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ORIGINAL ARTICLE

Down-regulation of lipids transporter ABCA1 increases the cytotoxicity of Nitidine

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Abstract
Purpose Nitidine (NTD) cytotoxicity is highly specific for A549 human lung adenocarcinoma cells. We hypothesized that this cytotoxicity involved the accumulation of NTD in intracellular organelles. However, there have been no reports of NTD transporting factors. In this study, we screened for an NTD transporter and evaluated its association with NTD cytotoxicity.
Methods Gene expression analyses were done for A549 and human fetal lung normal diploid fibroblast (WI-38) cells. We screened for ABC transporter, multi-drug resistance-associated genes. Gene expressions of ATP-binding cassette transporter A1 (ABCA1) were confirmed in 8 cell lines by quantitative PCR. The involvement of ABCA1 in NTD cytotoxicity was evaluated using siRNA-mediated ABCA1 gene silencing.
Results Gene expression analysis indicated that A549 cells expressed higher levels of ABCC1, ABCC2, ABCC3, and ABCG2 and a lower level of ABCA1 compared to WI-38 cells. NTD resistant cell lines uniformly showed higher ABCA1 expression levels. Gene silencing experiments showed that the down-regulation of ABCA1 resulted in increased sensitivity to NTD.
Conclusions These results indicated that NTD efflux is controlled by ABCA1 activity, suggesting that ABCA1 transports molecules other than lipids. Thus, there is a possibility that ABCA1 acts as a drug resistance transporter involved in the cytotoxicity of NTD derivatives. This also suggested that the expression level of the ABCA1 gene may be an indicator for the efficiency of NTD treatment.

Keywords Nitidine · ABCA1 · ABC transporter · Multi-drug resistance · Gene expression analysis

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Introduction

Nitidine (NTD) is a benzophenanthridine alkaloid that has been reported as an antitumor agent by its inhibition of topoisomerase I (TOPO-I) (Fig. 1) [1, 2]. TOPO-I is required for DNA transcription, synthesis, and replication [3] and, thus, is an attractive target for anticancer drug development.

Camptothecin (CPT) is one of the best known anticancer plant alkaloids that kills cells by inhibition of TOPO-I [4, 5]. The results of quantitative DNA cleavage assays using a human enzyme showed that the inhibitory potency on TOPO-I by NTD was significantly less than by CPT [6]. Cytotoxicity assays using various tumor and normal cell lines have also supported this observation [1, 7, 8].

In lung cancer treatment, chemotherapy plays a major role worldwide. The possibility of developing chemotherapeutic drugs has been shown by numerous studies. However, resistance to drugs has also been reported as a major

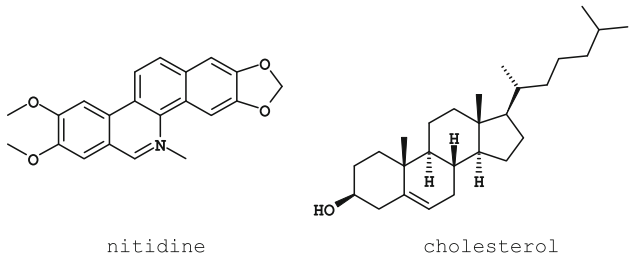

Fig. 1 Chemical structures of nitidine and cholesterol

obstacle to the treatment of many types of cancer including lung adenocarcinomas.

MDR1/ABCB1, MRP2/ABCC2, and BCRP/ABCG2 are well-studied members of the ABC transporter family of genes as multi-drug resistance-associated genes [9–13]. Their gene products actively export many type of drugs from cancer cells. The ABC transporters serve to protect cells through their recognition and energy-dependent removal of a large variety of natural toxic agents [14]. Thus, the efficacy of chemotherapeutic drugs depends not only on their mechanisms of action but also on the activities of transporters in tumor cells.

In a previous study, a strong multi-drug resistance phenotype was suggested for human lung adenocarcinoma A549 cells because of the over-expressions of MDR1/ABCB1, MRP2/ABCC2, and BCRP/ABCG2 genes [15]. For this reason, many previous studies have used A549 cells to screen for drugs that were not affected by multi-drug resistance transporters.

We have reported that NTD has highly specific cytotoxicity for A549 cells both in vitro and in vivo [16]. Although the relative TOPO-I inhibitory activity was less, NTD had cytotoxicity comparable to CPT as for A549 cells [16]. This report also suggested that NTD cytotoxicity was due to intracellular accumulations. It has been known that CPT is exported from cells by the action of BCRP/ABCG2, which is an over-expressed gene in A549 cells [17, 18]. From these results, one can deduce that NTD is not transported by major drug resistance transporters. However, there have been no reports regarding NTD transporters. Therefore, identifying the mechanisms of NTD transport is crucial to understand the A549-specific cytotoxicity induced by intracellular accumulations of NTD.

The present study was undertaken to identify NTD transporters associated with its specific cytotoxicity. We analyzed gene expression levels of ABC transporters in sensitive (A549) and resistant (WI-38) cell lines. We also applied small interfering RNA (siRNA) to knock down the expression of a candidate transporter gene and evaluated the sensitivity to NTD treatment.

Materials and methods

Gene expression analysis for A549 and WI-38 cells

A549 and WI-38 cells (1×10^6 cells/90 mm dish) were pre-incubated overnight. Gene expression analysis was done using the mRNAs from each cell lines without NTD treatment. The method of total RNA extraction is described elsewhere. This analysis essentially followed the procedures described in the Affymetrix Expression Analysis Technical Manual. The prepared cRNA was hybridized to an Affymetrix HG-U95Av2 chip. Data analysis used GeneChip software. Experiments were performed 3 times.

Cell culture

The A549, human lung adenocarcinoma cell line, and OUMS-36T-2F, normal human embryo fibroblast (hTRT gene) cell line, were cultured in Dulbecco's modified Eagle's medium (D-MEM) containing 10% fetal bovine serum (FBS). WI-38, a human lung normal diploid fibroblast cell line, was cultured in Eagle's minimum essential medium with Earle's salts (E-MEM) containing 10% fetal bovine serum (FBS). WI-38 VA13 sub 2 RA (WI-38 VA13), SV40 virus transformed human lung fibroblast cell line, was cultured in E-MEM containing 10% FBS, non-essential amino acids, and 1 mM sodium pyruvate. VMRC-LCP, human lung squamous carcinoma cell line, was cultured in E-MEM containing 10% FBS and non-essential amino acids. ACC-MESO-4, human malignant pleural mesothelioma cell line, PC-14, human lung undifferentiated adenocarcinoma cell line, and RERF-LC-KJ, Japanese lung adenocarcinoma cell line, were cultured in RPMI-1640 medium containing 10% FBS. Cells were cultured at 37°C in a humidified atmosphere with 5% CO_2. Exponentially growing cells were used throughout the experiments.

Comparisons of ABCA1 expression and NTD cytotoxicity in various human cell lines

Total RNAs were prepared from nearly confluent cells (A549, WI-38, WI-38 VA13 sub 2 RA, OUMS-36T-2F, VMRC-LCP, ACC-MESO-4, PC-14, and RERF-LC-KJ) cultured in 60-mm dish according to the methods described in "Total RNA extraction and cDNA synthesis" section. Cytotoxicity assays were performed in 96-well microtiter plates. Cells were cultured in an appropriate medium, and seeded at 1×10^3 cells (100 µl) per well, and pre-incubated in a humidified atmosphere with 5% CO_2 at 37°C for 24 h. After treatment with each concentration of NTD (30, 16, 4, 1, 0.25 µM) for 24 h, cell viability was evaluated according to the methods described in "Cytotoxicity tests" section.

Cytotoxicity tests

Cytotoxicity was determined using a commercial cell titer kit (CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay, Promega). All measurements were taken in triplicate, and cell cytotoxicity was expressed as the relative viability of sample treated cells against untreated controls. The 50% lethal dose (D_{50}) was defined as the concentration of compound that inhibited 50% of cell growth compared with untreated controls.

Total RNA extraction and cDNA synthesis

Total RNAs were isolated from each cells by illustra RNAspin mini kit according to the manufacturer's instructions (GE Healthcare UK Ltd.). The isolated total RNA, 1.85 ± 0.70 μg/6 well of 96-well plate and 160 ± 39 μg/60-mm dish, was reverse transcribed into cDNA using a PrimeScript first-strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. Briefly, 1 μg total RNA was combined with 50 μM oligo-dT primer and 10 μM dNTPs in DNase- and RNase-free water to a final volume of 10 μl. RNA, and primer were denatured at 65°C and then cooled immediately on ice. Reverse transcription was performed using 10 μl of a master mix containing the following: 5× PrimeScript buffer (Takara Bio), RNase inhibitor (40 U/μl), PrimeScript reverse transcriptase (200 U/μl; Takara Bio), and DNase and RNase-free water. Reactions were incubated at 42°C for 60 min and terminated by incubation at 95°C for 15 min.

Real-time quantitative PCR

The mRNA level of ABCA1 was measured by real-time PCR. Primers for real-time PCR were designed using Primer Express software version 3.0 (Applied Biosystems). The relative mRNA levels of the beta-actin (ACTB) gene was measured and used to normalize the mRNA levels of the ABCA1 gene. The primer sequences were: ABCA1, 5′-AAA AAC TGG AAC GGT GAA GGT GAC-3′ and 5′-AAG GGA CTT CCT GTA ACA ATG CAT CT-3′; beta-actin (ACTB), 5′-CCC TTC TTA TAC CCT AAG ATG AAG C-3′ and 5′-AGT GCA AAA ATA GAT CCC ATT ACA G-3′. Real-time PCR used Ex Taq (Takara) with iCycler-iQ real-time PCR equipment (Bio-Rad) and SYBR Green. The threshold cycle (Ct) was defined as the PCR cycle number at which the reporter fluorescence crossed the threshold reflecting a statistically significant point above the calculated baseline. The Ct of each target product was determined and normalized against that of the housekeeping gene, ACTB.

siRNA preparation and transfection

Seven 21-nt siRNA duplexes of the human ABCA1 gene used in this study were obtained from RNAi Co., Ltd. (Tokyo, Japan, rnai.co.jp/) (Table 1). Silencer negative control siRNA (non-targeting pool siRNA) was purchased from Dharmacon, Inc. (Chicago, IL). A549 and OUMS 36T-2F cells were used to evaluate the effects of ABCA1-targeted siRNA. For siRNA transfection, 2×10^3 cells were plated in 96-well plates and grown for 24 h, followed by transfection with siRNAs using DharmaFECT™ 1 reagent (Dharmacon, Inc.) according to the supplier's instructions. In brief, 10 μl of 2 μM siRNA solution in serum-free D-MEM was mixed with 10 μl of 4% DharmaFECT™ 1 reagent diluted by serum-free D-MEM, followed by incubation at room temperature for 5 min. Eighty microliters of serum-free D-MEM was added to the siRNA-DharmaFECT™ 1 reagent complex, followed by incubation at room temperature for 20 min. After removing culture medium from the wells, 100 μl of the transfection mixture was added to 9 wells (6 wells forRNA extraction and 3 wells for cytotoxicity tests). After 24 h, medium was changed to fresh culture medium. Total RNA extraction was performed after 72-h siRNA transfection. To evaluate the effect of ABCA1 knockdown under same conditions, 3 wells of A549 and OUMS-36T-2F cells were treated with 1 and 20 μM of NTD, respectively, for 48 h. Cytotoxicity was measured according to the methods described in "Cytotoxicity tests" section.

Statistical analysis

Results are given as mean ± SD. ABCA1 mRNA expression levels and cytotoxicity results were compared between

Table 1 Sequences of ABCA1 siRNA oligos

siRNA	Sequence
siR01 sense	5′-GGC AAU CCG GAC CAU AUC UCG-3′
siR01 antisense	5′-AGA UAU GGU CCG GAU UGC CUG-3′
siR02 sense	5′-GAC GGA GUC GGA AAG GAU UUU-3′
siR02 antisense	5′-AAU CCU UUC CGA CUC CGU CUG-3′
siR03 sense	5′-GAG CUG UUC ACC GAC AAU AAG-3′
siR03 antisense	5′-UAU UGU CGG UGA ACA GCU CCA-3′
siR04 sense	5′-GUA CGU ACG UAU AAG ACU AGA-3′
siR04 antisense	5′-UAG UCU UAU ACG UAC GUA CAU-3′
siR05 sense	5′-GAA UAU UAA CGC UAA AGG UGU-3′
siR05 antisense	5′-ACC UUU AGC GUU AAU AUU CAA-3′
siR06 sense	5′-CGC UAA AGG UGU AAG ACU UCA-3′
siR06 antisense	5′-AAG UCU UAC ACC UUU AGC GUU-3′
siR07 sense	5′-GGA UGU ACA ACG AAC AGU ACA-3′
siR07 antisense	5′-UAC UGU UCG UUG UAC AUC CAG-3′

groups by the Dunnett test. A value of $P < 0.05$ or $P < 0.01$ was considered statistically significant.

Results

Gene expression analysis of A549 and WI-38 cells

To identify a gene(s) associated with NTD transport, we analyzed the expression levels of about 12,000 human genes using oligonucleotide microarrays. The available gene expression levels for ABC transporters are summarized in Table 2. The ABCA1 gene expression level in A549 was markedly lower than in WI-38 cells. By comparison, ABCC1, ABCC2, ABCC3, and ABCG2 genes were highly expressed in A549 cells.

Comparisons of ABCA1 expression and NTD cytotoxicity in various human cell lines

We performed quantitative real-time PCR to determine the levels of ABCA1 gene expression in the following cell lines (Table 3): A549, WI-38, WI-38 VA13 sub 2 RA, OUMS-36T-2F, VMRC-LCP, ACC-MESO-4, PC-14, and RERF-LC-KJ. The ABCA1 gene showed significantly higher expressions in WI-38, OUMS-36T-2F, VMRC-LCP, and ACC-MESO-4 cells than in A549 cells.

NTD cytotoxicity tests were also done for these cell lines (Table 3). The cells of higher ABCA1 expressors (WI-38, OUMS-36T-2F, VMRC-LCP, and ACC-MESO-4) were uniformly resistant to NTD while the lower expressors (A549, PC-14, and RERF-LC-KJ) showed somewhat variable sensitivity to this agent.

Table 2 Genes differently regulated between A549 and WI-38 cells based on gene chip analysis

Gene symbol	Fluorescence signal value		Fold change
	A549	WI-38	A549/WI-38
ABCA1	10.2 ± 12.9	52.8 ± 10.7	0.2
ABCC1	263.2 ± 32.1	109.9 ± 36.6	2.4
ABCC2	181.2 ± 39.4	4.4 ± 4.2	40.9
ABCC3	321.8 ± 30.8	27.1 ± 11.8	11.9
ABCC4	51.6 ± 11.3	42.4 ± 4.3	1.2
ABCC10	47.8 ± 29.0	55.1 ± 8.9	0.9
ABCE1	66.0 ± 23.2	60.8 ± 6.0	1.1
ABCF1	218.7 ± 34.2	186.4 ± 22.5	1.2
ABCF2	113.4 ± 18.2	68.9 ± 6.8	1.6
ABCF3	84.0 ± 12.4	90.7 ± 16.8	0.9
ABCG2	73.6 ± 13.4	14.4 ± 4.0	5.1

Results are the mean ± SD of triplicate measurements

Table 3 ABCA1 gene expression levels and NTD cytotoxicity in various cell lines

Cell line	Expression ratio	D_{50} (μM)
A549	1.0 ± 0.3	0.9
WI-38	13.9 ± 0.0**	>30.0
WI-38 VA13 sub 2 RA	1.3 ± 0.9	1.7
OUMS-36T-2F	4.3 ± 1.7*	20.0
VMRC-LCP	7.4 ± 1.6**	>30.0
ACC-MESO-4	15.0 ± 5.4**	>30.0
PC-14	1.0 ± 0.2	15.4
RERF-LC-KJ	0.3 ± 0.1	5.1

Expression ratios are based on the level in A549 cells. Lethal dose 50 was evaluated by cell growth inhibition by MTS assay after 24 h of NTD treatment. Results are the mean ± SD of more than triplicate measurements

* $P < 0.05$; ** $P < 0.01$; compared with respective level of A549 cells

Test for down-regulation of ABCA1 expression by designed siRNA

To identify an efficient RNA target sequence to down-regulate the ABCA1 gene, seven different parts of the ABCA1 mRNA sequence were tested for their ability to down-regulate ABCA1 mRNA expression levels in A549 cells. Among these seven oligonucleotides, siR07 showed the most significant reduction in ABCA1 gene mRNA (Fig. 2).

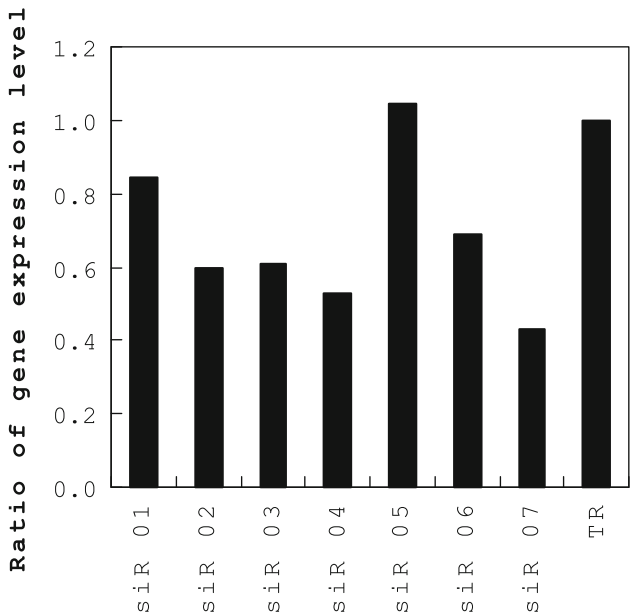

Fig. 2 Efficiency of designed siRNAs. Seven designed siRNAs were tested for ABCA1 mRNA reduction in A549 cells. Only one siRNA (siR07) reduced ABCA1 gene expression by at least >50%. Levels were quantified by real-time PCR and normalized by ACTB gene expression. Ratios of expression were based on the level in transfection reagent control (TR)

Enhanced NTD cytotoxicity by ABCA1 siRNA

To confirm the possible involvement of ABCA1 in NTD resistance, we inhibited its expression using siR07 and tested for changes in cell resistance to NTD. A549 and OUMS-36T-2F cells were transfected with siR07, or control (non-targeting) siRNA-transfected. Non-transfected cells with transfection reagent (column "TR") were used as controls (Fig. 3a, b). In this experiment, only treatment with transfection reagent did not affect the ABCA1 gene expression levels in each cells (data not shown). Then, relative ABCA1 gene expression levels in OUMS-36T-2F cells were about fourfold higher than A549 cells (Fig. 3a, b; Table 3). In these experiments, extent of ABCA1 gene knockdown was >70% in both cell lines. In contrast, significantly increased expressions were observed using non-targeting siRNA treatment in both cell lines.

To examine any alterations of NTD cytotoxicity after down-regulating the ABCA1 gene, cytotoxicity tests were performed using the same conditions. A549 and OUMS-36T-2F cells, treated with the concentration of D_{50}, 1 μM and 20 μM, respectively, showed significantly increased susceptibility to NTD cytotoxicity associated with ABCA1 down-regulation (Fig. 3c, d). These results showed that down-regulation of the ABCA1 gene was more effective in cells with higher expressions of the ABCA1 gene. ABCA1-siRNA did not alter the sensitivity of A549 cells to another chemotherapeutic agent camptothecin (data not shown), which may be an additional measure of specificity of this protein for NTD treatment.

Discussion

In our previous studies, we suggested that the cytotoxicities of benzo[c]phenanthridine derivatives, DHN and NTD, were associated with their accumulations in intracellular organelles [16, 19]. Based on our previous study of time-dependent changes in NTD fluorescence intensity, we hypothesized that a transporting factor was involved in importing and/or exporting NTD [16]. ABCA1 is a member of subfamily A of the ATP-binding cassette transporters, which cause familial HDL deficiency syndromes [20–22]. ABCA1 is a plasma membrane transporter that translocates cholesterol (Fig. 1) and phospholipids out of cells. Mutations in the ABCA1 gene results in Tangier disease, which is characterized by marked cholesterol accumulation in macrophages and other reticuloendothelial cells [20, 22, 23]. In most cells, cholesterol is not catabolized. Therefore, ABCA1 plays an important role of cellular sterol efflux by

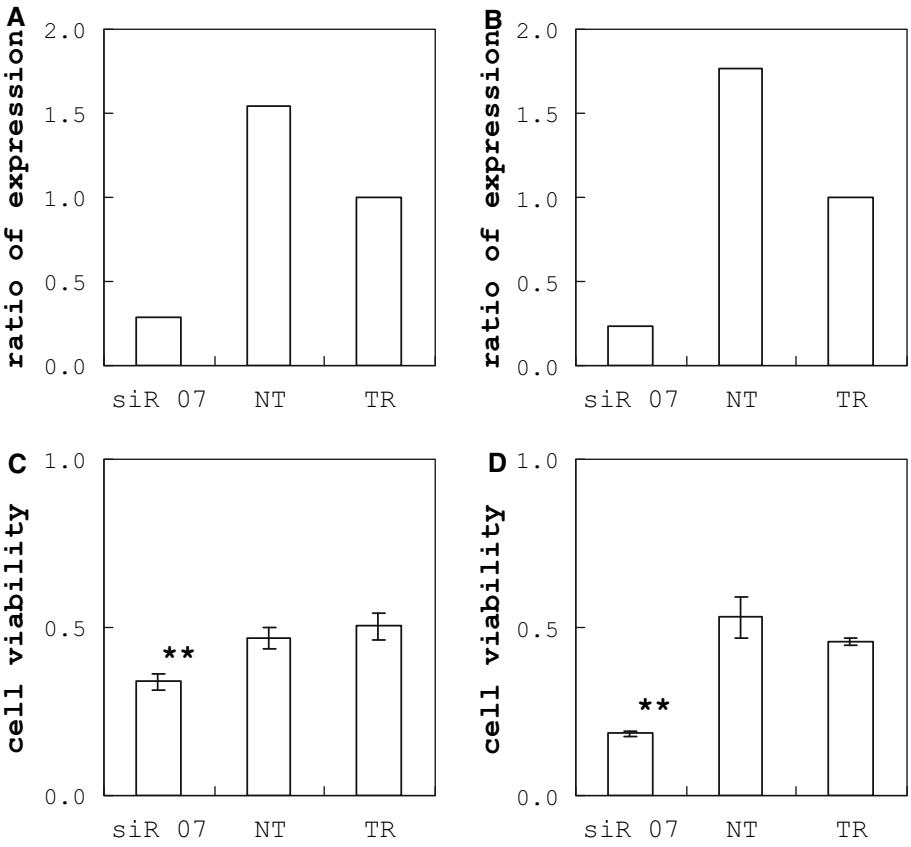

Fig. 3 Effects of ABCA1 knockdown on NTD cytotoxicity. Expression levels of ABCA1 in A549 (**a**) and OUMS-36T-2f (**b**) cells were quantified by real-time PCR and normalized by ACTB gene expression. Ratios of expression in each cell treated with siR07 mix or non-targeting pool siRNA (NT) were based on the level in transfection reagent control (TR). Cytotoxicity tests were performed after siRNA treatment. The A549 (**c**) and OUMS-36T-2f (**d**) cells were treated with 1 μM and 20 μM NTD, respectively, for 24 h. Cell viability were indicated based on control groups (cells without NTD treatment in each siRNA treatment). Results are the mean ± SD of triplicate experiments. ** $P < 0.01$ compared with cell viability with TR

constituting the initial step in the pathway of reverse cholesterol transport that ultimately leads to elimination of cholesterol from the body. Most reports regarding alterations of ABCA1 gene expression levels have identified relationships between its expression level and the efficiency of lipid metabolism.

In this study, gene expression analysis indicates that the MRP2/ABCC2 and BCRP/ABCG2 genes are highly expressed in A549 cells (Table 2). This observation supports a previous study [15]. It is important to note that although many transporters of this family were up-regulated in A549 cells, only ABCA1 was down-regulated. This suggests that lipids transport from intracellular compartments of A549 cells are relatively down-regulated compared with WI-38 cells. By association, no significant difference was also noted for the expression levels of other ABC transporter genes, ABCA2, ABCA7, ABCB6, ABCC6, ABCD3, ABCA3, ABCA8, ABCB8, ABCC8, ABCD4, ABCA4, ABCA12, ABCB9, ABCC9, ABCG1, ABCA5, ABCB1, ABCB11, ABCD1, ABCG4, ABCA6, ABCB4, ABCC5, ABCD2, and ABCG5 in these experiments using microarrays (data not shown).

In this study, we observed that down-regulation of ABCA1 gene expression increased the sensitivity to NTD cytotoxicity in A549 and OUMS-36T-2F cells, low and high ABCA1 expressing cell lines, respectively (Fig. 3b, d). These results suggest that ABCA1 exports NTD from cells that results in reduced cytotoxicity. With regard to the extent of suppressed cytotoxicity, effective alteration was limited to those cells that constitutively expressed the ABCA1 gene (Fig. 2d). This suggested that suppression of NTD cytotoxicity by ABCA1 required some threshold level of ABCA1 gene product activity, for example, a level exceeding the rate of NTD importation. However, studies on the effect of ABCA1 expression levels on NTD sensitivity in PC-14 and RERF-LC-KJ cells, compared to results for A549 cells, indicated some conflicting results (Table 3). These variable responses of the lower ABCA1 expressors may be explained by the differences in their capability to transport NTD into cells. Thus, intracellular concentration of NTD controlled by influx–efflux balance may differ between these cell lines. A selective transport and presence of receptor-mediated incorporation of protoberberine alkaloids have been reported in the cancer cells [24]. Our previous study also suggested that the variable sensitivity of cancer cells to NTD could be, in part, due to the difference in their capability to take up this agent from the extra cellular fluid [16]. Furthermore, it is alternatively possible that ABCA1 is not the sole protein involved in NTD transport. Quantitative kinetic studies of NTD accumulation associated with ABCA1 knockdown may shed more light into the involvement of this protein in NTD transport and are essential to withdraw a conclusion that NTD flux is largely controlled by ABCA1 activity. Thus, the variable responses of the lower ABCA1 expressors may be in line with the diverse transport system across the cancer cell membrane.

The results of this study have indicated an important role of ABCA1 as a drug resistance transporter. The results in previous studies of gene expression analyses with human tissues have shown that the ABCA1 transporter is highly expressed in macrophages, small intestine, liver, adrenal gland, stomach, lung, trachea, placenta, and uterus [25, 26]. Most of these cells or organs are known as proliferative cells. Consequently, these cells are easily targeted by chemotherapeutic agents that suppress cell proliferation. It is predicted that the proliferation of these organs that highly express the ABCA1 transporter would not be suppressed by NTD. The results of tumor suppression without apparent liver damages in our previous study also support this prediction [16]. Together, these results suggest that the ABCA1 transporter is as one of important factors to evaluate the side effect of the antitumor drugs.

In conclusion, although it remains to be determined whether there are specific mechanisms involved in the uptake of NTD, we expect that NTD and its derivatives will have applications for chemotherapeutic drugs.

References

1. Gatto B, Sanders MM, Yu C, Wu HY, Makhey D, LaVoie EJ, Liu LF (1996) Identification of topoisomerase I as the cytotoxic target of the protoberberine alkaloid coralyne. Cancer Res 56:2795–2800
2. Wang LK, Johnson RK, Hecht SM (1993) Inhibition of topoisomerase I function by nitidine and fagaronine. Chem Res Toxicol 6:813–818
3. Stewart L, Redinbo MR, Qiu X, Hol WG, Champoux JJ (1998) A model for the mechanism of human topoisomerase I. Science 279:1534–1541
4. Hsiang YH, Liu LF (1988) Identification of mammalian DNA topoisomerase I as an intracellular target of the anticancer drug camptothecin. Cancer Res 48:1722–1726
5. Hsiang YH, Hertzberg R, Hecht S, Liu LF (1985) Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 260:14873–14878
6. Holden JA, Wall ME, Wani MC, Manikumar G (1999) Human DNA topoisomerase I: quantitative analysis of the effects of camptothecin analogs and the benzophenanthridine alkaloids nitidine and 6-ethoxydihydronitidine on DNA topoisomerase I-induced DNA strand breakage. Arch Biochem Biophys 370:66–76
7. Sheng Ding F, Li Kai W, Sidney M, Hecht J (1993) Inhibitors of DNA topoisomerase I isolated from the roots of Zanthoxylum nitidum. Org Chem 58:5025–5027
8. Del Poeta M, Chen SF, Von Hoff D, Dykstra CC, Wani MC, Manikumar G, Heitman J, Wall ME, Perfect JR (1999) Comparison of in vitro activities of camptothecin and nitidine derivatives against fungal and cancer cells. Antimicrob Agents Chemother 43:2862–2868
9. Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2:48–58
10. Cole SP, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AM, Deeley RG (1992)

Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258:1650–1654
11. Borst P, Evers R, Kool M, Wijnholds J (2000) A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 92:1295–1302
12. Deeley RG, Cole SP (1997) Function, evolution and structure of multidrug resistance protein (MRP). Semin Cancer Biol 8:193–204
13. Litman T, Druley TE, Stein WD, Bates SE (2001) From MDR to MXR: new understanding of multidrug resistance systems, their properties and clinical significance. Cell Mol Life Sci 58:931–959
14. Sarkadi B, Müller M, Holló Z (1996) The multidrug transporters–proteins of an ancient immune system. Immunol Lett 54:215–219
15. Scharenberg CW, Harkey MA, Torok-Storb B (2002) The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. Blood 99:507–512
16. Iwasaki H, Okabe T, Takara K, Toda T, Shimatani M, Oku H (2009) Tumor-selective cytotoxicity of benzo[c]phenanthridine derivatives from Toddalia asiatica Lam. Cancer Chemother Pharmacol. doi:10.1007/s00280-009-1077-7
17. Kawabata S, Oka M, Shiozawa K, Tsukamoto K, Nakatomi K, Soda H, Fukuda M, Ikegami Y, Sugahara K, Yamada Y, Kamihira S, Doyle LA, Ross DD, Kohno S (2001) Breast cancer resistance protein directly confers SN-38 resistance of lung cancer cells. Biochem Biophys Res Commun 280:1216–1223
18. Brangi M, Litman T, Ciotti M, Nishiyama K, Kohlhagen G, Takimoto C, Robey R, Pommier Y, Fojo T, Bates SE (1999) Camptothecin resistance: role of the ATP-binding cassette (ABC), mitoxantrone-resistance half-transporter (MXR), and potential for glucuronidation in MXR-expressing cells. Cancer Res 59:5938–5946
19. Iwasaki H, Oku H, Takara R, Miyahira H, Hanashiro K, Yoshida Y, Kamada Y, Toyokawa T, Takara K, Inafuku M (2006) The tumor specific cytotoxicity of dihydronitidine from Toddalia asiatica Lam. Cancer Chemother Pharmacol 58(4):451–459 (Epub 2006 Feb 8)
20. Bodzioch M, Orsó E, Klucken J, Langmann T, Böttcher A, Diederich W, Drobnik W, Barlage S, Büchler C, Porsch-Ozcürümez M, Kaminski WE, Hahmann HW, Oette K, Rothe G, Aslanidis C, Lackner KJ, Schmitz G (1999) The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 22:347–351
21. Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, Yu L, Brewer C, Collins JA, Molhuizen HO, Loubser O, Ouelette BF, Fichter K, Ashbourne-Excoffon KJ, Sensen CW, Scherer S, Mott S, Denis M, Martindale D, Frohlich J, Morgan K, Koop B, Pimstone S, Kastelein JJ, Genest J Jr, Hayden MR (1999) Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 22:336–345
22. Rust S, Rosier M, Funke H, Real J, Amoura Z, Piette JC, Deleuze JF, Brewer HB, Duverger N, Denèfle P, Assmann G (1999) Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 22:352–355
23. Marcil M, Brooks-wilson A, Clee SM et al (1999) Mutations in the ABC1 gene in familial HDL deficiency with defective cholesterol efflux. Lancet 354:1341–1346
24. Marilyn MS, Angela AL, Tsai-Kun L, Hong-Yan W, Shyamal DD, Yong M, Eric HR, Edmond JL, Darshan M, Leroy FL (1998) Selective cytotoxicity of topoisomerase-directed protoberberines against glioblastoma cells. Biochem Pharmacol 56:1157–1166
25. Kielar D, Dietmaier W, Langmann T, Aslanidis C, Probst M, Naruszewicz M, Schmitz G (2001) Rapid quantification of human ABCA1 mRNA in various cell types and tissues by real-time reverse transcription-PCR. Clin Chem 47:2089–2097
26. Langmann T, Mauerer R, Zahn A, Moehle C, Probst M, Stremmel W, Schmitz G (2003) Real-time reverse transcription-PCR expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. Clin Chem 49:230–238