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ORIGINAL ARTICLE

Over-expression of *MDR1* in amrubicinol-resistant lung cancer cells

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Abstract
Purpose Amrubicin, a totally synthetic 9-aminoanthracycline anticancer drug, has shown promising activity for lung cancer, but little is known about the mechanism of resistance for this agent. This study was aimed to clarify the role of P-glycoprotein (P-gp) in amrubicinol, an active metabolite of amrubicin, resistance in lung cancer cells.
Methods Amrubicinol-resistant cell line PC-6/AMR-OH was developed by continuously exposing the small-cell lung cancer cell line PC-6 to amrubicinol. Gene expression level of *MDR1*, which encodes P-gp, and intracellular accumulation of amrubicinol were evaluated by PC-6 and PC-6/AMR-OH cells. The involvement of *MDR1* in amrubicinol resistance was evaluated by treatment with P-gp inhibitor verapamil and small interfering RNA (siRNA) against *MDR1*. Also, expression levels and single-nucleotide polymorphisms (SNPs) of *MDR1* in 22 lung cancer cell lines were examined, and the relationships between these factors and sensitivity to amrubicinol were evaluated.
Results The *MDR1* gene was increased approximately 4,500-fold in PC-6/AMR-OH cells compared with PC-6 cells, and intracellular accumulation of amrubicinol in PC-6/AMR-OH cells was decreased to about 15 percent of that in PC-6 cells. Treatment with verapamil and siRNA against *MDR1* significantly increased the sensitivity to amrubicinol in PC-6/AMR-OH cells with increased cellular accumulation of amrubicinol. Meanwhile, neither *MDR1* gene expression levels nor SNPs of the gene were associated with amrubicinol sensitivity.
Conclusions Results of this study indicate that increased *MDR1* expression and P-gp activity confer acquired resistance to amrubicinol. In contrast, neither expression level nor SNPs of *MDR1* are likely to be predictive markers for amrubicin activity.

Keywords Amrubicin · Amrubicinol · Drug resistance · Lung cancer · *MDR1* · P-glycoprotein

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Introduction

Amrubicin, a totally synthetic 9-aminoanthracycline anticancer drug, was approved in Japan for the treatment of lung cancer. In preclinical studies, amrubicin showed more potent antitumor activity than doxorubicin in several human tumor xenografts implanted in nude mice [1]. In general, anthracyclines are inactivated following conversion to the C-13 hydroxy metabolite by carbonyl reductase [2, 3]. As with other anthracyclines, amrubicin is also reduced to the C-13 hydroxy metabolite, amrubicinol. However, the cytotoxicity of amrubicinol is 10–100 times greater than that of amrubicin in vitro [4]. The superior anticancer activity of amrubicin in vivo is considered to depend on the anticancer efficacy of amrubicinol [5].

A phase II study of amrubicin showed overall response rates (ORRs) exceeding 75% and approximately 20% in previously untreated small-cell lung cancer (SCLC) and non-SCLC (NSCLC) patients, respectively [6, 7]. Recently, amrubicin treatment has been recognized to be an active and well-tolerated regimen in patients with previously treated SCLC [8, 9] and NSCLC [10]. Amrubicin can become a key drug for patients with lung cancer worldwide, but little is known about the mechanism of resistance and predictive biomarkers for the activity of this agent.

An amrubicinol-resistant lung cancer cell line was developed by exposure to amrubicinol, and over-expression of the *multidrug-resistance* (*MDR*) 1 gene was found in these cells. The *MDR1* gene encodes an integral membrane protein, P-glycoprotein (P-gp), whose function is the energy-dependent export of substances from cell membranes to the outside. Recently, Hira et al. [11] showed that P-gp was responsible for cellular accumulation and cytotoxicity of both amrubicin and amrubicinol using *MDR1* gene-transfected LLC-PK1 cells, suggesting that the anticancer effect of amrubicin could be affected by expression of P-gp in lung cancer. Therefore, the role of P-gp in amrubicinol resistance was investigated in lung cancer cells.

Materials and methods

Cell lines and chemicals

The following 22 human lung cancer cell lines were used in this study: 12 SCLC cell lines (PC-6, SBC3, NCI-H69, NCI-H1688, NCI-N417, SK-LC-17, DMS53, ACC-LC-5, ACC-LC-48, ACC-LC-80, ACC-LC-67, ACC-LC-73), 6 adenocarcinoma cell lines (PC-9, NCI-H23, A549, ACC-LC-94, ACC-LC-174, RERF-LC–MS), 2 squamous cell lung cancer cell lines (PC-10, QG56), and 2 large cell lung cancer cell lines (NCI-H460, SK-LC-6). PC-6 was kindly provided by Daiichi Pharmaceutical (Tokyo, Japan). Other cell lines were provided from Aichi Cancer Center or purchased from American Type Culture Collection. Cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum and 1% (v/w) penicillin/streptomycin, and the medium was changed every 4–7 days. Amrubicinol-resistant cell lines were established in our laboratory in the following manner. PC-6 cells were cultured in the presence of amrubicinol, starting at a concentration of 1 nmol/l and then increased in incremental steps to 200 nmol/l over 6 months. Finally, the amrubicinol concentration was maintained at 200 nmol/l, and a resistant clone was isolated (PC-6/AMR-OH). Amrubicinol, SN-38, doxorubicin, and gemcitabine were provided by Nihon Kayaku (Tokyo, Japan), Yakult Honsha (Tokyo, Japan), Kyowa Hakko Kogyo (Tokyo, Japan), and Eli Lilly Pharmaceuticals (Indianapolis, IN, USA), respectively. Cisplatin, etoposide, and paclitaxel were provided by Bristol Myers (Tokyo, Japan). Verapamil was purchased from Wako Pure Chemical Industries (Osaka, Japan).

Total RNA extraction and quantitative real-time RT-PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Quantitative real-time RT-PCR was performed in a volume of 20 µl with the Taqman One-Step RT-PCR Master Mix Reagents Kit (Applied Biosystems, Foster City, CA, USA) using the StepOnePlus Real-Time PCR System (Applied Biosystems) according to the manufacturer's suggestions. The cycling condition was as described previously [12]. The experiment was performed in triplicate. The primer and Taqman probe sets (Taqman Gene Expression Assays, Inventoried) for *MDR1* (Hs00184491_m1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Hs99999905_m1) were purchased from Applied Biosystems (sequences not disclosed). The expression values of each sample were normalized to those of the housekeeping gene *GAPDH*.

Chemosensitivity assay

Cells were cultured at 5,000 cells/well in 96-well tissue culture plates. In the inhibition study, 20 µmol/l verapamil was added to the medium as described previously [13]. To assess cell viability, stepwise tenfold dilutions of the anticancer drug were added 2 h after plating. After 72 h, 20 µl of MTS [3-(4,5-dimethylthiazol-2-yl)–5-(3-carboxymethoxyphenyl)–2-(4-sulfophenyl)-2H-tetrazolium, inner salt] solution (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) was added, and the cells were incubated for a further 4 h, after which the absorbance was measured at 490 nm using an ELISA plate reader. The experiment was performed in triplicate. The chemosensitivity was expressed as the drug concentration for 50% cell survival (IC_{50}), as determined from the concentration-effect relationship using GraphPad Prism version 4 (GraphPad Software, San Diego, CA, USA).

Modification of amrubicinol cytotoxicity by RNA interference

Approximately 2×10^5 cells were plated in 6-well plates in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum. After 24 h, the cells were transfected with small interfering RNA (siRNA) oligonucleotides for *MDR1* or negative-control siRNA using X-tremeGene (Roche Diagnostics, Indianapolis, IN, USA) to result in a final RNA concentration of 5 nmol/l, according to the manufacturer's instructions. Total RNA was extracted 24 h after transfection. To assess sensitivity to amrubicinol, the medium was changed, and the cells were seeded at 5,000 cells/well in 96-well tissue culture plates 24 h after being transfected. Stepwise tenfold dilutions of amrubicinol were added 2 h after plating. After 48 h, MTS solution was added, and after 4 h, the absorbance was measured at 490 nm using an ELISA plate reader. SiRNA oligonucleotides for *MDR1* (predesigned siRNA, ID s10418) and negative-control siRNA were purchased from Ambion (Austin, TX, USA).

Intracellular drug accumulation

Approximately 5×10^5 cells were cultured on 60-mm dishes and increased in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum for a few days until there were approximately 1×10^6 cells per dish. At the end of pre-incubation, the medium was changed to RPMI 1640 with 20 μmol/l of amrubicinol following a previous report [11], and the cells were incubated for 1 h. In the inhibition study, 20 μmol/l verapamil was added to the medium in combination with amrubicinol. After the drug treatments, the medium containing drug was aspirated, and the cells were washed by ice-cold phosphate-buffer saline. To extract the intracellular drugs, the cells were immersed in 1 ml of 50% methanol for 1 h at room temperature. The extract solutions were centrifuged at 13,000 rpm for 5 min and stored at −80°C until analysis. Amrubicinol concentrations were quantified by a high-performance liquid chromatography (HPLC) assay.

Genomic DNA extraction and detection of SNPs in the *MDR1* gene

Genomic DNA was extracted from the aforementioned 22 lung cancer cell lines using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Three SNPs of the *MDR1* gene [C1236T in exon 12, C3435T in exon 26, and G2677T/A in exon 21] were detected using the StepOnePlus Real-Time PCR System (Applied Biosystems) and Taqman Drug Metabolism Genotyping Assays (Applied Biosystems), according to the manufacturer's instructions. The primer and Taqman probe set for each SNP was purchased from Applied Biosystems. The assay ID of each SNP was as follows: C1236T, C_7586662_10; C3435T, C_7586657_20; G2677T, C_11711720D_40; G2677A, C_11711720C_30.

Statistical analysis

Statistical analysis was performed using Student's *t* test. The correlation between *MDR1* gene expression levels and IC_{50} values for amrubicinol was evaluated by Spearman's test.

Results

Characteristics of the amrubicinol-resistant cell line

The amrubicinol-resistant SCLC cell line PC-6/AMR-OH was established by continuously exposing PC-6 cells to amrubicinol. PC-6 cells displayed rounded-shaped morphology, whereas PC-6/AMR-OH cells displayed spindled one. Doubling time of PC-6/AMR-OH cells was approximately 24 h, which was not significant difference from that of PC-6 cells. The sensitivities of PC-6 and PC-6/AMR-OH cells to a panel of chemotherapeutic drugs are summarized in Table 1. The IC_{50} values for PC-6 cells and PC-6/AMR-OH cells treated with amrubicinol were 0.58 and 934 nmol/l, respectively. Thus, PC-6/AMR-OH cells were observed to be approximately 1,600-fold more resistant to amrubicinol than PC-6 cells. PC-6/AMR-OH cells also showed resistance to doxorubicin, paclitaxel, SN-38, and etoposide, but not to cisplatin and gemcitabine.

Expression of *MDR1* gene in PC-6/AMR-OH cells and inhibition of expression by siRNA

The *MDR1* gene expression levels in PC-6 and PC-6/AMR-OH cells were examined using quantitative real-time RT-PCR. As shown in Fig. 1a, *MDR1* gene expression was increased approximately 4,500-fold in PC-6/AMR-OH cells compared with PC-6 cells. To confirm that the alteration of amrubicinol cytotoxicity was induced by increased *MDR1* gene expression, PC-6/AMR-OH cells were transfected with siRNA directed against *MDR1*. PC-6/AMR-OH cells transfected with negative-control siRNA were used as controls. The *MDR1* gene expression level in PC-6/AMR-OH cells transfected with siRNA against *MDR1* was clearly decreased compared with that in PC-6/AMR-OH cells transfected with negative-control siRNA (Fig. 1b). It was confirmed that transfection of negative-control siRNA did

Table 1 Drug concentrations for 50% cell survival (IC_{50}) for various cytotoxic drugs in PC-6 and PC-6/AMR-OH cells

	PC-6		PC-6/AMR-OH		RR
	IC_{50} (nmol/l)	95% CI	IC_{50} (nmol/l)	95% CI	
Amrubicinol	0.58	0.48–0.68	934	636–1,373	1,610.0
Doxorubicin	28.04	22.96–34.24	1,329	810.6–2,178	47.4
Paclitaxel	0.040	0.026–0.061	1,152	832.4–1,594	28,800.0
SN-38	0.00032	0.00072–0.0014	1.59	0.619–3.706	4,968.8
Etoposide	76.5	60.2–100.2	2,179	1,439–3,301	28.5
Cisplatin	975.5	769.4–1,237.0	825.4	500–1,363	0.85
Gemcitabine	2.38	1.89–2.99	2.68	2.04–3.51	1.13

RR resistance ratio

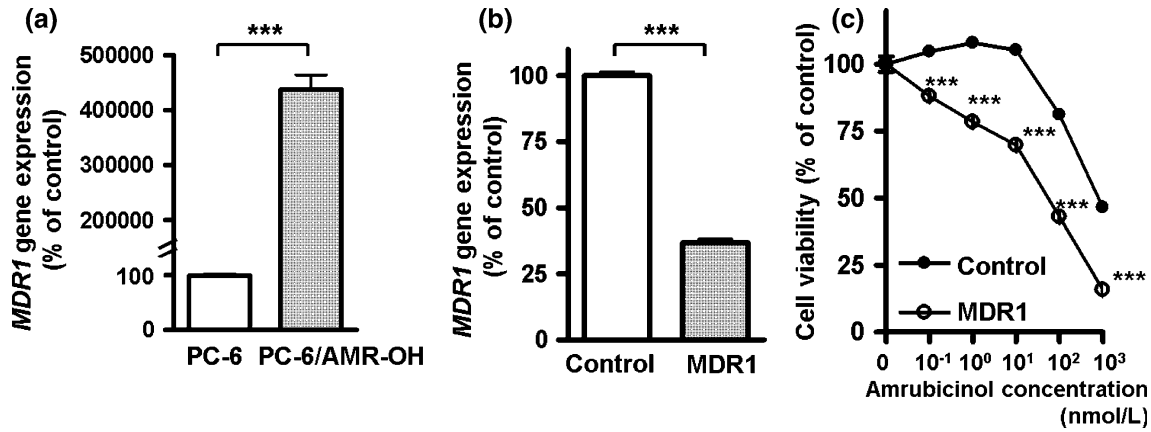

Fig. 1 Expression of *MDR1* gene in PC-6/AMR-OH cells and inhibition of expression by siRNA. **a** Gene expression levels of *MDR1* in amrubicinol-resistant cell line PC-6/AMR-OH and its parent cell line PC-6 were examined using quantitative real-time RT-PCR. Data are presented as percentages based on the value in PC-6 cells. **b** PC-6/AMR-OH cells were transfected with siRNA directed against *MDR1* or negative-control siRNA. *MDR1* gene expression levels in each transfected cell were examined using quantitative real-time RT-PCR. Data are presented as percentages based on the value in PC-6/AMR-OH cells transfected with negative-control siRNA. **c** Cytotoxicity to amrubicinol in each transfected cell was assessed by MTS-assay 72 h after siRNA transfection. Each point represents the mean ± SD of four independent measurements. ***$P < 0.001$

not meaningfully affect *MDR1* gene expression in PC6/AMR-OH cells (data not shown). Furthermore, the cytotoxicity to amrubicinol in PC-6/AMR-OH cells transfected with siRNA against *MDR1* was increased compared with that in PC-6/AMR-OH cells transfected with negative-control siRNA (Fig. 1c). These results indicate that overexpression of *MDR1* gene is induced by exposure of amrubicinol and confers resistance to the agent in PC-6/AMR-OH cells.

Alteration of intracellular accumulation of amrubicinol and cytotoxicity to amrubicinol by verapamil

To confirm increased P-gp function of excreting amrubicinol in PC-6/AMR-OH cells, intracellular accumulation of amrubicinol in PC-6 and PC-6/AMR-OH cells was examined. The accumulation was examined after exposing PC-6 and PC-6/AMR-OH cells to 20 μmol/l amrubicinol for 1 h. As shown in Fig. 2a, accumulation of amrubicinol in PC-6/AMR-OH cells was clearly lower than that in PC-6 cells. Alteration of amrubicinol intracellular accumulation in PC-6/AMR-OH cells by treatment with verapamil, a P-gp inhibitor, was next examined. Treatment with 20 μmol/l verapamil increased the accumulation of amrubicinol in PC-6/AMR-OH cells (Fig. 2a). Furthermore, the cytotoxicity to amrubicinol in PC-6/AMR-OH cells was recovered by the same treatment (Fig. 2b). These results indicate that the increased P-gp function of excreting amrubicinol in PC-6/AMR-OH cells actually plays a role in acquired resistance to amrubicinol.

Relationship between sensitivity to amrubicinol and the *MDR1* gene expression level in lung cancer cell lines

As previously shown in Fig. 1b and c, inhibition of *MDR1* gene expression by siRNA increased cytotoxicity of amrubicinol in PC-6/AMR-OH cells, suggesting that differences in *MDR1* gene expression levels affect amrubicinol sensitivity. To investigate the possibility of *MDR1* gene expression as a predictive marker for amrubicinol activity, *MDR1* gene expression levels and IC_{50} values for amrubicinol were examined in the aforementioned 12 SCLC cell lines and 10 NSCLC lines. As shown in Fig. 3a, IC_{50} values for amrubicinol were significantly higher in NSCLC cell lines than in SCLC cell lines. *MDR1* gene expression was relatively low in almost all cell lines, and it was not detected in NCI-N417 cells. In 21 cell lines, except for NCI-N417, there was no difference between *MDR1* gene expression levels in NSCLC cell lines and in SCLC cell lines (Fig. 3b). Furthermore, no significant correlation between *MDR1* gene expression levels and IC_{50} values was seen (Fig. 3c).

Relationship between sensitivity to amrubicinol and *MDR1* gene SNPs

MDR1 expression [14] and protein folding [15] are influenced by SNPs in *MDR1* gene. The relationships between three major SNPs [16] [C1236T in exon 12, C3435T in exon 26, and G2677T/A in exon 21] of the *MDR1* gene and IC_{50} values for amrubicinol were examined in the aforementioned 22 lung cancer cell lines. The relationships

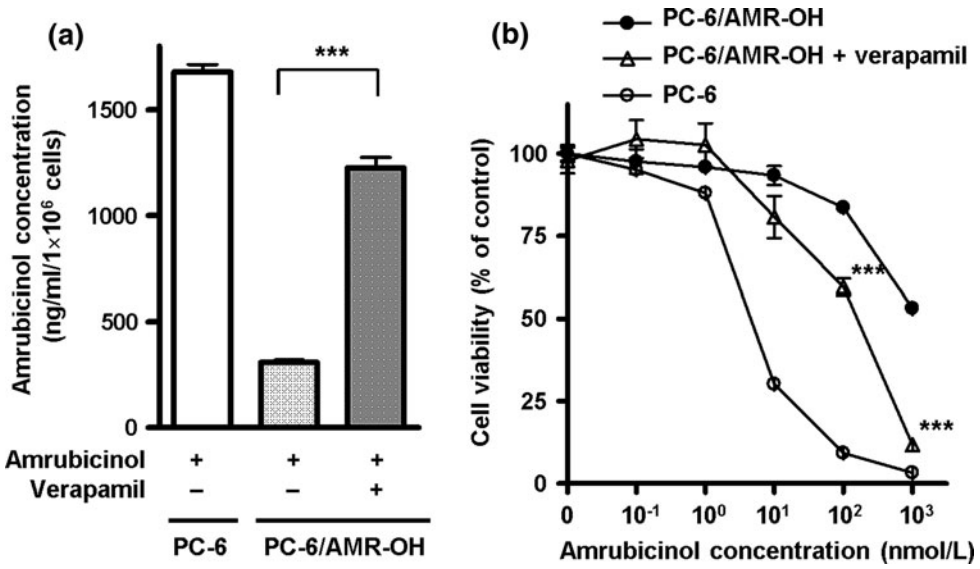

Fig. 2 Alteration of intracellular accumulation of amrubicinol and cytotoxicity to amrubicinol by verapamil. **a** To examine intracellular accumulation of amrubicinol, cells were incubated in 20 μmol/l of amrubicinol for 1 h. In PC-6/AMR-OH cells, 20 μmol/l verapamil was also added in combination with amrubicinol to assess P-gp function. After drug treatment, the cells were immersed in methanol to extract the intracellular drugs. Amrubicinol concentrations of the extract solutions were measured by HPLC. **b** Cytotoxicity to amrubicinol in PC-6, PC6/AMR-OH, and PC6/AMR-OH cells treated with 20 μmol/l verapamil was assessed by MTS-assay. Each point represents the mean ± SD of four independent measurements. ***$P < 0.001$

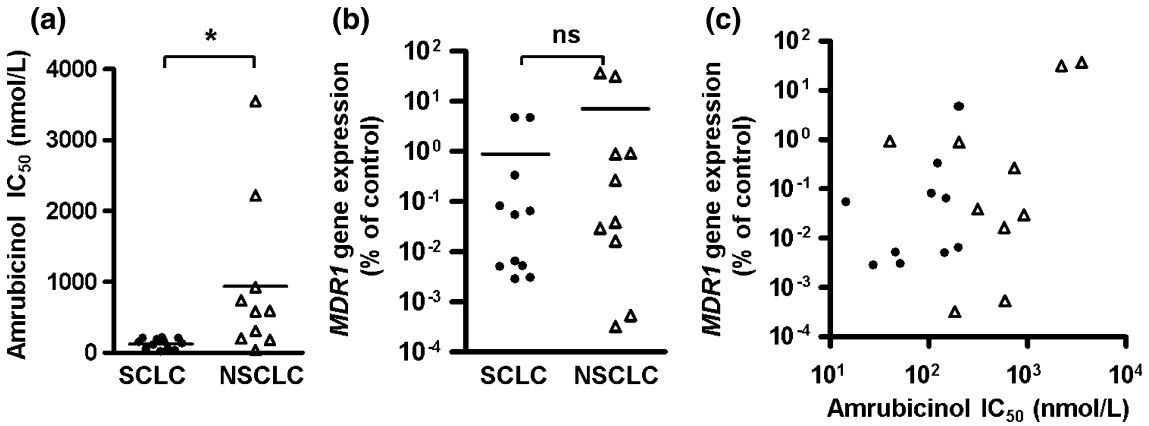

Fig. 3 Relationship between basal expression levels of *MDR1* gene and amrubicinol sensitivity in lung cancer cell lines. **a** Cytotoxicity to amrubicinol was evaluated using MTS-assays in 12 SCLC and 10 NSCLC cell lines. IC_{50} was calculated from dose–response curves. The IC_{50} values for amrubicinol in SCLC and those in NSCLC cell lines are compared statistically. *$P < 0.05$. **b** *MDR1* gene expression levels in the same cell lines were examined using quantitative real-time RT-PCR, and the levels in SCLC and those in NSCLC cell lines are compared statistically. In NCI-N417, which was included among the SCLC cell lines, the gene was not detected. Data are shown for these cell lines, with the exception of this NCI-N417 cell line. *NS* not significantly different. **c** The association between gene expression levels of *MDR1* and IC_{50} values in 21 cell lines in which the gene *MDR1* was detected was examined. No significant correlation is seen between the two. Statistical significances of the correlations were determined by Spearman's test. *Closed circle* SCLC cell lines, *open triangle* NSCLC cell lines

between each genotype and IC_{50} levels for amrubicinol are shown in Fig. 4. Cells carrying the homozygous wild-type allele showed lower IC_{50} levels for amrubicinol than cells that were homozygous or heterozygous for each mutant-type allele, but significant differences were not seen. There were also no significant correlations between the genotypes of each SNP and *MDR1* gene expression levels (data not shown).

Discussion

The *MDR1* gene was dramatically increased in amrubicinol-resistant cells produced by exposure to amrubicinol, and it was confirmed that the P-gp function of excreting amrubicinol was increased in the resistant cells. These results indicate that increased P-gp activity confers acquired resistance to amrubicinol. In contrast, neither

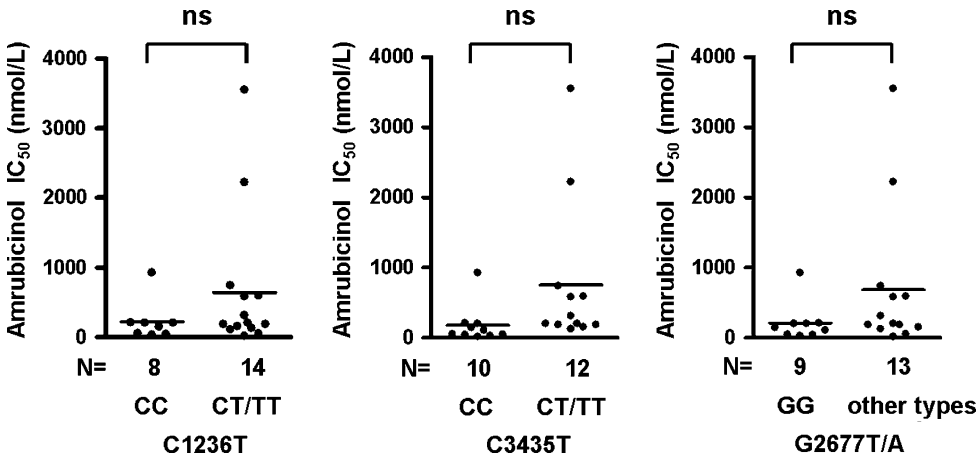

Fig. 4 Relationship between amrubicinol sensitivity and SNPs of *MDR1* gene. Three SNPs of the *MDR1* gene [C1236T in exon 12, C3435T in exon 26, and G2677T/A in exon 21] were assessed in DNA extracted from the aforementioned 22 lung cancer cell lines. The IC_{50} values in cell lines homozygous for each wild-type allele and those in cell lines homozygous or heterozygous for each mutant-type allele are compared statistically. *NS* not significantly different

expression levels nor SNPs of *MDR1* gene were associated with the amrubicinol IC_{50} values in lung cancer cell lines, suggesting that these factors are unlikely to be predictive markers for amrubicin activity.

Members of the ABC super-family of transporter proteins have been implicated as major contributors to the multidrug-resistance phenotypes observed in tumor cells. P-gp plays an important role in multidrug resistance by expelling several structurally and functionally unrelated anticancer drugs. Previously, paclitaxel, SN-38, and doxorubicin were reported as substrates for P-gp, but cisplatin and gemcitabine were not recognized as a substrate for P-gp [17, 18]. In this study, PC-6/AMR-OH cells showed approximately 1,600 times more resistance to amrubicinol compared with the parent cell line PC-6. Furthermore, PC-6/AMR-OH cells showed cross resistance to doxorubicin, paclitaxel, and SN-38, but not to cisplatin and gemcitabine. This pattern of cross resistance is compatible with the assumption that P-gp plays a principal role in this resistance.

Amrubicin is converted to its active 13-hydroxy metabolite, amrubicinol, and the anticancer activity of amrubicin is considered to be closely related to the tumor concentration of amrubicinol [19]. Carbonyl reductase, which is an enzyme that converts amrubicin to amrubicinol, is widely distributed in human tissues, such as the liver, kidney, epithelial cells of the small intestine, and the endothelium of blood vessels [20]. Therefore, the blood concentration of amrubicinol increases quickly after amrubicin administration [21]. Furthermore, the cellular uptake of amrubicinol was reported to be superior to that of amrubicin and doxorubicin [5]. Amrubicinol was also reported to show better susceptibility to P-gp than amrubicin [11]. The mechanism of the difference in the function of P-gp between amrubicin and amrubicinol has not been clarified, but a connection between alcohol metabolites and susceptibility to P-gp was considered by several authors [11, 22]. In this study, amrubicinol was used to develop an in vitro model of amrubicin resistance, and increased *MDR1* gene expression and P-gp function were shown in amrubicinol-resistant cells.

To improve clinical outcomes in advanced lung cancer patients, clinical integration of molecular biomarkers that predict responses to chemotherapeutic agents may be indispensable. For amrubicin, there have been few reports of predictive biomarkers [23]. In the present study, suppression of *MDR1* gene expression by siRNA increased cytotoxicity to amrubicinol in PC-6/AMR-OH cells, which showed that the *MDR1* gene expression level affects amrubicinol cytotoxicity. However, there was no significant correlation between *MDR1* gene expression levels and IC_{50} values for amrubicinol in lung cancer cell lines (Fig. 3c). Lai et al. [24] reported that *MDR1* gene expression levels were low or undetectable in almost all lung cancer cells. The present results are consistent with their results in terms of the low gene expression level. One of the reasons that we could not get the expected result may be dependent on the low *MDR1* gene expression in almost all lung cancer cell lines. Meanwhile, several authors have reported that the frequency of *MDR1* gene expression in clinical samples is higher than that in cell lines in in vitro culture [25, 26]. Indeed, Triller et al. [27] showed that P-gp expression levels in clinical samples of lung cancer correlated with the antitumor effects of cisplatin/etoposide chemotherapy. Thus, further investigations may be needed to determine the clinical role of P-gp. Another problem is that P-gp expression levels in tumors could become up-regulated on relapse after having been exposed to chemotherapy [28, 29], because amrubicin treatment is often selected after first-line chemotherapy [10]. It also remains a possibility that P-gp expression can affect amrubicin efficacy in previously treated lung cancer patients.

SNPs of *MDR-1* gene are also recognized as important factors that can affect P-gp function and expression [15]. There are a number of reports of the correlation between three SNPs [C1236T in exon 12, C3435T in exon 26, and G2677T/A in exon 21] and therapeutic effectiveness or side

effects of substrate agents for P-gp. In lung cancer, G2677T/A [30] and C3435T [31] polymorphisms were reported as predictive markers for treatment response. In the present study, a possible association between these three SNPs of the *MDR1* gene and amrubicinol sensitivity was investigated in lung cancer cell lines. However, there was no significant correlation between the two (Fig. 4), suggesting that these SNPs may not directly affect the response to amrubicin in vitro. Though many studies have addressed the correlation between these SNPs and activity toward specific anticancer agents, their effect on therapeutic efficacy remains controversial [32]. P-gp is expressed in a wide variety of normal tissues, such as intestine, liver, kidney, placenta, and the blood–brain barrier [17], so that SNPs of *MDR1* gene may affect the pharmacokinetics and toxicities of drugs that are substrates for P-gp. Indeed, a correlation between the homozygous variant for *MDR1* 1236TT-2677TT/TA/AA-3435TT and erlotinib toxicity in NSCLC patients has been reported [33]. To assess the involvement of *MDR1* gene polymorphisms for amrubicin treatment, further studies, including those that examine clinical outcomes, are needed.

Amrubicin is highly active and is one of the most potent anticancer drugs against lung cancer. Understanding the molecular mechanisms of resistance to amrubicinol may lead to treatment strategies for amrubicin-resistant tumors and to the improvement of lung cancer patients' survival. The present study indicates that P-gp plays an important role in acquired resistance to amrubicinol. Further investigations are needed to identify a predictive biomarker for amrubicin treatment.

Conflict of interest None declared.

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