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SHORT COMMUNICATION

MDR1 C2005T polymorphism changes substrate specificity

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Abstract
Purpose The current study is to determine the alterations of efflux transport activity to Rh123 and sensitivity to anticancer agents mediated by a *MDR1* C2005T polymorphism.
Methods Expressions of mRNA and protein of *MDR1* were measured by real-time PCR and immunoblotting, respectively, and localization of P-glycoprotein (P-gp) by confocal microscopy. Cell cytotoxicity and efflux transport activity were determined by MTT and Rh123 transepithelial permeability assay, respectively.
Results *MDR1* C2005T polymorphism did not affect the expression level of the *MDR1* mRNA and protein and had no effect on the trafficking of P-gp to plasma membrane. A cytotoxicity study showed that *MDR1wt* and $MDR1_{2005T}$ cells exhibited similar resistance, as measured by IC_{50} values, to vinblastine (30.3 ± 2.5 vs. 32.5 ± 1.7 nM) and vincristine (104.1 ± 1.9 vs. 110.3 ± 3.5 nM). However, $MDR1_{2005T}$ cells were less resistant to paclitaxel (28.2 ± 2.1 vs. 91.8 ± 3.5 nM; $P < 0.05$) and etoposide (119.7 ± 6.5 vs. 546.8 ± 9.5 nM; $P < 0.05$). The apparent transepithelial permeability ratios of Rh123 in $MDR1_{wt}$ and $MDR1_{2005T}$ cells were 2.12 ± 0.46 and 3.64 ± 0.78 ($P < 0.05$), respectively.
Conclusions The *MDR1* C2005T polymorphism alters the transepithelial permeability of a fluorescent substrate and sensitivity to select cytotoxic agents, which may influence drug disposition and the therapeutic efficacy of some P-gp substrates.

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Introduction

The pharmacokinetics of commonly used drugs varies from person to person, reflecting differences in absorption, distribution, metabolism, and excretion of the drug. Membrane transporters affect drug disposition and response by determining whether or not the level of the drug is maintained within the therapeutic index. Of the known human transporters, P-glycoprotein (P-gp) is an important efflux transporter that can influence the pharmacokinetics and pharmacodynamics of many drugs, including structurally and functionally divergent drugs that are in common clinical use [1–3]. P-gp belongs to the ATP-binding cassette (ABC) superfamily [4] and is encoded by the human *ABCB1* gene (also known as multidrug resistance 1 gene [*MDR1*]). P-gp is expressed in many tissues, including the intestine, liver, kidney, blood–brain barrier, and placenta, which suggests its broad physiological role [5, 6]. P-gp functions by pumping cytotoxic drugs and xenotoxins out of cells into the intestinal lumen, bile, and urine, and thus limits the distribution of such compounds to other organs.

Variability in drug response is widely observed for P-gp substrates. Genetic variation of the *MDR1* gene may be a potent determinant of inter-individual variability in the resistance to multiple drugs, including anticancer agents. Two factors stand out: the number of P-gp transporters on the cell membrane and the level of P-gp transport function that controls the apparent activity of P-gp. Numerous *MDR1* single-nucleotide polymorphisms (SNPs) have been identified. However, the correlation of SNPs with *MDR1*

expression and P-gp function in clinical pharmacokinetics has been inconclusive. The high-frequency *MDR1* SNPs include C1236T (exon 12), G2677T or A (exon 21), and C3435T (exon 26). Some of these SNPs are genetically linked. Their effects on protein function have been studied [7]. A synonymous 3435C>T SNP has been heavily studied, but its function remains under debate [8–12].

A polymorphism that affects P-gp activity at the protein level most likely will be an amino acid that changes a nonsynonymous variant. Amino acid changes may alter key domains necessary for substrate binding, ATP hydrolysis, or protein folding. SNPs were prioritized for functional analysis by two criteria: the degree of evolutionary conservation [13] and the biochemical severity of the alteration. The severity of missense changes was estimated using the Grantham scale [14], which formulates the difference in codon substitutions based on the chemical dissimilarity of the encoded amino acids. Grantham values range between 5 and 215, with higher values indicating more radical chemical changes. The 2005 C>T SNP of *MDR1* was particularly interesting because this Arg-to-Cys substitution had the highest Grantham value (180) [14], and this SNP was observed twice in the African American population where it exhibited a 1% allele frequency. In this report, we have characterized a nonsynonymous SNP, C2005T, which is located in the loop domain of P-gp. Using stable recombinant LLC-PK1 host cells expressing either *MDR1* wild-type ($MDR1_{wt}$) or $MDR1_{2005T}$, we have systematically characterized the functional differences between these two alleles. The data from these studies suggest that the C2005T polymorphism has altered function that is substrate-specific.

Materials and methods

Materials

Vincristine, vinblastine, etoposide, and paclitaxel were purchased from Sigma (St. Louis, MO, USA). The antibodies against P-gp and β-actin were from Sigma and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Cell culture media and reagents were obtained from Invitrogen (Carlsbad, California, USA). All other reagents were of molecular biology grade and from Sigma or Amresco (Solon, Ohio, USA).

Site-directed mutagenesis

Total RNA was extracted from the *MDR1*-overexpressing cell line, MES-SADX5, and full-length *MDR1* cDNA was generated using a high fidelity protocol developed previously [15]. The isolated *MDR1* cDNA was cloned into a linearized pcDNA3.1 TA vector (Invitrogen) containing cytomegalovirus and T7 promoters capable of transcription. The plasmid stock was designated as *MDR1* wild-type ($MDR1_{wt}$). The C2005T base pair change was generated with site mutagenesis using the QuikChange XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, California, USA) with the following primer: 5′-GATCCAGTCTAATA AGAAAAAGATCAACTTGTAGGAGTGTCCGT-3′.

Cell culture and transfections

LLC-PK1 cells (passages 10–40) were maintained in Medium 199 supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and gentamicin at 37°C in a humidified atmosphere (95% air, 5% CO_2). LLC-PK1 cells were seeded into a six-well plate and transfection was conducted when the cells reached approximately 90% confluence. In brief, cells were transfected with pcDNA3.1 plasmids containing the reference ($MDR1_{wt}$) or mutant *MDR1* cDNA ($MDR1_{2005T}$), or empty vector by Lipofectamine 2000 (Invitrogen) following the manufacture's protocols, respectively. Three days after transfection, stable clones were selected in media containing 500 μg/ml G418. After 10–14 days, individual stable clones were isolated and positive clones were further selected by immunocytochemistry.

RNA extraction and real-time PCR analysis

The cells were harvested and total RNA was isolated using a Trizol reagent (Invitrogen). One microgram of the total RNA was reverse-transcribed using the Reverse Transcription System Kit (Promega, Madison, Wisconsin, USA) according to the manufacturer's instructions. The real-time PCR was carried out in a Stratagene Mx3000p Multiplex Quantitative PCR System (Stratagene) using SYBR GreenER qPCR Supermix Universal Kit (Invitrogen) according to the manufacturer's instructions. The primers used to amplify *MDR1* were 5′-GCCTGGCAGCTGGAAG ACAAATAC-3′ (forward) and 5′-ATGGCCAAAATCAC AAGGGTTAGC-3′ (reverse); and the β-actin control were 5′-TGGCACCCAGCACAATGAA-3′ (forward) and 5′-CTAAGTCATAGTCCGCCTAGAAGCA-3′ (reverse). The cycle threshold value was defined as the PCR cycle number at which the reporter fluorescence crossed the threshold. The cycle threshold value of each product was determined and normalized against that of the internal control, β-actin.

Lysate preparation and western blot analysis

The cell lysate was prepared by lysing the cells in RIPA buffer at 4°C for 30 min, followed by centrifugation at

10,000×*g* for 10 min at 4°C. Protein concentration was measured using a bicinchoninic acid microplate assay protocol, and all samples were diluted to the same concentration using the lysis buffer and mixed with a 5× loading buffer containing 0.1% SDS and β-mercaptoethanol. The cell lysates were then separated using 7.5% SDS polyacrylamide gel electrophoresis and transferred to a PVDF membrane (Millipore, Bedford, Massachusetts, USA). The membrane was then blocked with 5% nonfat-dry milk in Tris-buffered saline/Tween 20 buffer (20 mM Tris–HCl, 0.9% NaCl, 0.05% Tween 20, pH 7.6). Immunoblotting was performed with the anti-P-gp monoclonal antibody F4 (Sigma). P-gp was detected on the blot using infrared-labeled secondary antibody visualized at the 800 nm fluorescence channel. The blot was developed and quantified by the Odyssey Infrared Imaging System (LI-COR Biosciences, http://www.licor.com) following the manufacturer's protocol.

Confocal microscopy imaging

Prior to immunostaining and between each immunostaining step, the cells were washed twice with PBS that was supplemented with 0.1% BSA. The cells were first fixed for 0.5 h with 4% paraformaldehyde at room temperature. The presence of P-gp was detected by labeling with the F4 monoclonal antibody for 1 h at 4°C. Cells were washed once with PBS/0.1% BSA followed by incubation with fluorescein isothiocyanate-conjugated donkey anti-mouse antibody at 4°C for another 1 h. The cells were washed three times with blocking solution and the cell nuclei were counterstained with DAPI (4′,6-diamidine-2-phenylindole) for 10 min. The coverslips were mounted on the slides before viewing with a confocal microscope. Samples were analyzed by using a Bio-Rad MRC-1024 confocal microscope.

Evaluation of cancer drug resistance

The effect of mutations on *MDR1*-mediated drug resistance was determined using a methyl thiazolyl tetrazolium assay. In brief, the cells (vector control, wild-type, or $MDR1_{2005T}$) were seeded at a density of 1×10^4 cells/well in a 96-well plate format in 100 µl of culture medium overnight. The medium was replaced with 100 µl of medium containing a dilution series of selected chemotherapeutic agents. The culture was maintained at 37°C with 5% CO_2 in a humidified incubator for 72 h. Cell viability was measured with a CellTiter-Glo cell viability assay kit (Promega). The incubation was continued for 4 h at 37°C and the absorbance was measured at 490 nm in a Multiskan Ascent 354 microplate reader (Thermo, Labsystems). The absorption value was determined by Ascent Software (Thermo, Labsystems, Helsinki, Finland) and the half maximal inhibitory concentration (IC_{50}) values were obtained from the dose–response curves. A relative resistance factor was obtained by dividing the IC_{50} value of the cells stably transfected with wild-type or mutant *MDR1* by the IC_{50} value of the cells stably transfected with the vector controls from at least six independent experiments.

Transepithelial transport assay

LLC-PK1 control and recombinant *MDR1* cells were plated at a density of 2×10^6 cells/24-mm well on permeable supports (Transwell; 0.45-µm membrane pore size; Corning) and grown for 4 days. The medium was refreshed after 2 days in culture. Fresh medium was added to the cells 1 h before the initiation of the experiment, and transepithelial electrical resistance (TEER) values were measured with a Millicell-ERS (Millipore). For assessing the transport of Rh123 across the epithelial cells, 5 µM Rh123 in Opti-MEM (Invitrogen) was added to either the apical or basolateral compartment with fresh Opti-MEM medium added to the opposite side. Inhibition was caused with 5 µM cyclosporin A. Aliquots of 50 µl were taken from the apical and basal compartments at 1, 2, 3, and 4 h. The fluorescence intensity of Rh123 was measured with a Gemini XS microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA) with SoftMax Pro software (Molecular Devices); the excitation and emission wavelengths were set at 488 and 535 nm, respectively. Apparent permeability (P_{app}) was calculated in the apical-to-basolateral direction (P_{app} A−B) and in the basolateral-to-apical direction (P_{app} B−A), as described previously [16]. Briefly, $P_{app} = [1/(A \times C_0)] \times (dQ/dt)$, where A is the surface area of permeable support, C_0 is the initial concentration in the donor compartment, and dQ/dt is the rate of transfer of the compound into the acceptor compartment. The ratio of P_{app}B−A/P_{app}A−B was also calculated to evaluate P-gp-mediated directional efflux.

Statistical analysis

Statistical analysis was performed with SPSS software (version 11.0 for windows, SPSS Inc., Chicago, IL, USA). The comparison of differences among groups was performed using the Student's *t*-test. All data were presented as mean ± standard deviation (SD). A P value less than 0.05 was considered significant.

Results

mRNA and protein expression levels in LLC-PK1 cells transfected with *MDR1* mutant

To determine whether a known SNP affects *MDR1* expression, the C2005T mutation was recreated in wild-

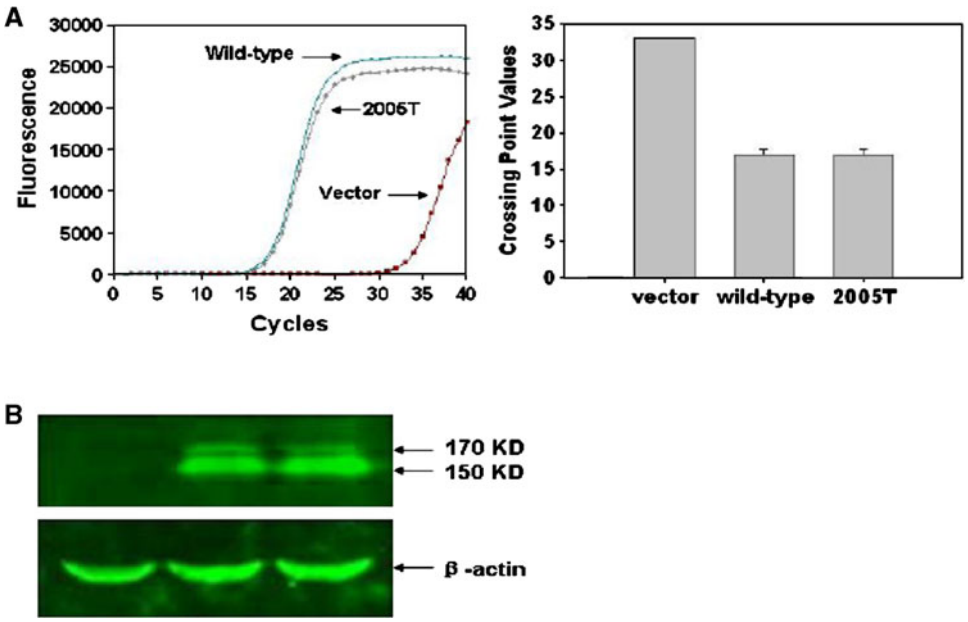

Fig. 1 mRNA levels and P-gp expression in the LLC-PK1 vector control and recombinant *MDR1* cells. LLC-PK1 cells were stably transfected with vector control, wild-type *MDR1*, or 2005T *MDR1*. **a** Analysis of vector only, wild-type *MDR1*, and the 2005T *MDR1* with real-time quantitative RT-PCR. Crossing-point values for the graph on the *left* were plotted in the histogram. **b** One-color immunoblot detection was used to quantify the level of P-gp compared to the level of *β*-actin, a loading control for the western blot analysis. The mature fully glycosylated (~170 kDa) and immature P-gp bands (~150 kDa) were marked by *arrows*

type *MDR1* cDNA by site-directed mutagenesis and stably transfected into LLC-PK1 cells. The expression levels of the wild-type and mutant C2005T were determined by real-time PCR and western blot for comparison. As shown in Fig. 1, there are no significant differences in expression levels of the mRNA and protein between the wild-type and mutant *MDR1* cells. Thus, the mutation does not affect the expression level of the *MDR1* protein.

Subcellular localization of wild-type and mutant *MDR1*

To further determine whether the C2005T mutation influences the trafficking of *MDR1* to the cell surface, we detected the subcellular localization of the wild-type and C2005T mutant proteins in stably transfected LLC-PK1 cells through the process of immunostaining. As shown in Fig. 2, strong plasma membrane staining was observed with all cells that were transfected with either the wild-type or mutant C2005T, but not in the vector-transfected control cells. It seems that the mutation does not affect the normal subcellular localization and that it may have no effect on the trafficking of human *MDR1* to the plasma membrane.

Drug resistance profiles of wild-type and mutant *MDR1*

To further determine whether the C2005T polymorphism affects the substrate specificity of *MDR1*, we chose different types of anticancer drugs. We addressed the relative resistance of the P-gp C2005T polymorphism with the structurally similar drugs, vinblastine and vincristine. Because P-gp can confer cellular resistance to a variety of cytotoxic drugs, we also tested whether the P-gp polymorphism C2005T might exhibit different resistance

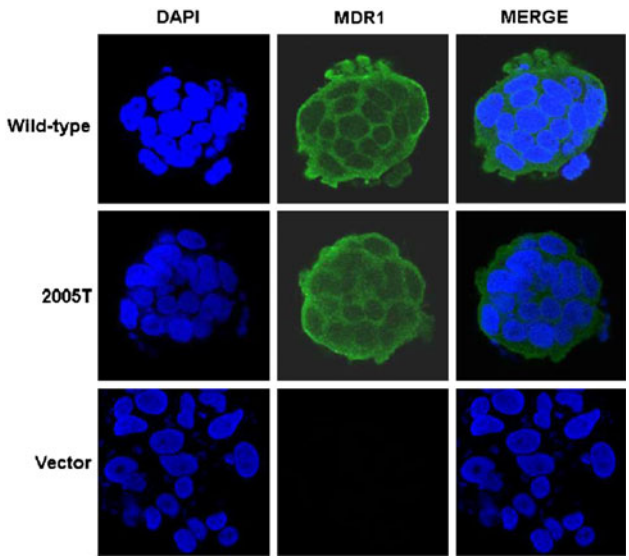

Fig. 2 Effect of 2005T polymorphism on subcellular localization of multidrug resistance protein 1 (*MDR1*). LLC-PK1 cells were stably transfected with vector control, wild-type *MDR1*, or 2005T *MDR1*. The cells were fixed and immunostained for *MDR1* using the monoclonal antibody F4 and an fluorescein isothiocyanate conjugated secondary antibody. Nuclei were counterstained with DAPI

profiles with other P-gp substrates, including paclitaxel and etoposide, which are structurally dissimilar from vinblastine and vincristine. The results from these studies are shown in Table 1. All cells expressing wild-type or $MDR1_{2005T}$ have substantially higher IC_{50} values or higher resistance to all drugs when compared with the vector-transfected control cells. Insignificant differences were found between $MDR1_{wt}$ and $MDR1_{2005T}$-expressing cells when the cells were treated with vinblastine or vincristine.

Table 1 Effects of *MDR1* 2005 C>T variation on cytotoxicity of recombinant LLC-PK1 cells to chemotherapeutic agents

Drug (nM)	Recombinant LLC-PK1 cell				
	Control: IC_{50}	$MDR1_{wt}$		$MDR1_{2005T}$	
		IC_{50}	R value	IC_{50}	R value
Vinblastine	4.5 ± 1.5	30.3 ± 2.5*	6.7	32.5 ± 1.7*	7.2
Vincristine	3.8 ± 0.5	104.1 ± 1.9**	27.6	110.3 ± 3.5**	29.0
Paclitaxel	8.3 ± 1.3	91.8 ± 3.5**	11.0	28.2 ± 2.1*#	3.4
Etoposide	19.6 ± 3.8	546.8 ± 9.5**	26.9	119.7 ± 6.5*#	6.1

IC_{50}: half maximal inhibitory concentration; *MDR1*, multi-drug resistance protein 1; WT, wild-type; control, empty vector-transfected cells. Relative resistance (*R* value) = IC_{50} of wild-type or $MDR1_{2005T}$ transfected cells/IC_{50} of control cells

* $P < 0.05$ and ** $P < 0.001$ compared to control; # $P < 0.05$ compared to $MDR1_{wt}$-expressing cells

However, the effects of the 2005 C>T transition significantly altered the drug resistance profiles for paclitaxel and etoposide. Recombinant $MDR1_{2005T}$-expressing cells exhibited an approximately fourfold reduction in resistance to paclitaxel (IC_{50} = 28.2 vs. 91.8 nM) and etoposide (119.7 vs. 546.8 nM) compared to $MDR1_{wt}$ cells (Table 1). Taken together, these data indicate that the C>T transition at the 2005 nucleotide position of *MDR1* with a predicted Arg > Cys change at amino acid 669 may be important in the reduced cellular resistance to these drugs. Collectively, these data suggest that the influence of 2005 C>T on drug resistance, as well as the magnitude of the effect, appears to be drug specific.

Transepithelial transport

The differences in transcellular directional permeability in epithelial cells expressing $MDR1_{wt}$ and $MDR1_{2005T}$ were evaluated by growing cells on filter supports, which help to establish polarized monolayers and tight junctions. P-gp is a directional transporter expressed on the apical membrane of epithelial cells and effluxes substrates in a basolateral-to-apical direction. The integrity of the monolayer and the formation of tight junctions were verified before an experiment, and the TEER values measured 350 ± 27.8 Ω cm^2. The directional efflux transport was evaluated with a fluorescent substrate, Rh123. The permeability ratios (P_{app}B−A/P_{app}A−B) were calculated for comparison. Cells expressing $MDR1_{2005T}$ seem to have an increased directional efflux and permeability ratio of Rh123 when compared to $MDR1_{wt}$ cells. In the presence of cyclosporin A, directional transport is eliminated, suggesting that the directional efflux is P-gp-mediated (Table 2).

Table 2 Apparent permeability ratios for rhodamine-123 transport in LLC-PK1 control and recombinant MDR1 cells

LLC-PK1 cell	P_{appB-A}/P_{appA-B}	
	Rh123	Rh123 + cyclosporin A
Control	0.96 ± 0.22	1.04 ± 0.30
$MDR1_{wt}$	2.12 ± 0.46##	0.68 ± 0.16
$MDR1_{2005T}$	3.64 ± 0.78*##	0.90 ± 0.24

* $P < 0.05$ compared to $MDR1_{wt}$-expressing cells

$P < 0.01$ compared to control cells (empty vector-transfected cells)

Discussion

The importance of P-gp-mediated efflux transport in drug delivery and disposition has become increasingly appreciated given P-gp's broad substrate specificity and localization in a variety of tissues. It is not surprising that this transporter has received the greatest attention in terms of the identification and characterization of single-nucleotide polymorphisms (SNPs). The search for key genetic determinants, including *MDR1* genetic polymorphisms, that alter the effectiveness of drugs that are P-gp substrates or inhibitors has just begun. Toward this end, we have developed stable recombinant LLC-PK1 cells expressing either $MDR1_{wt}$ or $MDR1_{2005T}$ and evaluated the significance of the C2005T polymorphism. The 2005C>T variant has an allele frequency of only 1% in African Americans, but was chosen because the amino acid substitution causes a drastic chemical change (Grantham value = 180). Grantham values were determined for the nonsynonymous variants to determine how drastic the amino acid change is in terms of chemical properties [14].

Alterations in the transepithelial transport and chemoresistance of P-gp due to the C2005T transition have been observed in our recombinant expression system. Variation in chemoresistance due to the C2005T polymorphism was also observed (Table 1). Cells expressing $MDR1_{2005T}$ seem to be less resistant to paclitaxel and etoposide than cells expressing $MDR1_{wt}$; however, $MDR1_{wt}$ and $MDR1_{2005T}$ recombinant cells displayed similar resistance to vinblastine and vincristine. Site-directed mutagenesis studies have also shown that modifications in the nucleotide sequence of

MDR1 alter substrate efflux of some anticancer agents but do not cause a change in efflux of others [17–20]. Mutational analysis was intensively used to investigate P-gp structure–function relationships. In particular, multiple mutations in regions of *MDR1* that encode transmembrane domains 5, 6, 11, and 12 alter P-glycoprotein substrate specificity [21, 22]. However, mutations in regions of *MDR1* that encode other domains, such as the intra- or extracellular loops or ATP-binding sites also alter the transporter's substrate specificity. Residue 669 is located in the intracellular loop adjacent to the 11th transmembrane domain (TMD11), which has been shown to be important for drug binding and ATP hydrolysis. It is possible that an amino acid change at this site may alter important functions of TMD11. The observed differential sensitivity to cytotoxic agents due to C2005T may be important in modulating the efficacy of chemotherapy. Although this is plausible, a Cys in the same position may also alter the overall conformation of P-gp and lead to the fourfold IC_{50} reduction of paclitaxel and etoposide. This reduced resistance in the $MDR1_{2005T}$-expressing cells would indicate that P-gp-mediated transport is reduced in these cells. The reduction may be due to increased intracellular accumulation, and therefore increased cytotoxicity. The variations in P-gp activity due to C2005T we found in our recombinant expression system are not comparable with those reported for $MDR1_{2005T}$ expressed in HEK293 cells using a transient expression system [23]. In this transient expression system, HEK293 cells expressing $MDR1_{2005T}$ had intracellular BODIPY-FL-paclitaxel levels within 5% of the reference, indicating increased P-gp function. However, the fluorescent bodipy modification on the P-gp substrates in this study may influence the ability to detect functional variability due to *MDR1* polymorphisms. Addition of a bulky fluorescence probe (or side chains) to P-gp substrates may alter the overall molecular spatial conformation and hydrodynamic volume. Such structural modification to the P-gp substrate may influence the transporter recognition and binding for the investigated substrate, and as such it might influence the ability to detect functional variability. However, in the absence of the crystal structure of the P-gp protein, the conformational details at the atomic level remain elusive. Using stable transfection in the LLC-PK1 cell line, we found that the C2005T mutation had no effect the expression of *MDR1*. Therefore, the SNP may not be essential to the stability of *MDR1* mRNA and protein. A Saccharomyces-based assay tested nine nonsynonymous P-gp variants for cellular resistance to the antibiotic valinomycin and the anticancer drugs daunorubicin, doxorubicin, and actinomycin D [24]. The R669C variant showed highly increased function for all substrates. Evidence from the Saccharomyces-based study highlights the importance of considering substrate-specific effects of P-gp variants. These data suggest that the influence of 2005 C>T on drug resistance, as well as the magnitude of the effect, appears to be drug specific.

Mammalian epithelial host cells similar to LLC-PK1, including MDCKII and Caco-2, could potentially be used for evaluating the significance of *MDR1* polymorphisms [25–28]. In theory, the same *MDR1* and variant constructs can be used to generate recombinant expression systems with these cells. Nevertheless, the described stable recombinant system expressed in LLC-PK1 cells has provided a unique method for evaluating *MDR1* genetic polymorphisms. Because LLC-PK1 recombinant cells can be grown continuously in culture for several passages, they may serve as an in vitro tool to detect potential drug–drug interactions in drug development. Variation in transepithelial transport was demonstrated with the fluorescent substrate Rh123; cells expressing $MDR1_{2005T}$ displayed increased efflux of Rh123 compared with $MDR1_{wt}$ cells. Permeability and directional efflux transport studies with the epithelial cells demonstrated that $MDR1_{2005T}$ recombinant cells exhibited a higher P_{appB-A}/P_{appA-B} ratio for Rh123 (Table 2). Regardless of the efficiency of transepithelial transport, cells expressing either $MDR1_{wt}$ or $MDR1_{2005T}$ were both inhibited to the same degree by a P-gp inhibitor, cyclosporin A. This indicates that the observed differences were due to the influence of an *MDR1* polymorphism on P-gp function. Changes in drug permeability may impact the absorption, bioavailability, and distribution into target tissues, including the central nervous system.

In summary, we have developed a stable expression system for *MDR1* in epithelial cells and have demonstrated that C2005T causes functional variance in drug resistance and transepithelial transport. We found that the *MDR1* C2005T polymorphism alters cellular sensitivity and transepithelial permeability in this recombinant cell system. However, the occurrence and magnitude of the effect of C2005T appeared to be drug specific. *MDR1* C2005T displayed significantly reduced drug resistance to paclitaxel and etoposide, but had no effect on drug resistance to vinblastine and vincristine. Elucidating the significance of the C2005T polymorphism is the first step. Our system may have substantial impact in providing a method to consistently express other MDR1 SNPs and haplotypes to characterize the functional significance of *MDR1* polymorphisms.

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Conflict of interest statement None.

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