasugi et al. 1998). Considering these pharmacokinetic significances of renal Pgp, the expression levels and activity of Pgp in the kidney would be a key factor for the optimal dosage regimen of digoxin.

The present findings represent a wide interindividual variation of MDR1 mRNA levels in the human kidney cortex (about 140-fold), and no relationship between the ten common SNPs of the *MDR1* gene and its mRNA expression levels. It was reported that the interindividual variation of MDR1 expression also existed in the human small intestine and liver, suggesting that expression variation has been responsible for interindividual

Table 1 *MDR1* genetic variants in 24 Japanese nephrectomized patients

Location	Position	Allele	Effect	Frequency	Genotype	Frequency	Goto et al. ^a
Intron 1	Exon 2-1	G	Initiation of translation?	100	G/G	100	100
		A		0	G/A	0	0
Exon 2	cDNA 61	A	21 Asn	100	A/A	100	100
		G	21 Asp	0	A/G	0	0
Exon 5	cDNA 307	T	103 Phe	100	T/T	100	100
		C	103 Leu	0	T/C	0	0
Intron 6	Exon 6 + 139	C	?	33.3	C/C	8.3	10.3
		T		66.7	C/T	50	52.9
Exon 11	cDNA 1199	G	400 Ser	100	T/T	41.7	36.8
		A	400 Asn	0	G/G	100	100
Exon 12	cDNA 1236	C	Wobble	33.3	G/A	0	0
		T		66.7	A/A	0	0
Intron 12	Exon 12 + 44	C	?	100	C/C	8.3	10.1
		T		0	C/T	50	49.3
Intron 16	Exon 17-76	T	?	68.8	T/T	41.7	40.6
		A		31.3	C/C	100	100
Exon 21	cDNA 2677	G	893 Ala	35.4	C/T	0	0
		T	893 Ser	45.8	T/T	0	0
Exon 26	cDNA 3435	A	893 Thr	18.8	T/A	45.8	51.5
		C	Wobble	47.9	A/A	8.3	5.9
		T		52.1	G/G	0	22.9
					G/A	29.2	5.8
					G/T	41.7	39.1
					T/A	8.3	15.9
					T/T	20.8	15.9
					A/A	0	1.4
					C/C	25	30.4
					C/T	45.8	50.7
					T/T	29.2	18.8

^aThese values are from our previous study with Japanese recipients of living-donor liver transplantation

variation of drug absorption and disposition (Goto et al. 2002; Hashida et al. 2001; Schuetz et al. 1995). A nuclear receptor, pregnane X receptor (termed as steroid and xenobiotic receptor), predominantly expressed in the liver and small intestine, was reported to regulate *MDR1* expression in these tissues as a part of the regulatory mechanisms by various compounds including endogenous steroids and xenobiotics (Synold et al. 2001). Therefore, the nuclear receptor may contribute, at least in part, to the large interindividual variability of the expression level of *MDR1* in the liver and small intestine. However, there is no information predicting the expression regulation of renal Pgp. The elucidation of the nuclear receptors and SNPs in the transcriptional regulatory region of the *MDR1* gene would clarify interindividual variation of renal Pgp content.

During the last few decades, the incidence of RCC has steadily increased (Chow et al. 1999). Obesity, hypertension, gender, smoking, and several drugs such as diuretics, phenacetin, and aspirin are suggested to be associated with RCC (Dhote et al. 2000). Furthermore, various genetic polymorphisms were also reported to be related to the disease (Nakamura et al. 2002; Tanaka et al. 2002). Recently, Siegmund et al. (2002) reported that the frequency of T/T genotype at *MDR1* cDNA 3435 was significantly higher in patients with RCC than in the control Caucasians, suggesting that this SNP

would be a risk factor for RCC in Caucasian. In this study, the T/T was observed in seven of 24 RCC patients (29.2%). Since this frequency is not significantly different from healthy Japanese (20%) in the data reported by Schaeffeler et al. (2001) with χ^2 statistics ($P > 0.540$), the T/T genotype at *MDR1* cDNA 3435 might not be a risk factor for RCC in Japanese. Further research is needed to elucidate the association between the cDNA 3435 SNP and RCC in Japanese. Chow et al. (1999) reported the incidence of RCC in black subjects was higher than in Caucasian subjects. On the other hand, the frequency of the T/T genotype at the cDNA 3435 is reported to be markedly lower in black subjects than in Caucasian and Japanese subjects (Schaeffeler et al. 2001). The T/T frequency in black subjects with RCC should be interested. Siegmund et al. (2002) also represented that Pgp expression levels in renal noncancerous segments were significantly lower with the T/T genotype at cDNA 3435 than with the C/C genotype by using the quantitative immunohistochemistry method. This suggests that renal Pgp expression levels influence susceptibility to the development of renal epithelial cancers. Our work illustrates that *MDR1* levels were not affected by the cDNA 3435 T/T genotype. In the future, not only to determine the predominant factor(s)/material(s) developing RCC but also to clarify its renal handling, including the contribution of Pgp, are necessary for

Fig. 3 Comparison of mRNA levels in the normal kidney cortex, renal cell carcinoma (RCC) segments, or their variation by carcinogenesis with the *MDR1* genotype. The *MDR1* mRNA levels in the normal kidney cortex (open box) and RCC segments (gray box) were compared with the five polymorphic SNPs. Simultaneously, the variation of the expression levels of *MDR1* mRNA were assessed in comparison with the *MDR1* genotypes between normal and RCC segments. After statistical analyses by Mann-Whitney *U* test, *P* values less than 0.05 were shown

analysis of the relationship between RCC and the SNP of the *MDR1* gene.

RCC displays an intrinsically high degree of resistance to chemotherapy (Hartmann and Bokemeyer 1999). Fojo et al. (1987) and Kakehi et al. (1988) represented that the resistance of RCC against anticancer drug vinblastine was associated with *MDR1* expression using cell lines. The present study clarified that *MDR1* expression levels showed a downward tendency by malignant transformation to RCC and that expression in the RCC was still relatively high. These results are compatible with other groups (Fojo et al. 1987; Kakehi et al. 1988). Taken together with the fact that various anticancer drugs are substrates of Pgp, it is suggested that this phenomenon is, at least in part, involved in the chemoresistance of RCC. The downregulation of *MDR1* mRNA in the kidney cortex by transformation to RCC was dependent on the T allele at exon 17–76

(Fig. 3). Despite the small number of patients carrying the A/A genotype at exon 17–76 ($n=2$), the mRNA expression of *MDR1* in the kidney might be downregulated by carcinogenesis in patients with the T allele. Therefore, further studies would clarify the effect of SNPs in the *MDR1* gene on the reducing rate of its mRNA level in renal tissue by carcinogenesis. In addition to *MDR1*, glutathione S-transferase, topoisomerase II, and *MDR*-associated protein are suggested to contribute to the chemoresistance of RCC (Volm et al. 1993; Kim et al. 1996). Elucidation of not only renal handling of anticancer agents but also function and expression levels of these proteins in RCC, and of their relations with SNPs, give information for the adequate selection of anticancer agents for individual RCC.

In summary, we estimated the copy number of *MDR1* mRNA in the human kidney cortex and RCC and found that there is a wide interindividual variation in renal *MDR1* expression levels and that *MDR1* mRNA levels tend to decrease by malignant transformation to RCC in the human kidney. In addition, the ten common polymorphisms of the *MDR1* gene were examined, and the effect of SNPs on expression levels of the transporter mRNA was not observed in the normal kidney cortex and RCC. To our knowledge, this is the first report representing the measurement of renal *MDR1* mRNA and correlation of the expression and SNPs of *MDR1*.

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