C421A Polymorphism in the Human Breast Cancer Resistance Protein Gene Is Associated with Low Expression of Q141K Protein and Low-Level Drug Resistance[1]

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Abstract

Breast cancer resistance protein (BCRP) confers multidrug resistance to cancer cells against agents such as SN-38 (an active metabolite of irinotecan), mitoxantrone, and topotecan. Among 59 human tumor cell lines tested, 6 cell lines, A549, NCI-H460, KM-12, HT-29, OVCAR-5, and RPMI8226, showed high BCRP expression. *BCRP* cDNA was isolated from 11 cancer cell lines and three variant cDNAs [G34A substituting Met for Val-12 (V12M), C421A substituting Lys for Gln-141 (Q141K), and 944–949 deletion lacking Ala-315 and Thr-316 (∆315-6)] were identified. G34A and C421A variants were polymorphisms, and 944–949 deletion was a splicing variant. C421A *BCRP*-transfected PA317 cells showed markedly decreased protein expression and low-level drug resistance compared with wild-type *BCRP*-transfected cells when transfectants expressed similar levels of *BCRP* mRNA. G34A or 944–949-deleted *BCRP*-transfected PA317 cells showed similar or somewhat lower protein expression and drug resistance compared with wild-type *BCRP*-transfected cells. Of 124 healthy Japanese volunteers, 67 were wild-type, 48 were heterozygous, and 9 were homozygous for the C421A allele. These results suggest that some people possess the C421A polymorphic *BCRP* gene and express low amounts of Q141K BCRP. In addition to that, C376T polymorphism in exon 4 substituting stop codon for Gln-126 was found in 3 of the 124 general Japanese population. This C376T polymorphism may also have high impact because active BCRP protein will not be expressed from the C376T allele. Therefore, people with C376T

and/or C421A polymorphisms may express low amounts of BCRP, and this low BCRP expression might result in hypersensitivity of normal cells to such anticancer drugs as irinotecan and mitoxantrone.

Introduction

ABC[3] transporters, such as *MDR1* gene product P-glycoprotein and MRP1, are involved in multidrug resistance by pumping out a variety of antitumor drugs from cells (1–3). Expressions of these transporters in cancer cells are important determinants for the efficacy of anticancer agents. BCRP, the second member of the G family of ABC transporters (ABCG2) and also called ABCP or MXR, is one such ABC transporter, mediating resistance to SN-38 (an active metabolite of irinotecan), mitoxantrone, and topotecan concurrently (4–8). Our recent study suggested that BCRP mediates the transport of estrogens from the placenta to a mother's body (9). In addition to that, BCRP is presumed to play a protective role against toxic substances and metabolites in the mother-placenta barrier, the digestive tract, and the blood-testis barrier.

Identification of single nucleotide polymorphisms has become important work because single nucleotide polymorphisms in various genes might not only be simple genomic markers but may also have certain significance in the expression and/or function of their product proteins. For example, C3435T polymorphism in exon 26 of the *MDR1* gene was shown to be closely associated with low expression levels of P-glycoprotein and high plasma digoxin levels (10).

This study aimed to investigate whether variation of the first structure of the *BCRP* gene present in nondrug-treated cell lines and a normal population might influence the expression and function of the protein. First, we screened BCRP expression in a panel of 59 cancer cell lines in the anticancer drug screening program of the NCI (Bethesda, MD). Next, the whole coding sequence of *BCRP* cDNA was determined in 11 cell lines, 5 of which highly expressed BCRP protein. We identified three variant *BCRP* cDNAs, G34A, C421A, and 944–949 deletion, and investigated functional outcomes. Incidences of G34A and C421A polymorphisms were examined in healthy Japanese volunteers. We report that C421A polymorphism is very frequent in the general Japanese population and may be associated with decreased protein expression and low-level drug resistance.

[3] The abbreviations used are: ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; NCI, National Cancer Institute; RT-PCR, reverse transcription-PCR.

Materials and Methods

Cell Lines and Blood Samples. Murine fibroblast PA317 cells were grown in Dulbecco's modified essential medium supplemented with 10% fetal bovine serum at 37°C with 5% CO_2. Peripheral blood nucleated cells were obtained from healthy Japanese volunteers after obtaining written informed consent for genetic analysis.

Western Blot Analysis. Frozen cell pellets of 59 cell lines in the NCI anticancer drug screening were obtained from the NCI (11). Cell pellets for MDA-N in the 60 cell line panel were not available. Western blot analysis of BCRP was performed as described previously (12). Briefly, cell pellets were solubilized in a lysis buffer [10 mM Tris-HCl (pH 8.0), 0.1% Triton X-100, 10 mM $MgSO_4$, 2 mM $CaCl_2$, 1 mM 4-(2-aminoethyl)-benzenesulfonylfluoride with or without 1 mM DTT]. Cell lysate was solubilized with 2% SDS, 50 mM Tris-HCl (pH 7.5), in the presence or absence of 5% 2-mercaptoethanol, and resolved by SDS-PAGE. After electrophoresis, proteins were transferred onto nitrocellulose membranes and incubated with an anti-BCRP polyclonal antibody (12). Then, the blots were incubated with a peroxidase-conjugated donkey anti-rabbit secondary antibody (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom), and membrane-bound peroxidase was visualized using ECL Plus chemiluminescence detection kit (Amersham).

Sequence Analysis of the *BCRP* Gene. The entire coding region of *BCRP* cDNA was generated by RT-PCR from total RNA by using RNA LA PCR kit (Takara, Kyoto, Japan) according to the manufacturer's instructions. The primer set used was 5′-CGGATCCTCCTGAGATCCTGAGCCTTTG-GTT-3′ and 5′-CGCTCTAGAGATGGCAAGGGAACAGAAA-ACAACA-3′. PCR products were inserted into pCR2.1 TA cloning vector (Invitrogen, Carlsbad, CA), and three to eight clones were sequenced with ABI Prism377 automatic sequencer (Applied Biosystems, Foster City, CA). Mutations observed in more than two clones were subjected to analysis of other clones derived from an independent RT-PCR product to exclude PCR-induced mutation.

Next, the exon-intron structure of the *BCRP* gene was determined by aligning the complete *ABCP* cDNA coding sequence (GenBank accession no. AF103796) and *Homo sapiens* BAC clone RP11-368G2 from 4 (GenBank accession no. AC084732). The genomic structure of the *BCRP* gene that we determined was the same as reported in the literature (13). Exon 2 that covers the 34th nucleotide of *BCRP* cDNA was amplified by PCR with the primer set 5′-GCAATCT-CATTTATCTGGACTA-3′ and 5′-TGTGAGGTTCACTGTAG-GTAAA-3′. Exon 5 that covers the 421st nucleotide of *BCRP* cDNA was amplified by PCR with the primer set 5′-CCT-TAGTTATGTTATCTTTGTG-3′ and 5′-GAAACTTCTGAAT-CAGAGTCAT-3′. Exon 9 that includes the 944–949th nucleotides at the 5′-end was amplified with the primer set 5′-TTAGGGAAGCATCCAAGAAAG-3′ and 5′-GAAGCAGATG-ATAACAGAACC-3′. PCR products were either sequenced after TA subcloning or directly sequenced as described.

Establishment of Mutant BCRP-expressing Cells. G34A, C421A, and 944–949-deleted *BCRP* cDNAs without any other mutations were inserted into a pHaL-IRES-DHFR bicistronic retrovirus vector plasmid. PA317 cells were transfected with those vectors by using a Mammalian Transfection kit (Stratagene, La Jolla, CA) and selected by exposure to 120 ng/ml methotrexate. Hundreds of drug-resistant colonies were pooled and used as *BCRP*-transfected cells. PA317 cells transfected with wild-type, G34A, C421A, and 944–949-deleted *BCRP* cDNAs were designated PA/WT, PA/V12M, PA/Q141K, and PA/Δ315-6 cells, respectively.

Northern Blot Analysis. Twenty μg of total RNA was fractionated on a 1% agarose-formaldehyde gel and transferred to Hybond-N+ (Amersham). The blot was hybridized with a 456-bp fragment from 574th to 1029th nucleotides of *BCRP* cDNA by using AlkPhos Direct Labeling kit according to the manufacturer's instructions (Amersham). Chemiluminescence signal generation and detection were performed with CDP-Star detection reagent according to the manufacturer's instructions (Amersham).

Growth Inhibition Assay. The sensitivity of cells to SN-38, mitoxantrone, and topotecan was evaluated by measuring cell growth inhibition after incubation of cells at 37°C for 5 days in the absence or presence of various concentrations of anticancer agents. Cell numbers were determined with a Coulter counter. IC_{50}s (drug dose causing 50% inhibition of cell growth) were determined from growth inhibition curves, and the degrees of resistance were calculated by dividing IC_{50}s of *BCRP*-transfected cells by those of parental PA317 cells.

Measurement of Intracellular Topotecan Uptake. The effect of mutant BCRP on cellular accumulation of topotecan was determined by flow cytometry. Cells (5×10^5) were incubated with 30 μM topotecan for 30 min at 37°C, washed in ice-cold PBS, and subjected to fluorescence analysis using FACSCalibur (Becton-Dickinson, San Jose, CA).

Results

Western Blot Analysis of Tumor Cell Lines. BCRP expression in 59 human tumor cell lines was examined by Western blotting in the absence of reducing agents. Under this condition, BCRP migrated as a homodimer and was detected as a M_r 140,000 band (12). Of the 59 cell lines analyzed, 6 cell lines (lung cancer, A549 and NCI-H460; colon cancer, HT-29 and KM-12; ovarian cancer, OVCAR-5; and leukemia, RPMI8226) showed considerably high BCRP expression (Fig. 1). The other 53 cell lines showed marginal or no BCRP expression. Western blotting of representative 6 cell lines with low or no BCRP expression was also shown in Fig. 1.

Isolation of *BCRP* cDNA Variants. To identify *BCRP* cDNA variants and to examine the possible effect of the mutations on BCRP expression and function, *BCRP* cDNA clones were isolated from 11 human tumor cell lines, breast cancer MCF-7 and MDA-MB-231, lung cancer A549, NCI-H23, and NCI-H460, colon cancer HCT-116, HT-29, and KM-12, and ovarian cancer OVCAR-5, OVCAR-8, and SK-OV-3. *BCRP* cDNA could not be obtained from NCI-H226 cells that did not express BCRP protein. mRNA expression in MCF-7, MDA-MB-231, NCI-H23, HCT-116, OVCAR-8, and SK-OV-3 cells was very low as determined by RT-PCR, in accordance with very low or undetectable BCRP protein expression by Western blot analysis (Fig. 1). In cDNA sequence analysis, C421A mutation that substitutes Lys for
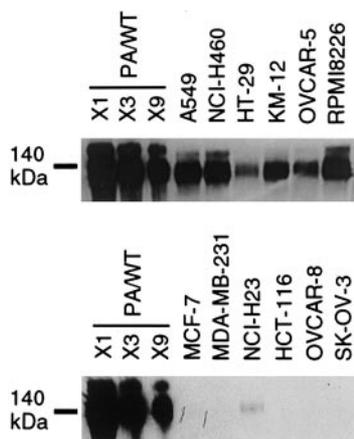
Fig. 1. Western blot analysis of BCRP expression in 12 tumor cell lines of the NCI anticancer drug screening. Western blot analysis was performed under a nonreducing condition, so that the dimeric form BCRP was observed as a band at ~140 kDa. A serial dilution of PA/WT cells (PA317 cells transfected with wild-type *BCRP* cDNA) were used as a positive control. Six cell lines in the *top lane* showed considerably high BCRP expression.

Table 1 *BCRP* cDNA variants identified in this study

Variant	Amino acid change	Cell line
G34A	Val-12 to Met	MCF-7[a]
C421A	Gln-141 to Lys	MDA-MB-231[a]
		A549[a]
		HCT-116[a]
Deletion of 944–949	Deletion of Ala-315 and Thr-316	MCF-7
		A549
		HT-29
		SK-OV-3

[a] Heterozygous allele.

Gln-141 was found in MDA-MB-231, A549, and HCT-116 cells (Table 1; Fig. 2). G34A mutation that substitutes Met for Val-12 was found in MCF-7 cells. Deletion of 944–949 resulting in the loss of Ala-316 and Thr-316 was noted in MCF-7, A549, HT-29, and SK-OV-3 cells (Table 1).

Next, the above-mentioned mutations/polymorphisms were confirmed by genomic DNA analysis. Aligning *ABCP* cDNA sequence and *Homo sapiens* BAC clone RP11-368G2 from 4 demonstrated that the 34th and 421st nucleotides were in exons 2 and 5, respectively. Nucleotides 944–949 were at the 5′-end of exon 9, after the splicing acceptor site. By genomic DNA analysis, G34A polymorphism in exon 2 was observed in MCF-7 cells. C421A polymorphism in exon 5 was noted in MDA-MB-231, A549, and HCT-116 cells (Fig. 2*C*). Deletion of nucleotides 944–949 was not observed with genomic analysis, suggesting that the 944–949-deleted mRNA is a splicing variant.

Western and Northern Blot Analyses of Mutant BCRP-expressing Cells. Western blotting of mutant *BCRP*-transfected PA317 cells demonstrated markedly low expression of Q141K BCRP in PA/Q141K cells compared with other *BCRP*-transfected cells. PA/WT, PA/V12M, and PA/Δ315-6 cells showed similar BCRP expression (Fig. 3*A*). The results
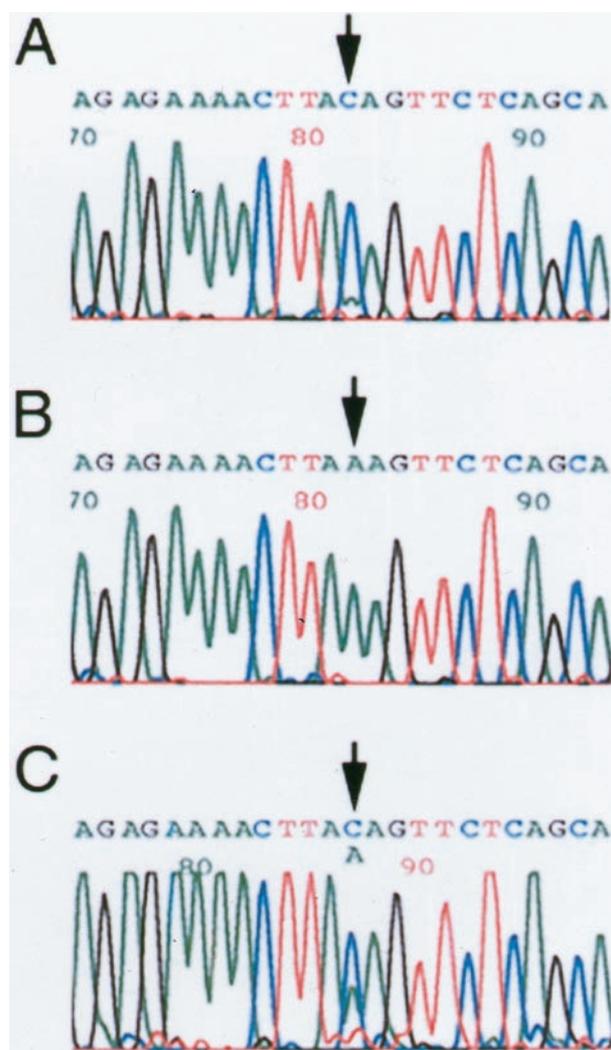

Fig. 2. Identification of C421A polymorphism in A549 cells. *A*, wild-type *BCRP* cDNA. The entire coding region of *BCRP* mRNA was amplified by RT-PCR, subcloned, and sequenced. The 421st base is cytosine (*arrow*). *B*, C421A *BCRP* cDNA. The 421st base was changed from cytosine to adenine (*arrow*) in five of eight clones analyzed. *C*, direct genomic sequencing of the 421st nucleotide. The mutation was confirmed by genomic DNA analysis. Exon 5 covering the 421st nucleotide was amplified by PCR and directly sequenced, demonstrating both the wild-type and C421A alleles (*arrow*).

were confirmed by a second, independent transfection experiment of PA317 cells. BCRP expression levels in the transfected cell lines were stable for 4 months without any drug selection. In contrast, Northern blotting demonstrated similar levels of *BCRP* mRNA in PA/WT, PA/V12M, PA/Q141K, and PA/Δ315-6 cells (Fig. 3*B*). These results suggest that Q141K BCRP is unstable when expressed in mammalian cells.

Growth Inhibition Assay of Mutant BCRP-expressing Cells. PA/WT cells showed 40-fold higher resistance to SN-38 and 10-fold higher resistance to mitoxantrone compared with parental PA317 cells (Table 2; Fig. 4). PA/WT cells also showed a greater than 10-fold resistance to topotecan
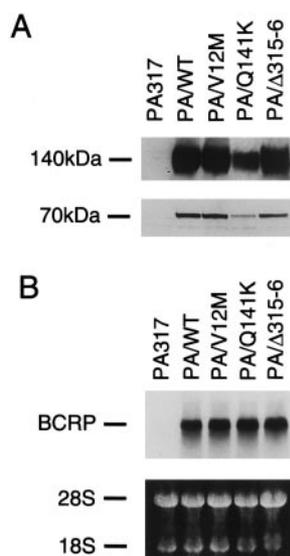
Fig. 3. Expression analysis of mutant *BCRP* transfectants. PA317 cells transfected with wild-type, G34A, C421A, and 944–949-deleted *BCRP* cDNAs were termed PA/WT, PA/V12M, PA/Q141K, and PA/Δ315-6, respectively. *A*, Western blotting of PA317 cells transfected with mutant *BCRP* cDNA in pHaL-IRES-DHFR plasmid. Twenty μg of protein was loaded in each lane. *Top panel*, Western blot analysis processed under a nonreducing condition. BCRP dimer was detected as a band at ~140 kDa. *Bottom panel*, Western blot analysis processed under a reducing condition. BCRP monomer was detected as a band at ~70 kDa. *B, top panel*, Northern blot analysis of PA317 cells and *BCRP* transfectants. Twenty μg of total RNA was loaded in each lane of the agarose-formaldehyde gel and transferred to Hybond-N+. The blot was hybridized with a *BCRP* cDNA probe. *Bottom panel*, ethidium bromide staining of total RNA after electrophoresis. 28S and 18S indicate 28S and 18S ribosomal RNA, respectively.

Table 2 $IC_{50}{}^a$ (ng/ml) of *BCRP*-transfected PA317 cells

	PA317	PA/WT	PA/V12M	PA/Q141K	PA/Δ315-6
SN-38	2.5	98	98	30	55
Mitoxantrone	0.060	0.58	0.63	0.25	0.42
Topotecan	17	>200	>200	100	190

^{a} IC_{50}s (drug dose causing 50% inhibition of cell growth) were determined from cell growth curves in each experiment.

(Table 2; Fig. 4). PA/WT and PA/V12M cells showed similar levels of resistance to the anticancer drugs (Table 2; Fig. 4*A*). PA/Δ315–6 cells were marginally more sensitive to the drugs than PA/WT cells (Table 2; Fig. 4*B*). In contrast, PA/Q141K cells showed a 12-fold greater resistance to SN-38 and a 4-fold greater resistance to mitoxantrone (Table 2; Fig. 4*A*). This means that PA/Q141K cells are 2–3 times more sensitive to these drugs compared with PA/WT cells. These results support the low expression of BCRP in PA/Q141K cells. Cross-resistance patterns of the transfectants were similar, suggesting that these polymorphisms/deletions did not affect substrate recognition of BCRP.

Measurement of Intracellular Topotecan Uptake. When PA317 cells were incubated with topotecan, a fluorescence peak shifted to the right in parental PA317 cells, indicating cellular uptake of topotecan (Fig. 5). The mean fluorescence channel number of PA317 cells increased 4.6-
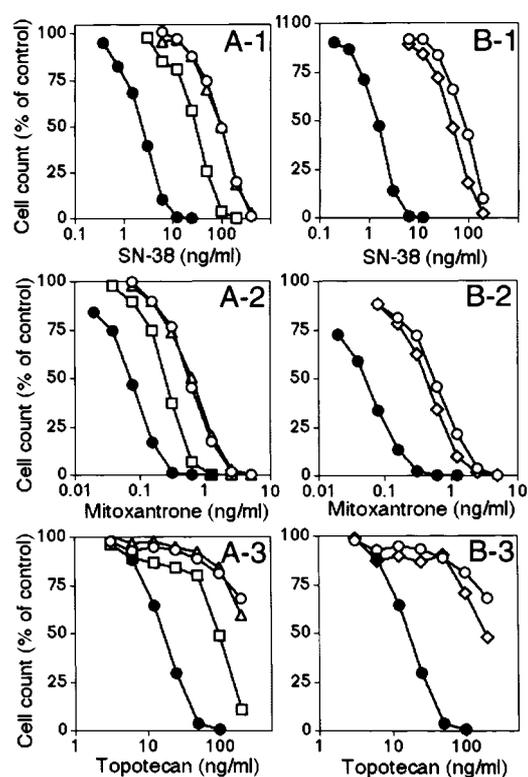

Fig. 4. Growth inhibition assay of mutant *BCRP*-transfected PA317 cells. PA317 cells transfected with wild-type, G34A, C421A, and 944–949-deleted *BCRP* cDNAs were termed PA/WT, PA/V12M, PA/Q141K, and PA/Δ315-6, respectively. *A*, sensitivity to SN-38 (*A-1*), mitoxantrone (*A-2*), and topotecan (*A-3*) of PA317, PA/WT, PA/V12M, and PA/Q141K cells. *B*, sensitivity to SN-38 (*B-1*), mitoxantrone (*B-2*), and topotecan (*B-3*) of PA317, PA/WT, and PA/Δ315-6 cells. ●, PA317; ○, PA/WT; △, PA/V12M; □, PA/Q141K; ◇, PA/Δ315-6.

fold in the presence of topotecan. In contrast, only a marginal shift occurred in PA/WT, PA/V12M, and PA/Δ315-6 cells (Fig. 5). Increases of mean fluorescence channel number in PA/WT, PA/V12M, and PA/Δ315-6 cells were 1.5-, 1.6-, and 1.5-fold in the presence of topotecan, respectively. There was a stronger peak shift to the right in PA/Q141K cells than PA/WT cells, showing that topotecan uptake in PA/Q141K cells was higher than that in PA/WT cells, and the increase of mean fluorescence channel number in PA/Q141K cells was 2.1-fold in the presence of topotecan (Fig. 5). This suggests that the topotecan efflux activity of exogenous BCRP is low in PA/Q141K cells.

Frequencies of G34A and C421A Polymorphisms. Frequencies of G34A and C421A polymorphisms in the *BCRP* gene were examined in a normal (noncancer) Japanese population. Twenty-nine samples were used in the first screening, and because of the possible importance of C421A polymorphism, an additional 95 samples were used only for the allele. The results are summarized in Table 3. Of 124 samples examined, 67 were wild-type, 48 were heterozygous, and 9 were homozygous for the C421A allele. C421A polymorphism exhibited high frequency in this normal Japanese population.
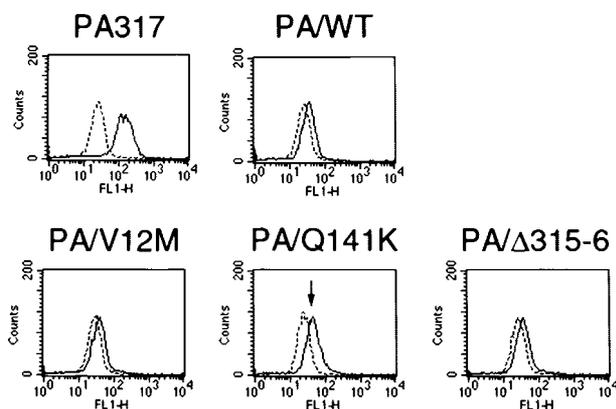
Fig. 5. Intracellular topotecan uptake of *BCRP*-transfected PA317 cells. PA317 cells transfected with wild-type, G34A, C421A, and 944–949-deleted *BCRP* cDNAs were termed PA/WT, PA/V12M, PA/Q141K, and PA/Δ315-6, respectively. Cells were incubated for 30 min with (———) or without (······) 30 μM topotecan, washed, and topotecan uptake was measured by FACS. In parental PA317 cells, a fluorescence peak shift to the right after the incubation with topotecan indicates cellular uptake of topotecan, whereas only marginal shifts occurred in PA/WT, PA/V12M, and PA/Δ315-6 cells. There was a stronger fluorescence peak shift to the right in PA/Q141K cells than the other transfectants (*arrow*).

Table 3 Frequencies of G34A, C376T, and C421A polymorphisms of the *BCRP* gene in the general Japanese population

	Wild-type	Heterozygous	Homozygous	Total
G34A	19 (66%)	9 (31%)	1 (3%)	29
C376T[a]	121 (98%)	3 (2%)	0 (0%)	124
C421A	67 (54%)	48 (39%)	9 (7%)	124

[a] C376T polymorphism in exon 4 substitutes stop codon for Gln-126.

In our subsequent genomic DNA analysis, C376T polymorphism in exon 4 substituting stop codon for Gln-126 was found in 3 (2.4%) of the 124 general Japanese population (Table 3). The 3 were all heterozygous for the C376T allele. This C376T polymorphism may have higher impact than C421A polymorphism because active BCRP protein will not be expressed from the C376T allele.

Discussion

In this study, we first examined BCRP expression by Western blotting in a panel of 59 cell lines in the NCI anticancer drug screening. Among these 59 cell lines, considerably high expression of BCRP was noted in 6 cell lines (10%), consisting of lung cancer, colon cancer, ovarian cancer, and leukemia cell lines, showing that BCRP was highly expressed in a variety of cancer cells without drug selection. BCRP is physiologically expressed in the placenta, digestive tract, genitalia, and hematopoietic stem cells (5, 14). Our result suggests that BCRP expression in cancer cells occurs irrespective of their primary origins and may be responsible for natural drug resistance of cancer cells.

In this study, we identified three *BCRP* cDNA variants. G34A and C421A are polymorphisms because they were observed with high frequencies in the general Japanese population. Nucleotides 944–949 followed the splicing acceptor site of exon 9, and genomic analysis did not reveal such deletion mutations. Therefore, the deletion of nucleotides 944–949 was considered to be a splicing variant between exons 8 and 9. This splicing variant mRNA is also expressed in normal individuals because the deleted cDNA has been also isolated from commercially available human placental cDNA (Marathone-ready cDNA; Clontech, data not shown).

PA/Q141K cells were significantly more sensitive to anticancer agents than the other *BCRP* transfectants. Intracel-lular topotecan accumulation of PA/Q141K was higher than that in the other *BCRP* transfectants. By Western blotting, BCRP expression in PA/Q141K cells was markedly lower than that in the other *BCRP* transfectants. Another transfection experiment of mutant *BCRP* cDNAs in KB-3-1 human epidermoid carcinoma cells also revealed markedly lower expression of Q141K BCRP compared with wild-type and V12M BCRP (data not shown). Although the 141st amino acid of BCRP is located in the functionally important ATP-binding region between Walker A and B, increased sensitivity to anticancer drugs was not because of functional alteration but because of decreased protein expression. In the transfection experiment, the expression of C421A *BCRP* mRNA was identical to those of mRNAs from wild-type, G34A, and 944–949-deleted *BCRP* by Northern blotting. Therefore, the increased sensitivity was considered to be a result of instability of Q141K BCRP. Because lysine and glutamine have different electronic charges, substitution of lysine for glutamine might alter the tertiary structure of BCRP protein, leading to greater susceptibility to degradation.

We first intended to correlate mutant cDNA with protein expression levels of cancer cell lines, but three variants were observed in either cell lines that highly expressed BCRP or those that did not express BCRP. For instance, BCRP was highly expressed in A549 cells that carry both the C421A and wild-type *BCRP* alleles. Protein expression levels can be influenced not only by mRNA sequences but also by genomic structures such as chromatin alterations, methylation, or acetylation. Increased BCRP expression in A549 cells could be explained by the high transcription rate of the wild-type *BCRP* gene.

In this study, we showed that 46% of a normal Japanese population carries the C421A allele and, in particular, 7% were homozygous. In the analysis of 59 tumor cell lines, 5 (8%) were heterozygous and 2 (3%) were homozygous for the C421A allele. Because most of these cell lines were established in Western countries, people with the C421A allele should exist at high frequency in Western countries as well. BCRP transports anticancer agents such as SN-38 and mitoxantrone. Irinotecan, a prodrug of SN-38, and mitoxantrone are used in practical chemotherapy for a wide variety of cancers. BCRP expressed in normal tissues of cancer patients may serve to reduce adverse effects of these drugs such as hematological toxicity and digestive tract disorders. Irinotecan in clinically administered dosages causes grade 3–4 leukopenia in ~30% and grade 3–4 diarrhea in 20% of cancer patients according to the World Health Organization criteria (15, 16). These severe toxicities are presently unpredictable. Because BCRP is expressed in both hematopoietic stem cells and enterocytes of the digestive tract and may

protect those cells against toxic compounds, administration of the drugs to patients with the C421A allele may cause severe side effects. We started additional studies to investigate the association of C421A polymorphism with side effects from irinotecan chemotherapy.

In our subsequent genomic DNA analysis, C376T polymorphism in exon 4 substituting stop codon for Gln-126 was found in 3 (2.4%) of the 124 general Japanese population. The 3 were all heterozygous for the C376T allele. This C376T polymorphism may have higher impact than C421A polymorphism because active BCRP protein will not be expressed from the C376T allele. Additional investigation is planned concerning mRNA expression of this C376T allele and possible implication of the C376T polymorphism in side effect of irinotecan.

In summary, C421A *BCRP* cDNA was associated with low protein expression and subsequent sensitivity to anticancer drugs compared with wild type. C421A polymorphism showed high frequency in the general Japanese population. Screening for C421A polymorphism in cancer patients before chemotherapy should be useful for the prevention of serious side effects of some anticancer drugs.

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